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In Vivo Formation of Tyrosine from *p*-Fluorophenylalanine*

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ABSTRACT: When DL-phenylalanine-3-^{14}C and DL-fluorophenylalanine-3-^{14}C are injected into rats, tyrosine-^{14}C is found incorporated into both plasma and pancreas proteins. No phenylalanine-^{14}C derived from fluorophenylalanine-^{14}C is found in proteins. The percentage of the protein radioactivity derived from tyrosine-^{14}C is higher when fluorophenylalanine is injected than when phenylalanine is injected. This would indicate a fast transformation of fluorophenylalanine into tyrosine.

p-Fluorophenylalanine, an analog of phenylalanine, is known to be incorporated into bacterial and mammalian proteins both *in vivo* and *in vitro* (Fruton, 1963). This is explained by its ability to replace phenylalanine in protein peptide chains. However, fluorophenylalanine is known to be very toxic to rats when added to the diet (Armstrong and Lewis, 1951a). This toxicity was attributed to the formation of fluoride ions (Armstrong and Lewis, 1951b).

Recently Kaufman (1961, 1964) has reported that, *in vitro*, fluorophenylalanine is transformed directly into tyrosine at one-sixth the rate of the conversion of phenylalanine to tyrosine. This reaction, which is catalyzed by the enzyme phenylalanine hydroxylase, produces equal amounts of L-tyrosine and fluoride ions. No phenylalanine is formed, and only synthetic pteridine derivatives can act as the cofactor in the transformation.

In our laboratory we are studying the metabolism of phenylalanine-3-^{14}C and fluorophenylalanine-3-^{14}C in rats. We have obtained incorporation of fluorophenylalanine into tissue proteins and wish to report evidence that the radioactive tissue proteins isolated contained two radioactive amino acids, tyrosine and fluorophenylalanine.

Experimental Section

DL-Phenylalanine-3-^{14}C (1 μc/mg) and DL-*p*-fluorophenylalanine-3-^{14}C (1 μc/mg) were injected intravenously to albino Wistar rats weighing about 150 g. Each rat received 1 mg of the radioactive amino acid in 0.5 ml of isotonic NaCl. The rats were killed by decapitation after 2, 6, and 24 hr. The blood was collected and certain organs were removed. The trichloroacetic acid insoluble proteins were obtained from the plasma and the pancreas after homogenization. The dry insoluble proteins were hydrolyzed under vacuum in 6 N HCl at 100° for 18 hr. The hydrolysates were analyzed

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TABLE I: Radioactivity of Protein Precipitates.[a]

	Plasma				Pancreas		
Time (hr)	0[b]	2	6	24	2	6	24
Phenylalanine-^{14}C (cpm/mg)	0.3	56.0	51.0	47.9	225.0	117.0	30.5
p-Fluorophenylalanine-^{14}C (cpm/mg)	0.4	38.9	57.8	33.4	136.0	113.0	18.5

[a] These results are for trichloroacetic acid insoluble proteins obtained from the pancreas after homogenization in distilled water or from the plasma. Samples were taken at 2, 6, or 24 hr after intravenous injection of 1 μc of the radioactive compounds, with six rats used in each group. [b] The zero-time control was obtained by adding 1 mg of the radioactive amino acid in isotonic NaCl to 2 ml of blood plasma. The plasma proteins were then isolated as described in the Experimental Section.

on the Technicon automatic amino acid analyzer. Fractions were collected from the amino acid analyzer, and the radioactivity was determined in a Nuclear Chicago Liquid Scintillation Counter.

Results and Discussion

Amino acid analyses of DL-phenylalanine-3-^{14}C and DL-*p*-fluorophenylalanine-3-^{14}C showed these compounds to be chromatographically pure. Phenylalanine-3-^{14}C contained no tyrosine; *p*-fluorophenylalanine-3-^{14}C contained no tyrosine or phenylalanine.

