Acta neuropath. (Berl.) 33, 91—103 (1975)

Originalarbeiten · Original Investigation · Travaux originaux

Experimental Cerebral Ischemia in Mongolian Gerbils

II. Changes in Carbohydrates

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Received July 14, 1975; Accepted August 20, 1975

Summary. A cerebral ischemia was produced by unilateral ligation of the common carotid artery in the neck of Mongolian gerbils (*Meriones unguiculatus*), which are frequently characterized by deficiencies in the circulus of Willis. Concentrations of glucose, lactate, pyruvate and glycogen were measured in the hemisphere on the side of occlusion and in the contralateral control hemisphere of animals sacrificed after 5, 15 and 30 min, as well as after 1, 3, 5 and 9 hrs of carotid clamping. Significant decrease of glucose, and increase in lactate and pyruvate concentration were found in the hemisphere ipsilateral to occlusion; the extent of the changes was proportional to the duration of the ischemia. After an initial fall, an increase in the glycogen content occurred in the later stages of ischemia. Glycogen, glucose, lactate and pyruvate were determined also at 1, 5, 20 hrs and 1 week intervals following release of an occlusion lasting for 1 hr. Return to normal values of glucose and pyruvate was seen at 1 hr after release. The lactate and glycogen levels were significantly raised on the occluded side after 20 hrs release. An increased level of glycogen was observed as long as 1 week after a 1-hr carotid occlusion.

Key words: Cerebral ischemia — Carbohydrates — Mongolian gerbils.

Introduction

A unique feature of the Mongolian gerbil is the frequency of anatomical anomalies (about 30%) in the circle of Willis. These have great usefulness in experimental brain ischemia, since they can result in infarctions of the ipsilateral hemisphere following occlusion of the common carotid artery in the neck. The simplicity of this procedure and the possibility of using large groups of animals subjected to varying periods of occlusion and of release offer special advantages for quantitative study of the biochemical dynamics of cerebral ischemia. The morphological background for such investigations has been provided by our previous light-microscopic observations on histopathological changes in this experimental model (Ito *et al.*, 1975). The present report describes changes in glucose, glycogen, lactate and pyruvate content of the brain regions ipsilateral and contralateral to the left common carotid artery after it has been occluded for

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varying periods of time. The brain carbohydrate status in gerbils with clinical signs (symptom-positive) and in those without clinical signs (symptom-negative) has been determined; changes in brain content of carbohydrate and its metabolites have also been monitored after reestablishment of cerebral circulation for different periods following a 1-hr occlusion.

Methods

Mature Mongolian herbils (50—60 g) were anesthetized with sodium pentobarbital (35 mg/kg) and the left common carotid artery was exposed through a ventral midline cervical incision, and closed with a clip for 5, 15, 30 min and 1, 3, 5 and 9 hrs, respectively. Also, the left common carotid was released after having been clipped for 1 hr, and the animals were allowed to survive for 1, 5 and 20 hrs and 1 week before termination of the experiments. Sham-operated animals were anesthetized and the left common carotid was exposed but not clipped, in order to evaluate the effect of operative stress on the brain.

When clinical signs of infarction (Kahn, 1972) became clearly recognizable in the sensitive gerbils subjected to 1 hr and longer periods of occlusion, the animals were accordingly divided into symptom-positive and symptom-negative groups. Non-selected animals with occlusions shorter than 1 hr were grouped according to results obtained. Since the data from these gerbils fell into two distinct biochemical profiles, they were classified into two groups: a) those showing a "full ischemic profile", and b) those failing to present such a profile. Thus, this investigation covers the following groups of gerbils: 1. control, non-anesthetized, non-operated gerbils; 2. sham-operated animals; 3. gerbils with "full ischemic profile" subjected to the left carotid occlusion for 5, 15, 30 and 60 min; 4. gerbils without "full ischemic profile", subjected to left carotid occlusion for similar periods; 5. symptom-positive gerbils occluded on the left side for 1, 3, 5 and 9 hrs; 6. symptom-negative gerbils occluded for the same period of time as in group 5; 7. symptom-positive gerbils, occluded for 1 hr and clip then released for 1, 5, 20 hrs and 1 week; and 8. symptom-negative gerbils treated as in group 7.

