The results are shown in the Table. High tyrosine hydrolylase activities were seen in the supernatant and in nerve endings (synaptosomes) and microsomes. The enzyme activity in mitochondria was significantly lower than that in nerve endings or microsomes. In contrast, monoamine oxidase activity was seen predominantly in mitochondria. The low activity of monoamine oxidase in nerve endings may be attributed to the mitochondria which are contained in the fraction. Granulated vesicles, which were isolated after hypotonic treatment of the nerve-ending fraction, contained relatively high tyrosine hydroxylase activity and low monoamine oxidase activity.

These results showed that there exist 2 forms of tyrosine hydroxylase in the homogenate of the bovine caudate nucleus; soluble and particle-bound enzymes. The particle-bound enzyme appears to be localized in nerve endings, and probably in granulated vesicles which stores catecholamines. These results agree with UDENFRIEND's scheme[16] that the enzymes necessary for catecholamine synthesis may actually be organized into a single particle which stores catecholamines[17].

Subcellular distribution of tyrosine hydroxylase and monoamine oxidase in the bovine caudate nucleus

Subcellular fractions	Tyrosine hydroxylase pmoles/mg protein/min (37 °C)	Monoamine oxidase
Homogenate	12	755
Myelin (A)[a]	3	320
Nerve endings (B)[a]	17	1300
Mitochondria (C)[a]	9	3030
Microsomes	20	800
Soluble fraction	17	0
Granulated vesicles[b]	18	390

[a] These subcellular fractions were isolated from crude mitochondrial fraction by the method of WHITTAKER[12]. [b] Granulated vesicles were isolated from crude mitochondrial fraction by the method of MAYNERT[13].

Zusammenfassung. Die subzelluläre Lokalisation von Tyrosin-Hydroxylase und Monoaminoxydase im Nucleus caudatus des Rindergehirns wurde untersucht. Tyrosin-Hydroxylase fand sich grösstenteils in den Synaptosomen, den Mikrosomen und dem Zytoplasma. Eine bedeutende Aktivität von Monoaminoxydase war in den Mitochondrien vorhanden.

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3,5-Dihydroxyphenylpropionic Acid, a Further Metabolite of Sinapic Acid

Sinapic acid, a widely distributed phenolic compound in plants[1,2] and a constituent of human dietary materials[3,4] has recently been reported[5] to give rise on oral administration to the rat to a number of phenolic metabolites including dihydrosinapic acid, 3-hydroxy-5-methoxyphenylpropionic acid and 3-hydroxy-5-methoxycinnamic acid. Further investigation has resulted in the identification of an additional metabolite, excreted by sinapic fed rats, which has been shown to possess identical chromatographic and spectral characteristics (Table) with synthetic 3,5-dihydroxyphenylpropionic acid. mp 125°, obtained from 3,5-dihydroxycinnamic acid[5] by sodium amalgam reduction.

The metabolite was isolated from the urines of 6 rats which had each received a single initial dose of 200 mg of sinapic acid in admixture with the standard diet[6]. After a collection period of 7 days, the phenolic metabolites were obtained from the seven 24 h urine samples of each animal by ethereal extraction[5] and the 3,5-dihydroxyphenylpropionic acid separated from the other metabolites by band chromatography in solvents B and D. The metabolite, 3,5-dihydroxyphenylpropionic acid was shown to be absent from the urines of a control group of 6 rats receiving the standard diet only.

Since earlier investigations had shown that other phenolic acids are dehydroxylated by the intestinal microflora[7,8], the effect of an oral antibiotic known to inhibit bacterial dehydroxylation[7] was studied. Administration of 30 mg chloramphenicol daily to each rat of an experimental and control group of 6 animals resulted in complete suppression of the metabolites 3,5-dihydroxyphenylpropionic acid, 3-hydroxy-5-methoxyphenylpropionic acid and 3-hydroxy-5-methoxycinnamic acid; the formation of dihydrosinapic acid under these conditions was reduced but not abolished indicating that the hydrogenation of sinapic acid is effected in part by tissue enzymes or a chloramphenicol resistant strain of intestinal microorganism.

