# Pathophysiological and Morphological Observation after 30 min Bilateral Occlusion of the Common Carotid Artery in Gerbils

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ABSTRACT

The 30 min cerebral ischemia was induced by bilateral occlusion of the common carotid artery in Mongolian gerbils under intraperitoneal pentobarbital anesthesia. The short duration unilateral carotid occlusion proceded the main ischemic insult to check anomalies of the Willis circle. Cortical bioelectric activity, arterial blood pressure, respiratory and cardiac function were continuously recorded during the ischemic period and recovery up to 9 hrs. Postmortem intra-cardiac dye infusion was performed to demonstrate communications between the vertebrobasilar and carotid circulations. In the other group, animals were sacrificed every hour after ischemia and prepared for the light microscopic observations. During ischemia blood pressure increased and electrocerebral silence was recorded. Release of the carotid arteries produced drop of blood pressure below the control values. Recovery of cerebral bioelectric activity took place between 50 min and 3 hrs after ischemia reaching in some cases the control recording. Afterwards the slow decline of electrocerebral activity appeared. The morphological alterations were observed already 1 h after ischemia. During recovery of cerebral bioelectric activity with tendency toward normalization, considerable progression of structural alterations existed with dominance of cytotoxic edema. They suppressed the cerebral function leading to the brain death.

KEYWORDS

Mongolian gerbils, unilateral carotid occlusion, bilateral carotid occlusion, cerebral ischemia, pathophysiological alterations, cerebrovascular anatomy, morphological alterations.

## INTRODUCTION

There is no more doubt that the cerebral bioelectric activity

can recover after a long period up to 30 min complete cerebral ischemia induced in normothermic conditions (Hinzen and co-workers, 1972; Hossmann and Zimmermann, 1974; Pluta and Kapuściński, 1980). The experimental study of cerebral ischemia demands animal models in which a known ischemic insult produces a consistent effect. Levine and Payan (1966) observed that the Mongolian gerbils (Meriones unguiculatus) were unusually susceptible to cerebral infarction following occlusion of one or both common carotid arteries. This was attributed to the lack of a significant posterior communicating artery (Levine and Sohn, 1969). Subsequently, many experiments were performed utilizing gerbils to demonstrate functional and anatomical communications between both anterior cerebral arteries and between the basilar and posterior cerebral arteries (Ito and co-workers, 1975; Crockard and co-workers, 1980; Levy and Brierley, 1974; Osburne and Halsey, 1975; Tamura, Horizoe and Fukuda, 1981; Yanagihara, 1978). Variability during the ischemic period related to differences in the number and size of these communicating vessels, may influence the outcome and interpretation of experiments utilizing carotid occlusion in the gerbil. Changes in arterial blood pressure during experimental procedures in gerbils are also important since they can produce residual blood flow at the beginning of ischemia or significantly prolong the predicted period of ischemic insult during recovery (Ames and co-workers, 1968; Harrison, 1975; Ito and co-workers, 1980). The respiratory disturbances after occlusion of carotid arteries play also an important role superimposing hypoxia and hypercapnia which is especially known as a worsening factor in recovery from cerebral ischemia. Definition of gerbils sensitivity to carotid occlusion based on clinical observations is not quantitative and in some cases unprecise. Many experiments utilizing gerbils have been performed without broad physiologic control because the small size of the animal causes serious technical problems. This was compensated by a large number of animals employed in a particular experimental model and/or predicted period of ischemia to receive statistically significant results. Thus, we decided to perform experiments on gerbils with the possible broad physiologic control during the ischemic period and recovery with postmortem documentation of anatomical variability of the Willis circle in each individual animal, to confirm pathophysiological observations with the morphological alterations. This study was undertaken in order to determine the effect of unilateral and bilateral occlusion of the common carotid artery in gerbils on the cerebral bioelectric activity, arterial blood pressure, cardiac and respiratory function as well as to evaluate anatomical variability of the Willis circle in the individual animals. The aim of the study was to evaluate recovery of the cortical bioelectric activity after 30 minute bilateral occlusion of the common carotid artery and to correlate its character with the morphological alterations in the brain.

## MATERIAL AND METHODS

The experiments were carried out in 22 adult Mongolian gerbils of both sexes under intraperitoneal anesthesia in dosis of 70 mg/kg. The animals were divided into two experimental groups.

The first group consisted of 10 animals in which physiological parameters were contiuously registered. In the second group, which consisted of 12 animals physiological control was omitted since they served for the assessment of morphological alterations. All surgical procedures were performed using 12.5 x binocular operating microscope. In the first experimental group two holes 1 mm in diameter were drilled symetrically in the temporal regions of the skull. Silver needle recording electrodes were fixed on the dura of the brain temporal regions and the reference needle electrodes were introduced into the muscles of both ears or chin. In three animals an additional electrode was fixed in the left occipital region. Electrocorticograms (ECoG) were recorded in the referential and bipolar montages: $T_4$ - $A_2$ - right hemisphere; $T_4$ - $T_3$ - interhemispheric linkage; $T_3$ - $A_1$ - left hemisphere, at the time constant of 0.03 sec, and a speed of paper from 10 to 60 mm/sec. Evaluation of ECoG during recovery from cerebral ischemia was based on a peak to peak amplitude and frequency expressed as percentage of the control values. Electrocardiogram (EKG) was recorded from the needle electrodes introduced into the right anterior and left posterior paw (second lead). Systemic arterial pressure (SAP) was continuously monitored from a catheter placed in the left femoral artery using a Statham P 23 pressure transducer and an EK 4 Farum electromanometer. The above physiological parameters were recorded on the eight-channel electroencephalograph (Acutrace 8, Beckman). Animals were tracheotomised, and during the period of respiratory disturbances supportive controlled ventilation with room air was applied by means of a Medipan mechanical respirator. An anterior midline cervical incision was made and both common carotid arteries were separated free from accompanying structures. All surgical procedures and preparations for the beginning of experiment took approximately one and a half hour. To check the existance and efficacy of the anterior communicating artery, the unilateral carotid occlusion was performed for the period of 3 minutes in the each side with a Heifetz clip. In the gerbils sensitive to unilateral occlusion after release of the second carotid artery the 15 min resting period was introduced before the main ischemic insult to allow recovery of bioelectric activity to the control values. Afterwards, bilateral common carotid artery occlusion was produced for the period of 30 min. Physiological parameters were continuously or periodically recorded up to 9 hrs after completion of cerebral ischemia. At the end of pathophysiological observation thoracotomy was performed and animals sacrificed by the opening of the right atrium. By means of a pressurized system, transducer and an electromanometer, physiological saline was injected into the left ventricle under the pressure of 80 mm Hg until the right atrial effluent was clear. During saline infusion the descending aorta was ligated. Afterwards the colored dyes were infused into the left ventricle under the pressure recorded in the control period. The brains were carefully removed, placed under binocular microscope and vascular anatomy was studied and photographically documented. Animals from the second experimental group were sacrificed in the same manner every hour after ischemia in groups of two up to 6 hrs, then perfused with saline and fixed in situ with 10 % buffered formalin under the perfusion pressure of 80 mm Hg. Brains were then removed and placed in 10 % formalin for further fixation

