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ANNOTATION

PYRIDOXINE AS AN ESSENTIAL GROWTH FACTOR OF LISTERIA MONOCYTOGENES

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Experiences over the past ten year show that *Listeria monocytogenes* must be regarded as a serious pathogenic agent for man. A number of cases of human Listeria infections — especially neonatal — were recorded. The study of the biological properties of this microorganism has not yet been completed.

In the present experiments the authors studied the growth requirements of *Listeria monocytogenes* in practically all the strains isolated during a neonatal epidemic in the Prague region.

The first authors to study the growth requirements of Listeria in semi-defined media were Porter and Pelczar,[1]) who found that on a basic medium composed of vitamin-free casein hydrolysate, inorganic salts and glucose, riboflavin, biotin and haemin were essential growth factors. According to these authors, thiamine is not essential but stimulates growth in the presence of riboflavin and biotin. These authors also tested 36 other substances (vitamins, purine and pyrimidine bases) and found that some of them improved growth but were not absolutely essential. With some strains, casein hydrolysate could be replaced by 25 amino acids, but 18 of the most important did not stimulate growth.

Hutner[2 3]) and Curry et al.[4]) formed a synthetic medium containing amino acids, salts, glucose and riboflavin, thiamine, biotin and thioctic acid (protogen, alpha-lipoic acid), which can be replaced by acetate or pyruvate. Growth was still further enhanced by casein hydrolysate, however.

None of these authors mention the essential influence of pyridoxine on growth of *Listeria monocytogenes.* Patočka and Schindler,[5]) who studied 30 strains of Listeria, found that on casein hydrolysate, containing glucose and inorganic salts and possibly thioglycolate, the essential growth factors were riboflavin, biotin and haemin and, in the case of six strains, pyridoxine, which markedly stimulated growth in the presence of riboflavin. These incomplete experiments were repeated with the aim of demonstrating the influence of pyridoxine as a basic growth factor and of correcting views on the stimulation of growth by some of the vitamins named above.

METHODS

The experimental strains were isolated from human cases of neonatal Listeria infection. All belonged to type I. Type I, II, III, IVa and IVb strains were also used. The strains were maintained on blood agar and were used in the S phase. When preparing the