Administration of 200 mg sinapic acid to a group of 3 rabbits under similar experimental conditions has been found to give rise to 3,5-dihydroxyphenylpropionic acid

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Chromatographic and spectral characteristics of metabolite S and 3,5-dihydroxyphenylpropionic acid

	Rf values Solvents A	B	C	D	E	Colour *p*NA	DSA	λ_{max} (nm) In methanol	Methanol + $AlCl_3$	0.1 *N* HCl	0.1 *N* NaOH
Metabolite S	0.33	0.05	0.07	0.62	0.79	Orange brown	Yellow brown	215, 276 283	215, 276 283	207, 273 279	220, 293
3,5-dihydroxyphenyl-propionic acid	0.34	0.05	0.07	0.63	0.79	Orange brown	Yellow brown	215, 276 283	215, 276 283	207, 273 279	220, 293

(A) Propan-2-ol-aq.-NH_3 (sp. gr. 0.88)–water (8:1:1 by vol.). (B) Benzene–acetic acid–water (6:7:3 by vol.). (C) Chloroform–acetic acid–water (2:1:1 by vol.). (D) 20% KCl (w/v). (E) n-Butanol–pyridine–water (14:3:3 by vol.). (*p*NA) Diazotized *p*-nitroaniline reagent. (DSA) Diazotized sulphanilic acid reagent.

in the urine where it occurs as the major metabolite of sinapic acid.

3,5-dihydroxyphenylpropionic acid was also formed from sinapic acid when the latter (10 mg) was incubated anaerobically with a heavy mixed inoculum of rat intestinal bacteria (obtained by sterile section of the large intestine) in 10 ml of a glucose/peptone/yeast extract medium buffered at pH 7.4[8] for 48 h at 37 °C. The metabolite was obtained following acidification of the centrifuged medium to pH 2, by ether extraction and subsequent chromatography in solvents B and D. Dihydrosinapic acid, a metabolite of sinapic acid in the intact animal was also formed in considerable amounts under these conditions and was characterized as described earlier[5]. The in vitro formation of 3,5-dihydroxyphenylpropionic acid from sinapic acid by intestinal bacteria and the suppression of metabolite formation by an orally administered antibiotic indicates that in the intact animal both the observed demethylation and *p*-dehydroxylation of sinapic acid is mediated by intestinal microorganisms.

Zusammenfassung. Aus Rattenurin wurde 3,5-Dihydroxyphenylpropionsäure als Metabolit der Sinapinsäure isoliert.

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Disc Electrophoresis of Fractions of Bovine Central Nervous System Myelin

Previous studies[1] have shown that treatment of cerebral white matter homogenates with 1 *M* NaCl allowed 50% of the lipids and proteins to be extracted with water. This cation-sensitive fraction will be called here the 'Na' fraction. In further studies[2] it has been found that one half of the remaining lipids and a smaller part of the proteins could be extracted with water after treatment with 1 *M* KCNS. This anion-sensitive fraction will be called here 'CNS', whereas the remaining residue after the two treatments and extractions will be designated as the 'R' fraction. Indirect evidence has been obtained[3] indicating that the 'Na' fraction corresponds to the electron microscopical intraperiod line of myelin, i.e. to the continuation of the part of the glial plasma membrane which faces the extracellular space; the 'CNS' and the 'R' fractions appear to correspond to the main dense line of myelin which continues that part of the membrane which faces the cytoplasm. In unpublished experiments WIENER examined by TLC the lipid constituents of the various fractions, but did not find any significant differences in constitution which could account for the different bonds, the electron-microscopic appearance, and other differences between the layers.

In the present study the protein constitution of various white matter fractions was studied by disc electrophoresis. 4 fractions were prepared from homogenized bovine white matter as previously described[2]: the 'T' (total), the 'Na', the 'CNS' and the 'R' fractions. All fractions were lyophilized and then 100 mg samples of each were dissolved in 1 ml of phenol-acetic acid-water solution (2:1:1, w/v/v) containing 2 *M* urea. Disc electrophoresis was done following the procedure of TAKAYAMA[4] for 2 h at 5 mA per column, at 4 °C. Samples of various fractions originating from the same homogenate were run simultaneously and stained together with 0.5% amido black in 7% acetic acid. The sample of the 'T' fraction was of 0.02 ml and it contained 305 γ of protein. The sample of 'Na' was also of 0.02 ml and contained 610 γ protein. The 'CNS' sample was of 0.1 ml and contained 955 γ protein, while the 'R' sample was of 0.02 ml and contained 610 γ protein.

It can be seen in the Figure that the 'Na' fraction is rich in acidic constituents which moved for a long distance from the starting line. Fractions 'CNS' and 'R' are, in comparison, poor in acidic proteins and contain more presumably basic proteins. This is in accordance with a previous observation[5] which showed that most of the experimental allergic encephalomyelitis antigen

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