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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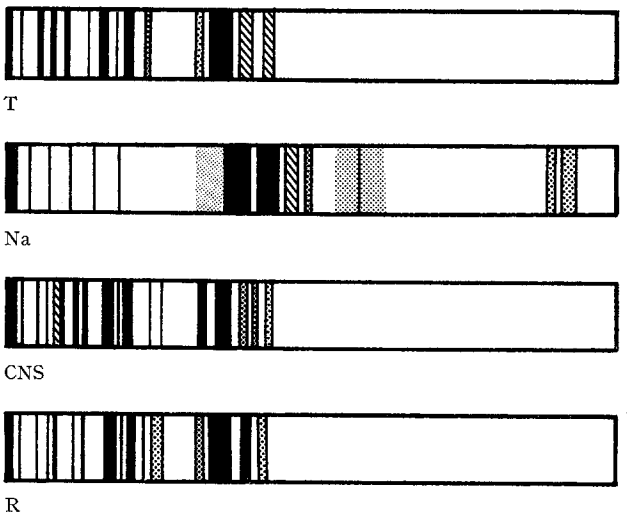
(which is known to be a basic polypeptide or a protein containing such a polypeptide) is concentrated in the 'CNS' and 'R' fractions. The presence of basic constituents in the 'CNS' and 'R' layer, in contrast to the presence of acidic constituents in the 'Na' layer, is in accordance with the previously expressed concept that the side of plasma membrane facing the extracellular space is dominated by fixed anionic charges, whereas the side facing the cytoplasm is dominated by fixed cationic charges and is less hydrophilic[6].



Electropherograms of samples of various fractions of white matter myelin. T, total white matter; Na, Na fraction; CNS, CNS fraction; R, residue fraction. Starting line on left.

Zusammenfassung. Fraktionen der weissen Hirnsubstanz, die offenbar zu den hauptsächlichsten Dichtelinien des Myelins respektive zur interperiodischen Linie gehören, wurden elektrophoretisch untersucht. Die Na-Fraktionen (interperiodische Linie; Membranseite zum extrazellulären Raum) enthalten meistens saure Proteine, während die CNS- und R-Fraktionen (Hauptlinie; Membranseite zum Zellinnern) zur Hauptsache alkalische Komponenten aufweisen.

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Inhibition of RNA-Polymerase by Arginine-Rich Histone Fractions

Several studies have established that the activity of DNA dependent RNA polymerase is inhibited if histones are added to the reaction mixture[1-6]. The interpretation of these studies is however hampered by the poor solbility of aggregated DNA-histone complexes which are formed when the added histone combines with the DNA template. It has therefore been suggested that the observed inhibition is the result of this aggregated state of nucleohistone particles, which will precipitate out of solution and make the template unavailable for transcription[7,8].

In most of the above mentioned studies, increasing amounts of histones were added to a constant amount of DNA and the results represented graphically by a linear plot of template activity or inhibition against DNA/histone ratio. Butler and Chipperfield[9] were e.g. able to demonstrate in this manner that although template activity decreased gradually with increasing histone/DNA ratios, the solubility of the DNA template dropped precipitously at a ratio of about 0.7, using a centrifugal force of $2000 \times g$ as criterium for the aggregated state. These results would seem to be contrary to the idea that the inhibition is due to insolubility of the DNA-histone complex.

In the present studies an attempt was made to resolve the question of whether or not a correlation exists between the activity and solubility of the DNA template under these conditions by plotting the logarithm of the percent inhibition of template activity against the amount of unaggregated DNA present in the reaction mixtures at various histone/DNA ratios. The histones used were highly purified subfractions of histone fractions f2a and f3[10]: GAR (glycine-rich arginine-rich), AL (arginine-rich lysine-rich) and AR-5 (arginine-rich fraction 5), generously provided by Dr. W. C. Starbuck (Dept. of Pharmacology, Baylor University Medical School, Houston, Texas). The weights of the histones were standardized against bovine serum albumin using the Lowry reaction[11]. Calf thymus DNA was isolated according to Kay et al.[12] and purified according to Gulland[13]. RNA polymerase from micrococcus lysodeikticus was purchased from Miles Laboratories. Uridine 5'-triphosphate-H^3 tetralithium and

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