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Studies on the Pathogenicity of *Listeria monocytogenes*

I. Protein Substance Isolated from Cells of *Listeria monocytogenes* Enhancing Listeric Infection

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With 9 figures

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The great number of listeric abortion and early infant death observed since 1952/53 in central Europe (of that more than 50 cases within 3 years in the Prague District) was one of the reasons which lead us to study the biologic properties of *Listeria monocytogenes*. Besides studying the pathogenesis of neonatal listeric infection, we have undertaken since 1956 the task of reviewing questions concerning the toxicity of this microbe and its factors of virulence, because accessible data are very incomplete.

Girard (1) described the isolation of an active hemolysin in *L. m.* grown on semi-solid media in an atmosphere with CO_2 according to Burnet's method. The hemolysin was lethal to mice and produced a dermonecrotizing reaction in guinea pigs. Potel obtained from the supernatant of a sonically disintegrated suspension of *L. m.* a substance producing in rabbits a severe dermal reaction. This property disappeared when papain was added, suggesting the protein nature of this toxic substance (2). We were not successful in demonstrating the toxicity of a polysaccharide fraction isolated by Stanley (3) from the cells of *L. m.* strain 109, though we used the same strain and employed the same methods. The lipid component MPA (monocytosis producing agent) also isolated by Stanley is a substance biologically very active, but has no influence on its pathogenicity.

In 1956 we have (4,5) determined a factor in organisms of *L. m.* which enhances listeric infection in white mice, for the lethality of these animals was substantially raised when to the infectious dose killed listeria organisms and in later experiments an aqueous extract of acetone-killed listeriae were added. The effect of the extract was enhanced by precipitation with ammonium sulfate. In further experiments the same active agent was found in glycine lysates of *L. m.* The effect of this agent was at first glance analogous with the

action of salmonella endotoxin in enhancing experimental salmonellosis. But as further studies clarified, this new substance greatly differs in its biologic activity, and, as we may so far conclude from our findings, even in its chemical composition.

It is the purpose of this paper to present the results of our experiments in isolating, purifying and determining the biologic and chemical properties of this active agent.

Material and Methods

Preparation of bacterial organisms:

Listeria monocytogenes strain ŠE, isolated from a case of human adnate listeriosis, was grown in 1 % glucose meat broth in 5 l flasks. Eighteen to twenty-four hour cultures were centrifuged on current flow separator. Centrifuged organisms were washed in saline at pH 7.2, again centrifuged at 10,000 r.p.m. (all at room temperature) and stored at −20° C. The strain was sustained in S-phase by occasional passage on white mice. Cultures before centrifugation were controlled microscopically for possible contaminants and rough forms of listeria. Control cultures were taken from every batch and before final storage of bacterial sediment.

Glycine lysis:

A 10 % suspension of listeriae (moist weight) was prepared in a sterile 1 M solution of glycine, and incubated at 37° C for 24 hours and occasionally agitated. The suspension, after being controlled microscopically, was shaken with sterile sand for 12 hours on an shaking machine (diameter of sand particles 0.5–1 mm, 350 strokes p. m.) and, following centrifugation at 10,000 r. p. m. dialysed over night against running tap water at 13° C. The obtained glycine lysate (GL) was stored at 4° C until used.

Precipitation:

Chilled centrifuged GL was precipitated with methanol at a temperature varying between −10 to 0° C and stored over night at −15° C. The next day the precipitate was centrifuged and dissolved in saline of pH 7.2. Ammonium sulfate precipitation was done at 4° C with solid ammonium sulfate to 50 % saturation. The following day the precipitate was centrifuged and redissolved in saline of pH 7.2, and freed of the remainder of ammonium sulfate by dialysis against tap water for 24 hours. Sodium chloride to a concentration of 0.94 % was added to the nondialysing fraction. The insoluble particles of the purificate were separated and discarded after centrifugation at 10,000 r. p. m. After determining the dry weight of macromolecular substances, solutions to be administered to animals were diluted with buffered saline to 0.1 % (vide infra).

Analyses:

A sample of 2.5 ml was taken and precipitated with equal volume of 25 % TCA. After 15 minutes the precipitate was centrifuged and non-precipitating polysaccharides were determined in the supernatant by Dische's alpha-naphthol method(6). For reasons of control we used in some samples the orcinol method by Heidelberger & Kendall (7), obtaining slightly lower values. The sediment was washed in 5 % TCA and again centrifuged; the precipitated proteins were then dissolved in 1 n NaOH and determined by the biuret reaction in Weichselbaum's modification (8). Nucleic acids were determined in a sample of 1.5 ml by diphenylamine test for desoxyribose according to Sevag, Lackmann & Smolens (9). Lipids were determined according to Swahn (10) with alciholether (3:1) and stained on chromatographic paper (W 1) with Ceres-Schwarz BN (Bayer-Leverkusen). Total phosphorus was determined according to Fiske & Subbarow (11) and nitrogen by kjeldahlizing in Marckham's apparatus (12). Colorimetric measurements were made with Zeiss' universal spectrophotometer using flint glass prism and caesium-antimony photo-cell MSVB. The readings were adjusted to the dry weight of organic substances obtained by usual methods following dialysis of the analysed samples.

Ultra-violet spectrum:

Adsorption maximums of the purified substances were also measured with Zeiss' universal spectrophotometer using sodium chloride prism, quartz cuvettes, and caesium-antimony photo-cell MSVB.

Electrophoresis:

Electrophoresis of the glycine lysate was made on Tiselius' apparatus at 340 V 14 mA in veronal-oxalate-citrate buffer of pH 8.6 and ionic strength 0.66. Resistance of the buffer was 331 ohms.

Determination of activity:

LD_{50} was determined on white mice weighing 13–16 g (strain Bioveta-Kut). Groups of 6 mice were inoculated with *L. m.* strain ŠE from 10^7 to 10^3 of microbes in 0.25 ml of saline. The number of microbes was adjusted according to our turbidity standard. The studied substances were added in the amount of 0.25 ml simultaneously with the inoculum. Each experiment was terminated on the 14th day. Every experiment had its own control groups in view of certain variations in determining LD_{50}, as OSEBOLD & SAWYER (13) already pointed out. Activity was determined by an index expressing the measure by which the LD_{50} is lowered under the influence of the studied substance. Considered as significant enhancement on the basis of many titrations the index = 50 was selected as the lower limit, which corresponds to the logarithm = 1.7. LD_{50} was calculated according to REED & MUENCH (14). In the control group the LD_{50} was determined 25 times in a period of 18 months, the values being: 15 × of the order 10^6, 6 × 10^5, 3 × 10^4, and once 10^7.

Anticomplementarity:

The influence of studied substances in concentrations of 50 to 250 μg per ml on guinea pig complement was determined in the amount of 1 ml according to WALTON et al. (15), using 2–3 units of complement.

Sensitisation:

An attempt at elicitation of SHWARZMAN reaction was made on groups of three rabbits (average weight 2500 g) according to THOMAS (16).

Histology:

Substances were injected intradermally into skins of rabbits in the amount of 0.1 ml, excisions were made at 24, 48, and 72 hours. Histologic examinations of excisions and organs were kindly furnished by Doc. Dr. B. BEDNÁŘ of the Ist Dept. of Pathology, Charles University.

Leukocyte count:

Blood was drawn in rabbits from v. marginalis, in mice by puncture of plexus orbitalis. Counts were made by the usual method in BÜRKER's cell (17).

Results

Effect of glycine lysate on listeric infection.

