strongly bound to the cation exchanger and, consequently, must have a higher positive charge.

Separate rechromatography of both active fractions of transketolase revealed that each isoform was eluted as an individual protein peak displaying enzymatic activity (Figures 1, b and c). Isoform I is always removed from the column at 0.1 *M* concentration of the eluting phosphate buffer and isoform II at 0.2 *M*. The specific activity of transketolase across the protein peaks is constant in all fractions, indicating that both isoforms are free from protein contamination. A nonactive peak of protein which is weakly bound to the cation exchanger appears to be an inactivated form of transketolase. It is also seen in the course of rechromatography of individual isoforms as a result of partial inactivation of the enzyme during its concentration, desalting and storage at pH lower than 6.0.

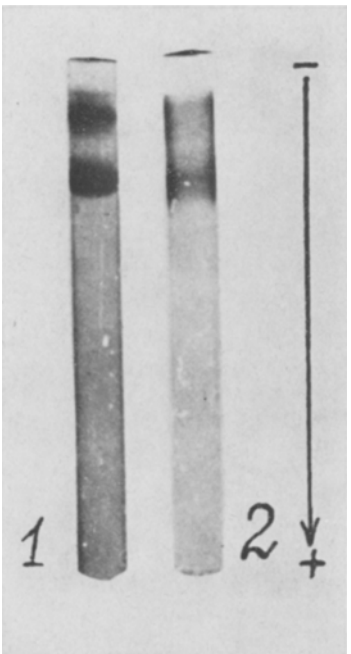

Fig. 3. Electrophoretic separation of transketolase apoenzyme (100 μg) in the anion system with subsequent determination of transketolase activity. *Tris* buffer (0.041 *M*, pH 8.9) containing 0.0063 *M* boric acid and 0.0017 *M* EDTA was used in the electrode vessels. 1. Protein stained with amido black. 2. Transketolase activity.

Phosphate is most important for chromatographic separation of the isoforms. Adequate separation was not observed when phosphate was substituted by acetate or when the concentration of phosphate buffer was decreased 15-fold (the ionic strength of the solution was kept constant by addition of KCl).

The existence of isoforms of transketolase first demonstrated by ion exchange chromatography was confirmed by electrophoresis (Figures 2 and 3). It can be seen in the figures that in both acidic and alkaline ranges the enzyme has 2 protein fractions.

Выводы. Методом ионообменной хроматографии с использованием ионообменных сефадексов показано существование двух изоформ транскетолазы пекарских дрожжей. Наличие двух активных изоформ фермента было подтверждено также при электрофоретическом разделении высокоочищенного препарата апотранскетолазы в полиакриламидном геле.

G. A. KOCHETOV and N. N. CHERNOV

Laboratory of Bioorganic Chemistry, Moscow State University, Moskwa W234 (USSR), 1 April 1970.

Absence of Dehydroxylation of Caffeic Acid in Germ-Free Rats

The dehydroxylation of caffeic acid (3,4-dihydroxycinnamic acid) to *m*-hydroxy derivatives following administration to animals was first reported by DEEDS et al.[1,2] *m*-Hydroxyphenylpropionic acid was shown to be the major metabolite of caffeic acid in rats and the finding that this metabolite was also excreted following the i.p. administration of caffeic acid suggested that dehydroxylation was not a result of the action of intestinal microorganisms. However, several subsequent studies with caffeic acid and related compounds have definitely implicated the intestinal microflora as the site of the dehydroxylation reaction[3–6]. The initial finding that dehydroxylation of caffeic acid occurred also after its i.p. injection has been shown to be dependent upon the biliary excretion of caffeic acid and/or its metabolites which can then undergo dehydroxylation in the intestine[7]. These findings in animals, which point to the intestine as the site of dehydroxylation, have been further substantiated in studies of the metabolism of caffeic acid by intestinal microorganisms[7–9]. The evidence now available thus indicates that the dehydroxylation of caffeic acid occurs in the intestine as a result of bacterial metabolism rather than in the tissues of the animal. However, unequivocal evidence in this regard is dependent upon studies in germ-free animals. No such studies with caffeic acid have hitherto been published and we therefore thought it would be of interest to report our findings on the metabolism of caffeic acid in germ-free rats.

The germ-free rats used were of the CDF strain and were reared according to techniques described previously[10]. The conventional rats were of the same strain and were the fourth generation after conventionalization.

[1] F. DEEDS, A. N. BOOTH and F. T. JONES, J. biol. Chem. *225*, 615 (1957).
[2] A. N. BOOTH, O. H. EMERSON, F. T. JONES and F. DEEDS, J. biol. Chem. *229*, 51 (1957).
[3] K. N. F. SHAW, M. GUTENSTEIN and J. B. JEPSON, Int. Congr. Biochem. (Ed. N. M. SISSAKIAN; Pergamon Press, Oxford 1963), vol. 9, p. 427.
[4] J. C. DACRE, R. R. SCHELINE and R. T. WILLIAMS, J. Pharm. Pharmac. *20*, 619 (1968).
[5] J. C. DACRE and R. T. WILLIAMS, J. Pharm. Pharmac. *20*, 610 (1968).
[6] J. DAYMAN and J. B. JEPSON, Biochem. J. *113*, 11P (1969).
[7] R. R. SCHELINE, Acta pharmac. tox. *26*, 189 (1968).
[8] A. N. BOOTH and R. T. WILLIAMS, Nature *198*, 684 (1963).
[9] A. N. BOOTH and R. T. WILLIAMS, Biochem. J. *88*, 66P (1963).
[10] T. MIDTVEDT and A. TRIPPESTAD, Acta path. microbiol. scand., in press.