prior to examination for one week. Thereafter they were cut into blocks coronally through the anterior portion of the basal ganglia, through the fully developed thalamus, and most caudally through the cerebellum and brain stem at the posterior edge of the pons. The tissue blocks were embedded in paraffine in a routine manner. Paraffine sections from a discontinuous series were stained with H.E. and according to Klüver-Barrera's method. Brains of 3 animals not subjected to any experimental procedure, served as control.

## RESULTS

### Pathophysiological Results

The presented figures are the original recordings from experimental animals illustrating control, ischemic and recovery periods. The narrow segments were cut out from the recording paper in particular periods of time to illustrate better the below discussed phenomena. The electrocardiographic recording under the speed of 60 mm/sec registered in the chosen periods of time was cut out and placed below the curve recorded under the lower speed. The abbreviations in the figures are the following: ECoG - electrocorticogram; EKG - electrocardiogram; SAP - systemic arterial pressure; $T_4$ - $A_2$ - right hemisphere; $T_4$ - $T_3$ - interhemispheric linkage; $T_3$ - $A_1$ - left hemisphere; $O_1$ - $T_4$ - left occipital-right temporal linkage.

Unilateral occlusion of the common carotid artery. In 4 out of 10 animals the unilateral occlusion of the common carotid artery resulted in suppression of cortical bioelectric activity in the ipsilateral hemisphere during the 3 min period after occlusion (Fig. 1, and Fig. 5.a, the second segment). However, despite the serious suppression of the amplitude and frequency of the ipsilateral hemispheric activity, we never noticed electrocerebral silence during the period of observation. In 2 animals after unilateral carotid occlusion the activity from the contralateral hemisphere was also slightly suppressed (Fig. 1). There was no difference in results depending on the side of occlusion. In 3 of these animals the slight increase of systemic arterial pressure was observed in ranges of 10 - 20 mm Hg above the control values (Fig. 1). In one animal despite unilateral suppression of the cortical bioelectric activity blood pressure did not increased (Fig. 5.a, the second segment). In 6 animals the unilateral occlusion of the common carotid artery had no influence on the cortical bioelectric activity in the ipsilateral hemisphere (Fig. 2). In 4 of them the slight increase of blood pressure was noticed after occlusion (Fig. 4.a, the second segment) and 2 showed no increase of blood pressure (Fig. 2).

Bilateral occlusion of the common carotid artery. Bilateral occlusion of the common carotid artery in 9 out of 10 animals produced a decline of cerebral bioelectric activity in both hemispheres during the first minute after occlusion. From the

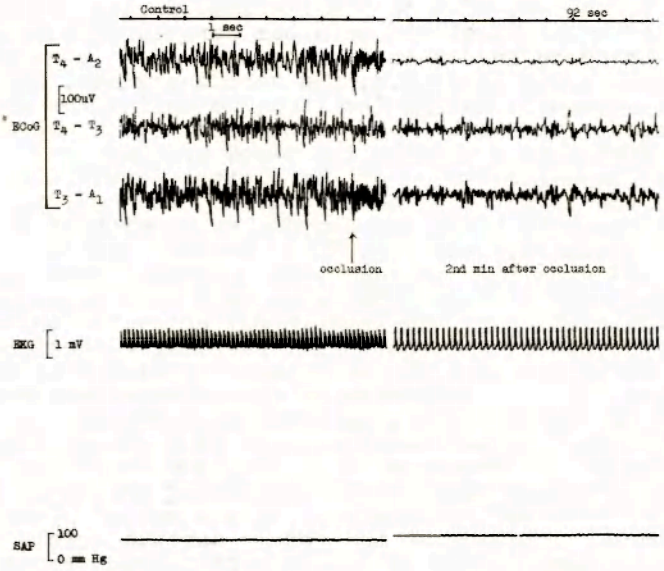


Fig. 1. Occlusion of the right common carotid artery.