When phenylalanine-^{14}C or fluorophenylalanine-^{14}C is injected intravenously the proteins obtained from the plasma or the pancreas after 2, 6, or 24 hr are radioactive, as shown in Table I.

When phenylalanine is injected the proteins of all of the organs studied have their highest specific activities 2 hr after injection. Following this the specific activity goes down with time. This decrease is much more important in the pancreas, the organ with the highest specific activity after 2 hr. The plasma, liver, kidney, and brain proteins all have a lower radioactivity.

On the other hand, when *p*-fluorophenylalanine is injected, the highest specific activity for plasma proteins is reached only after 6 hr, as shown in Table I. In the case of the pancreas, the highest specific activity is reached after 2 hr while the 6-hr value is only slightly lower than that obtained at 2 hr. In the first few hours there is no big decrease in activity as there was in the case of phenylalanine-^{14}C, which showed a 50% decrease. It seems evident from Table I that at first more phenylalanine-^{14}C than fluorophenylalanine-^{14}C is incorporated into proteins. From quantitative determinations we found that after 2 hr the amount of fluorophenylalanine-^{14}C in pancreas proteins is 7.6% of the total phenylalanine content while that in plasma proteins is 3.7%.

In each case when the radioactive proteins are hydrolyzed and the hydrolysates analyzed on the amino acid analyzer, two radioactive amino acids are found. In proteins obtained after injection of phenylalanine the two radioactive amino acids were found to be tyrosine and fluorophenylalanine. No radioactive phenylalanine was found. Figures 1 and 2 show typical radioactivity curves obtained from pancreas proteins. In Figure 2 the two radioactive peaks correspond to the position of tyrosine and fluorophenylalanine. There is about one-third more radioactivity in the phenylalanine peak of Figure 1 than in the fluorophenylalanine peak in Figure 2. However, there is slightly more radioactivity in the tyrosine peak of Figure 2 than in the one of Figure 1.

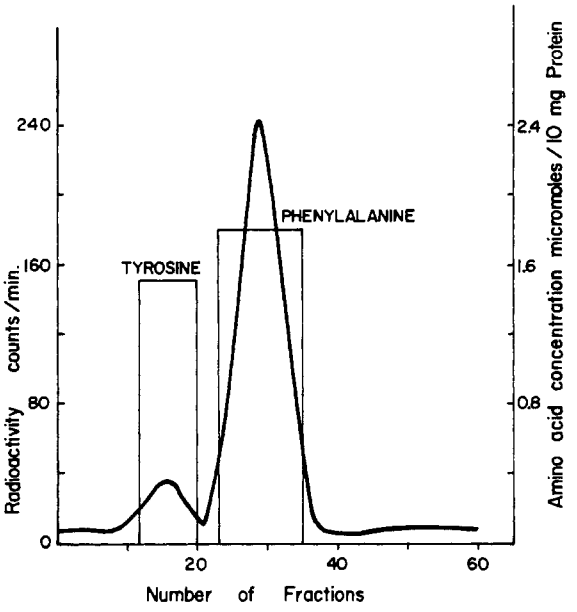

FIGURE 1: Results of the analysis of a pancreas protein hydrolysate on the amino acid analyzer. The proteins were obtained 2 hr after the injection of DL-phenylalanine-3-^{14}C into rats. Only the section of the chromatogram showing the position and concentration of tyrosine and phenylalanine is shown here. The curve was obtained by plotting the radioactivity contained in the fractions collected from the amino acid analyzer. The horizontal lines indicate the quantity of amino acids found in 10 mg of proteins.

