Synthesis of cyclododecan-1,4-dione (II) further confirmed the structures assigned. Cyclododecanol was tosylated and then refluxed with acetic acid-potassium acetate to furnish in excellent yield cyclododecene b.p. 108–110° (12 mm)[3]. Allylic oxidation of cyclododecene with SeO_2 in acetic acid-acetic anhydride[4] afforded an unsaturated diacetate b.p. 160° (1 mm) which was treated in ethereal solution with CrO_3 in dil. H_2SO_4 to eliminate the last traces of selenium.

The diacetate was reduced with Pt/H_2 in acetic acid, and then saponified with alkali to yield cyclododecan-1,4-diol (III) m.p. 145–147°. *Anal.* calcd. for $C_{12}H_{24}O_2$: C 71.95; H 12.08; found: C 71.98; H 12.02, identical to the diol obtained by $NaBH_4$ reduction of II. Oxidation of III gave the diketone II identical by m.p., mixed m.p., IR, thin-layer chromatography to II obtained from I.

Riassunto. Per irradiazione con luce ultravioletta del ciclododecanone in soluzione di *n*.esano si ottiene il biciclo[8.2.0]dodecan-1-olo. La struttura del nuovo composto viene dimostrata.

B. Camerino and B. Patelli

Laboratori Ricerche Farmitalia, Milano (Italy), February 27, 1964.

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Electrophoretic Fractionation of Chloroplast Fragments in a pH Gradient[1]

We have shown that a fraction isolated from sonically ruptured spinach chloroplasts which sediments between 1,000 and 50,000 × *g* exhibits higher Hill activity than intact chloroplasts or fractions sedimented at more than 50,000 × *g*[2]. The highest activity of this fraction resides in a subfraction which can be centrifuged between 20,000 and 50,000 × *g*[3]. The Hill reaction rate and chemical composition of this subfraction, CF_{20-50}, has also been briefly described.

It was reasoned that fragments sedimented on the basis of weight and size might not be chemically uniform, especially since the structure from which they were originally derived, the intact spinach chloroplast, is itself structurally heterogeneous. Thus, it was considered that it might be possible to isolate from the centrifugal fraction, CF_{20-50}, a chemically and structurally pure fraction which might be responsible for the high Hill activity of the parent mixture and would itself exhibit an even higher reaction rate. As an approach to this problem, electrophoretic separation of CF_{20-50} was performed by a modification of the pH and density gradient technique described by Kolin[4].

A simple glass U-tube with side chambers as electrode compartments was used. Electrodes were connected to a Hewlett-Packard model 711A power supply. The U-tube was filled to one third of its height with 50% sucrose-saturated acidic buffer. The subfraction, CF_{20-50}, which had been dialyzed 3 h in the cold against distilled water, was brought to 10 to 25% sucrose saturation; the sucrose concentration depended upon the experiment. The sample was layered above the acid-sucrose buffer in the right-hand arm of the electrophoresis cell. The height of the sample layer was equal to the inside diameter of the tube, as recommended by Kolin. Sucrose-free alkaline buffer was added above the sample layer to fill the tube and adjoining electrode chamber. The opposite arm and its electrode compartment (anode) were filled with sucrose-free acidic buffer; the cathode, then, was in contact with the basic buffer.

The buffer systems used were either Michaelis' universal veronal-acetate buffer[4] or citrate-phosphate buffer prepared according to Gomori[5]. Initially, experiments were performed at a current of about 5 ma. Subsequently, the ionic strength was adjusted with sodium chloride so that the current could be maintained at 20–30 ma, thus permitting good separations in about 5 min. At the end of a run, separated fractions were isolated by means of a 5-ml syringe with a long 18- or 20-gauge needle bent to 90° at the tip to facilitate withdrawal with minimal disturbance of the column.

Figure 1 depicts the results of the electrophoretic fractionations of the chloroplast fraction CF_{20-50}. The sample column in the electrophoretic cell at the beginning of an experiment (Figure 1a) and after 3 min at a current of 5 ma (Figure 1b) is shown.

Both the Michaelis and citrate-phosphate buffers were used with several pH gradients. The citrate-phosphate buffer system providing a gradient of pH 3.0 to 7.0 yielded the sharpest fractionation.

The concentration of suspended material had an effect on the degree of electrophoretic separation. Dilutions of CF_{20-50} with chlorophyll levels of 18, 30, and 60 μg per ml

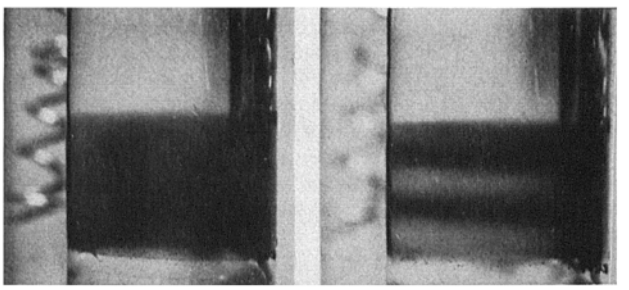

Fig. 1. Electrophoretic separation in Michaelis' buffer of CF_{20-50} from broken spinach chloroplasts. Left – sample column before separation; right – sample column after 3 min, 5 ma, pH 3.0–5.7.

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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.

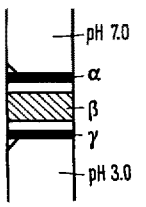

Fig. 2. Electrophoretic separation in citrate-phosphate buffer of CF_{20-50}.

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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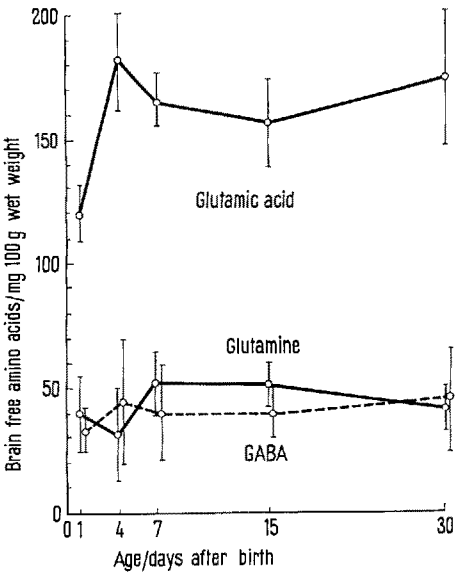
Amino Acid Changes in the Developing Feline Brain

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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