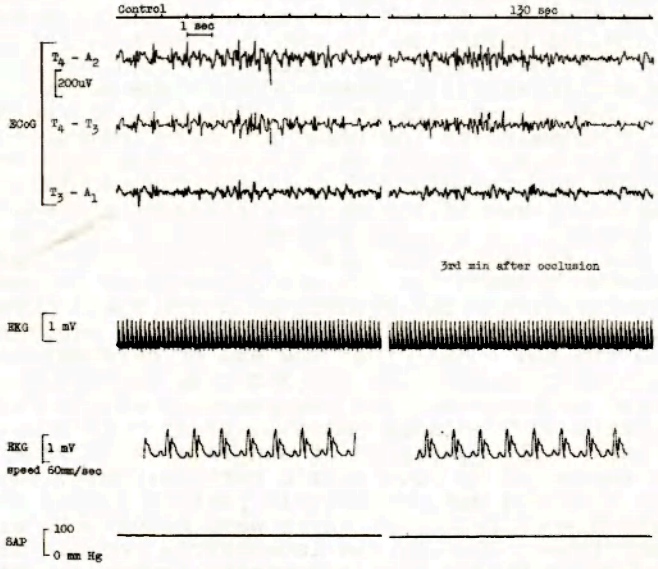


Fig. 2. Occlusion of the right common carotid artery.

second minute after occlusion the electrocerebral silence was recorded to the end of a 30 min ischemic period (Fig. 3.a, and Fig. 5.a). In the first minute after carotid occlusion systemic arterial pressure increased in ranges of 25 - 35 mm Hg above the control values (Fig. 3.a, the second segment, and Fig. 5.a, the third segment). During the ischemic period the elevated blood pressure descended very slowly and at the end of 30 min ischemic period in 7 animals the blood pressure was slightly above the control value or at the control level (Fig. 5.a). In two cases the level of blood pressure at the end of ischemia decreased slightly below the control value without necessity of pharmacological support (Fig. 3.a). In the first 10 minutes of cerebral ischemia in 9 animals with bilateral carotid occlusion respiratory disturbances developed. They were characterised by progressive slowing of the respiratory activity. Between 8 - 10 min after occlusion the controlled supportive ventilation was introduced and extended to the recovery period till the animals regained the control frequency of spontaneous respiration. Between the 10 and 30 minutes of cerebral ischemia progressive bradycardia was noticed reaching 47 - 63 % of the control heart rate at the end of ischemia (Fig. 3.a, and Fig. 5.a). It was transient in nature and disappeared during the early stages of recovery of cerebral circulation. In one out of 10 animals after bilateral occlusion of the common carotid artery despite the profound suppression of electrocorticogram, residual activity was periodically recorded during the ischemic period (Fig. 4.a). An increase of blood pressure after carotid occlusion in this animal was relatively slow and the peak level, equal to 80 mm Hg, appeared in the 10th min of the ischemic period (Fig. 4.a, the fourth segment). No respiratory disturbances had been observed in this animal, thus, the controlled ventilation was not applied during cerebral ischemia. The heart rate was very slow and did not change to the end of ischemia. In every animal release of the carotid artery occlusions resulted in a rapid drop of systemic arterial pressure below the control values (Fig. 3.b, the second segment, and Fig. 5.b. the second segment). In 7 animals the low blood pressure increased spontaneously during the early period of recovery reaching between the 15th and 20th min the control or higher then control values. In 3 animals release of the carotid arteries produced critical arterial hypotension and the pharmacological support of blood pressure was necessary. The heart rate and spontaneous ventilation increased between the 5th and 10th min of recovery period reaching subsequently the control or above the control values. The spontaneous cerebral bioelectric activity recovered slowly between 50 min and 3 hrs after the end of cerebral ischemia. The latency of recovery i.e. the interval between the end of the cerebral ischemia and the reappearance of the first cortical potentials in 9 animals amounted to 45 - 55 min. The first cortical potentials consisted of single slow waves with high amplitude separated by isoelectric periods. Then, the bursts of slow waves appeared and isoelectric periods diminished. Subsequently the higher frequency waves were noted with slowly increased amplitude. Finally the isoelectric periods disappeared and continuous activity with increasing frequency and amplitude was observed (Figs. 3.b, 4.b, and 5.b). The cerebral bioelectric activity regained the control amplitude and frequency only in 2 animals. In 8 of them the electrocorticographic re-

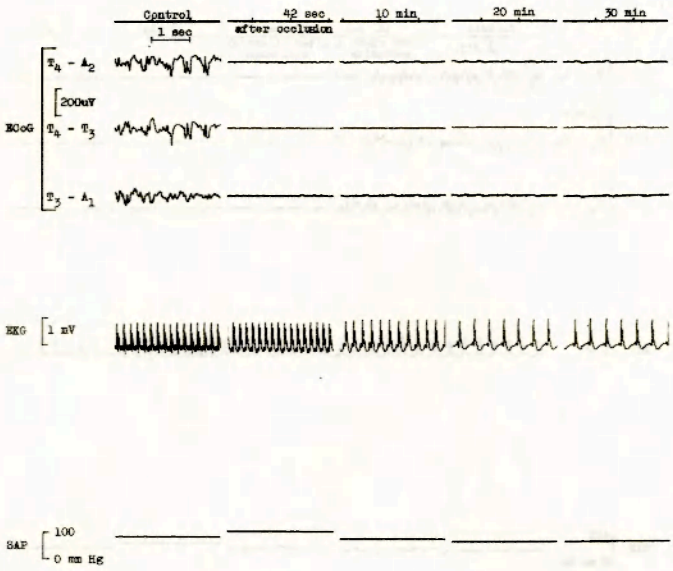


Fig. 3.a. Bilateral occlusion of the common carotid artery, ischemia.

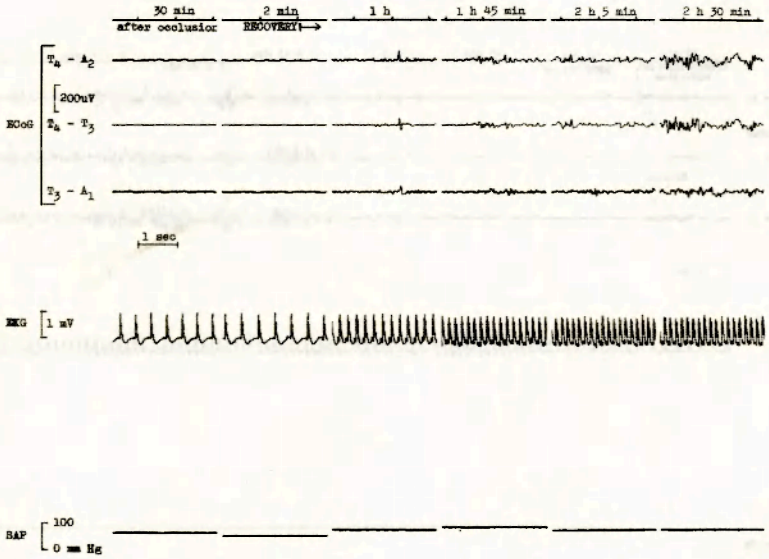


Fig. 3.b. Bilateral occlusion of the common carotid artery, recovery.

