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Systemic Factors and Epileptic Brain Damage

Prolonged Seizures in Paralyzed, Artificially Ventilated Baboons

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Prolonged electroencephalographic seizures were induced by the intravenous injection of bicuculline (0.5 to 1.4 mg/kg) in adolescent *Papio papio*, while they were paralyzed and artificially ventilated on air or oxygen. Physiological monitoring revealed an initial increase in cerebral blood flow. Arterial oxygen tension remained steady or decreased slightly. Rectal temperature rose, but did not exceed 40.0 C.

After perfusion-fixation of the brain, light microscopy revealed neurons with ischemic cell change in seven animals who had had seizures lasting three hours 25 min-

utes to seven hours 30 minutes. These changes predominated in the neocortex (small pyramidal neurons), thalamus (anterior, dorsomedial, and ventral nuclei), and hippocampus (Sommer sector and end-foolium).

Comparison with our previous studies in nonparalyzed baboons indicates that paralysis provides partial protection against neuronal damage in the neocortex and hippocampus. Cerebellar damage (related to hyperpyrexia and arterial hypotension) is almost totally prevented by paralysis.

(29:82-87, 1973)

Children or adults dying shortly after an episode of status epilepticus commonly show ischemic neuronal changes in the cerebral cortex, cerebellum, hippocampus, and thalamus.¹⁻⁴ It has not been possible in man to establish the relationship between these cerebral lesions and physiological changes occurring during or after the seizure. However Meldrum and Brierley⁵ have shown that prolonged seizures induced by

the alkaloid bicuculline in adolescent baboons, *Papio papio*, lead to ischemic neuronal changes with a pattern of distribution comparable to that found in man. Such seizures are associated with a variety of systemic changes, including hyperpyrexia, lactic acidosis, arterial hypotension, arterial hypoxia, and, sometimes, hypoglycemia.⁶ In order to evaluate the role of systemic factors in the causation of the brain damage, we have induced similar seizures in adolescent *Papio papio*, but in the presence of peripheral motor paralysis and artificial respiration. This greatly reduces the severity of the systemic changes and, as this study shows, modifies the pattern of epileptic brain damage.

Materials and Methods

Experiments were performed in *Papio papio* from Senegal, four males and four females, weighing 3.3 to 8.5 kg. They were anesthetized with halothane (Fluothane) (2% to 4% in air) and an arterial (femoral) and venous cannula inserted. Arterial pressure was recorded by a pressure transducer. Arterial pH, oxygen tension (Po₂) and carbon dioxide tension (Pco₂) were determined by means of appropriate electrodes and corrected to actual body temperature. In the four experiments in which cerebral blood flow was estimated, one or both common carotid arteries were exposed and the head mounted in a stereotaxic apparatus (prone, tilted slightly right side down). In the other four experiments the animal was in lateral decubitus (on the right side in No. 653, on the left side in Nos. 730, 670, and 748). Wounds and pressure points were infiltrated with 2% procaine hydrochloride. An endotracheal tube was inserted, halothane therapy discontinued, and gallamine triethiodide (2% solution) given intravenously until respiratory movements ceased. Mechanical ventilation (stroke volume 100 to 200 ml, 20 strokes per minute) was begun with air. In two cases (Nos. 670 and 748) 100% oxygen was subsequently used for ventilation. Atropine sulfate (0.25 to 0.5 mg) was given intravenously. Electroencephalographic activity was recorded on a four- or eight-channel EEG recorder by means of needle electrodes in the scalp, giving symmetrical fronto-rolandic and parieto-occipital derivations. The electrocardiogram was recorded by means of needle

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EEG Seizure Duration, Physiological Factors, and Regional Brain Damage in Paralyzed Baboons Given Bicuculline*

