The Development of Pyruvate Kinase, Glycerol Kinase and Phosphoenolpyruvate Carboxykinase Activities in Liver and Adipose Tissue of the Rat

Birth in the rat, as in other mammalian species, involves a sudden change in the diet, milk being a high fat diet[1]. In the suckling period carbohydrates are in short supply and a number of changes in enzyme activities observed in that period may be explained by that fact[2,3]. The 3 enzymes examined in this work were chosen for the following reasons.

(1) Phosphoenolpyruvate carboxykinase (E.C. 4.1.1.32) is decisive for gluconeogenesis from pyruvate and Krebs cycle intermediates forming phosphoenolpyruvate (PEP) and CO_2 from oxaloacetate. It was to be expected that the high fat milk diet would induce formation or activation of this enzyme.

(2) Pyruvate kinase (E.C. 2.7.1.40) serves the opposite purpose, being one of the rate-limiting enzymes of glycolysis and breaking down PEP to pyruvate. We expected low activity in the suckling period.

(3) Glycerolkinase (E.C. 2.7.1.30) forms glycerophosphate from glycerol and ATP and is important for the further metabolic fate of glycerol which shows a high level in the blood of suckling animals[1]. High activity in the suckling period was expected.

Material and method. All 3 enzyme activities were determined in the high speed supernatant (75,000 *g*, MSE centrifuge) of the liver and brown and white adipose tissues. PEP carboxykinase was determined according to [4], pyruvate kinase according to [5] and glycerol kinase according to [6]. The oxidation or reduction of NAD was measured with a Calbiometer instrument (Calbiochem California). Protein content was determined according to [7].

Results. PEP carboxykinase activity was not found in white adipose tissue. This is in agreement with other work[8,9]. Nevertheless we agree with the conclusion of LEVEILLE[9] that probably the assay conditions are not optimal and that in all probability this enzyme is present in white adipose tissue, as follows also from the incorporation of labelled pyruvate into glyceride glycerol[9] and our unpublished work.

The enzyme was found to be present in brown adipose tissue and activity is somewhat higher in the suckling period than later in life (Table).

In the liver PEP carboxykinase. activity is very low before birth and rises rapidly after birth, decreasing again after weaning (day 30). The rise after birth has been described previously (GREENBERG and CHRISTIANSEN, not published).

Pyruvate kinase activity in white adipose tissue shows no developmental changes. Surprisingly enough, in brown adipose tissue activity increases to a peak in the suckling period and then falls off again.

In the liver, activity is low in the suckling period and rises in older animals, as described by WEBER[10]. We find, however, that prenatally activity is relatively high, higher than soon after birth.

Glycerol kinase activity in white and brown adipose tissue has been described previously[11]. In fetal liver, activity is very low and a relatively rapid rise is observed after birth up to day 10. After weaning a further increase in activity is found.

Discussion. The results described here can be fitted into the general picture of metabolic development in relation to food composition. The liver, being the main source of blood glucose, is forced to produce relatively more of that substance after birth, since little carbohydrate is supplied in the milk. Glucose production from glycogen is limited after birth because liver glycogen stores are soon exhausted[1] and so gluconeogenesis remains as the main mechanism for glucose formation. In agreement with this, glucose breakdown in the liver

[1] P. HAHN and O. KOLDOVSKÝ, *Utilization of Nutrients During Postnatal Development* (Int. Ser. Monographs, Zool. Div., Pergamon Press, London 1967), vol. 33.
[2] P. HAHN, Experientia *21*, 634 (1965).
[3] P. HAHN and Z. DRAHOTA, Experientia *22*, 706 (1966).
[4] R. C. NORDLIE and H. A. LARDY, J. biol. Chem. *238*, 2259 (1963).
[5] S. P. COLOWICK and N. O. KAPLAN, *Methods in Enzymology* (Academic Press, New York 1955), vol. 1, p. 435.
[6] C. BUBLITZ and E. B. KENNEDY, J. biol. Chem. *211*, 951 (1954).
[7] H. O. LOWRY, N. Y. ROSEBROUGH and A. L. RANDALL, J. biol. Chem. *193*, 265 (1951).
[8] J. W. YOUNG, C. SHRAGO and H. A. LARDY, Biochemistry *3*, 1687 (1964).
[9] G. A. LEVEILLE, Life Sci. *5*, 421 (1966).
[10] G. WEBER, M. A. LEA, E. A. FISHER and N. B. STAMM, Enzym. biol. clin. *7*, 11 (1966).
[11] P. HAHN and R. GREENBERG, Life Sci. *7*, 187 (1968).