GL administered intraperitoneally to white mice together with an infectious dose of *L. m.* prominently lowers LD_{50} as against controls. This basic experiment was repeated in a period of 18 months with 5 different batches of lysate and the findings were again reviewed when controlling and comparing the activity of original preparations with purified ones. We found that GL, when given in larger doses, raised the effect expressed by the index, and log respectively as shown in Fig. 1. On the basis of these titrations we selected as the average test dose 250 μg dry weight of nondialysable substances in 0.25 ml, i. e. a 1 % concentration.

The active agent in GL is thermolabile. Incubation at 55° C for 20 min., and at 60° C for 60 min. lowers its activity as shown in Table 1. Boiling inactivates it completely. Deproteinization of GL with 15 % TCA likewise causes inactivation. These experiments suggest that the active agent in GL is a protein, or a thermolabile substance combined with a protein.

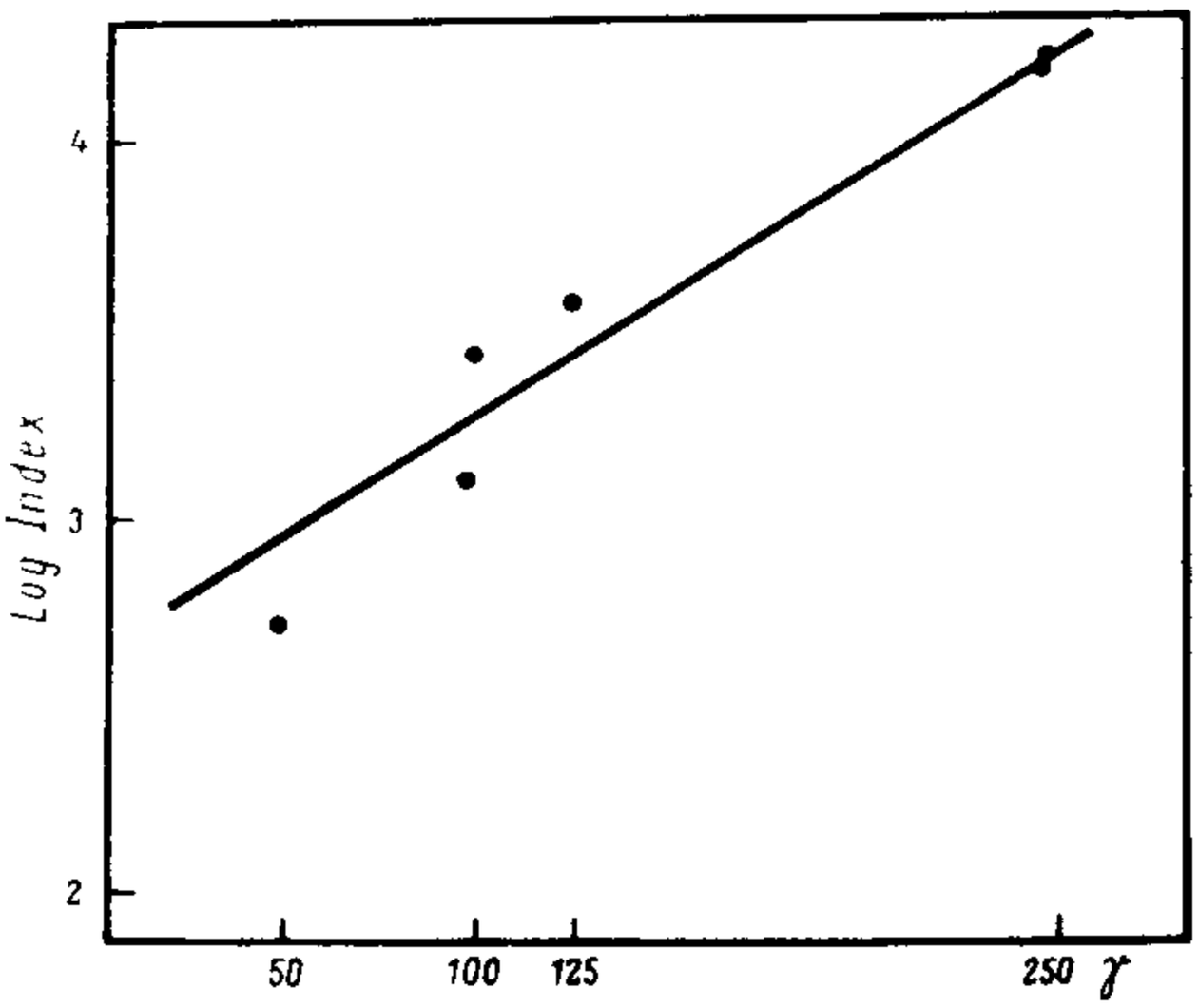

Fig. 1. Relationship of activity of glycine lysate to dosage. Determination repeated after 12 months

Precipitation.

On the supposition that one of the active components is of protein nature, we made an attempt to concentrate protein substances while diminishing the other components of GL. The amounts of basic components were determined in the crude lysate. Table 2 shows the composition of three preparations prepared in a period of 12 months.

Table 1

Influence of temperature and deproteinization on the activity of glycine lysate

Glycine lysate temperature °C	exposure min.	LD_{50} control	LD_{50} test	index	log
		5,728,000	336	16,980	4.231
55	20	1,580,000	3,890	396	2.597
60	60	3,597,000	34,430	104	2.019
deproteinized GL		5,890,000	161,000	29	1.462

Table 2

Chemical composition of crude glycine lysate

Batch No.	Proteins %	Polysaccharides %	DNA %	Total lipids %
1	55.9	15.4	7.0	3.1
2	55.3	12.8	6.1	4.1
3	58.0	17.8	6.5	3.6

The amounts of the determined components in repeated analyses were constant. Small variations in the amounts of polysaccharides are caused by varying amounts of insoluble polysaccharides in the bacterial wall mechanically disrupted.

Methanol precipitation was employed to concentrate the proteins. Table 3 shows that precipitation of GL with 2 volumes of methanol neither increases the amount of proteins, nor diminishes the amount of polysaccharides which do not dissolve in 66 % methanol. Nucleic acids also remain unchanged.

Table 3

Influence of methanol precipitation (2:1) on chemical composition of lysate

	Proteins %	Polysaccharides %	DNA %	Nitrogen %	Phosphorus %
GL	55.9	15.4	7.0	11.1	4.2
M (2:1)	55.5	16.0	6.8	11.9	4.3

Since this method did not lead to purification of proteins we then on employed one volume of methanol. After repeated precipitations nucleic acids gradually diminished, polysaccharides remained in the solution, and proteins were concentrated. The amount of lipids increased; this question will be the subject of another study.

Table 4

Influence of repeated methanol precipitation (1:1) on chemical composition of lysate

	Proteins %	Polysaccharides %	DNA %	Nitrogen %	Phosphorus %
GL	55.9	15.4	7.0	11.1	4.2
M_1*)	63.7	8.4	4.2	11.1	4.5
M_2	69.1	1.9	3.9	13.4	3.4
M_3	69.7	1.5	3.1	12.7	3.3
M_4	72.6	1.3	2.9	14.2	
M_9	85.1	1.8	4.9		

*) The index denotes the number of methanol precipitations.

Table 5

Influence of repeated ammonium sulfate precipitation (to 50 % saturation) on chemical composition of lysate

	Proteins %	Polysaccharides %	DNA %	Nitrogen %	Phosphorus %
GL	55.9	15.4	7.0	11.1	4.2
A_1*)	61.1	5.8	2.3	12.6	4.4
A_2	67.5	5.8	1.5	13.8	4.4
A_3	74.2	5.7	1.2	14.9	5.3
A_4	80.7	4.4	1.0	16.1	5.6

*) The index denotes the number of ammonium sulfate precipitations.

