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.

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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.

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Results. The results of mass and RNA determinations are shown in Figures 1 and 2, respectively. The dry mass of rat spinal anterior horn neurones after Carnoy fixation (reflecting essentially their protein content since lipids are extracted by the fixation procedure[3]) increases from 989 ± 53 pg at birth to 3570 ± 106 pg at 1 year of age. The total neuronal RNA content increases from 122 ± 3 pg to 507 ± 9 pg during the same period. Nearly adult mass and RNA values are attained already at 1 month of age after which the increase is very slow.

The simultaneous confidence levels for the confidence intervals in Figures 1 and 2 have been calculated without any assumptions on the shape of the curves representing the neuronal mass or RNA content as function of age. With weak regularity conditions, the shadowed areas can be considered as confidence bands for the curves on a confidence level exceeding 85%. On the basis of these calculations, it appears sound to assume that the curve representing the neuronal RNA content is convex-concave (S-shaped) with a very steep rise between the ages 5 and 15 days. A steep rise in neuronal mass appears also to occur during the same period.

Discussion. Our observations show that the intense accumulation of RNA and protein, demonstrated by HYDÉN[1] to occur in the neuroblasts of embryonal rat spinal cord, continues after birth. Rat spinal anterior horn neurones undergo a period of rapid chemical development during the first month of postnatal life, particularly from 5–15 days of age. This period coincides with DOBBING's[8] period II a ('growth spurt') of morphological central nervous system development. The important cytochemical changes observed in neurones in the present study thus apparently parallel their morphological maturation.

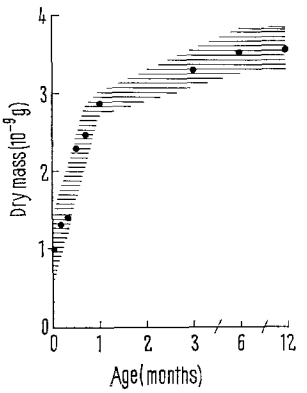

Fig. 1. The dry mass of Carnoy-fixed rat spinal anterior horn neurones plotted against age. Each point represents the mean value of 3 animals (about 100 neurones). The angular points of the upper and lower edges of the shadowed area give the observed upper and lower confidence limits, respectively, on a confidence level of 98% for the neuronal mass of the corresponding age. This implies that the simultaneous confidence level for all confidence intervals is over 85%.

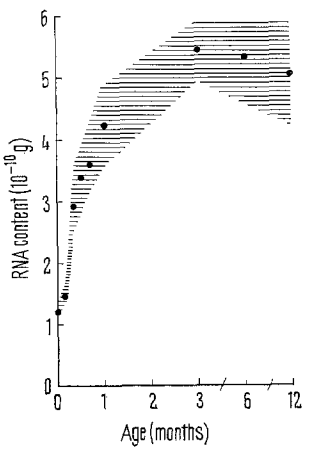

Fig. 2. The RNA content of rat spinal anterior horn neurones plotted against age. Statistical calculations as in Figure 1.

Nearly adult mass and RNA values are attained already at one month of age, before the main growth spurt of the whole body. This is in aggreement with observations on the increase in cell diameter[9] and volume[10] of rat spinal anterior horn neurones with advancing age. Postnatal changes in volume of cat spinal neurones were also found to follow a similar course[11]. The values obtained in the present study for neuronal mass after lipid extraction and neuronal RNA content of adult rats are consistent with previous experience on mass and RNA content of spinal anterior horn cells in other species[12,13]. The postnatal fourfold increase in the RNA content of spinal neurones is in close agreement with earlier observations on pyramidal cells of rat hippocampus[4]. It is of interest to note that the relative increase in neuronal RNA appears to exceed that in mass.

Considerable attention has recently been devoted to the hypothesis of vulnerable periods in central nervous system development[8]. It has been shown that e.g. myelination or cellular proliferation in rat brain may be irreversibly affected by undernutrition during early postnatal life[8,14]. Little is known, however, about the susceptibility of developing neurones to variations in the nutritional status of the individual, or other environmental influences. Because of the magnitude and rapidity of the postnatal cytochemical development of rat spinal anterior horn neurones, they may constitute a suitable model for experimental studies on the effects of environment on neuronal growth and maturation[15].

Zusammenfassung. Die postnatale Entwicklung der Trockenmasse und des RNS-Gehaltes isolierter spinaler Vorderhornzellen der Ratte wurden mit quantitativen Methoden untersucht, wobei eine postnatale, 3,6fache Vermehrung der Nervenzellmasse und eine 4,2fache des RNS-Gehaltes beobachtet wurde. Bereits im Alter von einem Monat werden nahezu endgültige Werte erreicht.

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[15] The results of the completed study will be published as a supplement in Acta physiol. scand. (1970). The authors are indebted to Mrs. LILLEMOR BERRY for expert technical assistance. This work was supported by a grant from the Swedish Medical Research Council No. B 69-12X-82-05 B.