The whole animals were rapidly frozen in liquid N_2; in gerbils subjected to carotid occlusion up to 1 hr the following brain regions of left and right (control) hemisphere were dissected out: cortex, caudate, thalamus and hippocampus. Since 1 hr of carotid occlusion induced profound changes in all mentioned brain structures, the brain was divided only into left and right hemisphere in other groups of animals with 1 hr and longer occlusion periods. Occasionally, the frozen animals were stored at $-50°$ C for 1—3 days. The brain tissue was homogenized according to the method of Brunner *et al.* (1971) and the content of glucose, lactate and pyruvate were determined by enzymic methods (Lowry *et al.*, 1964) from the neutral supernatant, while glycogen was measured enzymatically as glucose equivalent following acid hydrolysis (Lowry *et al.*, 1964). Total, free and bound glycogens in both brain hemispheres were also measured (Mršulja *et al.*, 1967) in animals which were ischemic for 9 hrs and sacrificed immediately without clip release.

In order to ascertain possible histopathologic changes in symptom-negative gerbils, an additional 20 animals without clinical signs of ischemic injury were sacrificed following 6 hrs of carotid occlusion and 1 week release. The brains were processed for light microscopic observations as previously described (Ito *et al.*, 1975). Also, the histochemical localization of the tissue-bound glycogen was performed in animals which were occluded for 6 and 9 hrs and sacrificed immediately after. Glycogen was demonstrated according to the method of Guth and Watson (1968) by perfusing the animals with paraformaldehyde, embedding the coronal blocks of the brain in paraffin and staining the 10 μ sections with the PAS stain after incubation in dimidon.

Results

Sham-Operated Animals

In comparison with the controls (non-anesthetized and not operated) the contents of glucose and glycogen of sham-operated animals were significantly higher in all brain regions: $P < 0.05$ after 5 min of anesthesia; $P < 0.001$ after

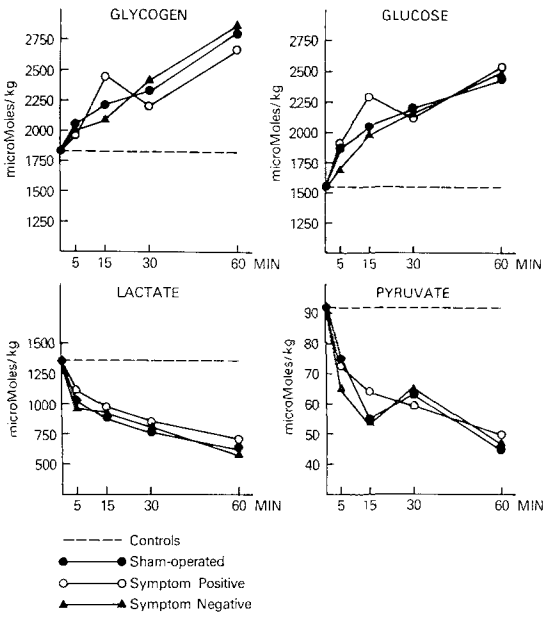

Fig. 1. Changes of glycogen, glucose, lactate and pyruvate in right non-ischemic caudate nucleus of gerbils exposed to unilateral ischemia on the left side. Each point is the average of 4 animals

15 min, and $P < 0.001$ after 30 and 60 min. On the other hand, the concentrations of lactate and pyruvate were significantly lower: from $P < 0.005$ to $P < 0.001$. There were no great differences in values of lactate/pyruvate (L/P) ratio between controls and sham-operated animals. Also, the values of glycogen, glucose, lactate, pyruvate and L/P ratio in regions of the left hemisphere of sham-operated animals were in the range of those on the right side, as well as in the range of the values obtained in brain regions of the right hemisphere in animals with carotid occlusion. Fig. 1 shows these changes in the caudate nucleus. Fig. 3 indicates that the effect of sodium pentobarbital anesthesia used in this experiment was not demonstrable after 3 hrs.

Gerbils with a "Full Ischemic Profile"

The "full ischemic profile" was characterized by a significant ($P < 0.001$ and 0.001) decrease of glucose and glycogen and increase of lactate and pyruvate concentration, as well as of L/P ratio within 5 min after carotid occlusion. These changes were pronounced in all brain regions of the hemispheres ipsilateral to occlusion, and run nearly parallel, with the exception of lactate, which showed markedly higher values in the caudate nucleus (Fig. 2).