The results with ammonium sulfate precipitation are given in Table 5. Precipitation with ammonium sulfate as against methanol is of advantage, because of the increase of proteins and the marked diminution of nucleic acids. However, the relative amount of phosphorus increases with the decrease of nucleic acids, suggesting the presence of other phosphorus-containing substances, likely of lipid character which both methods are unable to clear. A comparison of both purification methods is given in Fig. 2. The activity of the various purified preparations is outlined in Table 6.

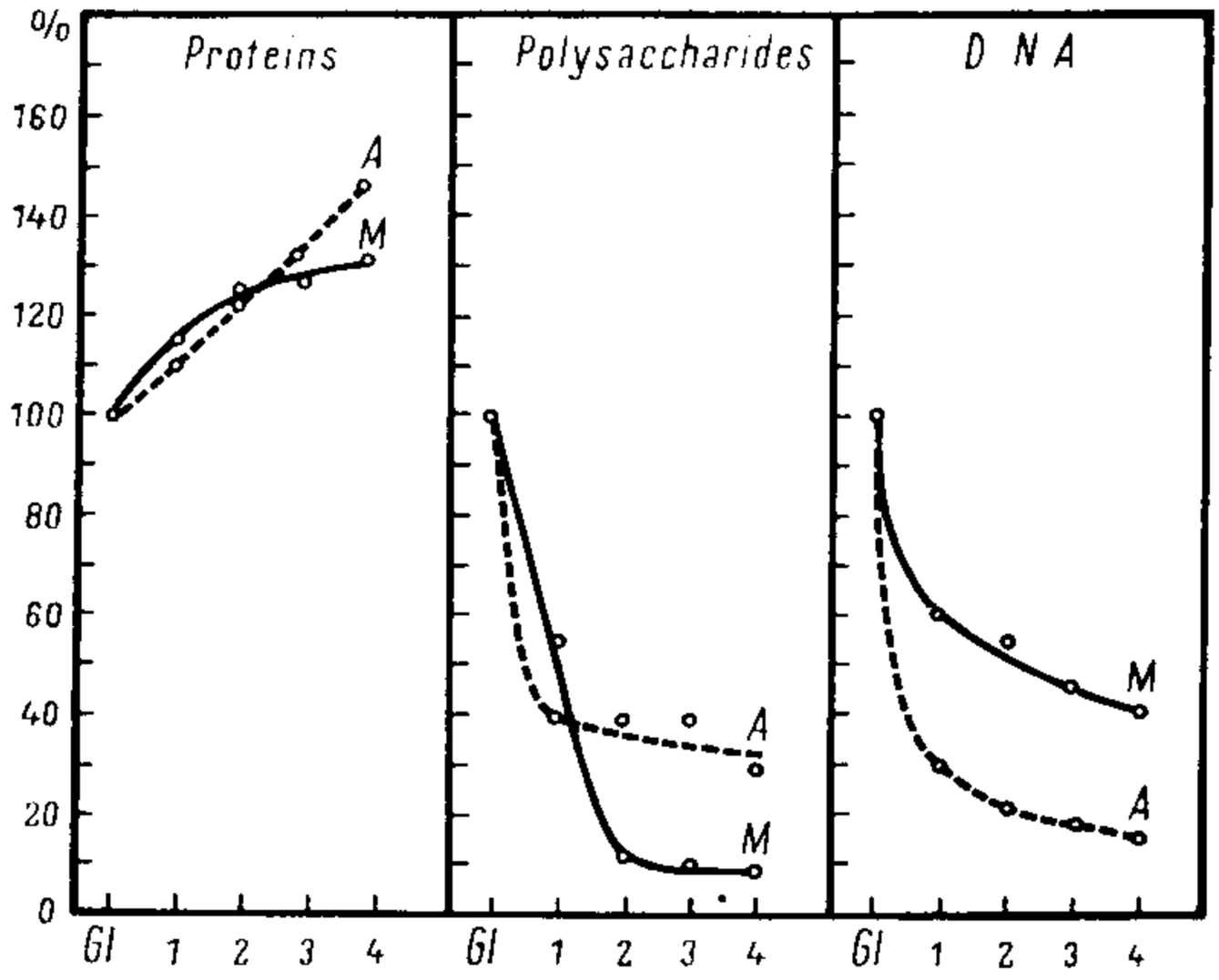

Fig. 2. Change of protein, polysaccharide and desoxyribonucleic acid composition of glycine lysate with ammonium sulphate to 50 % saturation (A) and methanol 1:1 (M). Initial quantities of substances equal 100 %.

Table 6

Activity of various purified preparations

Preparation	LD_{50} controls	LD_{50} test	Index	Log
GL	8,043,000	21,740	378	2.577
M_1 (1:1)	20,400,000	10,500	1,943	3.287
M_3	5,000,000	1,390	3,597	3.555
A_1	20,400,000	11,440	1,783	3.250
A_2	20,400,000	792	25,757	4.410

In accordance with the increase of proteins, and lessening of polysaccharides and nucleic acids the activity did not decrease, but almost in all instances it was enhanced. Certain varying results may be attributed to partial denaturation of the respective samples caused by prolonged preparation. The dependence of the effect on dosage remains similar as in the original preparation (Fig. 3). For these reasons we attempted to obtain a highly active preparation which would be possible to identify more closely. By combining both technics we attained the same degree of purity with only 3 precipitation cycles: in the first step precipitation with an equal part of methanol was employed, and ammonium sulfate to 50 % saturation in the following two. We thus obtained a more highly concentrated preparation of proteins than preparations after nine precipitations using methanol only, or four with ammonium sulfate. The percentage of polysaccharides and nucleic acids diminished in the same degree, or more, than in the preceding monotechnic. The chemical analysis and activity of this purificate designated as MA_2 is presented in Table 7.

