(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.

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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_2^3-3,5-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

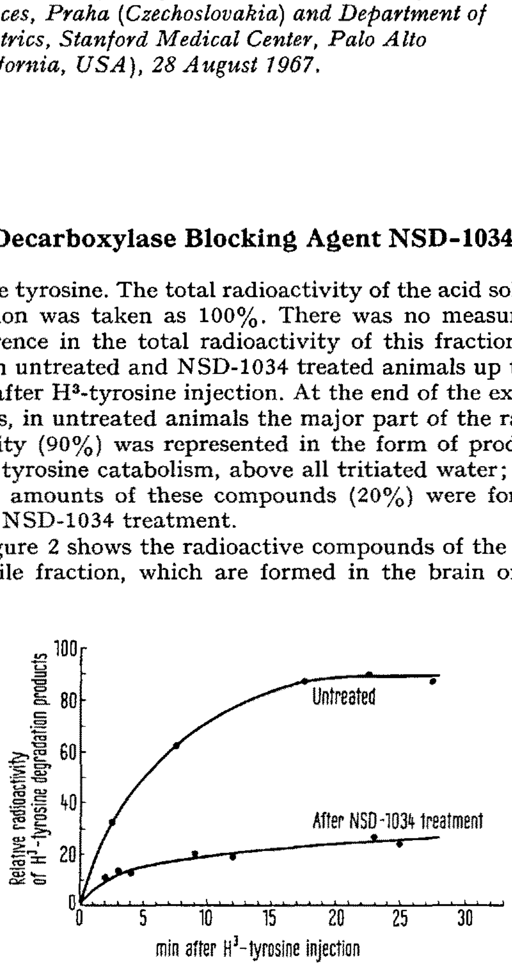

Fig. 1. Radioactivity of all H^3-tyrosine degradation products present in the acid-soluble fraction of venous blood (cat) after i.v. injection of H_2^3-3,5-L-tyrosine. The total radioactivity of the acid-soluble fraction was taken as 100%.

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during the 20 min following the administration of labelled tyrosine. Besides H^3-tyrosine radioactive glutamic, aspartic, γ-aminobutyric acid and catecholamines could be isolated in untreated animals. After NSD-1034 treatment, almost the total amount of radioactivity is present as tyrosine. The metabolites which could be observed in untreated animals are no longer detectable. Similar results were obtained with the other organs investigated.

Figure 3 compares the H^3-tyrosine content in suprarenals, brain stem and venous blood, which is markedly elevated after NSD-1034 treatement. The values reported for cats are higher than those for cocks. In studies with cats concerning another goal, blood vessels were ligatured. This procedure decreased the volume of circulating blood and may be the reason for the generally elevated H^3-tyrosine concentration in cats.

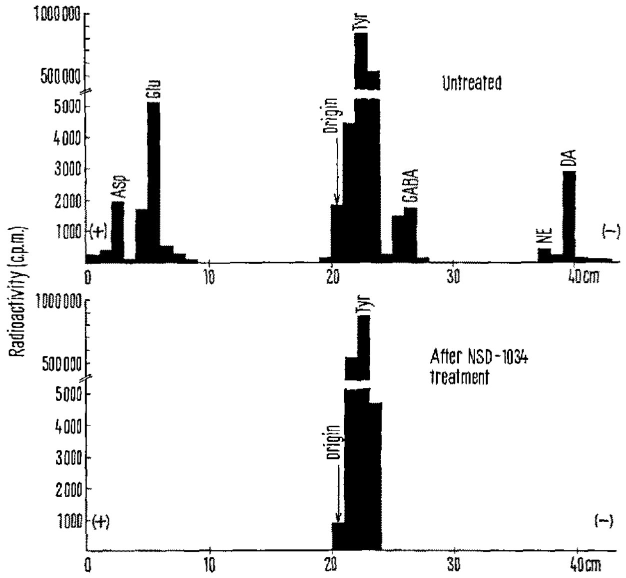

Fig. 2. Radioactive compounds in the non-volatile fraction of brain (cat), 20 min after i.v. injection of H^3-tyrosine. Paper high voltage electrophoretogram. Buffer: pyridine/acetic acid glacial/water (2:1:47 v/v), pH 5,1, 40 V/cm, -8°C, 180 min. Paper: SS 2043 bMgl.

