

NOTES

NUTRITIONAL REQUIREMENTS OF BACILLUS LARVAE¹

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Bacillus larvae, causal agent of American foulbrood of bees, is a fastidious organism requiring special media for successful cultivation. Crushed brood, egg yolk, yeast, carrot and turnip extracts, and chicken embryo have been used for this purpose, but no attempt to elucidate the specific nutritional requirements of this organism was made until 1942, when it was found to require thiamine for growth on an agar medium containing peptone, glucose, and inorganic salts (Lochhead: *J. Bact.*, **44**, 185). The peptone could not be replaced by casein hydrolyzate or a mixture of known amino acids. An attempt to develop a completely synthetic medium for this organism is briefly reported herein.

Ten growth factors and 18 amino acids (Katznelson and Lochhead: *J. Bact.*, **54**, 83) were added in different combinations to a basal salts glucose fluid medium, and the mixtures inoculated with heavy suspensions of *B. larvae* cells from a 48-hour peptone yeast extract glucose agar culture (Holst and Sturtevant: *J. Bact.*, **40**, 723). Although only fair growth was obtained in this medium, the requirement for thiamine was confirmed with all strains tested. The thiazole and pyrimidine fractions of this vitamin could not replace the intact molecule. None of the remaining growth factors was required; however, subsequent tests with streptogenin² indicated that it was essential for some, though not for all, strains of *B. larvae*. A purine base was necessary for growth of all strains, xanthine and guanine being interchangeable, though adenine was somewhat less effective.

Omission of each amino acid singly from the mixture of 18 in a basal medium containing a purine base in addition to the original ingredients showed that histidine was essential for the growth of all strains. In the absence of cystine, valine, leucine, aspartic acid, tryptophan, arginine, proline, or isoleucine growth was retarded. Considerable strain variation was observed in regard to requirements for these amino acids. The nine acids mentioned above supported fair growth of 12 strains. However, serial transfer was not achieved even in a fluid medium containing 18 amino acids. This was accomplished only on the addition of washed agar to give either a semisolid or solid medium.

Slightly different results were obtained in regard to amino acid requirements when a semisolid agar instead of a fluid medium was used. The omission of histidine or proline suppressed growth completely, as did the omission of tyrosine

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and phenylalanine together, though not singly; without arginine growth was very slight. The remaining amino acids found to be stimulatory in a fluid medium were omitted without effect from the semisolid agar medium.

The need for heavy inocula to initiate growth in both fluid and semisolid media and our inability to culture the organism serially in the liquid medium suggest additional growth essentials. This is borne out by the fact that washed cells grew better in a fluid peptone medium than in the synthetic medium and by the ability of the organism to grow on serial transfer in the former. The replacement of agar by other substances providing increased surface, such as glass wool, crushed glass, and glass beads, was ineffective in stimulating growth, as was aeration.

MIMA POLYMORPHA IN MENINGITIS

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A perusal of the bacteriological literature shows that the bacteria isolated from the spinal fluid in meningitis in sporadic cases may be classified into three groups: (1) a very high percentage of the pathogenic bacteria, (2) many bacteria that are not ordinarily considered pathogenic, and (3) new species that may or may not bear a resemblance to *Neisseria* or other known bacteria. Within the genus *Neisseria*, smear preparations are not sufficient for identification of the microscopically similar species. Murray (Urol. and Cutaneous Rev., **43**, 739) called attention to the importance of the cultural identification with respect to the meningococcus and the gonococcus and was confirmed later by Carpenter and Charles (Am. J. Pub. Health, **32**, 640). Branham (U. S. Pub. Health Service, Pub. Health Repts., **45**, 845) discovered *Neisseria flavescens* by the cultural method during a localized epidemic of meningitis. Pleomorphism is another factor that can confuse a diagnosis by smear preparations. This has been shown by the author (J. Bact., **38**, 119; Iowa State Coll. J. Sci., **16**, 471; J. Lab. Clin. Med., **28**, 710).

A patient (J. C.) was admitted to the hospital in a comatose condition. In the routine examination of the spinal fluid a gram-negative intracellular diplococcus was isolated that resembled the genus *Neisseria*. Since it was obtained from the spinal fluid, it was assumed for the moment to be *Neisseria intracellularis*. However, the cultural characteristics did not conform to that organism and it was given by Miss Margaret Bush to the author for identification. Further studies showed the organism to be *Mima polymorpha*. Cultures from the spinal fluid yielded this organism during the first week of illness, and during this time micro-

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