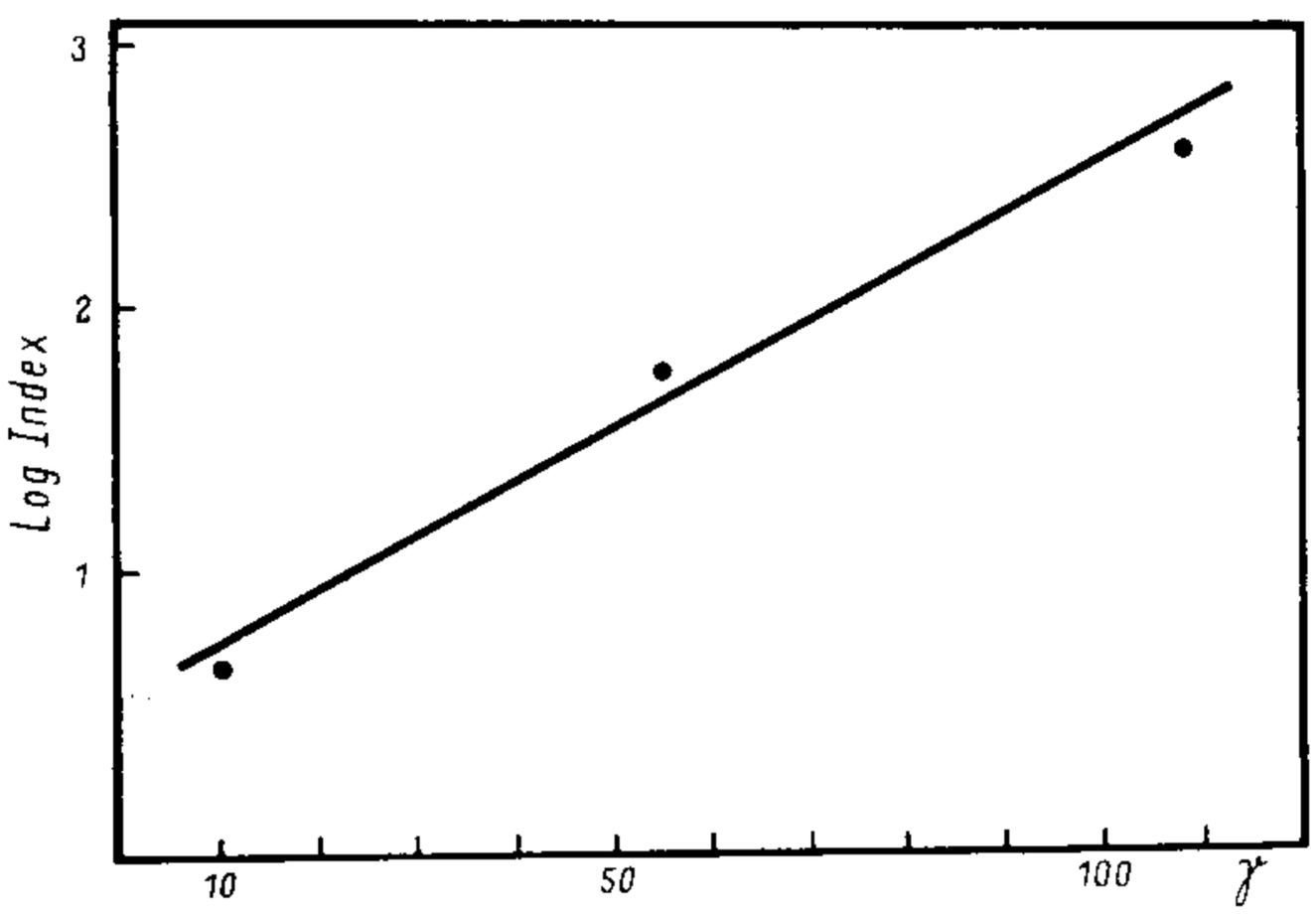

Fig. 3. Relationship of activity of purified glycine lysate $(M_5) - M_5$ (after fivefold methanol precipitation 1:1).

Table 7

Chemical composition of GL and MA_2

	Proteins %	Polysaccharides %	DNA %	Index	Log
GL	58.0	17.8	6.5	16,980	4.231
MA_2	82.5	2.5	4.6	15,870	4.198

Specificity of effect.

So far it seems the glycine lysate (GL) and purificate (MA_2) enhance lethality of white mice to infection by *Listeria monocytogenes*, being specific in their action. Experimental brucellosis and streptococcal or staphylococcal infections remained uninfluenced (Table 8).

Table 8

Influence of GL and MA_2 on various experimental infections in white mice

		Index	Log
Brucella abortus BrO	+ 250 µg GL	1,3	0.113
Micrococcus pyogenes 5112	+ MA_2	3,0	0.470
Streptococcus pyogenes C	+ MA_2	1,6	0.211

Brucella abortus and Streptococcus were injected intraperitoneally, Micrococcus intravenously.

Optimal effect.

The active agent in *L. m.* is effective only when administered simultaneously with the infectious dose. Administration of the agent 24 hours before or after inoculation does not influence the course of listeric infection (Table 9).

Table 9

Enhancement of listeric infection by GL in temporal relationship of its administration to the infection

Time	Index	Log
Simultaneously with infection	1416	3.151
24 hours before infection	16	0.226
24 hours following infection	13	0.131

Other biologic properties.

Neither GL nor partial purificate MA_2 is lethal on intravenous injection to rabbits, on intravenous and intraperitoneal injection to white mice, and on intraperitoneal injection to rats. Rabbits tolerate daily doses of 30 mg intravenously over a period of 6 days. White mice tolerate without any indication of response a single dose of 1 mg intravenously and intraperitoneally. Although a generalized toxic effect of GL and MA_2 was not demonstrated in our experiments, their parenteral administration certainly is not wholly indifferent to the macroorganism.

Intradermal injection of GL and MA_2 produces in rabbits in the site of inoculation erythema with firm infiltration rising above the level of the skin, lasting 3 to 4 days. Histological examination revealed an inflammatory reaction with a considerable number of eosinophils. Mononuclear cells were also found enlarged and in greater numbers. Granulomatous reaction did not develop.

Intravenous injection of 28 mg of GL produces in rabbits an elevation of rectal temperature of up to 4°C (Figure 4). A febrile response is also produced by MA_2 (Figure 5). Rabbits and white mice respond to MA_2 with leukocytosis (Figure 6 and 7). The elevation of circulating leukocytes reached its peak in 4 hours in rabbits. In mice on intraperitoneal injection the peak was reached on the third day with the elevation lasting for 6 to 7 days.