Baboon/Sex	Weight, kg	Dose, mg/kg	Seizure, Hours and Minutes	Ischemic Cell Change				Maximum Temperature, C	Minimum PO ₂ , mm Hg
				Thalamus	Cortex	C'blm.	Hippo.		
730 M	6.4	0.6	0.13	0	0	0	0	37.2	39
734 F	3.6	1.1	3.25	+	+	0	+	35.5	77
714 F	3.3	0.6	3.45	+	++	0	+	38.1	78
683 M	5.2	1.1	4.25	0	+	0	0	39.4	43
653 M	5.0	0.8	5.58	++	+	0	+	38.5	115
748 F	8.5	1.0	6.25	++	++	+	++	39.8	93
670 M	4.0	1.0	7.05	+	+	0	++	39.0	76
706 F	3.4	0.5	7.30	+	++	0	++	36.3	72

*Incidence of ischemic cell change is shown as += a few neurons; ++ = moderate number of neurons. Baboons 748 and 670 were ventilated on oxygen, the others on air.

Electrodes. Cerebral blood flow was estimated by isotope washout curves⁷ following the intracarotid injection of xenon Xe 133.^{8,9}

Bicuculline was dissolved in 0.1N hydrochloric acid, and subsequently neutralized with dilute sodium hydroxide solution. Doses of 0.5 to 1.4 mg/kg were injected intravenously.

Arterial blood was sampled intermittently for blood gas determination (synchronously with blood flow measurements when these were performed). Further doses of gallamine were given as required. In some cases glucose (5% or 10% solution) was administered intravenously after three to four hours of seizure activity.

At an interval from seizure onset, which varied between three hours 50 minutes and seven hours 45 minutes, the animal was given pentobarbital sodium intravenously (if required), and heparinized. A periaortic perfusion with heparinized saline was rapidly followed by perfusion fixation with a mixture of formaldehyde solution, glacial acetic acid, and methanol (1:1:8). The subsequent processing of the histological material was as described by Meldrum and Brierley.⁵

Results

Electroencephalographic Seizure Activity.—Within a few seconds of the injection of bicuculline (0.5 to 1.4 mg/kg) generalized seizure activity was seen symmetrically over both hemispheres. A brief tonic phase (with spikes at 10 to 20 per second) was followed by a sustained clonic phase (with polyspikes and waves at 2 to 3 per second). Occasional brief pauses in seizure activity (lasting 2 to 20 seconds) were followed by resumption either with tonic activity or with slow spikes and waves subsequently

merging into a pattern of polyspikes and waves. In one animal the injection of bicuculline, 0.6 mg/kg, produced a seizure lasting only 13 minutes; a second injection two hours 51 minutes later produced a seizure lasting 109 minutes. In the other seven animals seizures lasted between three hours 25 minutes and seven hours 30 minutes (Table) and might have lasted longer in four cases had the experiment not been terminated by perfusion-fixation of the brain. When seizure activity stopped spontaneously, it was followed by postictal depression. Recovery was followed for a significant period in only one animal (683). Isolated delta waves were seen after five minutes. This activity became continuous after about 30 minutes becoming more irregular in frequency and form over the following 40 minutes. The only other activity seen was a single burst of spikes lasting 60 seconds.

Blood Pressure.—There was an immediate rise in arterial pressure, giving peak systolic pressures of 180 to 280 mm Hg one to three minutes after seizure onset. Return to normal levels occurred 30 minutes to three hours later. Values slightly below control levels were sometimes seen late in the seizures but mean arterial pressure did not fall below 75 mm Hg (Fig 1 and 2).

Arterial Gas Tensions and pH.—In the six animals ventilated on air, control arterial oxygen tensions were slightly below normal (mean 83 mm Hg, range 59 to 106 mm Hg); with ventilation on oxygen, values of 390 and 539 mm Hg were found. Control arterial Pco₂ values were usually low

(mean 26 mm Hg, range 14 to 38 mm Hg). Arterial pH was correspondingly elevated (mean 7.52, range 7.35 to 7.60). During the seizure, arterial oxygen tension remained approximately at control level in three animals (734, 653, and 706) and fell in the other five, the lowest values being recorded in baboons 730 and 683 (Table). In general, the arterial Pco₂ level rose, the mean of the highest value being 39 mm Hg. Similarly pH level fell (mean of lowest values during seizure was 7.33 and, lowest individual value was 7.07 in baboon 670).