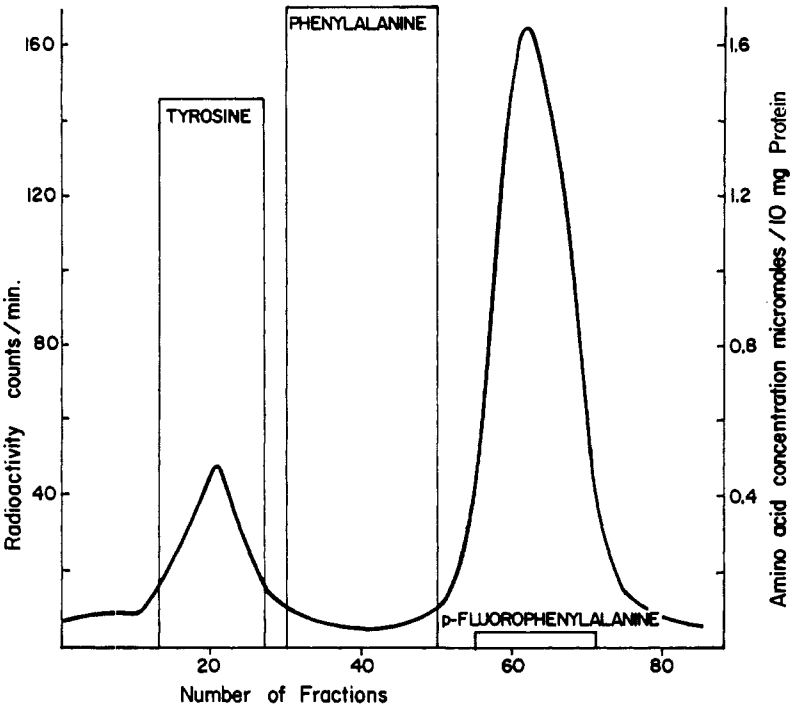

FIGURE 2: Amino acid analysis of a pancreas protein hydrolysate obtained 2 hr after the injection of DL-*p*-fluorophenylalanine-3-^{14}C into rats. The graph was prepared in the same way as that in Figure 1.

TABLE II: Ratios of Radioactivity[a] (Tyrosine/Phenylalanine or /Fluorophenylalanine).

	Plasma			Pancreas		
Time (hr)	2	6	24	2	6	24
Phenylalanine-^{14}C	0.28	0.33	0.29	0.15	0.18	0.22
p-Fluorophenylalanine-^{14}C	1.42	1.18	0.74	0.32	0.19	0.64

[a] These results are the ratios of the total radioactivity found in the tyrosine peak over the total radioactivity found in the phenylalanine or fluorophenylalanine peaks obtained from protein hydrolysates analyzed on the automatic amino acid analyzer.

In order to have some indication of the rate of transformation of phenylalanine or fluorophenylalanine to tyrosine, the ratios of the radioactivity of tyrosine over that of phenylalanine or fluorophenylalanine were determined. Udenfriend and Bessman (1953) have studied the conversion of phenylalanine to tyrosine in normal and phenylketonuric patients and in dogs. The ratios of specific activities of the two amino acids in dog plasma proteins 3, 6, and 24 hr after injection of phenylalanine-^{14}C were found to be 0.20, 0.24, and 0.29, respectively. About one-fourth of the protein radioactivity was provided by tyrosine derived from injected phenylalanine.

In Table II, ratios of the radioactivity of tyrosine over that of phenylalanine or fluorophenylalanine are given. When phenylalanine is injected, the ratio in plasma proteins is almost constant over 24 hr. In the pancreas, the ratio is lower at first, then increases slowly during the next 24 hr, indicating an increase in the percentage of radioactivity due to tyrosine-^{14}C.