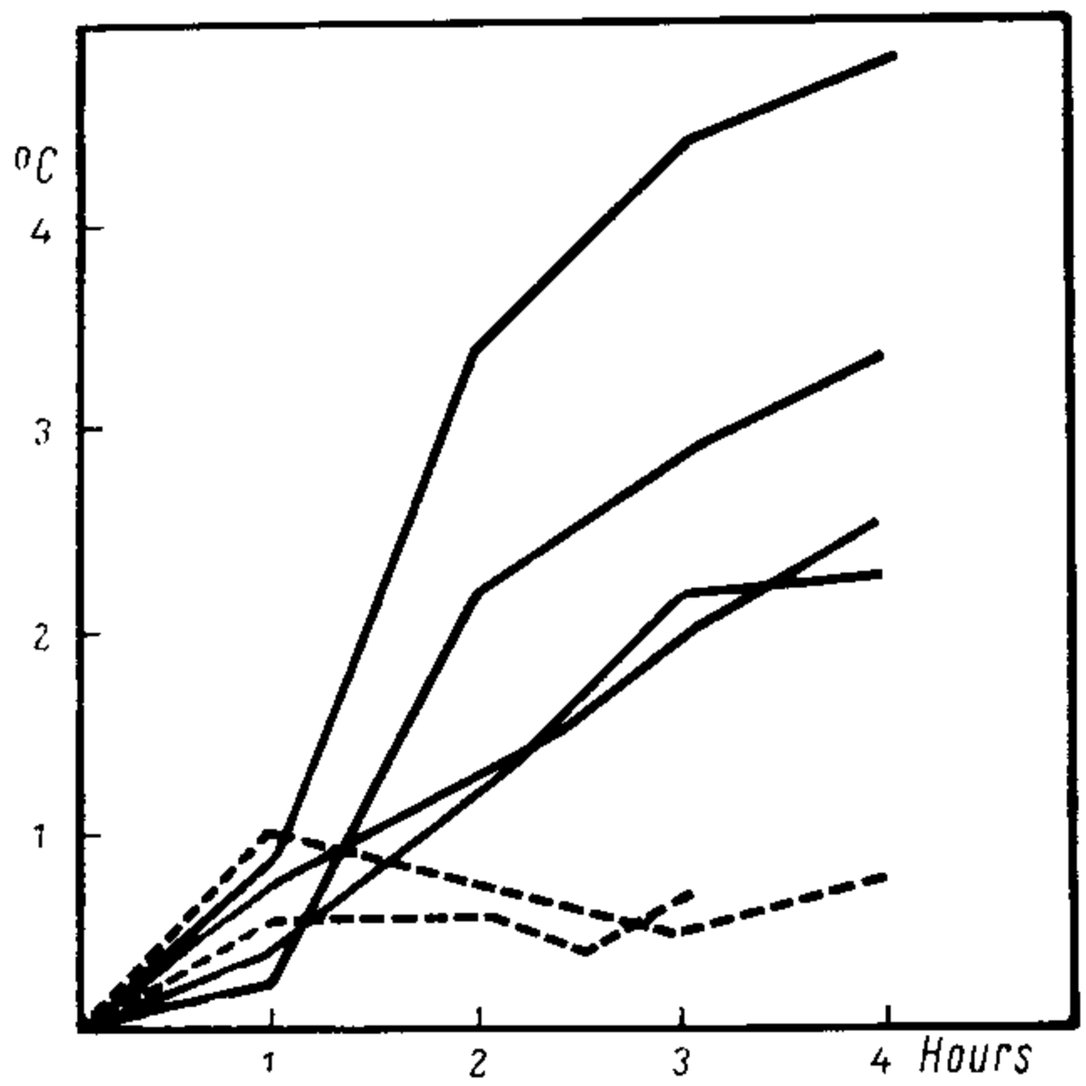

Fig. 4. Elevation of temperature in four rabbits following injection of 28 mg of glycine lysate intravenously. Controls with water are represented by the broken line.

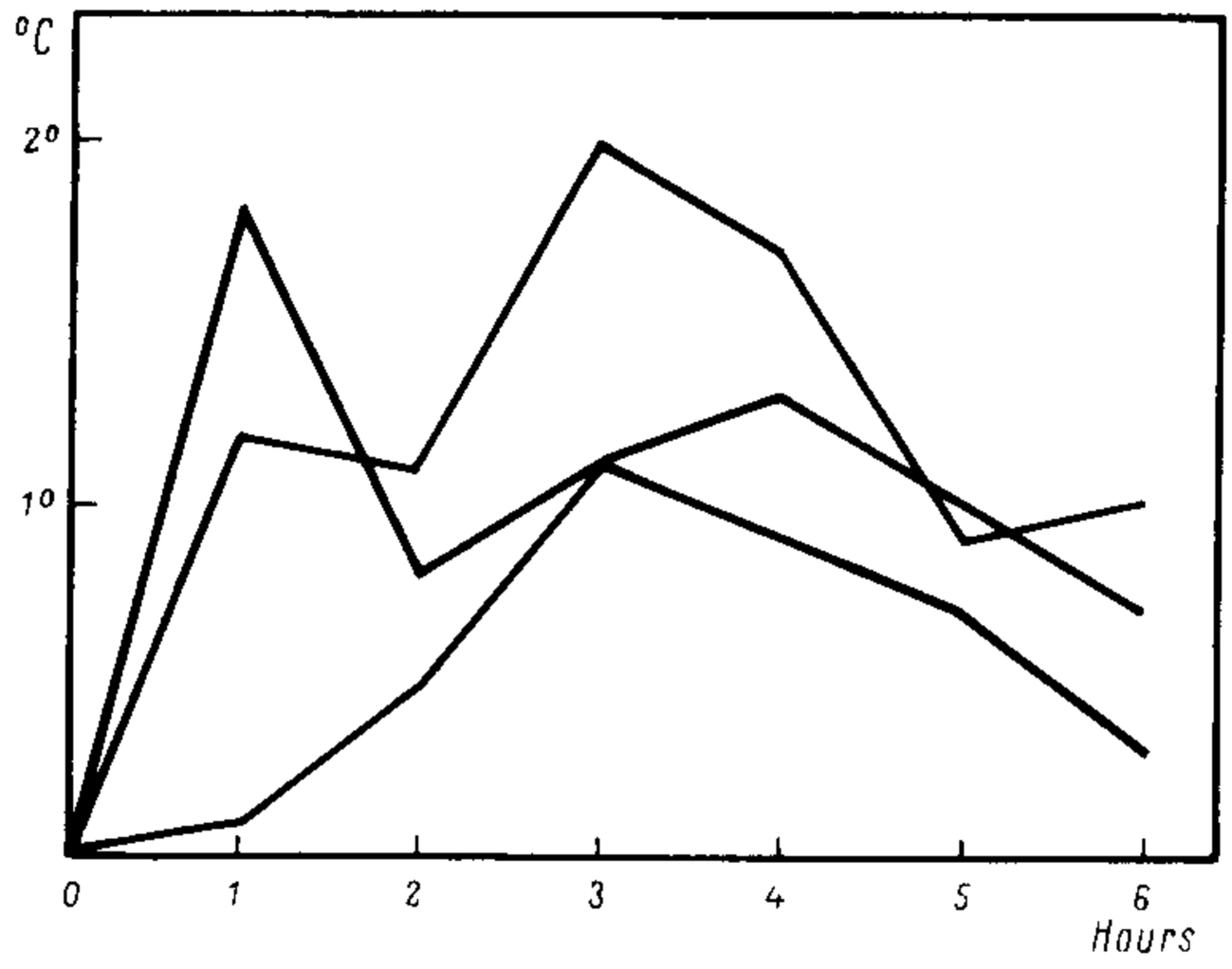

Fig. 5. Elevation of rectal temperature in three rabbits following injection of 45 mg of preparation MA_2.

The preparation of the described substances, their local action in tissues, and pyrogenic activity lead us to compare them with streptococcal endotoxin described by Stetson (18). We therefore attempted to produce hemorrhagic necrosis with epinephrine in skin following intravenous and intradermal injec-