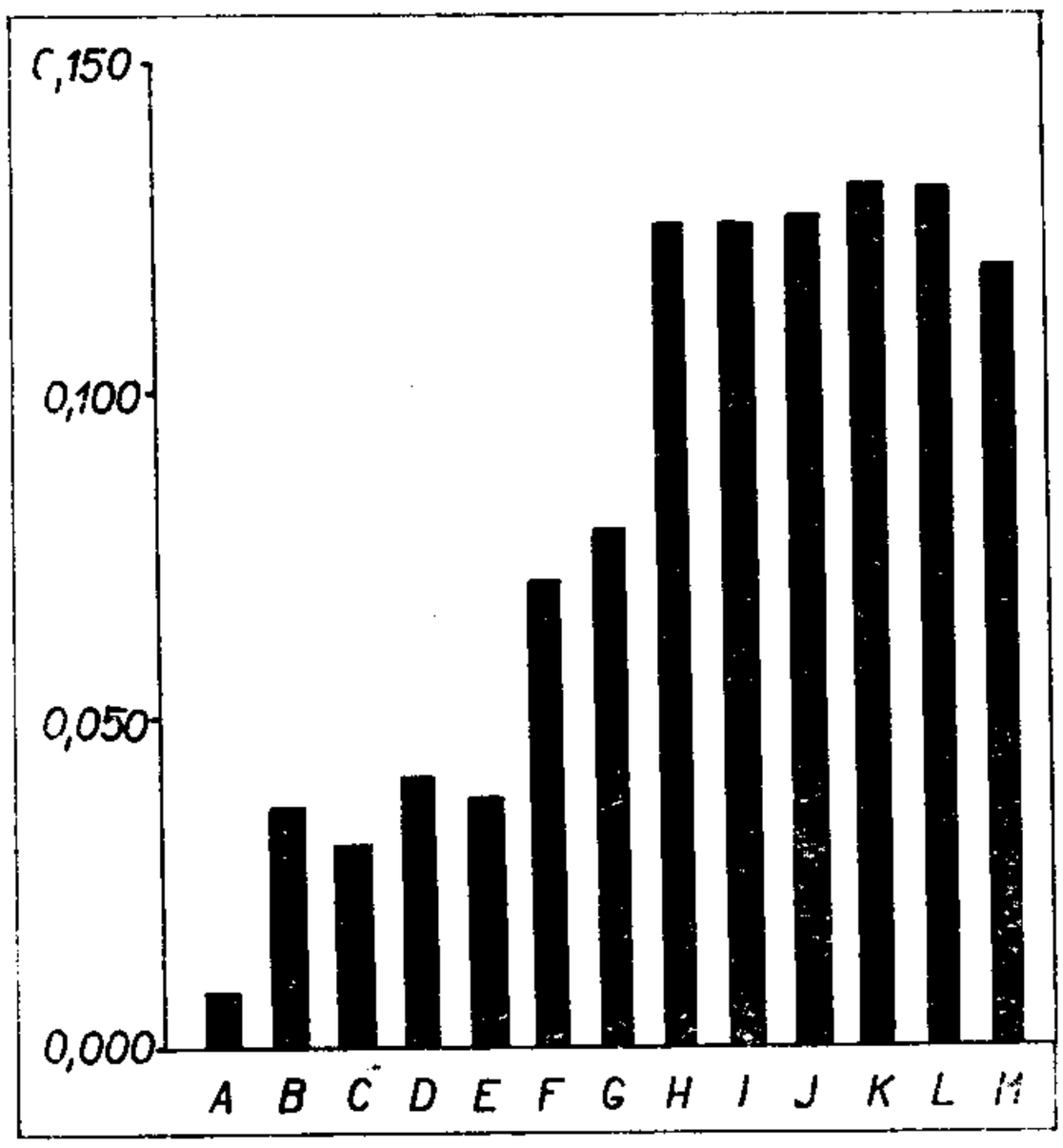

Fig. 1. Growth of 9 strains of *Listeria monocytogenes* (mean extinction-values). A. Glucosa and inorganic salts. B. Basal medium (BM). C. BM with thiamin. D. BM with biotin. E. BM with pyridoxin. F. BM with riboflavin. G. BM with riboflavin and thiamin. H. BM with riboflavin and biotin. I. BM with riboflavin and pyridoxine. J. BM with riboflavin, biotin and thiamin. K. BM with riboflavin, pyrodoxine and thiamin. L. BM with riboflavin, biotin and pyridoxine. M. BM with riboflavin, biotin, pyridoxine and thiamin.

inoculum the bacteria were left to proliferate for 48 hours in broth, after which they were centrifuged at 3,000 r.p.m. for 20 minutes and were washed twice in physiological saline. They were then left for 24 hours at 4^0 C.

The basic solution contained :

Vitamin-free caseo-amino acids (Difco) . . .	2 g.
Monobasic potassium phosphate	1 g.
Magnesium sulphate . 7 H_2O	1 g.
Sodium chloride	15 g.
Glucose	10 g.
Distilled water ad 1,000 ml.	

The chemicals were all analytically pure (p.a.).

The vitamins (Difco Merck) were prepared so as to give final concentrations of 0.1 mg.% riboflavin solution, 0.02 mg.% biotin solution, 0.05 mg.% thiamine solution and 0.05 mg.% pyridoxine solution.

The solutions were mixed, steam-sterilized three times and their pH was adjusted to 6.8 by adding NaOH. They were then filled into calibration tubes and autoclaved under 1 atm. pressure for 15 minutes. A suspension of washed resting cells was then added (0.1 ml. to 5 ml. medium). The initial inoculum contained 160 microorganisms. The cultures were incubated for 24 hours at 37^0 C and extinction was read off on a Hilger photometer using a green filter No. OB2,

RESULTS AND DISCUSSION

In the first experiment all combinations of the four vitamins (riboflavin, biotin, thiamine and pyridoxine) were compared. The results are given in Fig. I, which shows the average extinctions from the measurement of growth of nine