Cerebral Blood Flow.—In the four animals in which cerebral blood flow was measured by 133 Xe clearance the mean control flow, derived from the rapid monoexponential decay curve, was 65 ml/100 gm/min (range 59 to 74 ml/100 gm/min). The first measurement after seizure onset in each case showed a marked rise in flow (mean flow = 95 ml/100 gm/min). After one to two hours, cortical flow was still substantially increased. There was a slightly increased flow after three to four hours (75 ml/100 gm/min, mean of three baboons). Only after seven hours of seizure activity (Fig 1) was a value slightly below control obtained (55 ml/100 gm/min).

Temperature.—Once paralysis was established, and in the absence of EEG seizure activity, rectal temperature tended to fall. Two animals (734 and 706) had low temperatures before seizure onset. Rectal temperature fell transiently initially then rose during the major part of the seizure (mean rise 2.05 C, range 1.0 to 2.7 C). Ba-

boon 748 sustained a temperature of 39.8 C for more than three hours.

Cerebral Pathology. — Macroscopic examination of the perfusion-fixed brain did not reveal any significant brain swelling. Small hemorrhages were identified in two brains; in 734 these were focal in each anterior septal region; in 683 there were petechial hemorrhages scattered in the cingulate, orbital, and medial temporal cortex.

On microscopic examination the brain of 730 was normal, but the other seven brains showed evidence of ischemic cellular changes in neocortical neurons and elsewhere (Table). This involved small pyramidal neurons principally in the third but also in the fifth and sixth cortical laminae. Changes occurred diffusely throughout the cortex but with an accentuation occipitally in three cases. (There was no localization of this change to arterial boundary zones in the cortex, but one baboon [714] showed discrete foci in the occipital boundary zone, probably due to accidental carotid air embolism). Although simple ischemic cell change was the commonest finding, the earlier stage of microvacuolation and ischemic cell change was prominent in one baboon (653), and in five others it had partially evolved to the stage of ischemic cell change with incrustations (Fig 3).

Diffuse involvement of the temporal lobe was only slight but in five animals there was a concentration of ischemic neurons in the entorhinal cortex (Fig 4). In the hippocampus itself (Fig 5 and 6) changes were most marked in the Sommer sector (H_1) but were also evident in the endfolium (H_{3-5}). In five animals a high proportion of neurons in the H_1 sector did not show ischemic cell change but had pale cytoplasm with "scalloped" edges (Fig 7). (This change was seen in 683 in the absence of ischemic neuronal changes in H_1). The neuropile around the pyramidal layer commonly had a finely vacuolated appearance. Hippocampal changes, as with those elsewhere, tended to be symmetrical in the two hemispheres, but in two cases (670 and 706) the right side was slightly more severely af-