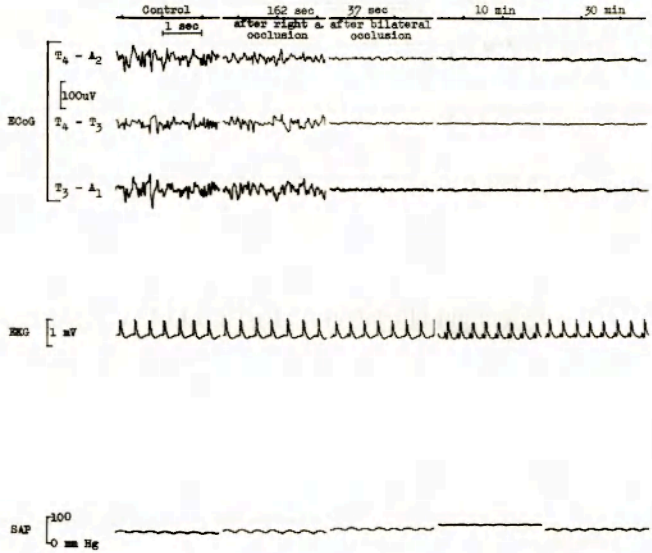


Fig. 4.a. Bilateral occlusion of the common carotid artery, ischemia.

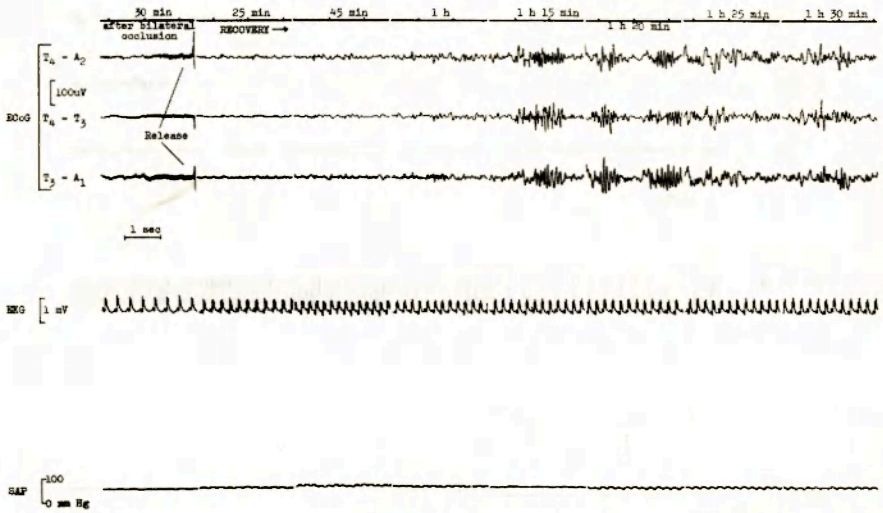


Fig. 4.b. Bilateral occlusion of the common carotid artery, recovery.

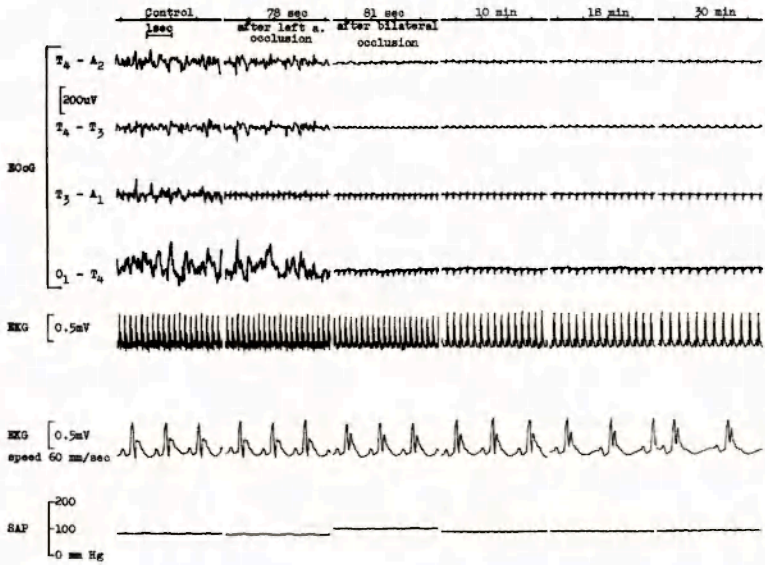


Fig. 5.a. Bilateral occlusion of the common carotid artery, ischemia.

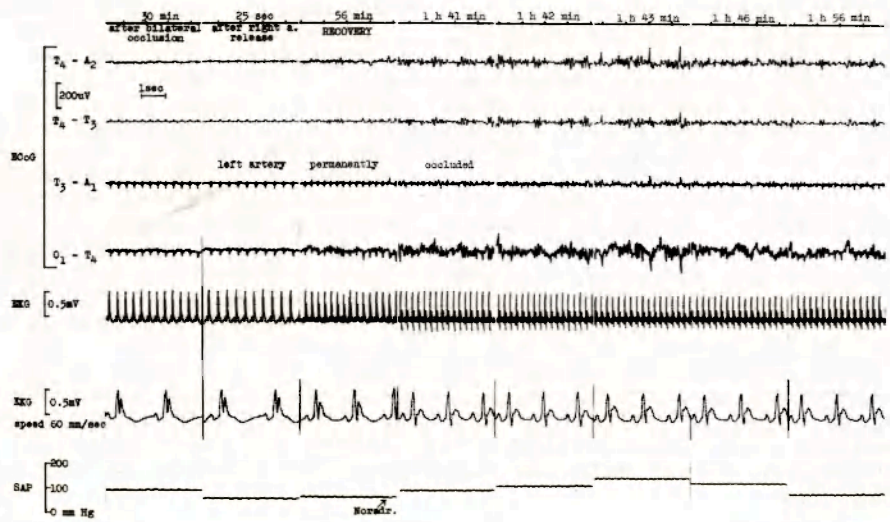


Fig. 5.b. Bilateral occlusion of the common carotid artery, recovery.