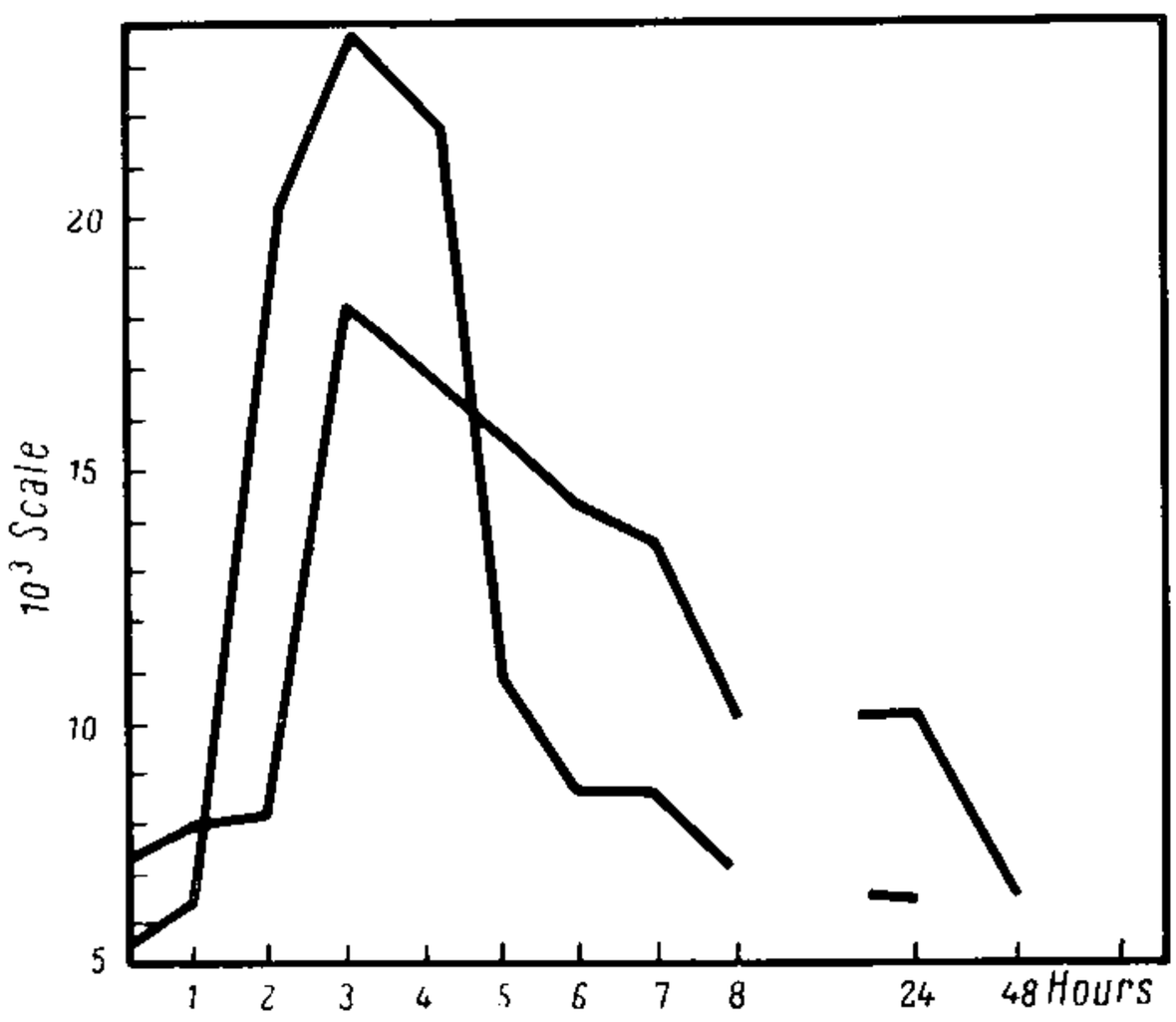

Fig. 6. Leukocyte count in two rabbits following injection of 20 mg of preparation MA_2.

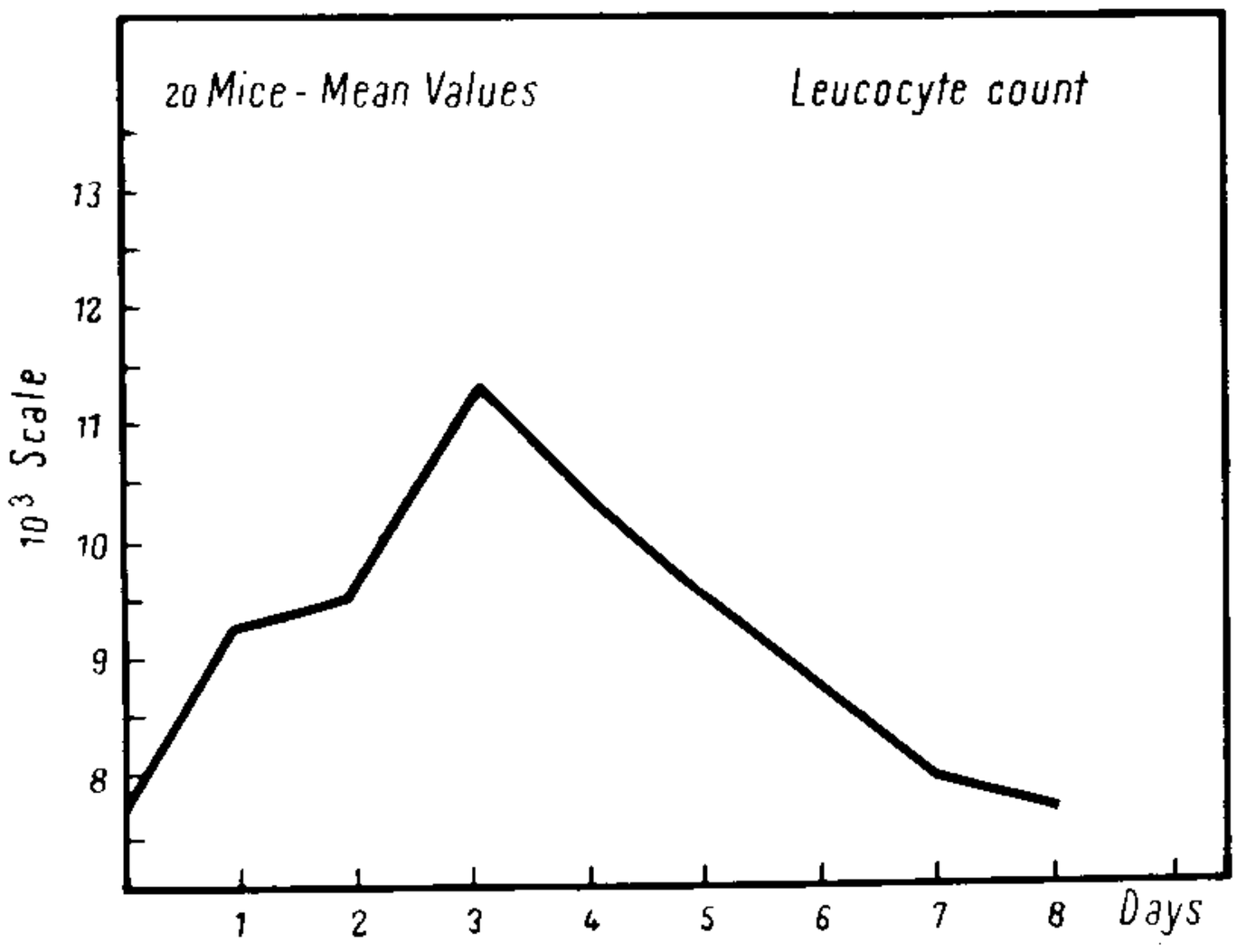

Fig. 7. Leukocyte response in white mice following intraperitoneal injection of preparation MA_2. The average value from 20 mice is given in the chart.

tion of MA_2 in rabbits (16). As against the streptococcal lysate neither GL nor MA_2 produce hemorrhagic necrosis after epinephrine or a local SHWARZMAN reaction. The listeric preparation MA_2 differs from STETSON's lysate also in the failure to produce a generalized SHWARZMAN reaction in a rabbit prepared with 30 mg of cortisone for 3 days in succession.

Chemical properties of the active agent.

The substances are undialyzable, thermolabile, readilly precipitated with TCA. On dialysis the greater part of the substances remain undissolved, but after raising the concentration of salts to 1 % dissolution takes place. The agent is stable in solution at 4° C for at least one month. Merthiolate (1 : 100.000) does not injure it. Solutions of the agent are brownish in color, or in some batches milky, and of great optical density in concentrations suitable for electrophoresis. On dilution the solutions are opalescent and foaming. Well centrifuged samples (10,000 r. p. m. in paper electrophoresis employing veronal buffer of pH 8.6 leave at the start a nonmoving (denatured ?) component.

Electrophoresis.

The electrophoreogram of the protein complex, contained in the preparation of GL combined with mechanical disruption, is presented in Figure 8. The relationship between activity and height of waves of protein components will be the subject of another paper.