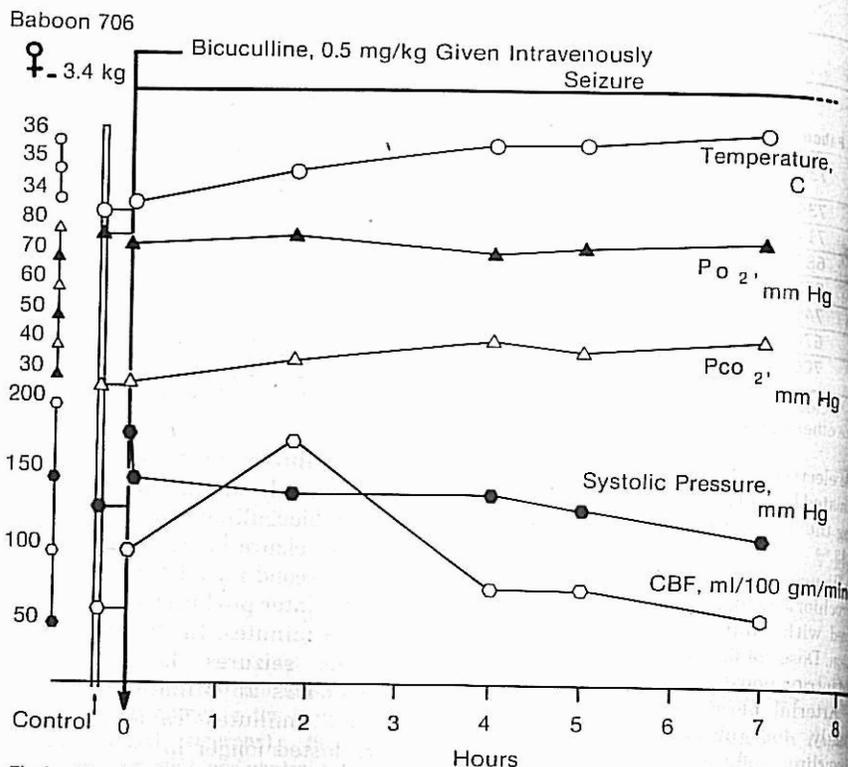


Fig 1.—Physiological changes in baboon 706 (female, weight 3.4 kg). Bicuculline, 0.5 mg/kg provoked EEG seizure activity lasting more than seven hours 30 minutes. CBF is value calculated from half-life of rapid monoexponential clearance curve of xenon Xe 133. The animal was ventilated on air. Arterial oxygen and carbon dioxide tensions (P_{O_2} and P_{CO_2}) were both initially low, but showed little subsequent variation. Rectal temperature was initially below normal.

Fig 2.—Graph of physiological changes in baboon 670 (male, weight 4 kg). Bicuculline, 1 mg/kg provoked EEG seizure activity lasting seven hours 5 minutes. Gallamine was given initially one hour before bicuculline, and the animal was subsequently ventilated on oxygen. Small doses of gallamine were repeated (arrows). Atropine, 0.25 mg/kg, was given intravenously 45 minutes after bicuculline. Glucose (10 ml of 10% solution) was given intravenously (arrows).