Enzyme activities in liver and adipose tissue of the rat during development

No. of days	PEP kinase μmol P/mg protein/6 min		Pyruvate kinase μmol NAD/mg protein/min			Glycerol kinase μmol NAD/mg protein/20 min
	Liver	Brown fat	Liver	Brown fat	White fat	Liver
19 (fetus)	0.06 ± 0.02		7.02 ± 1.2			
21 (fetus)	0.15 ± 0.014	0.5 ± 0.12	1.9 ± 0.4	6 ± 1.7	–	0.04 ± 0.003
1	0.22 ± 0.021	0.5 ± 0.10	1 ± 0.1	7 ± 1.3	–	0.04 ± 0.001
3	0.37 ± 0.041	0.5 ± 0.11	1 ± 0.05	10 ± 0.7	3.6 ± 0.9	0.14 ± 0.015
6	0.57 ± 0.05	0.5 ± 0.12	1.3 ± 0.06	10 ± 0.6	–	0.16 ± 0.012
10	0.31 ± 0.05	0.5 ± 0.04	1.0 ± 0.05	14 ± 0.8	3.8 ± 1.1	0.28 ± 0.011
18	0.12 ± 0.02	0.4 ± 0.09	3.1 ± 0.3	11 ± 1.0		0.26 ± 0.013
30	0.12 ± 0.03	0.5 ± 0.08	3.2 ± 0.2	8 ± 0.5	3.3 ± 0.5	0.24 ± 0.030
40	0.12 ± 0.02	0.2 ± 0.08	3.0 ± 0.3	8 ± 0.5		0.38 ± 0.018
90	0.12 ± 0.04	–	3.0 ± 0.2	–	3.4 ± 0.4	0.34 ± 0.012

4–10 determinations were made for each result shown.

(pyruvate kinase) is decreased after birth. It must be pointed out in this connection that WEBER's hypothesis of the genetic control of key glycolytic enzymes does not hold in the case of pyruvate kinase, since activity of this enzyme is high before birth. Hence it is unlikely that glucokinase, phosphofructokinase and pyruvatkinase are all controlled by the same gene.

The low activity of glycerol kinase in the livers of fetal and new-born rats explains our previous finding that glycerol is a poor precursor of liver glycogen in very young rats[1].

Zusammenfassung. Es wird gezeigt, dass in der Leber die Aktivität der PEP-Kinase und der Glycerolkinase nachgeburtlich ansteigt. Im braunen Fettgewebe fällt die PEP-Kinaseaktivität nach dem 40. Tag ab. Sie fehlt im weissen Fettgewebe. Die Pyruvatkinaseaktivität fällt in der Leber nach der Geburt ab, während sie im braunen Fettgewebe ansteigt.

PH. HAHN and R. GREENBERG

Institute of Physiology, Czechoslovak Academy of Sciences, Praha (Czechoslovakia) and Department of pediatrics, Stanford Medical Center, Palo Alto (California, USA), 28 August 1967.

Inhibition of Tyrosine Degradation in vivo by the Dopa Decarboxylase Blocking Agent NSD-1034

NSD-1034 (N-methyl-N-(3-hydroxybenzyl)hydrazine dihydrogen phosphate)[1] belongs to the most potent inhibitors of the aromatic L-amino acid decarboxylase[2–5]. It almost completely inhibits the decarboxylation of dopa but does not influence hydroxylation of tyrosine. NSD-1034 was therefore used in demonstrating dopa formation in vitro[6,7] and in our own experiments in vivo[8,9]. Besides this we found that tyrosine catabolism is extensively inhibited by this substance.

