0031-3998/86/2007-0581$02.00/0
PEDIATRIC RESEARCH
Copyright © 1986 International Pediatric Research Foundation, Inc.
Vol. 20, No. 7, 1986
*Printed in U.S.A.*

# $^{31}$P Nuclear Magnetic Resonance Study of the Effect of Hypoxemia on Neonatal Status Epilepticus

RICHARD S. K. YOUNG, RICHARD W. BRIGGS, SUSAN K. YAGEL, AND IDA GORMAN

*Department of Pediatrics (R.S.K.Y.), Yale University School of Medicine, New Haven, Connecticut 06510 and Department of Radiology and Biochemistry (R.W.B., S.K.Y., I.G.), The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033*

**ABSTRACT. Prolonged neonatal seizures are often accompanied or exacerbated by hypoxemia. To determine the effects of hypoxemia on neonatal status epilepticus, we determined cerebral blood flow and cerebral metabolic state in groups of neonatal dogs subjected to hypoxia, to seizures during normoxia, or to seizures during hypoxia. The compensatory increase in cerebral blood flow was greatest in animals subjected to seizures during normoxia and somewhat less pronounced in animals made hypoxic. However, blood flow failed to increase in forebrain structures when animals were subjected to the combination of seizures and hypoxia. Accordingly, levels of adenosine triphosphate in forebrain (measured both by *in vitro* enzymatic analysis and by *in vivo* phosphorus-31 nuclear magnetic resonance spectroscopy) were depleted to the greatest degree in animals who were seizing while hypoxic. In addition, brain glucose was significantly reduced only in the seizure-hypoxia group. Systemic factors such as hypoxemia may play a critical role in the disruption of cerebral energy balance during neonatal status epilepticus. (*Pediatr Res* 20: 581–586, 1986)**

**Abbreviations**

**$^{31}$P-NMR, phosphorus-31 nuclear magnetic resonance**
**CBF, cerebral blood flow**
**PCr, phosphocreatine**

Hypoxia, ischemia, seizures, and intraventricular hemorrhage are major sources of neurological morbidity in the human neonate (1, 2). Although these neurological insults may occur separately, they frequently occur in conjunction. Considerable controversy exists regarding the role of systemic factors in the production of epileptic brain damage. Initial experiments show that oxygenation greatly reduces neuropathological injury (3). However, more recent studies suggest that local factors secondary to the epileptic activity may be more important in the genesis of the lesions (4).

The paradox of epileptic brain damage with only small decline in energy state during prolonged seizures in the paralyzed and ventilated experimental animal is difficult to explain (5). The purpose of these experiments was to define the degree of metabolic perturbation imposed by hypoxia, by seizures during normoxia, and by seizures during hypoxia. These are topics of major clinical relevance since seizures in the neonate are frequently manifested by apnea and cyanosis (2).

In measuring cerebral metabolic state, we employed high resolution $^{31}$P-NMR spectroscopy, a powerful technique for noninvasive, sequential measurement of brain energy changes *in vivo* (6–9). These *in vivo* measurements were corroborated by *in vitro* determination of brain metabolite concentrations.

## METHODS

*Systemic changes.* Experiments were carried out in neonatal (1- to 10-day old) mongrel dogs as previously described (8). Halothane (0.5–3.0%) in oxygen was administered to allow tracheostomy. Halothane was then discontinued and the animals were paralyzed with pancuronium (0.2 ml administered intraperitoneally) and mechanically ventilated with a gas mixture of 30% $O_2$/70% $N_2O$. In addition to the use of nitrous oxide for analgesia, all sites of incision were infiltrated with local anesthetic (xylocaine, 1%). The femoral arteries were catheterized to continuously monitor blood pressure and to anaerobically withdraw samples for determination of blood gases. The femoral vein was catheterized to allow infusion of drugs. Wounds were periodically infiltrated with xylocaine.

Carbon dioxide tensions were normalized ($pCO_2$, 30–40 mm Hg) by adjusting minute ventilation. Thereafter, ventilator settings were not changed. Animals who had abnormal acid-base status (base deficit > 5 mmol $HCO_3$/liter) were not entered into the study. Following a 30-min normalization period, animals were randomly assigned to one of four study groups: control group, hypoxia group, seizure-normoxia or seizure-hypoxia group. The duration of hypoxia, seizure, or seizure and hypoxia was 45 min. Animals in the hypoxia group or seizure-hypoxia group were ventilated with a gas mixture containing 8% $O_2$/92% $N_2$. Animals in the control group or seizure-normoxia group were ventilated with a gas mixture consisting of 30% $O_2$/70% $N_2O$. Seizures were induced in the seizure-normoxia and seizure-hypoxia groups with an intravenous infusion of the $\gamma$ amino butyric acid blocking agent, bicuculline (Sigma Co.), 2 mg/kg intravenously. Animals in the control and hypoxia groups received an equivalent volume of 0.9% saline intravenously.