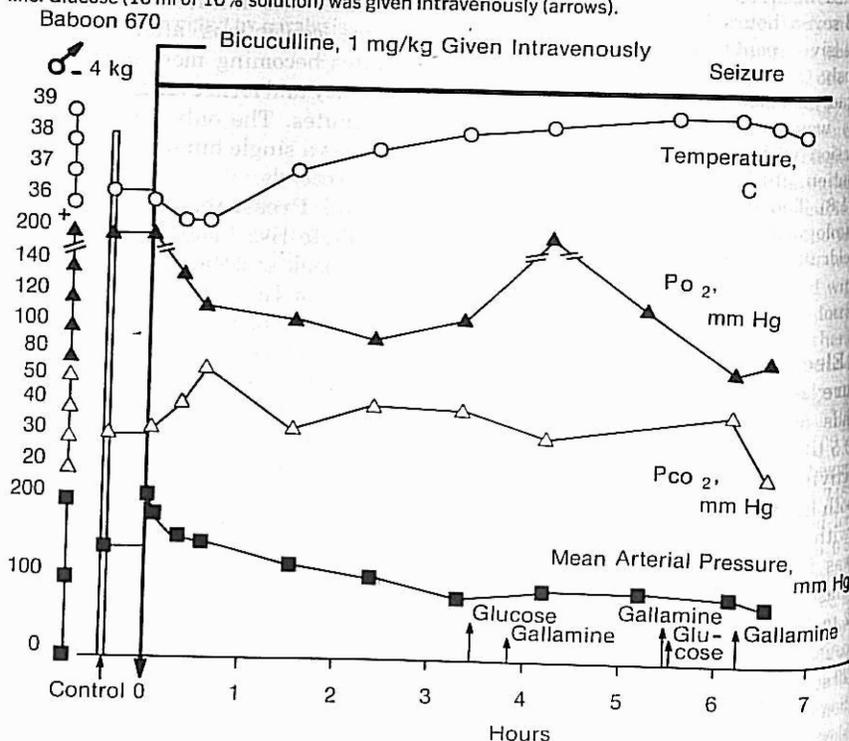


Fig 3.—Occ with incrustations

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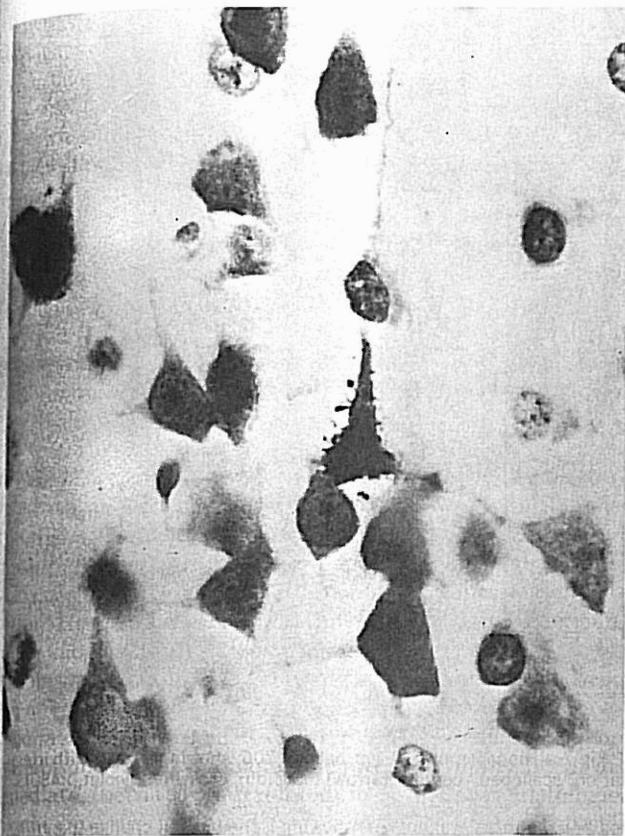


Fig 3.—Occipital cortex of baboon 683, showing ischemic cell change with incrustations (celloidin, cresyl fast violet, $\times 465$).

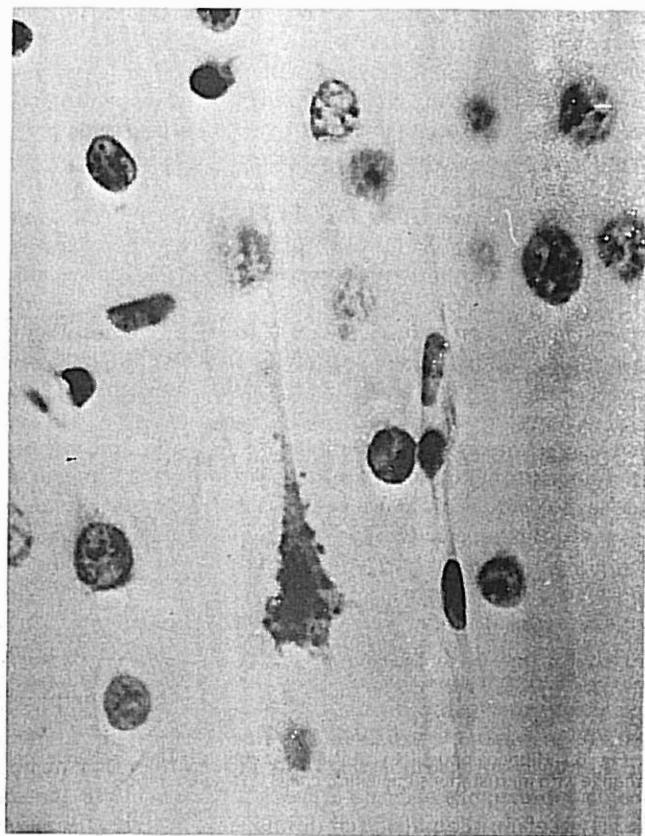
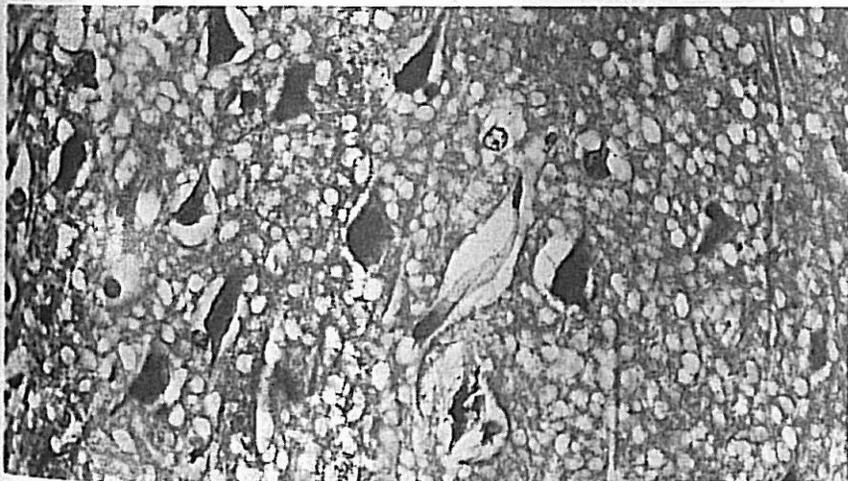