Gerbils without "Full Ischemic Profile"

These animals did not show any regional changes in concentration of glycogen, glucose, lactate and pyruvate after 5 min of carotid occlusion. On the other hand,

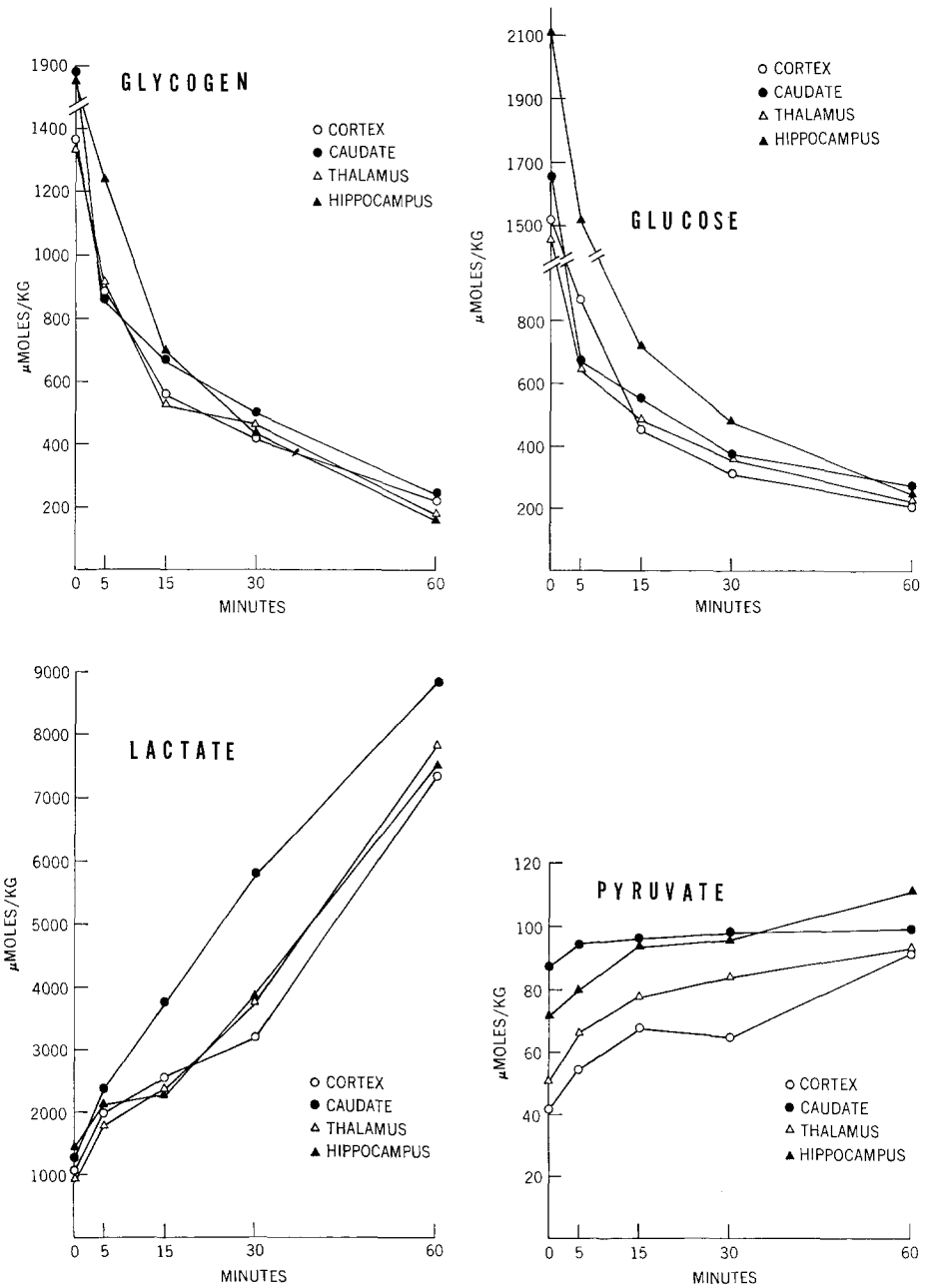

Fig. 2. Changes showing "full ischemic profile" in various brain structures on the left side in the gerbils exposed to the left carotid occlusion. The values are mean of 4 animals in each point

significant ($P < 0.01$) changes (decrease of glucose and glycogen, and increase of lactate and pyruvate) were obtained in the caudate nucleus ipsilateral to occlusion after 15 min, as well as in the thalamus after 30 min and in all brain regions after

