The experiments of CRAIN and PETERSON[10] and PETERSON et al.[11], through microelectrophysiological stimulation and recording techniques, indicate that neurons, if properly maintained in vitro, reveal a complex bioelectric activity resembling that of synaptic networks in vivo. It must be considered that synapses were already present at explantation of the nervous tissue, but the authors do not exclude the possibility that additional synapses developed in culture.

The evident difference of opinions on such an interesting problem led us to repeat and carry on the researches already made by some of us (STEFANELLI et al.[12]), trying to obtain long-term cultures and using the electron microscope for the specimen investigations. To overcome the difficult and undecided question of the pre-existence or neoformation of synaptic connections in vitro, we used eye cups of chicken embryos aged 4 days, disaggregated by trypsin; in such a way we dealt with a material that in vivo was still undifferentiated as far as nervous elements and neuronal interconnections. We chose the eye cup because, among the retinal layers, the synaptic types are morphologically well identifiable. The disaggregated cells were reaggregated as little balls of about 2 mm and cultured in rolling tubes. Our observations apply to cell aggregates maintained in culture for 25 days. The material was fixed and embedded for optical and electron microscopy.

We could show the differentiation of 3 cellular types: the Müller support cells, identifiable by their gliofilaments, by the microvilli and by the terminal bar at their free surface; the neurons, particularly rich in ribosomes and endoplasmic reticulum, but not distinguishable in bipolar, ganglion and amacrine cells; and lastly the photoreceptors, rods and cones not recognizable from each other.

Recently, EVANS[13] found that the distinctive feature between the two kinds of receptors in the chick retina lies in the presynaptic vesicles, which are rarer and smaller in the cones. But this peculiarity of the cones is not significant enough, and we failed to reveal any difference in our specimens; on the other hand, we never observed the characteristic pattern of double-membrane discs arranged to form a pile of the outer rod segments.

Furthermore, a thick neuropile with numerous synaptic connections showing the peculiar thickening of the membranes and the vesicles at the presynaptic side, was clearly differentiated (Figure 1, arrows).

A very interesting finding was the presence of 'synaptic ribbons', characteristic in the presynaptic sides of the contacts between photoreceptors and dendrites of the bipolar cells (Figure 2, arrows). These synaptic ribbons are common to rods and cones, therefore they could not be of a differential character. We think that our observations provide a valuable morphological basis for the future electrophysiologic verification of the synaptic connections established in vitro.

Riassunto. Cellule di abbozzo oculare di embrione di pollo di 4 giorni di incubazione sono state disgregate, riaggregate e coltivate in vitro per 25 giorni. Al m.e. è risultato un fitto neuropilo ricco di sinapsi tra cui i «synaptic ribbons» caratteristici delle cellule fotorecettrici. Questi risultati sono una prima sicura documentazione di sinapsi formate ex novo in vitro con nuova associazione di cellule.

A. STEFANELLI, A. M. ZACCHEI, S. CARAVITA, A. CATALDI, and L. A. IERADI

Centro di Neuroembriologia del Consiglio Nazionale delle Ricerche, Istituto di Anatomia Comparata della Università di Roma (Italia), 14th July 1966.