Experiments were performed with 4 cocks (weight approx. 1 kg) and 6 cats (weight 1.6–3.2 kg). The animals were injected i.p. (cock) or i.v. (cat) with NSD-1034 (100 mg/kg), which was dissolved in 2 ml 0.9% NaCl-solution. 20 min later 50 mc/kg H^3_2-3,5-L-tyrosine (spec. activity >15,000 mc/mMole, dissolved in 10 ml saline) was injected i.v. The radioactive tyrosine was synthetized according to BIRKOFER and HEMPEL[10]. 20–30 min after administration of the labelled tyrosine the animals were sacrificed and various organs (suprarenals, brain stem, cortex, heart, liver and blood) were removed. In addition, samples of venous blood were taken at different times after H^3-tyrosine injection. The cocks were anaesthetized with urethane (1 g/kg), the cats with ether. The controls were injected with H^3-tyrosine only.

The compounds soluble in 10% trichloroacetic acid (= acid-soluble fraction) were isolated and fractionated. In the following text the main steps of fractionation are summarized; details of the method are given elsewhere[9]. Firstly by extraction with ethyl acetate, compounds soluble in the organic phase were removed (ester fraction). Presumably this fraction included catabolites of H^3-tyrosine, such as *p*-OH-phenylpyruvic acid and homogentisic acid. No attempt was made to identify the individual compounds in this mixture. By evaporation to dryness the aqueous phase was divided into volatile (volatile fraction) and non-volatile compounds (non-volatile fraction). The volatile fraction consisted above all of water, including tritiated water derived from tyrosine catabolism. The non-volatile fraction contained nearly all H^3-tyrosine present in the original acid-soluble fraction and H^3-tyrosine metabolites, such as catecholamines, aspartic, glutamic and γ-aminobutyric acid. The compounds of the non-volatile fraction were separated by paper high voltage electrophoresis. Radioactivity was measured by liquid scintillation counting[9].

Figure 1 demonstrates the radioactivity of H^3-tyrosine degradation products in the acid-soluble fraction from venous blood at different times after i.v. injection of radioactive tyrosine. The total radioactivity of the acid soluble fraction was taken as 100%. There was no measurable difference in the total radioactivity of this fraction between untreated and NSD-1034 treated animals up to 30 min after H^3-tyrosine injection. At the end of the experiments, in untreated animals the major part of the radioactivity (90%) was represented in the form of products from tyrosine catabolism, above all tritiated water; only small amounts of these compounds (20%) were formed after NSD-1034 treatment.

Figure 2 shows the radioactive compounds of the non-volatile fraction, which are formed in the brain of cat

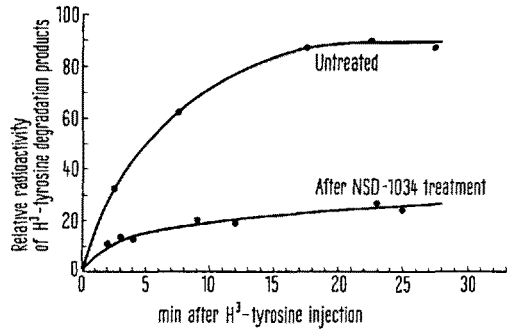

Fig. 1. Radioactivity of all H^3-tyrosine degradation products present in the acid-soluble fraction of venous blood (cat) after i.v. injection of H^3_2-3,5-L-tyrosine. The total radioactivity of the acid-soluble fraction was taken as 100%.

[1] NSD-1034 was kindly donated by Smith & Nephew Research Ltd., Harlow (England).
[2] E. HANSSON and W.G. CLARK, Proc. Soc. exp. Biol. Med. *111*, 793 (1962).
[3] D.J. DRAIN, M. HORLINGTON, R. LAZARE and G.A. POULTER, Life Sci. *3*, 93 (1962).
[4] E. HANSSON, R.M. FLEMING and W.G. CLARK, Int. J. Neuropharmac. *3*, 177 (1964).
[5] P. HOLTZ and D. PALM, Ergebn. Physiol. *58*, 41 (1966).
[6] T. NAGATSU, M. LEVITT and S. UDENFRIEND, Biochem. Biophys. Res. Commun. *14*, 543 (1964).
[7] T. NAGATSU, M. LEVITT and S. UDENFRIEND, J. biol. Chem. *239*, 2910 (1964).
[8] K. HEMPEL and H.F.K. MÄNNL, Experientia *22*, 689 (1966).
[9] K. HEMPEL and H.F.K. MÄNNL, Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. *257*, 391 (1967).
[10] L. BIRKOFER and K. HEMPEL, Chem. Ber. *96*, 1373 (1963).