Fig 4.—Entorhinal cortex of baboon 706, showing ischemic cell change with incrustations (celloidin, cresyl fast violet, $\times 465$).

Fig 5.—Hippocampus (H.) of baboon 706, showing spongy neuropile and ischemic cell change in pyramidal neurons. (paraffin, Luxol fast blue and cresyl fast violet, $\times 250$).



the striatum (670, 714, and 748) and two had involvement of the globus pallidus (706 and 714).

Comment

The neuronal damage found in these baboons was less severe, for comparable seizure durations, than was found in our previous study in unparalyzed baboons.⁵ Except for the sparing of the cerebellum, the selective pattern of damage was similar to that in the unparalyzed animals. The reduction in the severity of the secondary physiological consequences of the cerebral seizure was presumably responsible for the reduction of brain damage. The EEG seizure itself was sustained longer in the paralyzed animals, perhaps because the physiological status of the animals was better maintained.

Prominent among the physiological consequences of unmodified seizures was hyperpyrexia, resulting primarily from the excessive motor activity.⁶ The small rise in temperature, consis-

ected. The amygdala was spared in all cases.

The cerebellum was normal except that in one baboon (748) Purkinje cells at the boundary zone between the territories of the superior cerebellar and the posterior inferior cerebellar

arteries showed early ischemic cell change.

In the thalamus scattered neurons in the anterior, dorsomedial, and ventrolateral nuclei were variably involved (Fig 8). Three animals also showed ischemic neuronal changes in

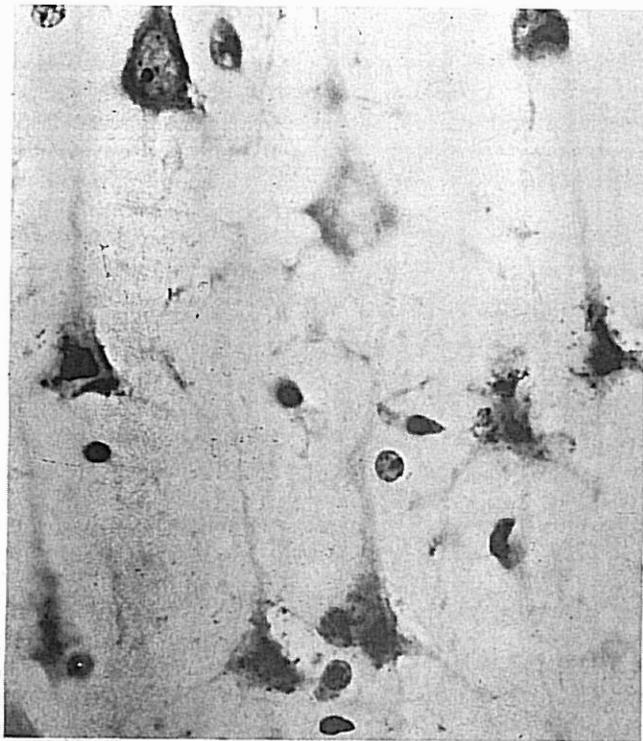


Fig 6.—Hippocampus (H.) of baboon 706, showing ischemic cell change with inclusions (celloidin, cresyl fast violet, $\times 465$).

