their functional significance have not been determined. It has been suggested that enriched environments would greatly expand learning experience, and these changes might reflect neuronal alterations associated with learning[12]. The brain slice cyclic AMP system is a logical extension of these studies because its responsiveness has been shown to undergo adaptation to altered synaptic input in vivo. Furthermore, the activity of neurohormone-sensitive cyclic AMP-generating systems would be expected to increase with number of synaptic concentrations. However, most EC-IC differences in brain parameters have been reported to be quite small (< 10%); such small differences in synaptic numbers would probably not be detectable in the brain slice system. The comparatively large (60–70%) alterations seen here in responsiveness of cyclic AMP-generating systems suggests that environmental enrichment may be altering chronic functional synaptic activation of adenylate cyclases responsive to certain putative neurohormones; i.e., prostaglandin and histamine. This possibility would not be unique: alterations in environmental lighting have been shown to control noradrenergic input to the pineal gland, producing corresponding hyper-responsiveness or hypo-responsiveness of post-synaptic cyclic AMP system[18]. Possible correlations of hyper- and hypo-sensitive brain cyclic AMP systems in EC and IC rats with behavioral profiles and drug effects on behavior are under investigation.

[18] J. AXELROD, Science *184*, 1341 (1974).

Brain Glycogen Following Experimental Cerebral Ischemia in Gerbils (*Meriones unguiculatus*)

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Summary. Cortical glycogen levels decreased after both 1 and 3 h of unilateral ischemia. After 1 h of recirculation, the levels of glycogen were restored to control values in both groups. Subsequently, glycogen increased above normal levels after 1 week of recirculation in the 1 h ischemic group, and after 5 h in the 3 h ischemic group. Thus, the onset of the excess glycogen accumulation appears to be dependent on the intensity of the ischemic insult.

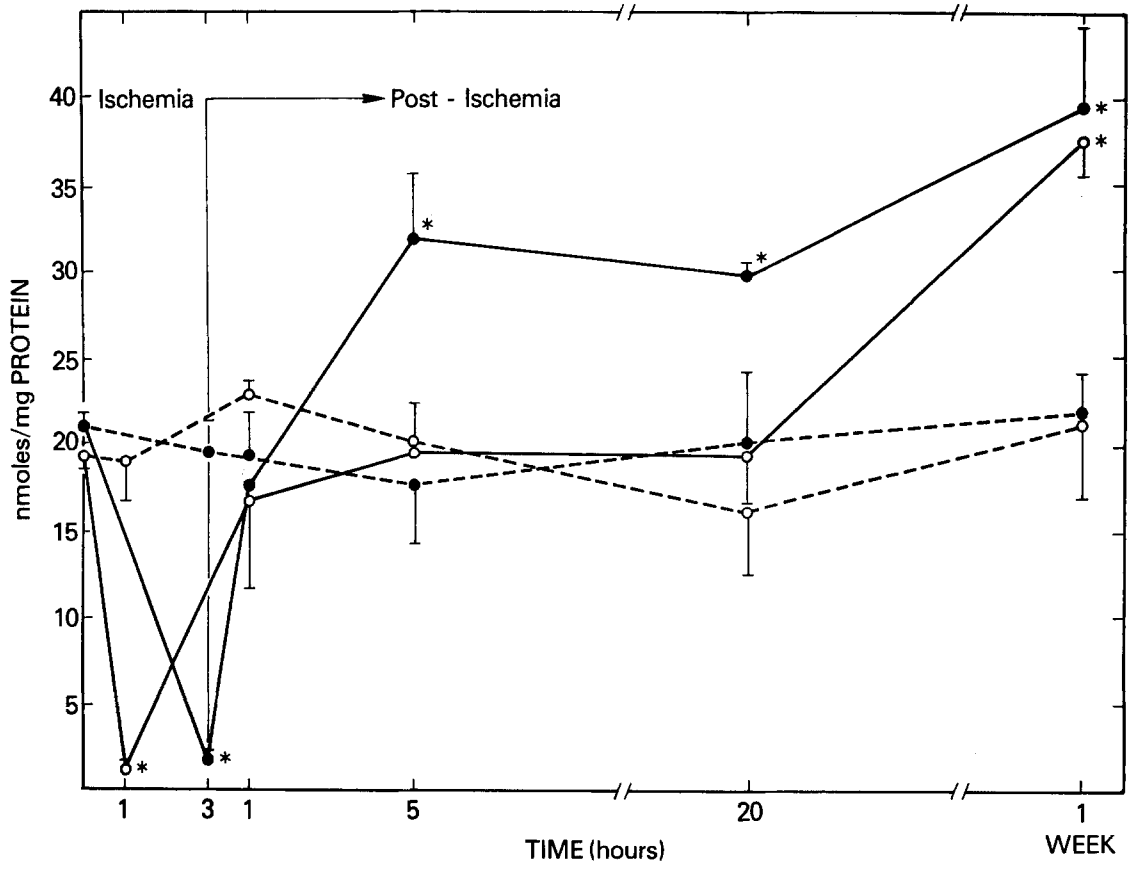
Glycogen accumulation in the central nervous system is a common occurrence following a variety of brain injuries. Abnormal deposits of glycogen have been shown in traumatized brains whether the tissue showed histological evidence of injury[2–7] or not[8,9]. Histochemical investigations have demonstrated that the glycogen deposits following brain injury were localized primarily in the astrocytes and neuropil[10,11].