cording reached 25 - 50 % of the control values up to 3 hrs during recovery period. Afterwards either no more improvement or slow deterioration of electrocerebral activity was observed leading to brain death. In the animal in which residual electrocerebral activity was recorded during the ischemic period, the latency of recovery of the first cortical potentials appeared at 25 min, and from 45 min of the recovery period the continuous activity was registered (Fig. 4.b). The relationship between the level of blood pressure and recovery of cerebral bioelectric activity as well as the efficacy of blood flow to the contralateral hemisphere was studied in vivo in one animal by the modification of experimental procedure. For this purpose after 30 min of cerebral ischemia only the right common carotid artery was released and the left artery remained permanently occluded. In the 56th min after ischemia when the right hemispheric activity was recovering and the left hemisphere was still silent, systemic arterial pressure was elevated by the injection of noradrenaline (Fig. 5.b, the third segment). The quick improvement of amplitude and frequency of the right hemispheric activity and the reappearance of activity in the left hemisphere was observed after rise of blood pressure up to 140 mm Hg (Fig. 5.b, the sixth segment). When blood pressure decreased to 80 mm Hg after 13 min, deterioration of cerebral bioelectric activity in both hemispheres was evident (Fig. 5.b, the eight segment).

Cerebrovascular Anatomy

Postmortem intra-cardiac dye infusion demonstrated peculiarities of the cerebrovascular anatomy in gerbils. The internal carotid arteries divided into posterior, middle and anterior cerebral arteries. In 6 animals in which the unilateral occlusion of the common carotid artery had no influence on the cortical bioelectric activity, the two anterior cerebral arteries had a definite anastomosis situated rostrally to the optic chiasm - the anterior communicating artery (Figs. 6, and 7). In 4 animals in which unilateral occlusion of the common carotid artery suppressed the cortical bioelectric activity, the anterior cerebral arteries were separated, and no significant communicating artery could be found. The basilar artery bifurcated into two superior cerebellar arteries. From the bifurcation of the basilar artery at the expected point of origin of the posterior communicating arteries, the small diameter arteries ramified anteriorly encircling the tuber cinereum. In 9 animals in which bilateral occlusion of the common carotid artery resulted in a decline of cerebral bioelectric activity there was no single posterior communicating arteries between the vertebral and carotid circulations (Fig. 6). In one animal in which after bilateral carotid occlusion the residual bioelectric activity was recorded, the communicating artery linked the bifurcation of the basilar artery with the right posterior cerebral artery (Fig. 7).

Morphological Results

Morphological abnormalities were found in all the experimental

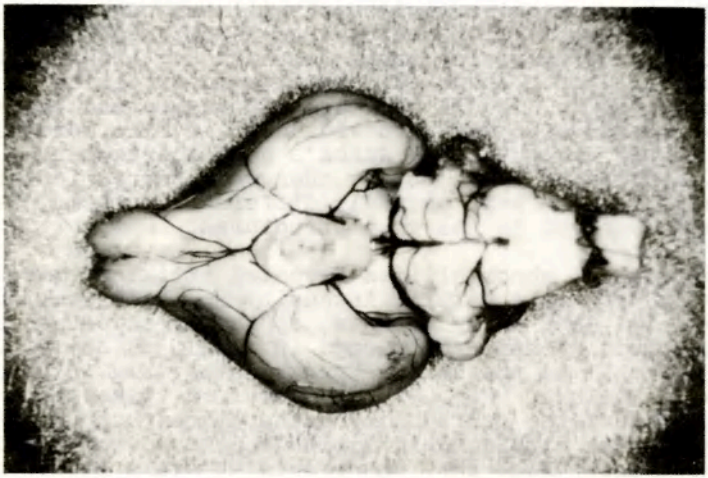

Fig. 6. Cerebrovascular anatomy of the gerbil outlined by intra-cardiac dye infusion. Base of the brain. The internal carotid arteries divide into posterior, middle and anterior cerebral arteries. The two anterior cerebral arteries join rostral to the optic chiasm. The basilar artery bifurcates into two superior cerebellar arteries. There is no posterior communicating artery. Brain of the animal presented in the Fig. 3., x 4.9.

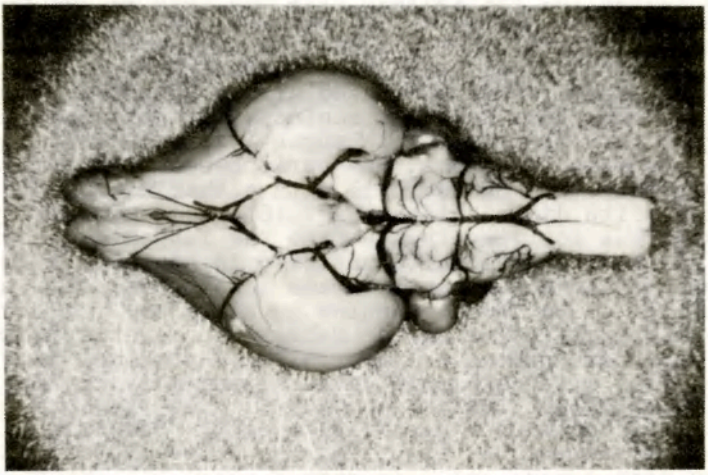

Fig. 7. The communicating artery links the bifurcation of the basilar artery with the right posterior cerebral artery. Brain of the animal presented in the Fig. 4., x 4.9.

animals. The changes varied considerably in their nature, intensity, extension and distribution. In all but one animal, structural alterations were bilateral, but in 3 cases they were symmetrical. In the remaining cases focal tissue abnormalities differed in their topography, advancement and extension in both cerebral hemispheres. Caudal hypothalamic region and thalami were the areas most frequently involved. Next in line were basal ganglia, first of all putamina, Ammon's horn and neocortex on the cerebral convexity. White matter changes appeared in later postischemic period and were entirely different in their nature from those observed in the gray structures.

