INCORPORATION OF SE^{75}-SELENOMETHIONINE INTO PANCREATIC JUICE PROTEINS IN VIVO

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The uptake of amino acids by the pancreas has been recognized for some time and it has been shown that the high degree of concentration in the pancreas is related to the synthesis of pancreatic enzymes (Hansson, 1959). Blau and Manske (1961) recently reported that Se^{75}-selenomethionine also was concentrated in the pancreas.

The present study was performed in order to find out if selenomethionine had the same properties as methionine and was incorporated into pancreatic juice proteins in the same way as has earlier been reported for the amino acid analogues, ethionine and p-fluorophenylalanine, (Hansson and Garzo, 1962).

The Se^{75}-selenomethionine used in these experiments was prepared by yeast biosynthesis (Blau, 1961) and supplied by the Squibb Institute for Medical Research, New Brunswick, N.J., U.S.A. The specific activity was 10 - 20 mC/mM. Chromatographic investigations showed a purity of more than 99 %. The selenomethionine was dissolved in physiological saline and 1 ml containing 25 μC (approx. 0.02 mg) was injected into the femoral vein of two cats (body weight 3.3 and 3.9 kg) which were fasted overnight.

Collection of pancreatic juice was performed by introducing a polythylene tube into the main pancreatic duct. Secretion was stimula-

ted by continous infusion of 10 I.U. secretin and 2 mg carbamylmethylcholine per kg body weight every hour into a femoral vein. Radioactivity was assayed in a Philips gamma ray spectrometer and corrected for background.

The proteins in the pancreatic juice were precipitated by adding an equal amount of 10 % trichloroacetic acid solution to the pancreatic juice. The appearance of proteinbound radioactivity in the pancreatic juice after intravenous injection of Se^{75}-selenomethionine in a cat is shown in fig. 1. The radioactivity showed a peak between one and two hours after injection. The radioactivity was mainly bound to the purified protein fraction and only during the first 15 minutes was a noticable part of the secreted radioactivity in the trichloroacetic acid-soluble fraction. The amount of Se^{75}-secreted into the pancreatic juice was 1.2 and 1.6 per cent of injected dose during a six hour period after injection.

Paper electrophoresis of the lyophylized pancreatic juice was also performed. The radioactivity on the electrophoretic strips and the paper chromatograms was then determined by making autoradiograms with X-ray film or by impulse counting. Five fractions appeared on the Amido Black 10 B stained strips. Radioactivity was found in all these fractions and the radioactivity was proportional to the intensity of the color of the strips.

The radioactive amino acid composition of the pancreatic juice proteins was determined by acid digestion and column chromatography. About 10 mg of pancreatic juice protein from a cat injected with Se^{75}-selenomethionine was digested with 50 ml 6N HCl at 105° C for 18 hours. The solution contained 50 mg of β-mercaptoethylamine as antioxidant and 7 mg carrier methionine. It was carefully deoxygenated and sealed before heating. Chromatography was done on Dowex 50 (Blau, 1961). Eightyfive per cent of the initial radioactivity appeared in a single peak at the position of selenomethionine. In control preparations of Se^{75}-selenomethionine digested along with non-radioactive protein, 95 % was

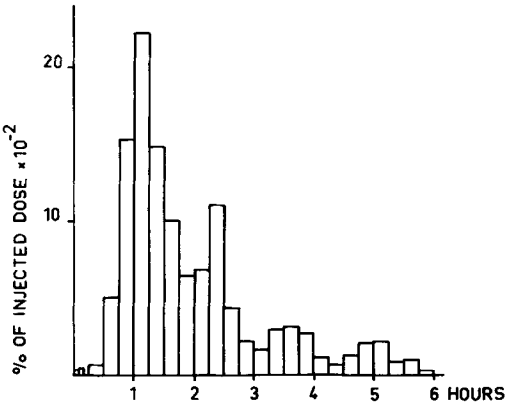

Fig. 1. Appearance of radioactivity in the protein fraction of cat pancreatic juice after intravenous injection of Se^{75}-selenomethionine. The pancreatic secretion was stimulated by continous infusion of secretin and carbamylmethylcholine.

recovered in this peak. When non-radioactive cat pancreatic juice protein was digested as above with $Se_2{}^{75}O_3^{=}$ there was no production of compounds which had amino acid-like chromatographic properties.

The results reported in the present study are in close agreement with the results earlier reported in studies with other amino acids and amino acid analogues. The Se^{75}-selenomethionine seems to be incorporated at the same rate and in the same manner as e.g. methionine, ethionine and p-fluorophenylalanine (Hansson and Garzo, 1962).

It has been known for a long time that selenium is associated with and incorporated into animal proteins. The logical explanation has been that it is incorporated into proteins as selenomethionine and selenocystine (cysteine), (Shrift, 1958). This investigation gives support to this theory by showing that selenomethionine can be incorporated intact into the pancreatic juice proteins without any prior metabolic changes. It is very likely that the selenomethionine replaces methionine since a compe-

tition between these amino acids has been observed in bacteria (Cowie and Cohen, 1957). It is interesting to notice that the selenomethionine is incorporated at almost the same rate as methionine. In this respect it differs considerably from e.g. ethionine, which seems to be discriminated in competition with methionine.

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