The susceptibility of Mongolian gerbils (*Meriones unguiculatus*) to unilateral ischemia has provided a suitable model for the biochemical investigation of prolonged ischemia, and also the long-term recovery process[12,13]. Using the gerbil model, ITO et al.[14] have shown histologically that certain pathological changes in brain appeared in the post-ischemic period. Further, the evidence indicated that the time of appearance of these histological changes in the post-ischemic period is related to the length of the ischemic insult; the briefer the period of ischemia, the longer the interval before pathological changes occur. The relationship between the duration of ischemia and the development of a detectable lesion has been described as the maturation phenomenon[14].

In this study we demonstrate that the onset of post-ischemic accumulation of glycogen also depends on the duration of the ischemic episode. The results provide biochemical evidence for the existence of the maturation phenomenon.

Materials and methods. Mongolian gerbils (Tumblebrook Farm, West Brookfield, Mass.) weighing 50–60 g were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and the unilateral ischemia was produced by occluding the left common carotid artery with an aneurysm clip. Those animals exhibiting neurological symptoms of cerebral infarction[15] at 1 or 3 h of ischemia were either frozen immediately in liquid nitrogen or at times ranging from 1 h to 1 week after the clip was removed. The outer 2–3 mm of cerebral cortex ipsilateral and contralateral to the occluded artery was excised separately in a cryostat maintained at −20 °C. Sham-operated animals were used for the control levels of glycogen. The frozen tissue was extracted in 0.03 *N* HCl and the glycogen was measured enzymically according to the method of PASSONNEAU and LAUDERDALE[16]. The protein concentrations were determined according to LOWRY et al.[17]. Statistical significance was determined by the Student's *t*-test.

[1] To whom the reprints should be requested.
[2] N. SHIMIZU and Y. HAMURO, Nature, Lond. *181*, 781 (1958).
[3] I. KLATZO, J. MIQUEL, C. TOBIAS and W. HAYMAKER, J. Neuropath. exp. Neurol. *20*, 459 (1961).
[4] L. GUTH and P. K. WATSON, Expl Neurol. *22*, 590 (1968).
[5] M. Z. M. IBRAHIM, E. PASCOE, S. ALAM and J. MIQUEL, Am. J. Path. *60*, 403 (1970).
[6] N. R. CLENDENON, N. ALLEN, T. KOMATSU, L. LISS, W. A. GORDON and K. HEIMBERGER, Arch. Neurol. *25*, 432 (1971).
[7] H. WATANABE and J. V. PASSONNEAU, Brain Res. *66*, 147 (1974).
[8] J. MIQUEL and W. HAYMAKER, in *Progress in Brain Research* (Eds. E. D. P. DE ROBERTIS and R. CARREA; Elsevier, Amsterdam 1965), p. 89.
[9] M. J. MOSSAKOWSKI, D. M. LONG, R. E. MEYERS, H. RODRIQUES DE CURET and I. KLATZO, Expl Neurol. *27*, 500 (1968).
[10] I. KLATZO, E. FARKAS-BARGETON, L. GUTH, J. MIQUEL and Y. OLSSON, Trans. 6th Int. Congr. Neuropath. (Masson et Cie, Paris 1970) p. 351.
[11] A. PRONASZKO-KURCZYNSKA, M. J. MOSSAKOWSKI, M. OSTENDA and J. KORTHALS, Pol. med. J. *10*, 991 (1972).
[12] W. D. LUST, B. B. MRSULJA, B. J. MRSULJA, J. V. PASSONNEAU and I. KLATZO, Brain Res. *98*, 394 (1975).
[13] B. B. MRSULJA, W. D. LUST, B. J. MRSULJA, J. V. PASSONNEAU and I. KLATZO, J. Neurochem., in press (1976).
[14] U. ITO, M. SPATZ, J. T. WALKER, JR. and I. KLATZO, Acta neuropath. *32*, 209 (1975).
[15] K. KAHN, Neurology, Minneap. *22*, 510 (1972).
[16] J. V. PASSONNEAU and V. R. LAUDERDALE, Analyt. Biochem. *60*, 405 (1974).
[17] O. H. LOWRY, N. J. ROSEBROUGH, A. FARR and R. J. RANDALL, J. biol. Chem. *193*, 405 (1951).