The morphological abnormalities revealed striking time dependence as far as their nature, intensity and distribution was concerned. The most early changes, appearing already one hour following brain ischemia consisted in focal, patchy vacuolization of neuropil in the caudal hypothalamus (Fig. 8) and lateral area of thalamus (Fig. 9). The rarefied tissue foci were ill-limited from the surrounding unchanged structures. The nerve cells present within their limits showed no abnormalities in their appearance (Fig. 10). The remaining brain structures were normal. In the next two hours the morphological picture did not become more diversified. However, both the hypothalamic (Fig. 11) and thalamic (Fig. 12) foci were more sharply confined. Some neurons within the rarefaction foci were shrunken and stained dark. Small, disseminated foci of neuropil vacuolization located in the brain midline within the septal nuclei were the new pathological feature (Fig. 13). Four hours after release of the carotid occlusion, the light microscopy of the brain revealed features of further advancement of the pathological process. Hypothalamic and thalamic foci were sharply demarkated (Fig. 14). Tissue rarefaction was more severe, there was an evident neuronal loss within the rarefaction foci and most of the remaining nerve cells showed features of nonspecific degeneration. Vacuolization of septal neuropil was more advanced and more extensive. Slight neuropil vacuolization appeared in the putamina, contrasting sharply with unchanged globus pallidus. Bipyramidal cells of Ammon's horn revealed eosinophilic degeneration and shrinkage (Fig. 15). The same changes involved quite a proportion of neocortical neurons. The parieto-occipital cortex showed slight vacuolization of neuropil with either laminar or irregular festoon-like distribution (Fig. 16). As a rule the 3rd cortical layer was most involved, while the 1st and 4th ones were spared. In the cerebral white matter pale staining of myelin and separation of nerve bundles were noted. Interfascicular oligodendroglial cells show features of acute swelling. In the 5th and 6th hours following the release of carotid arteries occlusion the above presented abnormalities remained more or less the same. They were only more advanced and more clearly visible. The new pathological feature consisted in an extensive pyramidal cell loss in Ammon's horn with a severe rarefaction of their tissue background (Fig. 17). The white matter abnormalities were more severe and wide-spread (Fig. 18). Sharply demarcated, irregularly distributed necrotic foci appeared in the parieto-occipital area of the cortex (Fig. 19). The tissue lesions foci showed neither the presence of leucocytic infiltration nor macrophagic activity throughout. There was no glial reaction either. In no case the tissue abnormalities were pre-

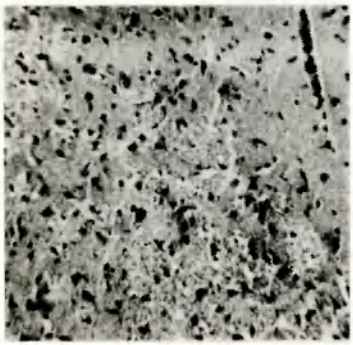

Fig. 8. One hr following brain ischemia. Delicate tissue rarefaction without sharp borders in the caudal hypothalamic area. H.E. x 200.

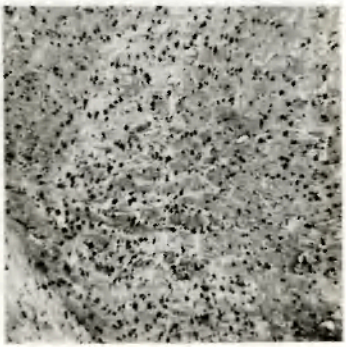

Fig. 9. One hr following brain ischemia. Slight tissue rarefaction within lateral thalamic region. H.E. x 100.

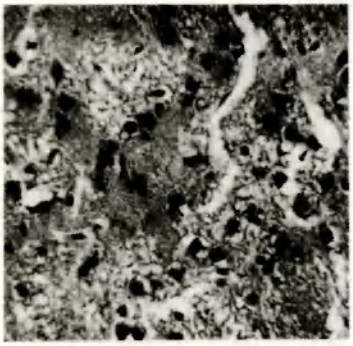

Fig. 10. One hr following brain ischemia. Confluent vacuolated foci within lateral thalamic nuclei. H.E. x 400

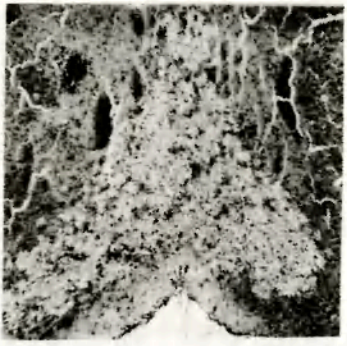

Fig. 11. Three hrs following brain ischemia. Symmetrical well limited focus of tissue rarefaction in the posterior hypothalamus. H.E. x 100.

Fig. 12. Three hrs following brain ischemia. Sharply limited foci of tissue rarefaction within thalamus. Note good preservation of Ammon's horn structures. H.E. x 20.

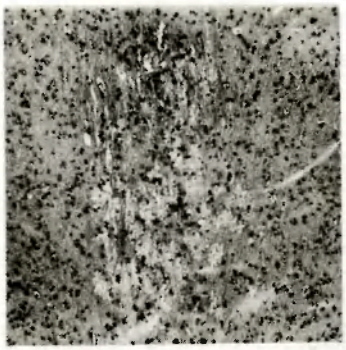

Fig. 13. Three hrs following brain ischemia. Confluent foci of vacuolated neuropil within septal nuclei. H.E. x 100.