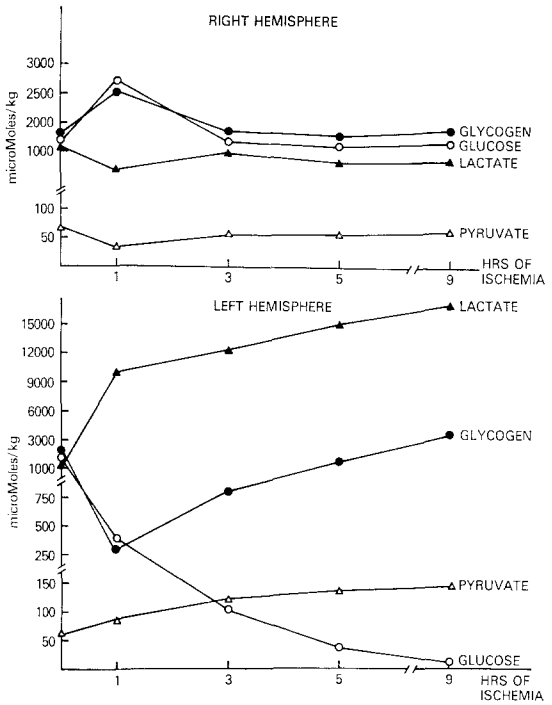

Fig. 3. Changes of glycogen, glucose, lactate and pyruvate in symptom-positive gerbils exposed to clipping of left common carotid for different time. Each point is the average of 4—7 animals

60 min of occlusion. Changes in brain structures were not so profound in these groups of animals as in previously described gerbils; thus, the gerbils with such changes were designated as animals without "full ischemic profile" (Table 1).

Symptom-Positive Gerbils with Carotid Occlusion of Different Durations

In this group 1 hr of carotid occlusion produced a marked fall in glycogen value ($P < 0.001$) in the ipsilateral hemisphere (Fig. 3). With the longer durations of ischemia, however, there was a progressive accumulation of glycogen reaching at 9 hrs of occlusion a significantly higher level ($P < 0.001$) than the normal control values. Total glycogen concentration after 9 hrs of ischemia was enhanced due to increase in the free form of glycogen (Table 2). The fall of glucose occurred progressively for the duration of the ischemia and it reached lowest values at 9 hrs of occlusion. The increase in lactate and pyruvate were proportional to the period of occlusion, lactate showing the sharpest increase in the first hour of ischemia.

Changes in the contralateral hemisphere were confined to slight increases of glycogen and glucose at 1 hr occlusion. Undoubtedly, these changes represented the effect of anesthesia.

Symptom-Negative Gerbils with Carotid Occlusion of Different Durations

In this group, the changes in the hemisphere ipsilateral to occlusion were characterized by a gradual return to normal values after maximal deviations were

Table 1. Changes in various brain structures, assayed

Duration of left carotid occlusion		Glycogen		Glucose	
		L	R	L	R
5 min	Cd.	1976 ± 28.1 (8)	1966 ± 29.6 (8)	1700 ± 31.2 (8)	1704 ± 24.4 (8)
	Th.	1548 ± 36.9 (8)	1427 ± 31.9 (8)	1556 ± 33.6 (8)	1626 ± 23.7 (8)
	Cx.	1492 ± 25.1 (8)	1531 ± 16.9 (8)	1713 ± 23.7 (8)	1683 ± 25.1 (8)
	Hp.	2006 ± 26.7 (8)	1960 ± 30.0 (8)	2016 ± 26.0 (8)	2027 ± 36.9 (8)
15 min	Cd.	1558 ± 19.2* (6)	2063 ± 37.7 (6)	1589 ± 19.4* (6)	1958 ± 34.7 (6)
	Th.	1667 ± 38.2 (6)	1695 ± 36.8 (6)	1880 ± 45.8 (6)	1808 ± 36.7 (6)
	Cx.	1852 ± 26.0 (6)	1878 ± 36.4 (6)	1745 ± 27.3 (6)	1782 ± 26.9 (6)
	Hp.	2210 ± 22.9 (6)	2398 ± 37.2 (6)	2186 ± 28.9 (6)	2298 ± 37.2 (6)
30 min	Cd.	1033 ± 26.8* (7)	2289 ± 44.1 (7)	974 ± 28.8* (7)	2170 ± 41.9 (7)
	Th.	1451 ± 31.8* (7)	1943 ± 31.7 (7)	1050 ± 31.4* (7)	1921 ± 23.5 (7)
	Cx.	1979 ± 38.4 (7)	1975 ± 47.3 (7)	1940 ± 36.0 (7)	1913 ± 35.6 (7)
	Hp.	2480 ± 39.3 (7)	2563 ± 54.0 (7)	2130 ± 24.7 (7)	2379 ± 33.4 (7)
60 min	Cd.	720 ± 18.4* (7)	2780 ± 32.5 (7)	550 ± 21.4* (7)	2480 ± 31.3 (7)
	Th.	855 ± 37.1* (7)	2170 ± 28.4 (7)	680 ± 22.5* (7)	2090 ± 24.8 (7)
	Cx.	1750 ± 27.4* (7)	2244 ± 31.2 (7)	1670 ± 28.5* (7)	2250 ± 37.2 (7)
	Hp.	1770 ± 37.2* (7)	2880 ± 38.2 (7)	1975 ± 31.4* (7)	2584 ± 38.9 (7)

The contents of glycogen, glucose, lactate and pyruvate are expressed as microMoles/kg of fresh tissue. The numbers indicate the mean value (M) ± S.E.M. The numbers of animals are given in parentheses. Abbreviations used: Cd = caudate; Th = thalamus; Cx = cortex; Hp = hippocampus; L = left; R = right.