Blood gases were measured with a Radiometer microblood gas analyzer. Plasma glucose levels were measured using a Beckman Glucose Analyzer II; plasma lactate levels were measured spectrophotometrically using standard enzymatic analyses (10). Plasma catecholamine concentrations were determined by radioenzymatic analysis (11).

*Cerebral physiology.* The EEG was continuously recorded with scalp electrodes and a Beckman polygraphic recorder. An auto-

Received November 25, 1985; accepted February 11, 1986.
Correspondence Dr. Richard S. K. Young, Department of Pediatrics, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510.
Supported by NIH Grant I R01 NS23315 (R.S.K.Y.) and were presented in part at the Child Neurology Society Meeting in Memphis,TN, October 1985. R.S.K.Y. is the recipient of a Clinician-Scientist Award from the American Heart Association.

radiographic technique was employed to measure regional CBF as previously described (8, 12). CBF was measured at the end of the 45-min experimental period. Briefly, 50 $\mu$Ci of ($^{14}$C)–iodoantipyrine were infused intravenously. During the infusion, samples of arterial blood were serially collected and later analyzed for radioactive content in a liquid scintillation counter. The blood circulation of the animal was stopped by intravenously injecting a solution of pentobarbital sodium and KCl. The brain was quickly removed, frozen in Freon 12, and sectioned in a cryostat. The sections were mounted on glass slides which were then applied to a single-emulsion mammography film with a set of ($^{14}$C) reference standards (Amersham). Calibration curves were obtained for each area of film by relating the optical density of each area of brain to ($^{14}$C)-iodoantipyrine concentrations.

*Cerebral metabolism.* Cerebral metabolic changes were monitored using $^{31}$P NMR, as previously described (8). Briefly, $^{31}$P NMR data were collected utilizing a transmitter-receiver coil tuned for phosphorus (32.5 MHz), a 1.89 Tesla superconducting magnet (Oxford Research Systems, 26-cm bore diameter), and a Nicolet 1280 computer with a 293C timer.

The coil (14 AWG copper magnet wire) was an open Helmholtz rounded to fit the animal's cranium. Each of the two loops was 3.3 cm long and 2 cm wide. The coil's sensitive volume thus encompassed approximately 3.5 cm in length, 4 cm in width, and 1.5 cm in depth, including both white and gray matter. Brain dimensions of 6- to 10-day old neonatal dogs are approximately 4 cm long and wide and 2.5 cm deep. Since negligible signal was obtained from scalp and cranial muscle, reflection or other cranial surgery was unnecessary (the rapid and complete demise of the PCr peak within minutes after death was the empirical criterion for judging the scalp and muscle contribution to be minimal).

The homogeneity of the magnet was optimized by observing the proton signal of water. A pulse width (36 $\mu$s) to provide optimal signal to noise (approximately 12.5:1 for ATP after 300 1-s scans) was utilized. Rapid pulsing was used to optimize sensitivity per unit time (13), even though comparisons of relative amounts of metabolites are thus invalid due to differential partial saturation of the peaks of interest. Serial concentration changes of individual metabolites may be reliably obtained if it is assumed that $T_1$ values are relatively independent of physiological state (6, 8, 13–16). Since PCr $T_1$'s of brain *in vivo* are in the range 2.2–4.4 s (15, 16), precise quantitation would require pulse delays of at least 6.6–13.2 s.

Control spectra were obtained for 30 min. Thereafter, the bicuculline infusion was begun in animals in the seizure-normoxia or seizure–hypoxia groups. The gas mixture was also switched from 30% $O_2$/70% $N_2O$ to 8% $O_2$/92% $N_2$ in the hypoxia and seizure-hypoxia groups. Three groups of three spectra were then computer averaged (0–15, 16–30, and 31–45 min after bicuculline). Curve fitting was performed using the Nicolet 1280 computer program "NMRCAP," to determine areas of individual resonances in spectral regions where peak overlap occurred. This deconvolution is necessary for accurate measurement of peak areas at low fields in crowded spectral regions. The NMRCAP program allows one to create theoretical spectra with individual peaks of variable intensity, position and width; the root mean square deviation between experimental and theoretical spectra is then minimized. We placed constraints of constancy of chemical shift and linewidth (± 10%) on all peaks except that of inorganic phosphate which could vary because of its pH-dependence.

