were tested. The best separation was achieved at the lowest concentration of sample. The ionic strength of the buffers also influenced the separations, values between 0.12 and 0.17 being most effective.

Three bands were obtained and designated as α, β, and γ (Figure 2). All bands were green and showed a fairly typical absorption spectrum when scanned in the Cary Model 14 recording spectrophotometer. The isoelectric pH's of the bands were: α, 6.6; β, 4.5; and γ, 3.7. The isoelectric points of the β- and γ-bands compare with previously reported values of 3.7–4.7 for chloroplastic material[6]. To the authors' knowledge, no previous report of a pH 6.6 band exists. Each isolated band, adjusted to pH 6.2–6.4 and assayed by the method previously used[2] showed Hill activity. However, insufficient amounts of each electrophoretic fraction were available for both physiological and spectral assays on the same sample. Consequently, no quantitative comparisons could be made.

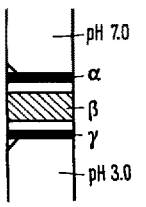

Fig. 2. Electrophoretic separation in citrate-phosphate buffer of CF_{20-50}.

The conclusions which can be drawn from these data are that the centrifugally differentiated fraction, CF_{20-50}, is probably not homogeneous, since it can be separated into several subfractions at their respective isoelectric points in an electrical field with a pH gradient. The differences in the isoelectric points might reflect structural as well as chemical changes, since the method is based on surface charge. Thus far, the existence of a high activity electrophoretic subfraction has not been ascertained.

Zusammenfassung. Durch Zentrifugierung isolierte Fraktionen fragmentierter Spinatchloroplaste von hoher Hill-Aktivität wurden in einem pH-Gradienten elektrophoretisiert. Bei Citrat-Phosphat-Puffer vom pH-Gradienten zwischen 3,0 und 7,0 wurden drei photoaktive Bänder von verschiedenem isoelektrischem Punkt gefunden. Dies deutet auf chemische oder strukturelle Differenzen in den Untereinheiten der Fraktion.

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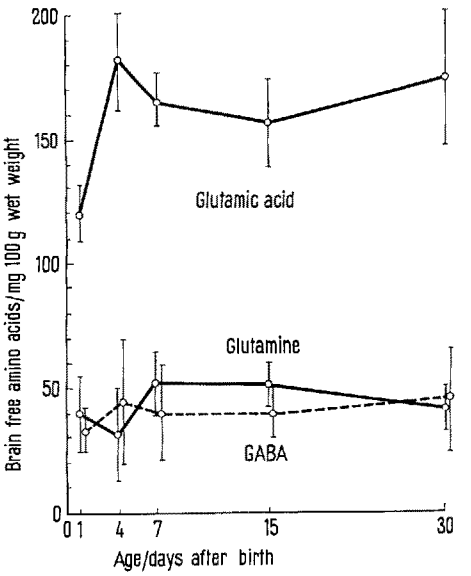
Amino Acid Changes in the Developing Feline Brain

Starting from the generally accepted assumption that glutamate-GABA system plays an important role in the maintenance of cerebral excitability, a series of experiments has been undertaken to investigate changes in concentrations of these amino acids at various stages of postnatal maturation of the cat brain and to correlate them with structural and physiological development. While this work was in progress, the paper was published by BERL and PURPURA on postnatal changes in amino acid content of kitten cerebral cortex[1]. Confirming in general their findings, we hope to supplement them with some additional information based upon the analysis of the whole feline brain and on a statistically more satisfying number of experiments.

A total of 35 kittens aged from one to thirty days was used. Animals were distributed in five age groups comprising seven kittens each, and sacrificed on the first, fourth, seventh, fifteenth and thirtieth postnatal day respectively. Litter-mates were killed at different postnatal days whenever this was possible. Glutamic acid, glutamine and GABA from the brain (portion rostral to the section at the collicular level) were separated and quantitatively estimated as previously described[2].

Contents of glutamic acid, glutamine and GABA found in the whole brain of kittens at various stages of cerebral maturation are presented in the Figure. In general, all amino acids studied attained the adult levels by the end of the first postnatal month. Concentrations of individual compounds, however, increased at different rates and reached the values encountered in the mature cat brain at different times. Thus, concentrations of glutamine remained practically unaltered throughout postnatal development. On the other hand, concentrations of GABA,



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[2] LJ. KRŽALIĆ, V. MANDIĆ, and LJ. MIHAILOVIĆ, Exper. *18*, 368 (1962).

although in an overall fashion closely parallel with changes of glutamic acid, attained the mature level apparently somewhat earlier. In newborn animals, glutamic acid amounted to approximately 65%, glutamine to 97% and GABA to 75% of values estimated in brains of adult animals. Closer scrutiny of individual results in the group of newborn kittens revealed values of GABA sometimes even higher than some of the concentrations found in adult animals. As could be seen in the graph, the greatest, the steepest and statistically highly significant increments of both glutamic acid (p 0.001) and GABA (p 0.01) occurred within the first week of postnatal development.