* $P < 0.01$, L in comparison with R.

reached at the time of 3 hrs occlusion (Fig. 4). The values of glycogen and glucose run parallel reaching the lowest dip at 3 hrs of ischemia and then returning to normal by 9 hrs of occlusion. In a similar fashion, the lactate and pyruvate concentration showed highest values at 3 hrs and returned to normal level at 9 hrs of occlusion.

The changes in the contralateral hemisphere were very slight and compatible with effects of anesthesia.

bilaterally, in gerbils without a "full ischemic profile"

Lactate		Pyruvate		L/P ratio	
L	R	L	R	L	R
954 ± 24.4 (8)	926 ± 34.4 (8)	63 ± 2.2 (8)	65 ± 3.1 (8)	15.2	14.2
858 ± 30.0 (8)	880 ± 28.5 (8)	44 ± 3.0 (8)	45 ± 3.4 (8)	19.5	19.6
830 ± 22.6 (8)	823 ± 32.9 (8)	35 ± 1.9 (8)	34 ± 2.9 (8)	23.6	24.2
1030 ± 43.7 (8)	1010 ± 53.2 (8)	59 ± 3.7 (8)	52 ± 2.8 (8)	17.3	19.4
1277 ± 23.2* (6)	895 ± 20.8 (6)	83 ± 2.1* (6)	53 ± 2.4 (6)	16.4	18.8
799 ± 14.7 (6)	750 ± 35.5 (6)	33 ± 1.7 (6)	31 ± 2.3 (6)	24.2	24.2
714 ± 22.8 (6)	684 ± 32.5 (6)	23 ± 2.6 (6)	26 ± 1.7 (6)	31.0	26.2
800 ± 33.0 (6)	810 ± 35.3 (6)	32 ± 2.3 (6)	34 ± 2.5 (6)	25.3	23.7
1759 ± 32.1* (7)	843 ± 30.6 (7)	85 ± 2.0* (7)	65 ± 1.8 (7)	20.7	12.9
1229 ± 29.5* (7)	583 ± 34.7 (7)	67 ± 2.4* (7)	36 ± 2.6 (7)	28.3	16.2
637 ± 23.5 (7)	740 ± 20.4 (7)	28 ± 1.7 (7)	29 ± 1.7 (7)	24.0	25.0
630 ± 22.8 (7)	671 ± 27.8 (7)	28 ± 2.1 (7)	32 ± 2.2 (7)	22.4	20.9
3280 ± 31.9* (7)	549 ± 32.0 (7)	94 ± 2.3* (7)	46 ± 2.1 (7)	34.8	11.9
2996 ± 32.0* (7)	656 ± 20.4 (7)	92 ± 3.1* (7)	34 ± 1.7 (7)	32.5	19.3
1520 ± 38.4* (7)	553 ± 20.7 (7)	46 ± 2.3* (7)	28 ± 1.7 (7)	33.1	19.7
3040 ± 47.1* (7)	483 ± 18.5 (7)	57 ± 1.2* (7)	30 ± 1.4 (7)	53.3	16.1

Table 2. Free, bound and total glycogens in ischemic and control cerebral hemispheres after 9 hrs of common carotid artery occlusion

Glycogen	Ischemic	Control	microMoles/kg ww
Free	2003 ± 27	329 ± 13	
Bound	550 ± 47	1376 ± 10	
Total	2553 ± 48	1705 ± 20	

The numbers represent the mean value of 4 experiments ± S.E.M.

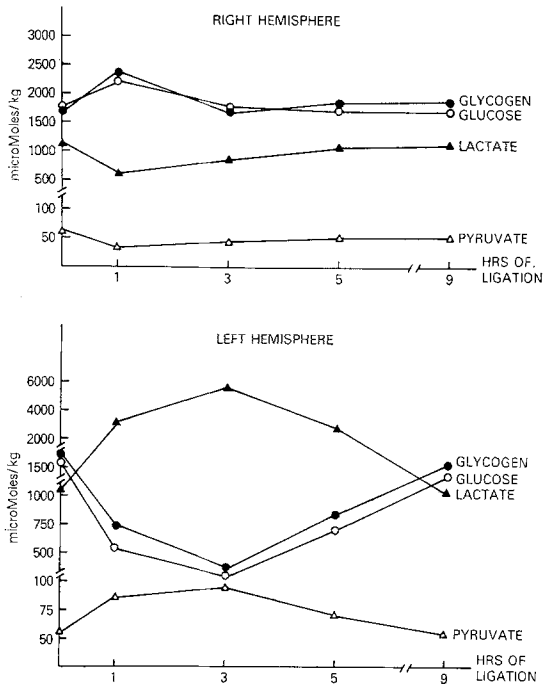

Fig. 4. Changes of glycogen, glucose, lactate and pyruvate in symptom-negative gerbils exposed to clipping of left common carotid for different time. Each point is the average of 4 animals