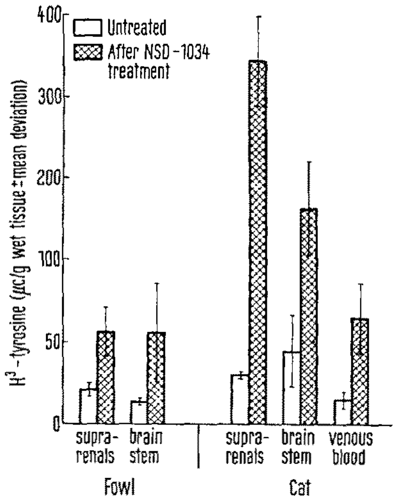

Fig. 3. H^3-tyrosine content in various organs 20 min after i.v. H^3-tyrosine injection (100 mc/kg), without and after NSD-1034 treatment (100 mg/kg, 20 min before H^3-tyrosine administration).

H^3_2-3,5-L-tyrosine degradation is initiated by transamination to H^3_2-3,5-*p*-OH-phenylpyruvic acid, which is transformed to H^3_2-4,6-homogentisic acid. Finally two C-4-units are formed: H^3-2-fumaric acid and H^3-4-acetoacetic acid. These compounds are metabolized through the citrate cycle leading to tritiated water. Also H^3-aspartic, H^3-glutamic and H^3-γ-aminobutyric acid (Figure 2) may be synthetized through this pathway. In animals treated with NSD-1034 the radioactivity of all these substances specified above, which were present in the different fractions, was markedly reduced (Figure 1) or not detectable (Figure 2). These results demonstrate that tyrosine degradation is almost completely inhibited by NSD-1034. This may be the reason for the elevated H^3-tyrosine concentration in treated animals (Figure 3).

In the following it will be discussed which enzymes are influenced by NSD-1034. The absence of labelled catecholamines (Figure 2) is explained by the well known blocking action of NSD-1034 on aromatic L-amino acid decarboxylase (dopa decarboxylase), a fact which could repeatedly be demonstrated[2,4,6,9]. The absence of the other H^3-tyrosine metabolites (Figure 2) cannot be explained by this mechanism, because the aromatic L-amino acid decarboxylase has only low enzymatic activity for tyrosine[11] and has no significance for tyrosine catabolism according to experiments with labelled tyrosine[12,13]. On the other hand, the inhibitory effect of NSD-1034 can be understood by an action on L-tyrosine transaminase. This may be possible because pyridoxalphosphate is coenzyme of aromatic L-amino acid decarboxylase and of L-tyrosine transaminase. NSD-1034 probably reacts with pyridoxalphosphate by aldehyde trapping, because it is a hydrazine derivative[14]. It is of interest to note that another hydrazine derivative MK-485 (DL-α-hydrazino-α-methyl-3,4-dihydroxyphenylpropionic acid), which belongs to the most potent dopa decarboxylase inhibitors, has blocking action on tyrosine catabolism, also[15]. Therefore it can be assumed that NSD-1034 may also inhibit other enzymatic steps if they require pyridoxalphosphate as coenzyme, and that it could have a multiple inhibitory effect[16].

Zusammenfassung. Der Stoffwechsel von H^3_2-3,5-L-Tyrosin wurde in mit NSD-1034 (100 mg/kg) vorbehandelten Versuchstieren (Hähnchen, Katzen) untersucht. Als Folge des durch NSD-1034 stark gehemmten Abbaus entstanden während der Versuchszeit (ca. 20 min) nahezu kein Tritiumwasser und keine aromatischen Abbauprodukte. Auch die sonst zu beobachtenden radioaktiven Metabolite des H^3-Tyrosins (Glutamin- und Asparaginsäure, Dopamin und Noradrenalin) waren nicht nachweisbar. Ausserdem war der Gehalt an H^3-Tyrosin in Organen behandelter Tiere höher als in Kontrollen.

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