Morphological investigations of the developing feline cortex showed that most remarkable changes, involving the maximal neuron growth and maturation of superficial and deep neuropil, accompanied by a decreased density of cells, take place during the first two weeks of postnatal development[3,4]. The extraneuronal space, during the same maturational period, was found to consist mostly of interstitial fluid and only a few glial cells[5,6]. The fact that the most dramatic changes in glutamic acid and GABA concentrations encountered in our experiments, coincided with morphogenetic stage characterized by the highest neuronal index, i.e. neuron/neuroglia density, indicate that the biochemical alterations observed should be related primarily to postnatal development of neurons and their processes. The mature level of glutamine in the newborn animal seems to justify the teleological inference that the detoxication of ammonia is of major significance for the neonatal animal[1], which, of course, should not preclude the importance of glutamine for glycolysis and protein synthesis in the early period of postnatal development[7].

Résumé. La teneur en glutamine dans le cerveau des chatons nouveau-nés au cours de la maturation cérébrale est comparable à celle des animaux adultes. Les concentrations en acide glutamique et en GABA augmentent et vers la fin du premier mois postnatal correspondent au niveau des adultes.

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Institute of Pathological Physiology, Faculty of Medicine, University of Belgrade (Yugoslavia), November 3, 1963.

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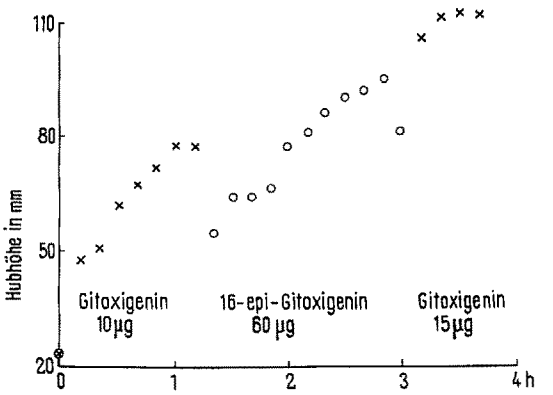
[7] This work has been supported by a grant from the Yugoslav Foundation for Scientific Research, Contract No. 490/1.

Einfluss der 16-Hydroxylgruppe auf die Aktivität der Kardenolide. Die inotrope Aktivität des 16-epi-Gitoxigenin

Beim systematischen Studium des kardiotonischen Prinzips von *Digitalis lanata* haben wir festgestellt, dass Lanatosid D am Katzenpapillarmuskel weniger wirksam ist als Lanatosid C[1]. Ein noch deutlicherer Unterschied wurde am Meerschweinchen mit der Knaffl-Lenz Methode gefunden. Lanatosid D unterscheidet sich vom Lanatosid C einzig durch die Anwesenheit der OH-Gruppe in der 16β-Position. Ein ähnlicher ungünstiger Einfluss der 16β-Hydroxylgruppe wurde für das Lanatosidenpaar A und B am Frosch und an der Katze, für Digitoxin und Gitoxin an der Katze und für das Paar Digitoxigenin und Gitoxigenin an der Katze[2] und am isolierten ungeschädigten Meerschweinchenvorhofpräparat gefunden[3]. Daraus ergibt sich, dass die 16β-Hydroxylgruppe der Kardenolide (aber nicht der Bufadienolide[3]) für die Senkung der kardiotonischen Aktivität und Toxizität von entscheidender Bedeutung ist. Die Frage stellt sich somit, inwieweit bei diesem ungünstigen Effekt, ausser der blossen Substituierung des C_{16}-Atoms, auch die sterische Orientation der Hydroxylgruppe beteiligt ist. Zu diesem Zweck wurde im Forschungsinstitut für Naturarzneimittel das 16-epi-Gitoxigenin (dessen OH-Gruppe in der 16α-Position steht) präpariert[4] und dessen inotrope Aktivität aus elektrisch gereizten Katzenpapillarmuskeln mit Gitoxigenin verglichen. Die Badeflüssigkeit war 25 ml, die Tyrodelösung (9 g NaCl, 0,42 g KCl, 0,12 g $CaCl_2$, 1 g Glucose, 0,12 g Na_2HPO_4, pH 7,2–7,4, Temperatur 38°C) wurde reichlich mit O_2 durchperlt. Die isotonischen Kontraktionen wurden photokymographisch registriert. Die Genine haben wir in steigenden Dosen von 2,5 µg appliziert (wechselweise Gitoxigenin und 16-epi-Gitoxigenin). Stets war die Dosis von 16-epi-Gitoxigenin 4–6mal höher als die vorangehende Dosis des Gitoxigenins.

Die Figur zeigt den charakteristischen Verlauf eines Teilversuches.



Inotrope Aktivität des Gitoxigenin und 16-epi-Gitoxigenin auf dem Katzenpapillarmuskel (Hubhöhe alle 10 min gemessen).

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