Symptom-Positive Gerbils with 1 hr Ischemia and Different Periods Following Release of Occlusion

In gerbils sacrificed 1 hr after release of the clip the lactate and pyruvate levels went down, whereas, those of glycogen and glucose markedly increased. As Fig. 5 indicates, at this time interval in comparison with the values of the control hemisphere, there were still significant differences in lactate ($P < 0.001$) and glycogen ($P < 0.01$), but not in glucose and pyruvate concentrations. The falling lactate levels were after 5 and 20 hrs release still significantly higher than controls ($P < 0.001$), reaching normal values 1 week after release. The glycogen content, rising slowly, reached a peak at 20 hrs after release when it was significantly higher than the control values ($P < 0.001$); 1 week after release the glycogen remained still higher ($P < 0.01$) than in the control hemispheres. The glucose and pyruvate levels assayed at 5, 20 hrs and 1 week following the release of occlusion did not differ significantly from the values in the control hemispheres.

Symptom-Negative Gerbils with 1 Hr Ischemia and Different Periods of Carotid Occlusion Release

One hour after release of carotid occlusion the concentrations of glucose, glycogen and pyruvate returned to normal values and remained as such during the longer release periods. The return to normal values of lactate was slower and it was demonstrated only after 5 hrs release of occlusion (Fig. 6).

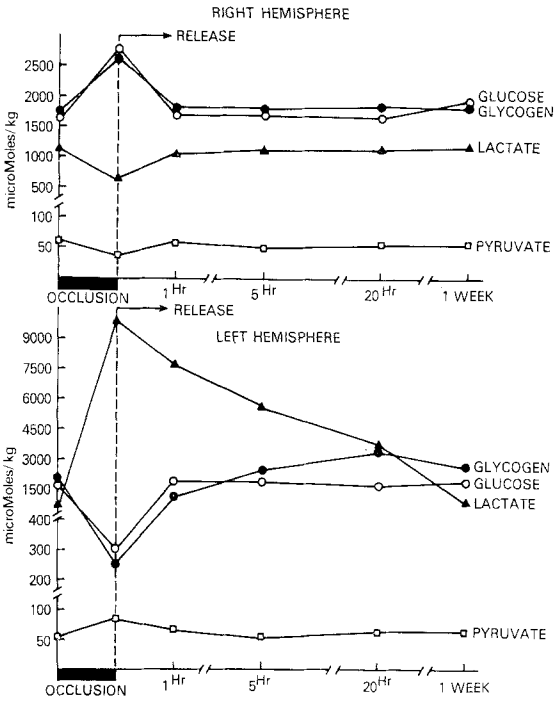

Fig. 5. Changes of glycogen, glucose, lactate and pyruvate in symptom-positive gerbils during clipping of left common carotid artery and following release for different periods. Each point is the average of 4—6 animals

Histological Examination of 20 Symptom-Negative Gerbils

Only one out of 20 gerbils in this group revealed histopathologic changes consisting of severe neuronal destruction confined to the H 2 sector of the hippocampus on the occluded side. The neurons of this sector were either absent or undergoing neuronophagia.

Histochemical Demonstration of Glycogen

The animals sacrificed immediately after 6 and 9 hrs of the carotid occlusion showed that areas with pronounced ischemic injury were completely devoid of glycogen. On the other hand, there was an intense accumulation of glycogen in the adjacent zones (Fig. 7). The glycogen demonstrable in the form of fine red granules was localized conspicuously around the blood vessels, within the astrocytic processes, or could be seen scattered within the neuropil.

Discussion

The data presented here on carbohydrate metabolism should be evaluated with background knowledge of the morphological changes appearing in the brain of ischemic gerbils.