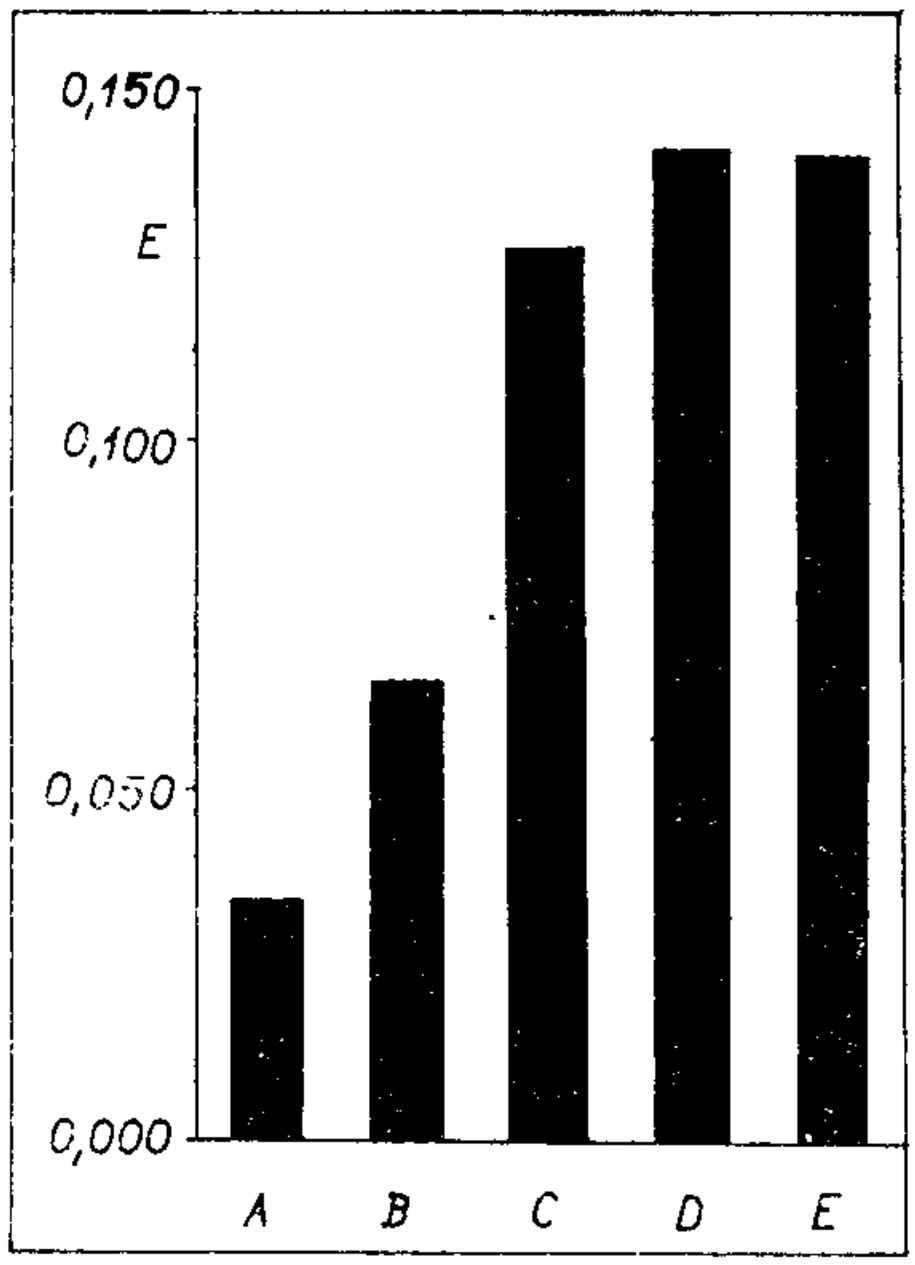

Fig. 2. Growth of 38 strains of *Listeria monocytogenes* (mean extinction-values). A. Basal medium (BM). B. BM with riboflavin. C. BM with riboflavin and biotin. D. BM with riboflavin and pyridoxine. E. BM with riboflavin, biotin and pyridoxine.

strains selected at random. The differences between absolute growth values in the individual strains were negligible. Growth was considerably stimulated by the addition of casein hydrolysate, thus confirming the data in the literature [Porter and Pelczar,[1]) Curry et al.[4])]. Greater stimulation of growth was obtained in all strains by adding riboflavin to medium containing casein hydrolysate. The other vitamins were not essential per se. The addition of biotin to medium containing riboflavin resulted in a very pronounced increase in growth of all the test strains. The same phenomenon was observed in every case, without exception, after adding pyridoxine. As seen from Fig. I, the simultaneous presence of biotin and pyridoxine did not enhance growth any further, indicating that pyridoxine substitutes, as it were, for biotin [Porter and Pelczar,[1]) Curry et al.[4])]. Thiamine did not stimulate growth of the majority of strains, either alone or when added to the medium together with the other vitamins. This contrasts with the findings of Porter and Pelczar[1]) and of Curry et al.[4]), who claim that it stimulates growth in the presence of riboflavin and biotin, but that it is not absolutely essential. Respiration of *Listeria monocytogenes* Še 77 was stimulated on a Warburg apparatus by the direct Warburg method, however, by adding riboflavin and thiamine.