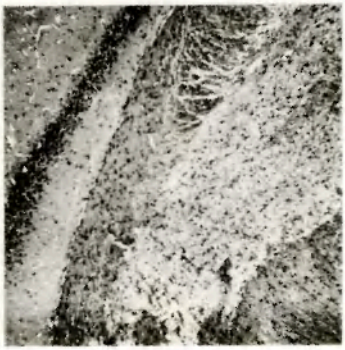

Fig. 14. Four hrs following brain ischemia. Sharp demarkation of the thalamic focus. H.E. x 60.

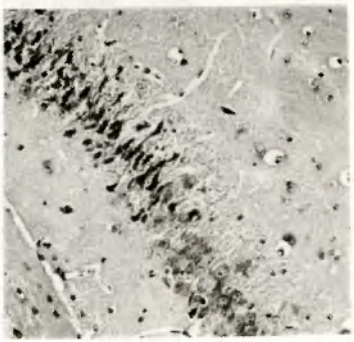

Fig. 15. Four hrs following brain ischemia. Degeneration of the Ammon's horn pyramidal cells. Note sharp border between changed and normal cell layer. H.E. x 200.

Fig. 16. Four hrs following brain ischemia. Irregular distribution of neuropil vacuolization within the cerebral cortex. H.E. x 100.

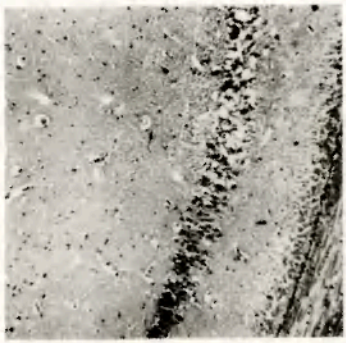

Fig. 17. Six hrs following brain ischemia. Neuronal loss and severe tissue rarefaction in the pyramidal cell layer of Ammon's horn. H.E. x 100.

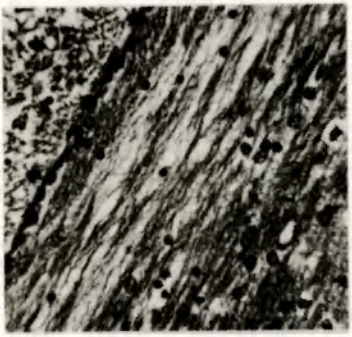

Fig. 18. Six hrs following brain ischemia. Widening of interfascicular spaces within the cerebral white matter. Oligodendrocytes with features of acute swelling. H.E. x 400.

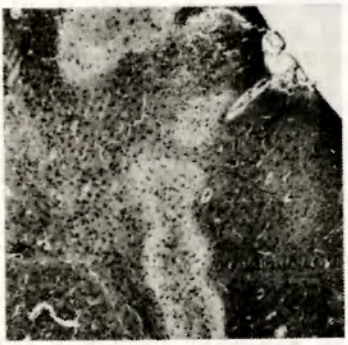

Fig. 19. Six hrs following brain ischemia. Sharply demarcated foci of cortical necrosis. H.E. x 60.

sent in the brain stem and cerebellum.

## DISCUSSION AND CONCLUSIONS

Unilateral occlusion of the common carotid artery produced suppression of the cortical bioelectric activity in the ipsilateral hemisphere in 4 out of 10 animals in our study what is in line with the results of the other authors (Ito and co-workers, 1975; Levy, 1975; Tamura, Horizoe and Fukuda, 1981; Yanagihara, 1978). Bilateral occlusion of these arteries in all but one animal resulted in bioelectric cerebral silence during the 30 min ischemic period. The quick cessation of cortical activity comparable to the results in other experimental models of ischemia (Hossmann and Zimmermann, 1974; Pluta and Kapuściński, 1980) implicated the completeness of hemispheric ischemia in our gerbils. However, we could not confirm this with certainty, since the cerebral blood flow was not examined in this experimental group. The effect of the complete versus incomplete cerebral ischemia on the outcome from the ischemic insult is still discussed. According to Hossmann and Zimmermann (1974) the ischemia "without blood" is not so damaging as compared to the "stagnant ischemia". In opposition to this opinion Steen, Michenfelder and Milde (1979) assume that the maintenance of a cerebral blood flow rate less than 10 % of control, extends the period of ischemia after which return to normal neurological function is better. The mechanism of progressive bradycardia observed in our studies especially during the second part of ischemic period is still obscure, but most probably it has the cerebral origin. The slow decrease of blood pressure during ischemic period was related to the developing bradycardia, but some relaxation of peripheral arterial system cannot be excluded. Blood pressure during the ischemic period was spontaneously maintained at a high enough level to protect blood supply to the brain stem. Periodical insufficient blood supply to the brain stem resulting in respiratory disturbances present between 8 and 10 min of ischemic period, additionally implicated the completeness of ischemia in cerebral hemispheres in our studies. The drop of blood pressure after release of carotid arteries observed in all of our animals was most probably reflexive and originating from carotid sinuses. This transient drop of blood pressure observed also by other authors (Harrison, 1975; Ito and co-workers, 1980) is mainly responsible for the "no-reflow" phenomenon which is not due to organic changes as has been suggested (Ames and co-workers, 1968). The latency of recovery of the first cortical potentials amounted to 45 - 55 min is in line with observations in rabbits which underwent the 30 min period of complete cerebral ischemia (Pluta and Kapuściński, 1980). The incomplete recovery of the cortical bioelectric activity in 8 animals up to 3 hrs after cessation of cerebral ischemia depended on the lenght of ischemic insult and the level of blood pressure in the early period after ischemia. The pharmacological induced rise of blood pressure above the control values immediately after cessation of cerebral ischemia improves the recovery of bioelectric activity (Hossmann and Zimmermann, 1974), however, an increase of systemic arterial pressure intensifies development of cerebral edema (Kapuściński, Mchedlishvili and Nikolaishvili, 1975). The lack of improvement or deterioration

of cerebral bioelectric activity after 3 hrs of recovery period was related to the progression of morphological alterations. After 3 hrs of recovery period we never noticed a profound arterial hypotension, although the systemic arterial pressure in a majority of cases was below the control values.