The histologic survey of 20 symptom-negative animals, subjected to 6 hrs of carotid occlusion and sacrificed 1 week later, revealed only one gerbil with evident ischemic injury, which was manifested by neuronal degeneration limited to the

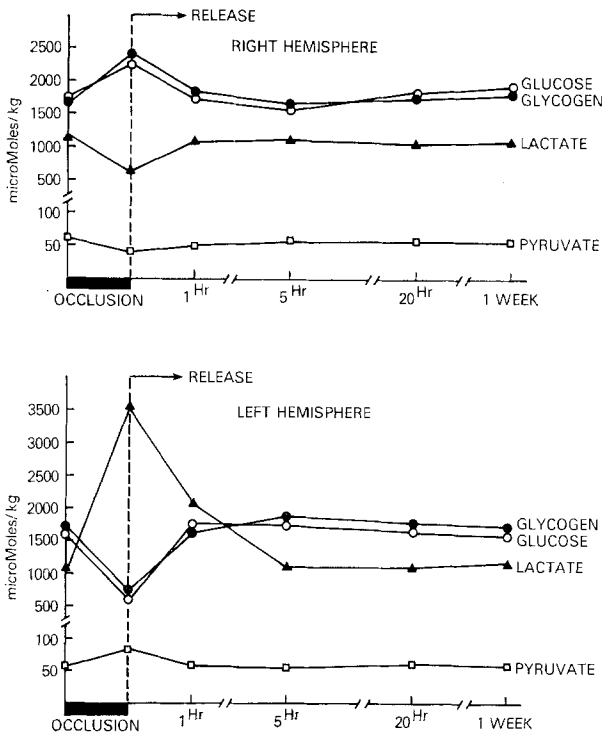

Fig. 6. Changes of glycogen, glucose, lactate and pyruvate in symptom-negative gerbils during clipping of left common carotid artery and following release for different periods. Each point is the average of 4 animals

H 2 sector of the hippocampus. Nonetheless, these symptom- and histopathologically negative gerbils showed pronounced carbohydrate changes in the hemisphere on the side of the occlusion (Fig. 4) indicating an ischemic effect, although obviously of lesser intensity. The comparison between the left hemispheres of symptom-negative (Fig. 4) and symptom-positive (Fig. 3) animals provides another example of the "maturation" phenomenon, first described in respect to the development of morphologic changes following the release of carotid occlusion and reestablishment of circulation (Ito *et al.*, 1975). Such a comparison indicates that the glycogen level in the symptom-positive gerbils falls promptly after occlusion, reaching its lowest value of 250 microMoles/kg at 1 hr of ischemia, whereas, in the symptom-negative animals, subjected to a weaker ischemic insult, the glycogen falls to a similar level but requires 3 hrs instead of one. Such shifting of the glycogen levels is in line with the main feature of the "maturation" phenomenon, indicating that the rate of "maturation" is directly related to the intensity of the ischemic insult, a lesser intensity resulting in slower development of lesions.

Earlier reports indicate that periods of complete cerebral anoxia or ischemia are followed by rapid depletion of the available energy reserves of the brain, in spite of energy production through increased anaerobic glycolytic processes.

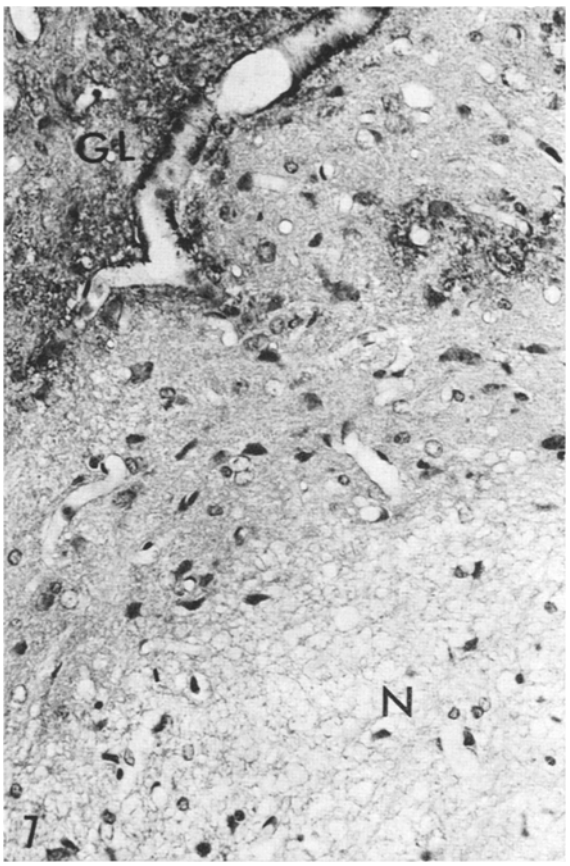

Fig. 7. Thalamus on the left side in a gerbil subjected to 6 hrs occlusion of the left common carotid artery. The necrotic region (*N*) appears to be devoid of histochemically demonstrable glycogen. The adjacent areas (upper part) show dense accumulation of PAS-positive granules (*GL*). PAS-stain after incubation in dimidon, ×280

