THE ABSENCE OF α-AMINOADIPIC ACID IN CHOLERA VIBRIO

By EMANUEL WINDSOR

(From the Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena)

(Received for publication, October 3, 1949)

Blass and Macheboeuf reported that they had isolated α-aminoadipic acid from cholera *Vibrio* (1). On repeating the experiment later, they tested the purity of the isolated product by means of paper chromatograms developed with phenol and retracted their earlier claim (2). However, Borsook *et al.* found that α-aminoadipic acid and glutamic acid gave the same spot on two-dimensional paper chromatograms developed with phenol and *s*-collidine (3). The retraction of Blass and Macheboeuf was therefore based on a method which does not differentiate between the two dicarboxylic amino acids.

By using the starch chromatographic method of Stein and Moore (4), Borsook *et al.* were able to separate α-aminoadipic acid from other amino acids, including glutamic acid (3). In this present study an attempt is made by the same technique to determine whether α-aminoadipic acid is present in the cholera *Vibrio*.

Method

Starch columns (0.9 cm. × 30 cm.) were prepared according to the Stein and Moore method. C^{14}-labeled α-aminoadipic acid[1] in a mixture of amino acids was put on a column and eluted with 1 part *n*-butanol, 2 parts *n*-propanol, and 1 part 0.1 N hydrochloric acid (1:2:1 solvent). The position of the radioactivity together with the intensity of the ninhydrin color in the eluate gave a reproducible peak at between 40 and 42 ml. for the α-aminoadipic acid. This is in good agreement with the results of Moore and Stein (5).

Later it was found that proline can be used as a marker, since it is eluted immediately after α-aminoadipic acid (peak at about 50 ml. of eluate) and can be detected by its yellow color with ninhydrin.

Washed and killed cholera organisms which had been raised on potato starch medium[2] were hydrolyzed by refluxing with 20 parts of 20 per cent

[1] The radioactive α-aminoadipic acid was obtained through the courtesy of Dr. Peter H. Lowy. The C^{14} used in this investigation was supplied by the Monsanto Chemical Company, Clinton Laboratories, and obtained on allocation from the United States Atomic Energy Commission.

[2] Supplied through the generosity of Dr. H. A. Dettwiler of Eli Lilly and Company, Indianapolis.

hydrochloric acid for 24 hours on an oil bath. The hydrolysate was filtered and evaporated nearly to dryness *in vacuo*. A quantity equivalent to 2.4 mg., dry weight, dissolved in 0.4 ml. of 1:2:1 solvent was put on a starch column, and the amino acids eluted with the same solvent on an automatic fraction-collector. The 0.5 ml. fractions were tested by the Moore and Stein photometric ninhydrin method (6). No evidence of the presence of α-aminoadipic acid was found.

In other experiments the dicarboxylic amino acids were separated from the cholera hydrolysate by precipitation with calcium hydroxide and absolute ethyl alcohol. Calcium was removed with oxalate and the amino acids chromatographed on a starch column. Again no α-aminoadipic acid was found.

Since there existed the possibility that the cholera *Vibrio* formed α-aminoadipic acid and excreted it into the culture medium, a sample of potato starch medium, after the harvesting of the cholera organisms for cholera vaccine,[2] was chromatographed on a starch column. As in the other experiments, there was no α-aminoadipic acid.

SUMMARY

Cholera *Vibrio* cultured on potato starch medium does not accumulate α-aminoadipic acid.

BIBLIOGRAPHY

1. Blass, J., and Macheboeuf, M., *Helv. chim. acta*, **29**, 1315 (1946).
2. Blass, J., and Macheboeuf, M., *Bull. Soc. chim. biol.*, **29**, 903 (1947).
3. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., *J. Biol. Chem.*, **176**, 1383 (1948).
4. Stein, W. H., and Moore, S., *J. Biol. Chem.*, **176**, 337 (1948).
5. Moore, S., and Stein, W. H., *J. Biol. Chem.*, **178**, 53 (1949).
6. Moore, S., and Stein, W. H., *J. Biol. Chem.*, **176**, 367 (1948).