The ratios obtained are about the same as those reported by Udenfriend and Bessman (1953). That more tyrosine is first incorporated into the plasma proteins may be explained by the facts that the phenylalanine hydroxylase is located mainly in the liver and that many of the plasma proteins are synthesized in the liver. Also it would seem that the newly formed tyrosine-^{14}C in the liver does not rapidly reach the pancreas. This may indicate the existence, in liver, of an amino acid pool for protein synthesis which is not in equilibrium with the plasma free amino acid pool. This existence of such a pool has been suggested recently by Schapira *et al.* (1962) and Ito *et al.* (1964). These authors have observed a difference in behavior between radioactive tyrosine derived from injected radioactive phenyl-

alanine and injected radioactive tyrosine. When fluorophenylalanine-^{14}C is injected, the percentage of radioactivity due to tyrosine-^{14}C (after 2 hr) is higher in the plasma proteins than in the pancreas proteins (Table II). The decrease in ratios first observed in the pancreas, between 2 and 6 hr, may be an indication that little tyrosine-^{14}C reached the pancreas in the first few hours. After 6 hr (as shown by the ratios) there is a big decrease in the percentage of tyrosine-^{14}C incorporated in plasma proteins and an increase in the pancreas proteins. The higher ratios obtained when *p*-fluorophenylalanine is injected are the result of a slightly lower incorporation of *p*-fluorophenylalanine-^{14}C and a higher incorporation of tyrosine-^{14}C. The increased availability of tyrosine-^{14}C for incorporation may be the result of a faster conversion of fluorophenylalanine into tyrosine or of a longer metabolic half-life for the fluorophenylalanine as compared to the phenylalanine.

The incorporation of fluorophenylalanine into proteins is well established, but its transformation into tyrosine *in vivo* has never been reported. The studies on this subject can be divided into three groups: (1) incorporation into bacterial proteins, particularly in *Escherichia coli;* (2) incorporation into mammalian proteins *in vitro;* (3) incorporation into mammalian proteins *in vivo*. The enzyme phenylalanine hydroxylase is not found in *E. coli*. It is probable that fluorophenylalanine is not transformed into tyrosine in these bacteria. As far as the second group of studies is concerned, most systems used were also devoid of phenylalanine hydroxylase. In ovalbumin obtained from hen's minced oviduct system, Vaughan and Steinberg (1960) have reported that 80% of the radioactivity was recovered as fluorophenylalanine. In hemoglobin isolated from rabbit reticulocytes *in vitro*, Kruh and Rosa (1959) have studied the distribution of radioactivity in amino acids using paper chromatography. They found no radioactivity in the tyrosine spot and only a little in the phenylalanine spot. This latter they had attributed to contamination. Using rabbit reticulocyte ribosomes, Arnstein and Richmond (1964) have reported that fluorophenylalanine is incorporated unchanged.

In vivo studies in mice, cats, and rabbits have been reported. In both mouse pancreas proteins (Hansson *et al.*, 1962) and in cat amylase (Hansson and Garzo, 1962) it was reported that on paper chromatograms the radioactivity was almost completely confined to the fluorophenylalanine spot. Westhead and Boyer (1961) have fed fluorophenylalanine-^{14}C to rabbits for a few weeks and then isolated two muscle enzymes, aldolase and glyceraldehyde 3-phosphate dehydrogenase. Radioactivity of the isolated aldolase (87%) was recovered in the fluorophenylalanine spot. Nonquantitative elution was blamed for the 13% loss. When amylase was treated by carboxypeptidase only 10% of the radioactivity which could be expected if fluorophenylalanine had completely replaced the C-terminal tyrosine was obtained. It was concluded that fluorophenylalanine cannot replace tyrosine in the peptide chains. These results could also be taken as proof of incorporation of tyrosine-^{14}C derived from fluorophenylalanine-^{14}C into amylase.

In rat protein hydrolysates, fractionated on the amino acid analyzer, we have found some tyrosine-^{14}C as described above. In some cases the quantity of tyrosine-^{14}C incorporated is higher than that of fluorophenylalanine-^{14}C. This may seem surprising, but one must remember that rat liver is known to contain a very highly active phenylalanine hydroxylase. Wang and Waisman (1963) have reported that rats have between 12 and 16 times as much liver phenylalanine hydroxylase as do other species.

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