Complex alteration of glycogen metabolism was demonstrated on the infarcted side and in the adjacent cortex of the contralateral hemisphere of anoxic-ischemic rat brain (Clendenon *et al.*, 1971). These changes in brain glycogen reflected several concurrent processes: the known reduction of oxidative metabolism in the anoxic-ischemic hemisphere, accompanied by decrease in glycogen concentration and increased lactate formation, followed by glycogen accumulation at a time when respiratory activity was returned to normal.

Our results show that depletion of glycogen content during unilateral ischemia in gerbils was followed by accumulation of glycogen, while at the same time, the contents of lactate and pyruvate were very high and that of glucose almost undetectable. Evaluation of the ratios between the free and the bound glycogen in gerbils subjected to 9 hrs of occlusion indicates that the increase in glycogen found in the ischemic hemispheres is primarily due to an increase in the free glycogen with concommitant reduction in the bound form of this compound (Table 2).

These data correlate fairly well with our histochemical observations on glycogen carried out in a similar group of animals using a procedure which apparently visualizes only the bound glycogen. Histochemical preparations revealed a conspicuous pallor indicative of quantitative loss of glycogen in the areas of ischemic injury, whereas there was a demonstrable accumulation of glycogen-positive granules in the immediately adjacent zones (Fig. 7).

The source and the mechanism for the occurrence of the progressively increased glycogen content observed in ischemia following an initial drop must remain a matter of conjecture. It can be due to either a step-up in synthesis or a decrease in the breakdown rate of the compound. Radioautographic and quantitative assays using ^{3}H and ^{14}C glucose in rats subjected to brain stab wounds (Klatzo *et al.*, 1970) seemed to provide sufficient evidence for the assumption of increased synthesis in this pathological condition. Also, histochemical observations in newborn monkeys subjected to asphyxia implied that glycogen accumulation was correlated with increase in activity of glycogen-synthesizing enzymes and this was especially pronounced in metabolically very active areas of the white matter undergoing myelination (Mossakowski *et al.*, 1968). On the other hand, Watanabe and Passonneau (1974) could not relate an increased deposition of glycogen, demonstrable 24 hrs after a brain stab wound, to an increased activity of glycogen synthetase in the active form; the increased level of glucose in these experiments was explained by the authors on the basis of increased glucose transport. The phenomenon of increase in facilitated glucose transfer from blood to brain has been reported in post-ischemic hemispheres of gerbils by Spatz *et al.* (1974). Nonetheless, it would be difficult to relate the accumulation of glycogen to oversupply of glucose by increased transport in view of the marked reduction in the levels of that compound shown in this study.

The L/P ratio during ischemia was significantly increased, proportionally to the period of circulatory arrest, and due to a great accumulation of lactate. The increased L/P ratio was previously reported in situations in which oxidative metabolism is depressed and hydrogen is accumulated in the cytoplasm (Hohorst *et al.*, 1959; Forsander *et al.*, 1965), and this also is correlative with the deficit in oxygen supply to the tissue. The low levels of glucose when combined with the high levels of lactate and pyruvate at the time of glycogen accumulation clearly indicate a depression of oxidative metabolism in the ischemic brain. The existence of metabolic depression is further supported by an increased L/P ratio, which was found in symptom-positive gerbils even 20 hrs after the release of a 1-hr carotid occlusion. The persistence of significantly elevated lactate levels in such animals appears to implicate this metabolite as playing an important role in the pathomechanism of ischemic injury which seemingly continues to develop after reestablishment of the circulation.

The reduction of oxidative metabolism was also indicated in symptom-negative gerbils by the fall of glycogen and glucose, and increase of lactate and pyruvate, as well as of the L/P ratio. The recovery from these changes, found only after a certain period of occlusion, was probably due to a compensatory mechanism. It is possible that the left hemisphere accommodated to the low metabolic requirements which developed after longer periods of carotid artery occlusion, and therefore, the substrates were found within normal limits. The regional differences in the observed

shifts among carbohydrate compounds might be explained by a different blood supply to various brain regions in gerbils, or by the fact that each brain structure might have a different metabolic rate or total energy requirement (Chesler and Himwich, 1944; Gatfield *et al.*, 1966).

The profiles of carbohydrate changes presented here constitute our first report on the biochemical substrate of cerebral ischemia in gerbils and they should serve as the baseline for assessing various other factors which could influence the dynamics of ischemic injury.

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