

Uptake of C^{14} -*para*-Fluorophenylalanine in the Tissues of Mice

It was reported recently¹ that ethionine showed almost the same tissue distribution pattern as that found after the administration of methionine. The methionine analogue was accumulated in the pancreas and liver as well as in the tissues of the foetuses. Furthermore it turned out that the analogue existed mainly in the TCA soluble fraction and that only a very small portion was incorporated into proteins even after 24 h. Contrary to this, it was found that in the case of a naturally occurring amino acid, methionine, the specific radioactivity in the proteins of the investigated organs was at least 10 times higher than in the TCA (trichloroacetic acid) soluble fractions even after 1 h.

According to these findings, it seemed of some interest to investigate the uptake and incorporation into protein of another amino acid analogue, *p*-fluorophenylalanine which is known to be incorporated into animal tissue^{2,3}.

Two days before the expected parturition, pregnant mice weighing about 30 g, were injected with C^{14} -(3)-*DL*-*p*-fluorophenylalanine (Centre d'Études Nucléaires de Saclay, France) intravenously. The specific radioactivity of the substance was 3.5 mC/mM. It was dissolved in physiological saline and 0.2 ml of this solution containing 0.2 mg *p*-fluorophenylalanine (3.8 μ C) was injected intravenously into each animal. The mice were sacrificed 15 min, 1, 4, and 24 h later. In one series the animals were subjected to autoradiography, according to a technique described earlier⁴. In another series, the foetuses, as well as the pancreas and liver of the mothers, were removed and homogenized in 10% TCA and the radioactivities in the TCA soluble and the washed and purified protein fractions were investigated as previously described^{1,5}.

The autoradiograms (Figure) showed a distribution pattern which was very similar to that which is obtained

after injection of naturally occurring amino acids⁶. The radioactivity accumulated rapidly in pancreas and in other organs which are the site of a rapid protein synthesis, such as the gastro-intestinal mucosa, liver, salivary glands and bone marrow.

The analysis of the TCA soluble and the purified protein fractions showed a pattern fairly similar to that obtained with natural amino acids^{1,6,7}. The variation with time of specific radioactivity is given in the Table. The specific activity of the protein fraction of the pancreas was, after 15 min, about equal to that of the TCA soluble fraction and after 1 h the ratio was about 10 to 1.

Paper chromatography was performed after acid hydrolysis of the purified protein fraction and the radioactivity on the chromatograms was determined by means of autoradiography. The radioactivity was recovered mainly in the spots corresponding to *p*-fluorophenylalanine.

Judging from our results obtained by radioactivity analysis with C^{14} -labelled compounds, *p*-fluorophenylalanine behaves very much like a natural amino acid both regarding the cell uptake and the incorporation in tissue proteins. In these respects it differs considerably from ethionine, which is incorporated to a much lower degree into proteins.

There is a theoretical risk that the values obtained reflect the uptake and incorporation of a product succes-

¹ E. HANSSON and T. GARZO, *Exper.* 17, 501 (1961).

² M. VAUGHAN and D. STEINBERG, *Biochim. biophys. Acta* 40, 230 (1960).

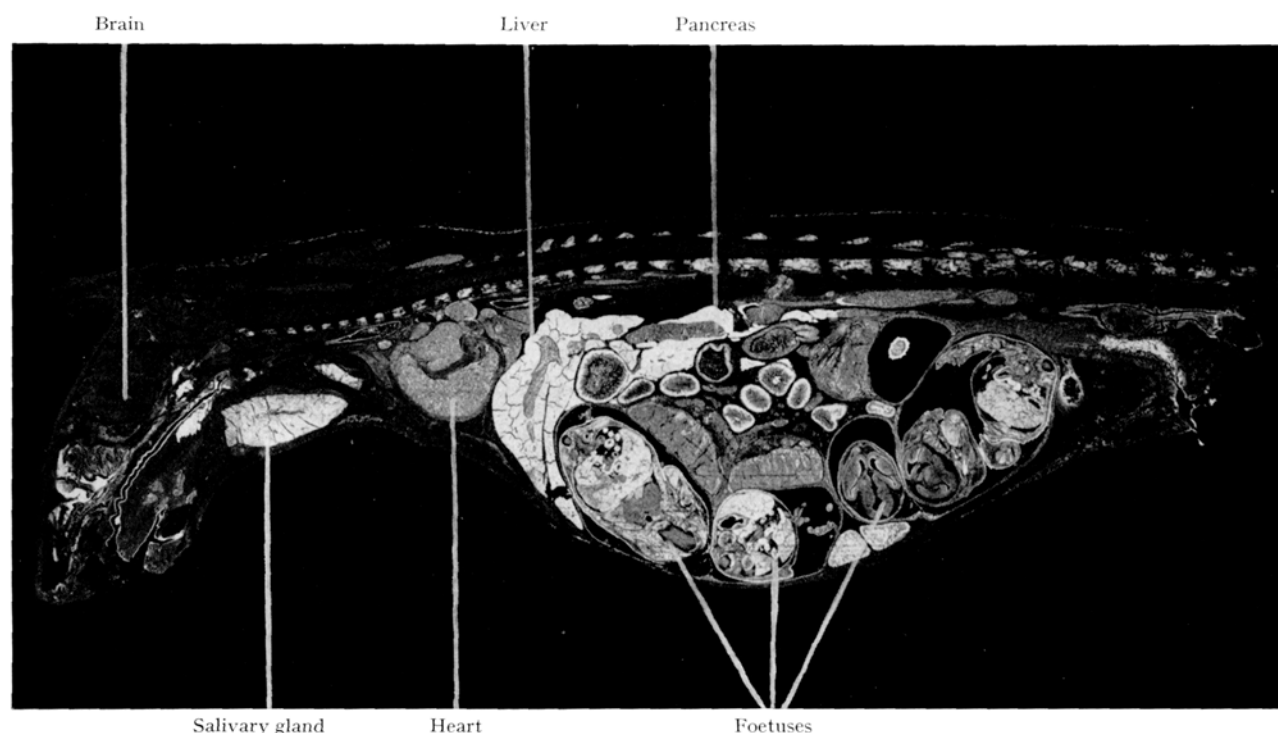
³ J. KRUH and J. ROSA, *Biochim. biophys. Acta* 34, 561 (1959).