2 separate experiments were carried out each with 3 germ-free and 3 conventional rats weighing from 150 to 170 g. 10 days before and during the study all animals were given a pellet diet, autoclaved at 120 °C for 20 min[11]. Caffeic acid (approximately 25 mg/animal) was given orally as a suspension in water following sterilization by autoclaving. Chromatograms of suspensions of caffeic acid so treated showed only unchanged compound. The animals were allowed free access to food and water during the experimental period. The urines from the conventional rats were collected for 3 days in containers placed in solid carbon dioxide while the urines from the germ-free rats were collected at room temperature for the same period but removed from the isolators every 24 h. The urines were stored frozen until analyzed by the extraction and thin-layer chromatographic methods described previously[7] except that a β-glucuronidase containing sulphatase preparation (approximately 2000 units/ml, type H-1, Sigma Chemical Co.) and 20 h incubation time were used.

The most pronounced difference between the chromatograms of the urines from conventional and germ-free rats was the complete absence of *m*-hydroxyphenylpropionic acid in the latter urines. On the other hand, this compound produced the most prominent spot on the chromatograms of urines from conventional rats where it was nearly exclusively found in the unconjugated fraction. Caffeic acid was excreted by both groups of rats and it was detected in both the unconjugated and conjugated fractions. The largest amounts were found in the unconjugated fraction from the germ-free animals. Both groups of rats excreted large amounts of ferulic acid which was found mainly in the conjugated fraction. A fairly large number of chromatographic spots arising from dietary substances made the search for minor metabolites difficult. The present results therefore conclusively show that the dehydroxylation of caffeic acid is a reaction carried out exclusively by the intestinal microorganisms[12].

Zusammenfassung. Nachweis, dass die Dehydroxylierung von Kaffeesäure zu *m*-Hydroxy-phenyl-propionsäure durch intestinale Mikroorganismen erfolgt, wie Fütterungsversuche mit normalen und keimfreien Ratten zeigten.

R. R. SCHELINE[13] and T. MIDTVEDT[14]

Department of Pharmacology, University of Bergen, Bergen (Norway), and
Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt, Rikshospitalet, Oslo (Norway), 8 April 1970.

[11] O. M. BAKKE and T. MIDTVEDT, Experientia *26*, 519 (1970).

[12] The authors wish to thank Mrs. ELI TEPSTAD for technical assistance.

[13] Department of Pharmacology, MFH-Bygget, University of Bergen, Bergen (Norway).

[14] Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt, Rikshospitalet, Oslo (Norway).

Mass and RNA Content of Rat Spinal Anterior Horn Neurones During Postnatal Development

Early UV-spectrographic studies on embryonal rat anterior horn neurones led to the conclusion that young nerve cells, in contrary to other somatic cells, pass through a 'second period of growth' characterized by continuous accumulation of nucleolar and cytoplasmic nucleotides and proteins[1]. Similar observations were made on the mass and nucleic acid content of anterior horn neurones of chick embryos[2]. No quantitative information is available, however, on the later phases of the chemical differentiation of spinal anterior horn neurones. Studies on rabbit retinal ganglion cells[3] and pyramidal cells of rat hippocampus[4] demonstrated differences in neuronal nucleic acid and protein content between young and adult animals but, because of discontinuous sampling, only limited conclusions could be made on the rate of change at different ages.

Systematic quantitative cytochemical information on the postnatal development of the nerve cell is desirable both for a better understanding of the normal growth and maturation of the nervous system, and as a baseline for studies of neuronal development under experimentally varied conditions. In the present report preliminary results of mass and ribonucleic acid (RNA) determinations on isolated rat spinal anterior horn neurones are presented which illustrate the timing and magnitude of the postnatal developmental changes.

Material and methods. Male Sprague-Dawley rats were fostered in litters of 8 pups, weaned at 21 days of life, and thereafter given standard diet ad libitum. The rats were sacrificed by decapitation at the following ages: at birth, 5, 10, 15, 21, 30, 90, 180, 360 days. The cervical spinal cord was rapidly dissected out, fixed in Carnoy's solution for 2 h, dehydrated, and embedded in paraffin. Sections were cut from the 7th cervical segment (at the level of the attachment of the posterior root on the spinal cord) at 40–80 μ, depending on the age of the animal. The sections were deparaffinized and hydrated with 0.01 *N* acetic acid. Large anterior horn neurones were dissected out from the anterolateral nuclei using a de Fonbrune micromanipulator[5].

For mass determination the direct X-ray absorption method of ROSENGREN[6] was used. The cells were placed on a thin supporting polyester film[7] on an aluminium holder. The X-ray absorption by the cells, in the wavelength range of 8 to 10 Å, was measured directly with the aid of a proportional counter tube and conventional pulse counting equipment. Cell mass was calculated from these values.

The total RNA content of the anterior horn neurones was determined according to EDSTRÖM[5]. The cells were extracted with ribonuclease (Worthington Biochemical Corporation, Freehold, New Jersey) solution, the RNA containing extracts evaporated on quartz slides, redissolved in a buffer solution to form lens-shaped droplets, and photographed in UV-light at 2570 Å. The amount of RNA per droplet was determined by a densitometric procedure.

[1] H. HYDÉN, Acta physiol. scand. *6*, suppl. 17 (1943). P. SOURANDER, Acta path. microbiol. scand. suppl. 95 (1953).

[2] A. HUGHES, J. Embryol. exp. Morph. *3*, 305 (1955).

[3] S.-O. BRATTGÅRD, Acta radiol. Suppl. *96* (1952).

[4] U. RINGBORG, Brain Res. *2*, 296 (1966).

[5] J.-E. EDSTRÖM, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1964), vol. I.

[6] B. H. O. ROSENGREN, Acta radiol. Suppl. 178 (1959).

[7] H. HALJAMÄE, Acta physiol. scand., in press (1970).