ISOLATION OF A PEPTIDE IN GUINEA PIG LIVER HOMOGENATE AND ITS TURNOVER OF LEUCINE*

Sirs:

Leucine was synthesized with C^{14} in the carboxyl group. 10 mg. of the radioactive amino acid (DL) and 0.66 gm. (wet weight) of guinea pig liver homogenate were added to a reaction mixture containing 1.3 per cent of an amino acid mixture corresponding to the composition of casein and 0.005 M fumarate, all in a final volume of 4 ml. of isotonic saline solution[1] at pH 7.4. The reaction was carried out under oxygen for 6 hours at 38°.

After precipitation of the proteins by boiling at pH 5.0, the non-protein filtrate was chromatographed on starch with a solvent mixture consisting of 1 part 0.1 N HCl, 2 parts of N-propanol, and 1 part of N-butanol according to the method of Moore and Stein.[2] A radioactive fraction was found which emerged from the column before the leucine. This fraction was concentrated and purified by chromatography on a smaller starch column. The distribution of the color on treatment with ninhydrin and the radioactivity gave a single zone of coinciding ninhydrin color and radioactivity.

The fraction was hydrolyzed with boiling 20 per cent hydrochloric acid for 24 hours, the hydrolysate chromatographed on starch, the fractions analyzed with ninhydrin, and their radioactivity determined as before.

Whereas before hydrolysis only one ninhydrin zone was obtained, after hydrolysis there were many zones. All the radioactivity was in the leucine fraction.

Once the point of emergence of the peptide had been determined by its radioactivity, the non-protein filtrate of guinea pig liver (prepared without prior incubation or any additions) was chromatographed. A peptide was found which emerged in the same position as did that from the incubated digest.

The identity of the amino acids giving the different ninhydrin zones after hydrolysis was determined by chromatography on filter paper[3] with known amino acids as references. The presence of the following was established:

* This work is part of that done under contract with the Office of Naval Research, United States Navy Department.

The C^{14} used in this investigation was supplied by the Monsanto Chemical Company, Clinton Laboratories, and was obtained on allocation from the United States Atomic Energy Commission.

[1] Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, **171**, 363 (1947).

[2] Moore, S., and Stein, W. H., *Federation Proc.*, **7**, 174 (1948). Stein, W. H., and Moore, S., *Federation Proc.*, **7**, 192 (1948).

[3] Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).

alanine, aspartic acid, glutamic acid, glycine, leucine, methionine, phenylalanine, and proline. Other amino acids are present; complete amino acid analysis awaits collection of a sufficient amount of the peptide.

The leucine "turnover," estimated from the radioactivity of the leucine obtained after hydrolysis of the peptide, was 40 per cent in 6 hours.

We have obtained evidence by methods similar to the above of other peptides in guinea pig liver.

The authors are indebted to and wish to thank Dr. S. Moore and Dr. W. H. Stein of The Rockefeller Institute for Medical Research for their generosity and courtesy in giving the details of their method of starch chromatography and ninhydrin colorimetry prior to publication.

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Received for publication, May 14, 1948