The positive results of potentiation of growth by pyridoxine were confirmed by comparing growth of 38 strains on medium containing casein hydrolysate,

glucose and salts with growth on this medium after adding riboflavin, riboflavin and biotin, riboflavin and pyridoxine and all three vitamins together. The results are given in Fig. 2. This shows the average extinctions of measurement in all 38 strains. Better growth with riboflavin was observed in all. The stimulant effect of biotin and pyridoxine was also manifested, in relative values, in all the strains tested. These experiments confirmed that riboflavin is an absolutely essential factor for all strains of Listeria. The known fact that biotin is important for growth was confirmed in all strains, with the qualification that it requires the presence of riboflavin. Pyridoxine had the same effect in practically all the strains (as distinct from previous experiments), while the simultaneous presence of biotin and pyridoxine in the medium in addition to riboflavin did not stimulate growth any further. Respiration of strains L.m.Še 77, L.m. 109 and T I was not stimulated by pyridoxine, irrespective of whether the medium contained casein hydrolysate or not.

Both the latter vitamins appear to influence in the actual process of proteosynthesis in growth of Listeria monocytogenes on semidefined media, whereas riboflavin together with thiamine stimulates the activity of energy mechanisms in the degradation of sugars.

SUMMARY

1. The authors studied growth of 38 strains of *Listeria monocytogenes* of human origin on a semi-defined medium containing glucose, inorganic salts and vitamin-free casein hydrolysate, with special reference to the influence of the vitamin B group.

2. The only vitamin which stimulated growth alone on this medium was riboflavin. Biotin stimulated growth only in the presence of riboflavin.

3. Another growth factor, which can be substituted for biotin, is pyridoxine. Like biotin, it is effective only in the presence of riboflavin.

4. The influence of thiamine on growth of *Listeria monocytogenes* was not demonstrated.

5. No differences in relative values were observed between the individual strains.

RÉSUMÉ

1. Nous avons suivi chez 38 souches de Listeria monocytogène d'origine humaine la croissance sur milieu mi-défini avec glucose, des sels anorganiques, avec de la caséin-hydrolysate sans vitamines avec le but de constater l'influence des vitamines du groupe B.

2. L'unique vitamine qui à elle-même augmente la croissance sur ce milieu est la riboflavine, tandisque la biotine augmente la croissance seulement en la présence de la riboflavine.

3. Un autre facteur de croissance qui peut substituer la biotine est la pyridoxine agissant de la même manière que la biotine seulement en présence de la riboflavine.

4. Nous n'avons pas réussi à confirmer l'influence de la thiamine sur la croissance de Listeria monocytogène.

5. Les différences entre les souches particulières dans les valeurs rélatives n'ont pas été observées.

ZUSAMMENFASSUNG

1. Wir haben bei 38 Stämmen von Listeria monocytogenes humanen Ursprunges das Wachstum auf einem halbdefinierten Medium mit Glukose, anorganischen Salzen und vitaminlosem Kaseinhydrolysat unter Einstellung auf den Vitamineinfluss der Gruppe B beobachtet.

2. Das einzige Vitamin, das das Wachstum auf diesem Nährboden allein steigert, ist das Riboflavin, wogegen das Biotin das Wachstum bloss in dessen Anwesenheit steigert.

3. Ein weiterer Wachstumsfaktor, der imstande ist das Biotin zu substituieren, ist Pyridoxin, das gleich wie Biotin bloss in Anwesenheit von Riboflavin wirkt.

4. Einen Thiamineinfluss auf das Wachstum von Listeria monocytogenes konnten wir nicht bestätigen.

5. Unterschiede zwischen den einzelnen Stämmen in den relativen Werten wurden nicht beobachtet.

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