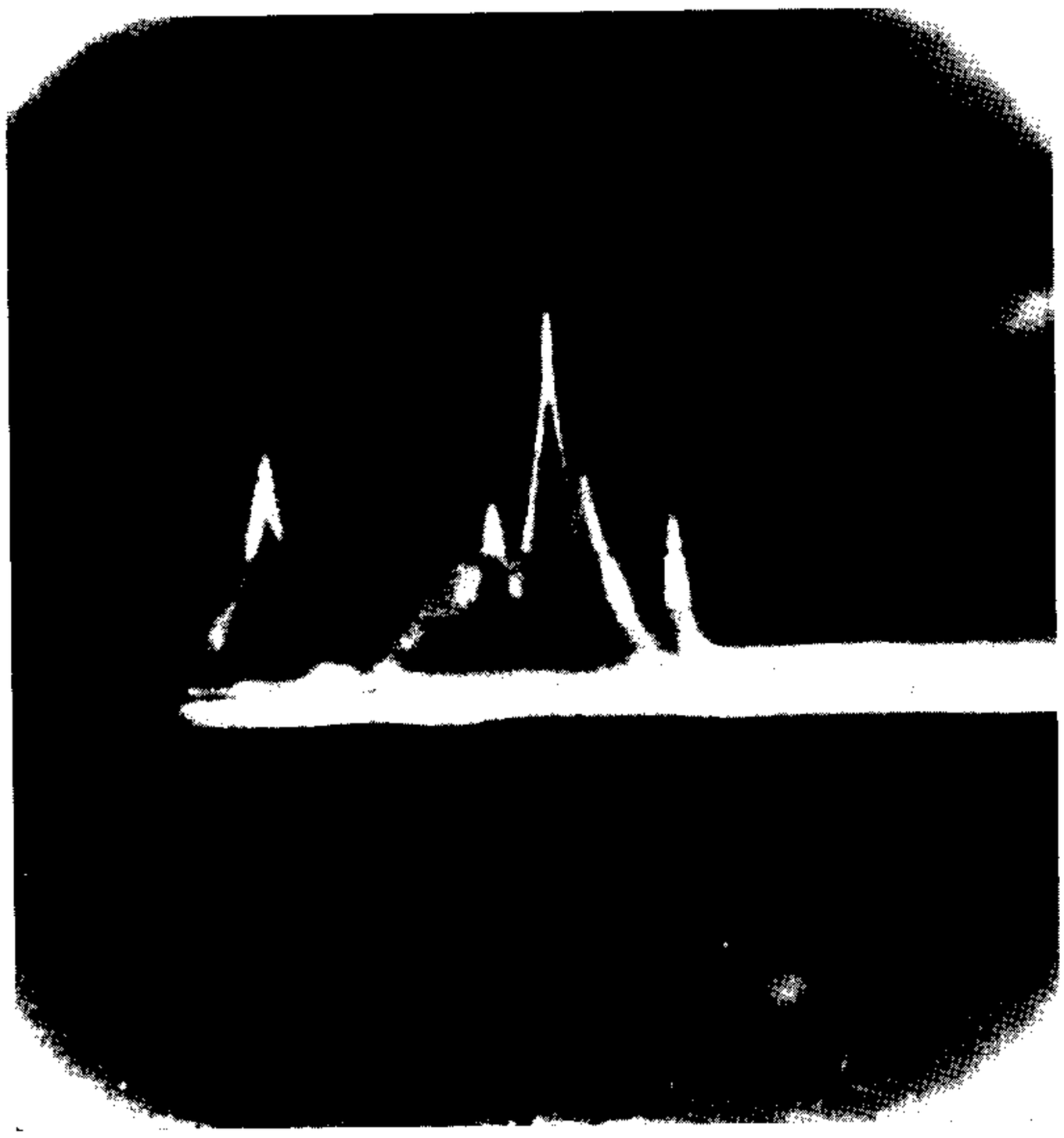

Fig. 8. Electrophoresis of glycine lysate combined with mechanical disruption. Veronal-oxalate-citrate buffer pH = 8.6, ionic strength = 0.06, resistance 331 ohms. Descendent electrophoreogram.

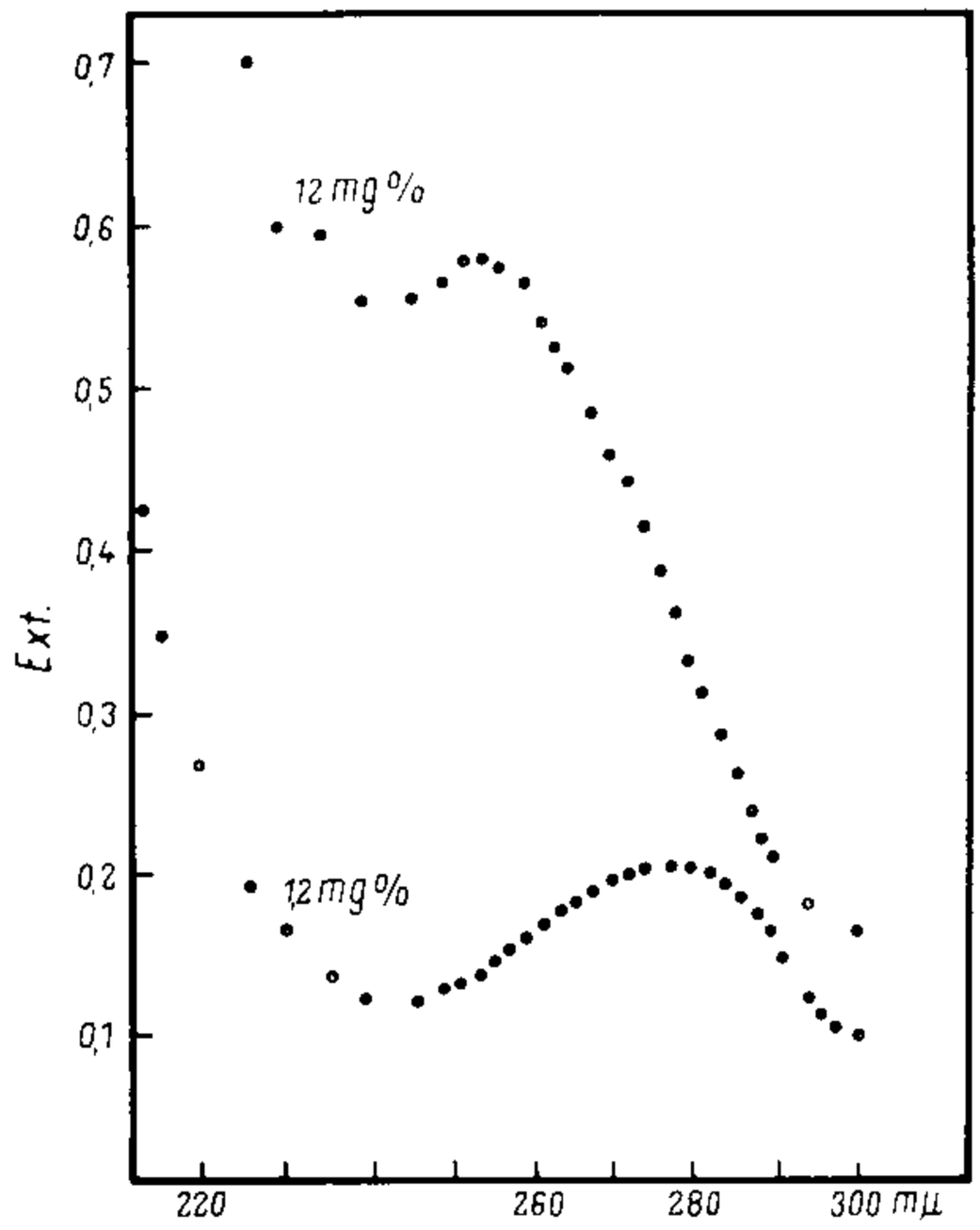

Fig. 9. Absorption curve of preparation MA_2 in ultra-violet range. Concentrations given in figures of undialysable substances.