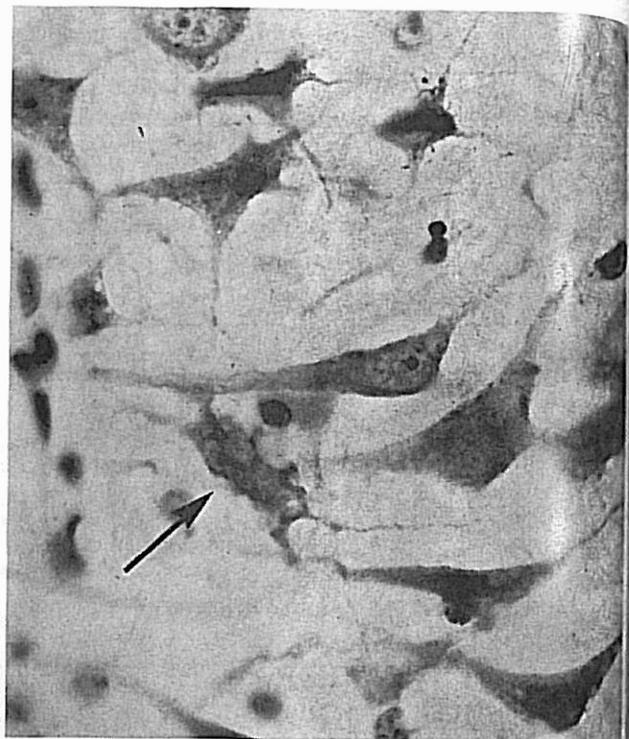
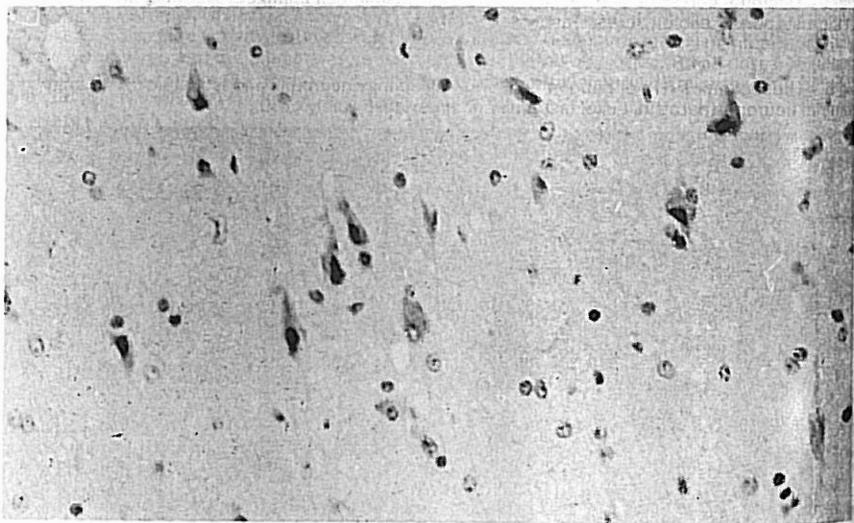


Fig 7.—Hippocampus (H.) of baboon 706, showing neuron with irregular or "scalloped" contour (arrow) (celloidin, cresyl fast violet, $\times 480$).

Fig 8.—Thalamus of baboon 748. Ventrolateral nucleus showing ischemic cell change (paraffin, hematoxylin-eosin, $\times 180$).



tently seen in the paralyzed animals, was probably due to increased heat production by the brain, heart, and liver, but reduced heat loss as an autonomic component of the seizure is also possible. In unparalyzed baboons Meldrum and Brierley⁵ observed a correlation between the severity of hyperpyrexia during the seizure and the severity of subsequent cerebellar damage. In man, hyperpyrexia in the absence of seizures can be followed by cerebellar damage.^{10,11} The absence of cerebellar damage in the present series (except for the animal with the most severe rise in temperature) emphasizes the role of pyrexia in facilitating cerebellar epileptic damage.