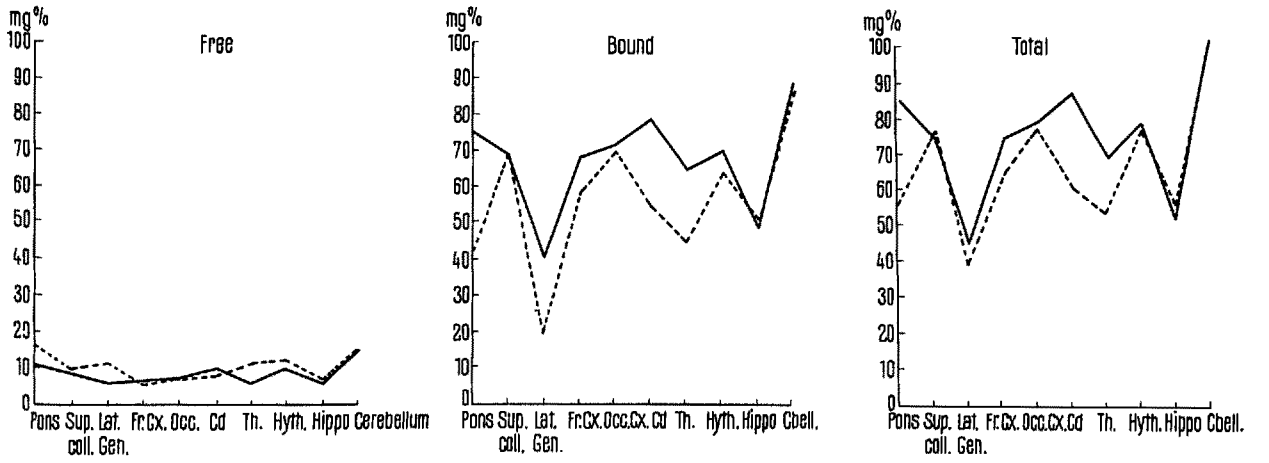
[10] S. M. CRAIN and E. R. PETERSON, J. cell. comp. Physiol. *64*, 1 (1964).
[11] E. R. PETERSON, S. M. CRAIN and M. R. MURRAY, Z. Zellforsch. mikrosk. Anat. *66*, 130 (1965).
[12] A. STEFANELLI, A. M. ZACCHEI and V. CECCHERINI, Atti Accad. naz. Lincei Rc. *30*, 818 (1961); Acta Embryol. Morph. exp. *4*, 47 (1961).
[13] E. M. EVANS, Z. Zellforsch. mikrosk. Anat. *71*, 499 (1966).

The Influence of Deprivation of Paradoxical Sleep on Glycogen Content in Various Brain Structures of the Cat

The role of carbohydrate metabolism in brain function has been emphasized many times. Brain tissue has exceptionally great potential and capacity to metabolize glucose, which represents one of its most important energy sources[1]. Glycogen, free and bound (residual), is an integral part of neuronal structure, and suffers considerable changes and quantitative variations dependent upon the functional state of the brain tissue. These variations are most intensive in structures which have the biggest metabolic rate[2]. Recent sleep studies point out the metabolic and enzymatic nature of the mechanism of sleep, especially its paradoxical phase (PS). Our previous experiments have shown that PS deprivation in cats leads to considerable changes in concentration of GABA-GA-AA and that these changes have very specific and selective regional character, limited to certain brain structures[3]. Starting from the fact that the quantity of glycogen reflects the metabolic and at the same time the functional state of the CNS, and that PS deprivation is a specific phenomenon causing prolongation of the paradoxical phase of sleep in the recuperative period, we considered it of interest to examine the quantitative changes of glycogen (total, free and bound) in various brain structures in order to find out the possible correlation between the regional metabolism of glycogen and structures exhibiting neurophysiological evidence for involvement in PS mechanism.

Experiments were carried out on 12 adult cats. 6 cats served as control, while the other 6 were submitted to selective and instrumental PS deprivation[4] for 96 h before being sacrificed. Quick decapitation was done by a

[1] R. BALÁZS and D. RICHTER, in *Regional Biochemistry* (Ed. S. S. VETY and J. ELKES; Pergamon Press, Oxford, London, New York, Paris 1961), p. 49.
[2] A. CHESLER and H. E. HIMWICH, Archs Neurol. Psychiat., Chicago *52*, 114 (1944).
[3] D. MIĆIĆ, V. KARADŽIĆ and LJ. RAKIĆ, Nature, in press (1966).
[4] D. JOUVET, P. VIMONT, J. F, DELORME and M. JOUVET, C. r. Séanc. Soc. Biol. *158*, 756 (1964).



Changes in glycogen content: —— = control; ···· = deprivation.

specially designed guillotine and the animals' heads fell into liquid air. The various brain regions were taken out from the frozen tissue in the cool chamber.

Free, bound (residual) and total glycogen were determined by the combined and slightly changed[5] methods of BLOOM and RUSSEL[6] and SEIFTER et al.[7]. Free fraction of glycogen was extracted by TCA, precipitated with ethanol and then determined by anthrone reagent. After digestion of the lipoprotein residue in 60% KOH, the glycogen was first precipitated by ethanol-chloroform (2:1) and then washed with methanol-chloroform (1:2) for the purpose of cerebroside elimination. From aqueous solution, glycogen was determined spectrophotometrically by the anthrone reagent[7]. The total glycogen is expressed as the sum of free and bound glycogen.