Glycogen levels at various post-ischemic intervals following either 1 or 3 h of unilateral ischemia. Mongolian gerbils were treated and the cerebral cortex was removed and extracted as described in the materials and methods. The left common carotid artery was ligated for either 1 (○) or 3 (●) h except for the sham-operated animals. The glycogen values for the contralateral (control) cortex are represented by the dashed lines and for the ipsilateral (injured) cortex by the solid lines. The zero time levels are the values determined for the sham-operated animals. The asterisk (*) represents those values which are significantly different ($p < 0.01$) from the controls. Each point represents the mean of at least 4 determinations and the vertical bars the SEM. The time scale in post-ischemia is the time after release of the arterial occlusion.

Results and discussion. The mean glycogen levels for all time periods in the contralateral cerebral cortex, were 19.3 ± 2.5 nmoles/mg protein and were essentially the same as those observed in the cerebral cortex from sham-operated gerbils. Occlusion of the common carotid artery for either 1 or 3 h significantly reduced the levels of glcyogen in the ipsilateral cerebral cortex to 1.5 ± 0.4 and 1.8 ± 0.4 nmoles/mg protein, respectively (Figure). These results suggest that the ischemic insult was limited to the ipsilateral cortex.

The rate of glycogen recovery following either 1 or 3 h of ischemia was the same during the first hour of recirculation. The glycogen levels were not significantly different from the control values at 1 h of recovery in both groups. However, at 5 h after a 3 h period of ischemia, the concentration of glycogen was increased in the ischemic cortex to 32.2 ± 3.4 nmoles/mg protein. The elevation of glycogen following 3 h of ischemia persisted at 20 h and 1 week following the insult; the levels were 29.4 ± 0.6 and 39.7 ± 4.6 nmoles/mg protein, respectively. A similar increase of glycogen was observed after 1 h of ischemia, but the elevation to 37.2 ± 2.0 nmoles/mg protein was manifested only after 1 week of recovery.

Our results show that the reduction of glycogen during unilateral ischemia is followed by a reaccumulation of this polysaccharide during post-ischemia to concentrations greater than those of control. Both the magnitude of the ischemia-induced decrease in glycogen and the time necessary for complete recovery (1 h of post-ischemia) were essentially the same in both groups. Thus the glycogen response during ischemia and during the first hour of recirculation did little to indicate a difference in degree of ischemic insult (or damage) between the two groups. However, the levels of glycogen continued to increase in the 3 h ischemic group to levels significantly greater than control after 5 h of recirculation. In contrast, the glycogen level in the 1 h ischemic group remained constant from 1 to 20 h of recirculation after which the levels increased and were significantly greater than control at 1 week of recirculation. These data are consistent with previous findings that the biochemical events during recirculation more accurately reflect the severity of the ischemic insult than those occurring during ischemia[13]. In previous studies, the levels of ATP, phosphocreatine, glucose and glycogen decreased during ischemia but were not totally depleted[12,13]. Furthermore, once these metabolites reached a new steady state level, they remained unchanged for up to 6 h of ischemia. It was only during recirculation that different responses were manifested. The time required for metabolite restoration during recirculation appeared to be proportional to the length of the ischemic insult.

[18] I. KLATZO, in *The Basic Neurosciences* (Ed. D. B. TOWER. Raven Press, New York 1975), vol. 1, p. 313.