Morphological abnormalities observed in our material deserve a short comment. They were present already 1 hr after cerebral ischemia and in the further time intervals they became more severe, extensive and generalized. Two periods in their development could be distinguished. The first one, comprised between the 1st and 3rd postischemic hours was characterized by "local" lesions, involving practically only three structures - caudal hypothalamic region, septal nuclei and thalami, the other brain structures remaining unchanged. In the second period, involving the 4th - 6th hrs after ischemia, the pathological process became more generalized. The early changes, which in the meantime had evolved to full necrosis, were overimposed by more recent abnormalities involving other brain structures such as basal ganglia, predominantly putamina, Ammon's horn and neocortex. Pathological pattern of tissue abnormalities was evolving. The most early changes consisted in ill-defined pachy foci of neuropil vacuolization, which in the next stage underwent severe tissue rarefaction, developing further towards demarkated necrotic foci. Regardless in what time after ischemia the pathological process had involved a particular brain structure, its initial stage consisted always in characteristic neuropil vacuolization. This type of change was considered by Klatzo (1975) as evidence of the development of cytotoxic brain edema. The earliest neuropil involvement could probably be related to the well known sensitivity of dendritic processes to ischemia. Degeneration of nerve cells perikarya and their breakdown was somewhat delayed, as compared with neuropil abnormality. In the most early period, even within the foci of severe tissue rarefaction neuronal perikarya were well preserved, and only in further evolution of the pathological process they were undergoing degeneration and eventually disappeared. Even neurons as sensitive as the pyramidal cells of Ammon's horn and those of the third cortical layer showed the first signs of degeneration not earlier than 4 hrs following brain ischemia. Neuronal loss was seen between the 5th and 6th hours of the postischemic period. At the end of the observation period, most of the neuronal population showed no microscopic alterations. The relatively late neuronal involvement appears to conform to the so-called "maturation phenomenon" described by Klatzo (1975), where a biphasic development of metabolic abnormalities induced by ischemia is assumed. Our observations confirmed those of Ito and coworkers (1975) concerning different vulnerability of Ammon's horn neuronal population. Smiałek (1977), who studied oxygen consumption with the microdiver technique, suggested the difference to be due to different oxygen requirement of those cells. Tissue abnormalities in the cerebral white matter appeared latest. Their morphological nature was indicative of the development of brain edema and their appearance coincided with that of necrotic foci within the grey structures of the brain.

The topography of brain lesions requires special attention. They prevailed in the posterior portions of the brain. Although

the topographic distribution of the cerebral arteries in Mongolian gerbils is not known in detail, these regions with all probability belong to the vascularization area of the posterior artery, which originates from the internal carotid artery (Levine and Sohn, 1969; Levy and Brierley, 1974). Repeated location of the most early and most severe pathological changes in the hypothalamic area, septal nuclei and thalamus suggests the involvement of some additional factors in the selective vulnerability of these areas. One can assume that they belong to the so-called border-line zones between the areas vascularized either by larger arteries originating from the carotid artery or by the arteries branching from carotid and vertebral systems. In light of a poor and unstable branching of the basilar artery directed rostraly (Levy and Brierley, 1974; Tamura, Horizoe and Fukuda, 1981), the later seems to be more possible. The border-line zones of vascularization are known to be the site of the most severe local hemodynamic disturbances (Zülch, 1955). Postischemic hypotension observed in most of the animals, although not critical, could facilitate hemodynamic abnormalities in these areas, extending locally the duration of ischemia and therefore leading to most severe and most early damage. The thalamic areas, corresponding to the damaged areas described here, were previously found to be the sites of profound microcirculation disturbances during the postischemic period in Mongolian gerbils subjected to unilateral carotid artery ligation (Mossakowski and Gadamski, 1978). On the other hand the unilateral or assymetrical lesions in the most frequently involved areas of the brain observed in some of the animals, may be attributed to the variability and instability of the basilar blood supply to some of the cerebral structures (Tamura, Horizoe and Fukuda, 1981). The topography, dynamics and time sequences of the brain tissue lesions are clearly indicative of their complex mechanism. In addition to the metabolic effects of longlasting ischemia with all its sequele, the other factors to be taken into coside-ration are: selective vulnerability of particular brain structures depending in a significant degree on their metabolic properties, hemodynamic disturbances resulting from both local and systemic factors, differences in angioarchitectonic pattern in various brain areas and structures, and local postischemic abnormalities in microcirculation. The latter appear in the form of disseminated foci of venous hyperemia or ischemia, and are present during the whole postischemic period up to 24 hours (Mossakowski, 1978; Wise and co-workers, 1973).

In summary, our results confirmed that spontaneous bioelectric activity may recover even after 30 min of cerebral ischemia in normothermic conditions. In a majority of cases the cerebral bioelectric activity progressively deteriorated leading finally to brain death. Abnormalities in brain circulation that occured during the recovery period may play an important role in the pathomechanism of irreversible tissue lesions. The above data clearly show that during recovery of cerebral bioelectric activity with a tendency toward normalization, considerable progression of structural alterations existed with dominance of cytotoxic edema. They suppressed the bioelectric activity for the second time leading to brain death. After longer periods of cerebral ischemia even normalization of cerebral bioelectric activity seems to be of no prognostic value. The widespread

microscopic damage can be predicted by quite different sets of clinical criteria for brain death (Black, 1978). The clinical evaluation considering the duration of cerebral ischemia seems to be the crucial survival prognostic factor.

ACKNOWLEDGEMENT

The skillful technical assistance of Mr. Sławomir Januszewski is gratefully acknowledged.

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