The results represented in the Figure show that the total quantity of glycogen in PS-deprived animals is significantly decreased in Pons (Pn, 34%), caudate nucleus (Cd, 30%) and thalamus (Th, 23%) (in all cases $p < 0.01$). However, free glycogen is increased in PS-deprived animals in Pn (47%), Th (84%) and lateral geniculate body (GL, 84%) (in all cases $p < 0.01$). Bound glycogen is decreased in the majority of brain structures in PS-deprived animals, i.e. in Pn (46%), GL (54%), Cd (32%), Th (30%) and frontal cortex (FrCx, 21%) (in all cases $p < 0.01$).

The results obtained indicate that the fall in total glycogen in certain brain structures (Pn, Cd, Th) in PS-deprived animals primarily occurs on account of bound glycogen, while in the same structures free glycogen is increased. It seems that the structures in certain physiological states lose most of their brain glycogen reserves through the transfer of bound to free glycogen. Free glycogen has to maintain its high level for the purpose of more rational energetic utilization.

When compared with neurophysiological data, our results offer the possibility of speculation about the possible connection of glycogen content in brain structures with their role in the PS mechanism. There are firm neurophysiological findings suggesting that the Pons is the seat of origin and maintenance of PS[8], where, as our results have shown, the biggest fall in glycogen content in the brain of PS-deprived animals occurs. The fall in glycogen content is also highly significant in Cd and Th, for which there are no positive data about their participation in PS. The fall in glycogen content in these structures might imply their role in the mechanism of origin of PS, for both Cd and Th are known for their strong inhibitory capacity[9,10]. However, there was no significant fall in total glycogen in GL and hippocampus (Hippo), the structures which change the pattern of their electrical activity in PS. There was only a fall in bound and an increase in free glycogen content in LG.

Functional states of the CNS where the fall in glycogen content was observed (hypoglycaemia, hypoxy, anaesthesia) exhibit the blockade of auditory evoked potentials in reticular formation without alterations in classical specific pathways. Also, those states are characterized by increased arousal threshold[10]. PS exerts identical neurophysiological characteristics as in the states mentioned above, and the fall in glycogen content in PS-deprived animals could be explained in connection with the neurophysiological findings mentioned. Perhaps the reason for the prolonged duration of PS after PS deprivation lies in the slow rebinding of glycogen, which synthesis in the brain is a slow process[11,12].

Zusammenfassung. Nach selektiver Deprivation des paradoxalen Schlafes wurde der Spiegel des freien und gebundenen Glykogens in kortikalen und subkortikalen Hirnstrukturen der Katze bestimmt. Es wurde eine Intensitätskorrelation zwischen der paradoxalen Phasenaktivität einiger Strukturen und deren Veränderung der Glykogenfraktionen festgestellt. Der Gesamtglykogengehalt zeigt eine signifikante Senkung in Pons, Caudatus und Thalamus.

B. B. MRŠULJA, LJ. M. RAKIĆ and M. RADULOVAČKI

Departments of Biochemistry and Physiology, Medical School, Institute for Biological Research, Belgrade (Yugoslavia), 8th September 1966.

[5] B. B. MRŠULJA, V. K. JORGA and LJ. M. RAKIĆ, Symposium of Yugoslav Neurophysiologists, Belgrade (1964), p. 36.
[6] W. L. BLOOM and J. A. RUSSEL, Am. J. Physiol. *183*, 345 (1955).
[7] S. SEIFTER, S. DEYTON, B. NOVIC and E. MUNTWYLER, Archs Biochem. *25*, 191 (1950).
[8] M. JOUVET, in *Progress in Brain Research, Sleep Mechanism* (Ed. K. AKERT; Elsevier Publishing Co., Amsterdam, London, New York 1965), vol. 18, p. 20.
[9] H. H. JASPER and F. J. DROOGLEEVER, Res. Publs Ass. Res. nerv. ment. Dis. *26*, 272 (1947).
[10] LJ. M. RAKIĆ, Israel Med. J. *1*, 1376 (1965).
[11] A. GEIGER, in *Neurochemistry* (Ed. K. A. C. ELLIOTT; C. H. C. Thomas, Springfield 1962), p. 128.
[12] This work was supported by grants from the Republic Scientific Fund, Belgrade, and by U.S. Public Health Service Grant No. 6X9803 from the National Institutes of Health.