Ultra-violet spectrum.

UV spectrum of purified preparation MA_2 in two different concentrations presents a pattern characteristic for nucleic acids at 260 mμ, which in higher concentrations obscure the characteristic zone for proteins around 280 mμ (Figure 9).

Anticomplementarity.

Finally, we tested the purified agent for anticomplementary action which could in part elucidate its activity. The purificate MA_2, however is not anticomplementary *in vitro* even in a concentration of 250 μg per ml, using 2–3 hemolytic units.

Discussion

The described experiments are a continuation of our earlier studies. We found that the bodies of virulent listeriae contain a protein, or an agent combined with proteins, which by a hitherto unknown mechanism enhances lethal listeric infection in white mice. The method, used in testing the activity of this agent over a period of 2 years, furnished such regular results that they are unequivocal.

So far we have verified the activity of a combined chemicomechanical lysate of listeric cells and its partial purificate which contained an increased amount of proteins while the amount of bacterial polysaccharides was substantially lesser. Our findings also suggested that the activity of the studied agent is not connected with a nucleoprotein component of the bacterial cell. It remains to be decided whether the infection-enhancing action may be interpreted as synergy of a substance having the character of a mild endotoxin administered with the infection (19), or whether an anti-immunogenic activity of the agent ought not be presupposed, e. g. diminution of the level of complement or properdin. Already, the substantial diminution of polysaccharides in the partially purified preparation (MA_2) suggested that an analogy with the action of endotoxins cannot be drawn, moreover, the existence of such a substance could not be demonstrated either directly or indirectly. The agent also does not produce leukopenia characteristic for endotoxins, but on the contrary produces an elevation of circulating leukocytes. Comparison with Stetson's streptococcal endotoxin (18) shows it to be much less toxic, produces leukocytosis, and does not sensitize to produce a Shwarzman reaction.

In view of the mode of isolation one may be dealing with a complex of active substances of which some may after all have some properties of endotoxin. So far we have not succeded in obtaining a homogenous preparation. Until then it is not possible to define more closely the chemical composition of the active agent. Another paper will deal with the activity of other homogenous and more easily isolated fractions extracted from listeriae.

The purpose of this paper was to describe a new active agent in *L. m.* and to define its properties. The mechanism of the agent's activity will still have to be studied.

Thanks are due to PhMr M. Šťastný for carrying out electrophoresis, to Prof. J. Košťíř for reviewing the chemical data in the report, and to Dr. M. Chýle for the translation and assistance in the preparation of the manuscript.

Summary

By chemical and mechanical disintergration of virulent culture of Listeria monocytogenes a protein agent, partially purified by methanol and ammonium sulfate precipitation, was obtained. This substance is specific in its action and prominently enhances listeric infection in white mice (diminution of LD_{50}). The agent is thermolabile, and so far as determined heterogenous. It does not produce the characteristic effect of endotoxin of Gram-negative bacteria, or of Stetson's streptococcal endotoxin. On intradermal injection the agent produces a transient inflammation, on intravenous injection a febrile response with a rapid elevation of leukocytes. The agent seems to be one of the hitherto unknown factors of virulence or pathogenicity of Listeria monocytogenes.

Zusammenfassung

Durch chemische und mechanische Desintegration virulenter Kulturen von Listeria monocytogenes wurde eine durch Methanol- und Ammoniumsulfat-Fällung teilweise gereinigte proteinhaltige Substanz erhalten. Diese Substanz ist spezifisch in ihrer Wirkung und steigert deutlich die Infektionsfähigkeit der Listeria monocytogenes für die Maus. (Herbsetzung des LD_{50}). Das Agens ist hitzestabil und so weit wie festgestellt heterogen. Es zeigt keine charakteristische Wirkung wie das Endotoxin von Gram-negativen Bakterien oder Stetson's Streptokokken-Endotoxin. dermaler Injektion ruft es eine vorübergehende Rötung hervor, bei intravenöser Injektion eine leichte Fieberreaktionmit schnellem Anstieg der Leukocyten. Das isolierte Agens scheint einer der bisher unbekannten Faktoren der Virulenz und Pathogen ität der Listeria monocytogenes zu sein.

Résumé

Recherches sur le pouvoir pathogène de Listeria monocytogenes
I⁰ Une substance protidique isolée des cellules de Listeria monocytogenes accroît le pouvoir infectieux de Listeria

F. Patocka, J. Schindler et M. Mára

Sous l'effet d'une désintégration chimique et mécanique de cultures virulentes de Listeria monocytogenes, les auteurs ont obtenu une substance à teneur en protéine purifiée par précipitation au méthanol et sulfate d'ammonium. Cette substance est spécifique dans son action et accroît nettement le pouvoir infectieux de Listeria monocytogenes à l'égard de la souris (abaissement de LD_{50}). L'agent est instable à la chaleur et, dans la mesure où l'on l'a constaté, hétérogène. Il ne manifeste, contrairement à l'endotoxine, aucune action caractéristique. Lors de l'injection intradermique, il provoque une inflammation passagère et lors de l'injection intraveineuse une faible réaction fébrile accompagnée d'une ascension rapide des leucocytes. L'agent, une fois isolé, apparaît comme étant l'un des facteurs jusqu'ici inconnus de la virulence et du pouvoir pathogène de Listeria monocytogenes.

Resumen

Estudios sobre la patogenicidad de la Listeria monocytogenes
I. Sustancia proteica aislada de las células de Listeria monocytogenes intensificadora de la infección listérica

F. Patock, J. Schindler y M. Mara

Por medio de desintegración química y mecánica de cultivos virulentos de Listeria monocytogenes se obtiene una sustancia proteica parcialmente purificada por precipitación mediante metanol y sulfato amónico. Esta sustancia es específica en su acción y eleva claramente la virulencia de la Listeria monocytogenes para el ratón (supresión del LD^{50}). El agente es termolábil y hasta cuanto puede determinarse, heterógéneo. No presenta ninguna acción característica como la endotoxina. En inyección intradermica ocasiona una inflamación progresiva, en inyección imtravenosa, una reacción febril leve con rápido ascenso de los leucocitos. El agente aislado parece ser uno de los factores, anteriormente desconocidos de la cirulencia y de la patogenicidad de la Lysteria monocytogenes.

Изучениепатогенетичности Listeriae monocytogenes. I. Выделенное из клеток Listeriae monocytogenes протеиновое вещество усиливает заразительность Listeriae.

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Резюме

Путем химической и механической дезинтеграции вирулентных культур Listeriae monocytogenes было при помощи оседания метанолем и аммоний-сульфатом добыто частично очищенное вещество, содержащее протеин. Это вещество обладает специфическим действием и явно усиливает заразительную способность Listeriae для мыши (понижение LD_{50}). Этот агент теплолабилен и, насколько удалось установить, гетерогене. Он не отличается характерным действием как эндотоксин. При внутрикожном впрыскивании он вызывает проходящее воспаление, при внутривенном введении — легкую лихорадку с быстрым увеличением числа эритроцитов. По-видимому, этот выделенный агент является одним из до сих пор незнакомых факторов вирулентности и патогенетичности Listeriae monocytogenes.

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