In spite of the paralysis and the mild secondary physiological changes, ischemic cellular changes occurred selectively in neurons in the neocortex, thalamus, and hippocampus. Such changes are the end result of disturbance of cellular energy metabolism. Their morphology and chronological evolution in the primate brain have been described by Brierley et al¹² after arterial hypotension or hypoglycemia. However, dur-

ing the prolonged seizure studied here these two factors cannot have been responsible for the neuronal changes. The variable fall we observed in P_{O_2} was probably due to autonomic components of the seizure modifying tracheo-bronchial secretion or pulmonary hemodynamics. However, only in one animal was the fall severe enough to have played a

significant part in the initiation of neuronal changes. Only slight metabolic acidosis occurred and there is no evidence that this can contribute to ischemic changes. It is thus not possible to attribute the changes observed to known systemic consequences of the seizure and local consequences of the excessive discharges must be considered.

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That seizure discharges lead to increased cerebral energy consumption has been demonstrated for seizures lasting a few minutes.^{13,14} Also, it is known that the increase in cerebral blood flow at the beginning of a seizure tends to compensate for the increased metabolic requirement.^{6,15} What is not yet known is the change in cerebral metabolic rate and cerebral blood flow that occurs late in prolonged status epilepticus. In our experiments the cerebral blood flow was approximately normal late in the seizure, but we do not know whether the cerebral metabolic rate was still increased. Local seizure activity could lead to a depletion of total energy charge within neurons because of a failure of oxygen and glucose supply to keep pace with energy consumption. However, because of the good physiological state of our animals, it is more probable that there was a two-stage process. Within neurons the first stage could be excessive consumption of a critical metabolic intermediate (perhaps one related to γ -aminobutyric, glutamic, or aspartic acid, or other substance released during nervous activity) or depletion of a metabolic cofactor, leading to a failure of a particular metabolic pathway and, secondarily, disturbing energy metabolism. The first stage could also

be a failure of astrocytic function. The extracellular accumulation of potassium or of glutamate or other amino acids released from neurons could overwhelm the uptake capacity of the astrocytes (particularly in the presence of increased cerebral venous pressure).⁶ Swelling of astrocytic end-feet around capillaries and neurons has been described¹⁶ after pentylene-tetrazol (Metrazol)-induced seizures and is the probable explanation of the fine spongy state of the neuropile observed by us in the hippocampus. This could lead to a local failure of transport of glucose and other compounds to the neurons or to a local failure of tissue perfusion. Secondarily, there would be impairment of intraneuronal energy metabolism. The interaction of these various neuronal, astrocytic, and local vascular factors probably shows regional variations and may account for features in the pattern of selective vulnerability.

It should be borne in mind that many brain areas and cell types appear histologically normal after 7½ hours of sustained seizure activity. For example, the large Betz cells in the motor cortex remained histologically normal. This observation is at variance with the claim of Epstein and O'Connor¹⁷ that after three hours' seizure activity in paralyzed,

ventilated cats Betz cells became incapable of demonstrating oxidative metabolism. Our findings are, however, entirely consistent with recent reports of the absence of irreversible changes in the rat brain or cat hippocampus after seizures lasting up to 1½ hours.^{18,19} The problem that still requires a biochemical and ultrastructural answer is, "What is it, happening after three to seven hours of seizure activity, that leads selectively to irreversible neuronal changes?"

These experiments provide an important guide to clinicians concerned in the management of status epilepticus. Energetic maintenance of respiratory and cardiovascular status, prevention of hypoglycemia, reduction of body temperature, and, if desired, muscular paralysis is likely to delay the occurrence of brain damage, but these conditions probably cannot prevent its ultimate appearance if the cerebral seizure continues. Thus, none of these procedures should be allowed to obscure the primary objective, which must be the early arrest of the seizure discharge itself.

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