Histological studies on Mongolian gerbils revealed that lesions not only develop during ischemia, but even after restoration of the circulation[18]. The two variables which determine lesion formation are the intensity of the ischemic episode and the duration of the post-ischemic interval. Once the ischemic threshold is reached, the time necessary for the development of lesions is a function of the initial ischemic interval. In this study, glycogen levels after 3 h of ischemia exceeded control values by 5 h of recirculation. Following 1 h of ischemia the elevation of glycogen was apparent only after 1 week of recirculation. The glycogen response during recovery supports the histological findings that the emergence of lesions occurs more rapidly with longer periods of ischemia.

Notes on the Sperm Morphology of *Ctenomys maulinus* (Rodentia, Octodontidae)[1]

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Summary. A morphometric study of the sperm of *Ctenomys maulinus* Philippi 1872 was carried out. A process of the postacrosomic region that probably corresponds to a permanent structure in the sperms of these rodents, and is characteristic of the genus, was observed.

Several authors[3-7] have stated that the form and diameters of mammalian spermatozoa are characteristic for each species. This has been observed even among strains[8].

There are few studies dealing with neotropical species. Among them, because of peculiar filogenetic and evolutionary traits of the group[9], those concerned with Octodontidae are of particular interest. In the progress of observations on spermatozoa of chilean rodents, it was found that one species, *Ctenomys maulinus* Philippi 1872[10] differed, compared with other known mammalian species, in the morphology of the postacrosomic region.

Fig. 1. Scheme of the sperm head of *Ctenomys maulinus*

In order to define this region, initially observed on slides obtained from macerated testis, the sperms were studied in 'im pronta' specimens from the cauda epididymis. Smears of spermatozoa obtained from 4 animals were airdried, fixed in 10% formaldehyde and stained with basic fuchsin, a technique which gave the best results.

The spermatozoa were screened under the microscope and measured using an ocular micrometer at magnifications of 250× or 2,250×. The following axes were measured: total length, length of the head, length of the acrosome, caudal edge of the anterior segment of the acrosome to the posterior ring, length of the postacrosomic process and maximal width of the head.

The acrosomic tip in the spermatozoal head of *C. maulinus* is rounded and the caudal edge of the postacrosomic region is concave (Figure 1). Thus the head of

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[2] We are indebted to Dr. E. BUSTOS-OBREGÓN for reading the manuscript.
[3] G. F. FRIEND, Q. Jl. microsc. Sci. *78*, 419 (1936).
[4] M. W. H. BISHOP and C. R. AUSTIN, Endeavour *16*, 137 (1958).
[5] H. F. HIRTH, J. Morph. *106*, 77 (1960).
[6] R. L. HUGHES, Aust. J. Zool. *13*, 533 (1965).
[7] A. V. LINZEY and J. N. LAYNE, Am. Mus. Novitates *2532*, 1 (1974).
[8] A. W. H. BRADEN, Aust. J. biol. Sci. *12*, 65 (1959).
[9] M. GALLARDO, Tesis de Grado, Facultad de Ciencias, Universidad Austral de Chile, Valdivia (1974).
[10] W. H. OSGOOD, Field Mus. nat. Hist. *30*, 1 (1943).

Measures of 50 sperms of *C. maulinus* ± SE

I	HL	AL	CE–PR	PPL	TL	WH	HL:AL
A	11.02 ± 0.07	8.61 ± 0.07[a]	8.39 ± 0.06	12.34 ± 0.16	86.0 ± 0.32	5.66 ± 0.04	1.27 ± 0.0079
B	10.83 ± 0.05	8.34 ± 0.05	8.49 ± 0.04	13.89 ± 0.13		5.82 ± 0.03	1.29 ± 0.0061
C	10.49 ± 0.08	8.15 ± 0.07	7.80 ± 0.05	13.42 ± 0.13[a]		5.34 ± 0.03	1.28 ± 0.0067
D	9.78 ± 0.07	7.64 ± 0.04	7.51 ± 0.04	12.86 ± 0.10		5.49 ± 0.03	1.27 ± 0.0065

I, individuals; HL, head length; AL, acrosome length; CE-PR, caudal edge of the anterior segment of the acrosome – posterior ring; PPL, postacrosomic process length; TL, total length; WH, width of the head; HL:AL, head length:Acrosome length. [a] 49 measures.