⁴ S. ULLBERG *Acta radiol. Suppl.* 118 (1954).

⁵ E. HANSSON, *Acta physiol. scand. Suppl.* 161 (1959).

⁶ F. FRIEDBERG, H. TARVER, and D. M. GREENBERG, *J. biol. Chem.* 173, 355 (1948).

⁷ H. BORSOOK, *Physiol. Rev.* 30, 206 (1950).



Autoradiogram showing distribution of radioactivity in a pregnant mouse 4 h after intravenous injection of C^{14} -*para*-fluorophenylalanine. Note a high concentration in the liver, the pancreas and the salivary glands of the mother and in the foetuses.

Specific radioactivity in the TCA soluble and in the protein fraction of foetus, maternal liver and pancreas after intravenous injection of C^{14} -*para*-fluorophenylalanine in pregnant mice

Mouse	Time after administration of C^{14} - <i>para</i> -fluorophenylalanine	TCA soluble radioactivity		Cpm/mg dry weight maternal pancreas	Protein bound radioactivity. Cpm/mg protein		
		foetus	maternal liver		foetus	maternal liver	maternal pancreas
1	15 min	1826	2800	3256	750	2366	3427
2	1 h	1163	920	890	982	2325	8310
3	4 h	507	560	332	2630	2794	8426
4	24 h	51	160	279	2176	2124	2366

sively defluoridated in the body, but earlier findings^{2,8} indicate that defluoridation of *p*-fluorophenylalanine occurs only to a very low extent. The great difference in *p*-fluorophenylalanine and ethionine which has appeared does not seem to be explainable by the differences in relation between the injected doses of the analogues and the body pool of corresponding free natural amino acid. The most probable explanation seems to be the degree of discrimination of the antimetabolite during the competition with the corresponding natural amino acid. *p*-fluorophenylalanine seems to resemble its corresponding natural amino acid more than does ethionine in its tendency to become incorporated into proteins, which may possibly be explained by stereostructural relationships⁹.

Zusammenfassung. Trächtigen Mäusen wurde 2 Tage vor dem Ende der Schwangerschaft 3- C^{14} -*para*-Fluorphenylalanin, eine dem Phenylalanin analoge Aminosäure, i.v. injiziert. Die Verteilung der Substanz im Organismus und ihr Einbau in die Proteine wurde gleichzeitig autoradiographisch und chemisch untersucht.

Aus den Autoradiogrammen geht hervor, dass *para*-Fluorphenylalanin im Organismus wie eine normale Aminosäure verteilt wird. Die chemischen Untersuchungen zeigen, dass ein grosser Teil der Substanz in die Proteine eingebaut wird.

Wir schliessen daraus, dass sich *para*-Fluorphenylalanin im Stoffwechsel gleich oder sehr ähnlich verhält wie die physiologisch vorkommende Aminosäure Phenylalanin.

T. GARZO¹⁰, E. HANSSON, and S. ULLBERG

Department of Pharmacology, Kungl. Veterinärhögskolan, Stockholm (Sweden), October 30, 1961.

⁸ R. MUNIER and G. N. COHEN, *Biochim. biophys. Acta* 31, 378 (1959).

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¹⁰ Present address: Chemical Institute of the Medical University, Budapest (Hungary).

Action of Sodium pyruvate upon Nucleated and Enucleated Fragments of the Parasitic Ciliate *Opalina ranarum*

Sodium pyruvate has a remarkable cytolysing effect upon enucleated fragments of *Amoeba proteus*, in contradistinction to nucleated fragments¹. As we obtained similar results with *Stentor coeruleus*², it was desirable for us to perform a corresponding investigation with the parasitic Ciliate *Opalina ranarum* to establish whether we are possibly concerned with a phenomenon of more general importance. For this purpose the specimens immediately after removal from the intestine of the frog *Rana esculenta* were cut into nucleated and enucleated fragments with a glass needle. As in *Amoeba*¹ and *Stentor*² whole cells of *Opalina* as well as nucleated fragments were found to tolerate concentrations of sodium pyruvate of up to 2.5×10^{-3} mM/ml for 24 h without appreciable changes in movement or morphology. Within the first 10 h exposure to this concentration about 90% of the enucleated fragments were cytolysed. The synthesis of ribonucleic acid and protein is dependent on the nucleus and consequently the increased toxicity of the sodium pyruvate to the enucleated halves might reflect an intracellular increase in lactic acid, caused by a change in the reduction of the diphosphopyridine nucleotide diphosphopyridine ratio as a result of insufficient consumption of chemical bound

energy^{3,4}. BRACHET demonstrated a similar increase in sensitivity of the enucleated fragments towards natural precursors of nucleic acid and proteins⁵. As the results obtained in the parasite *Opalina* correspond very well with the findings obtained in *Amoeba*¹, *Stentor*², and *Acetabularia*⁵, the cytolysing action of sodium pyruvate should probably be considered to be of more general importance.

Zusammenfassung. Natrium-Pyruvat zeigt eine cytoliierende Wirkung auf enukleierte Fragmente der schmarotzenden Ciliate *Opalina ranarum*.

A. STOLK

Histological Laboratory, Free University, Amsterdam (The Netherlands), September 27, 1961.

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