Intracellular pH was determined by noting the frequency of the inorganic phosphate peak with respect to that of phosphocreatine, which is constant over the physiological range of pH. Literature values of cerebral intracellular concentrations of ions and metabolites (18–20) were used to prepare a standard titration curve at 37° C for the inorganic phosphate position *versus* pH. Solution composition and best-fit values have been previously reported (8). More information of the details of measuring pH by $^{31}$P NMR can be found in several excellent reviews (21–23).

The second type of measurement of cerebral metabolites was made using a modification of the funnel freezing method (24). Immediately after collection of NMR spectra, brains of animals were frozen *in situ* with liquid nitrogen and stored at −80° C. Samples of cerebral cortex and adjacent subcortical white matter (approximately 250 mg) were extracted into perchloric acid and spectrophotometrically assayed for concentrations of glucose,

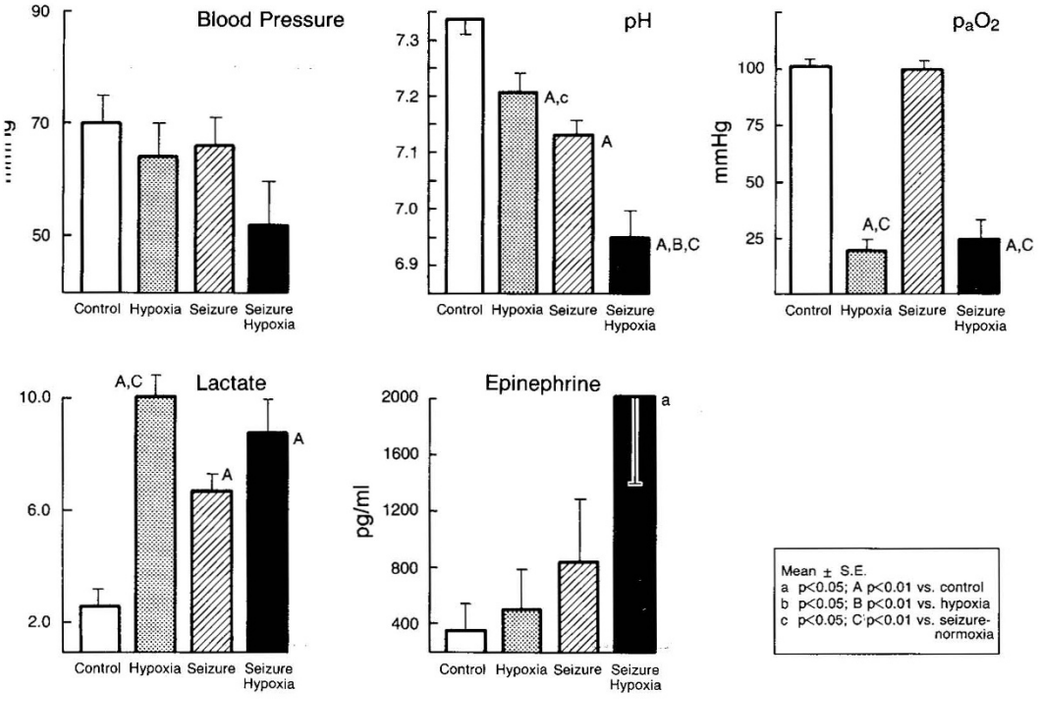


Fig. 1. Systemic changes after 45 min: animals in the seizure-hypoxia group develop the greatest degree of acidosis and the highest epinephrine levels.

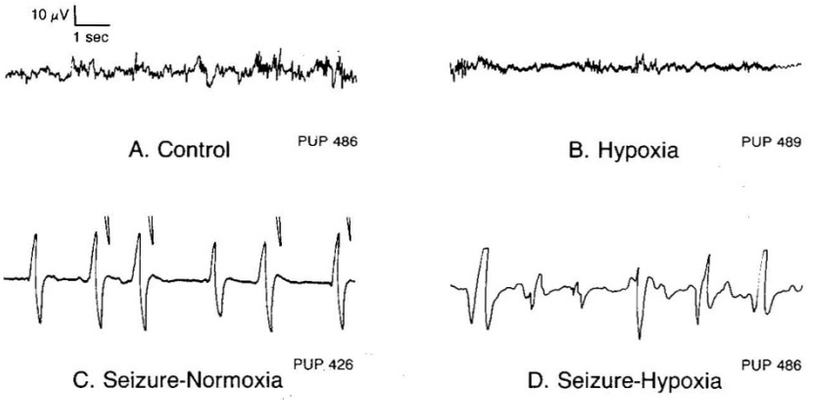


Fig. 2. Representative electroencephalograms: the EEGs of control animals and hypoxic animals are similar. Both the seizure-normoxia and seizure–hypoxia animals develop continuous high voltage spike or spike-and-wave discharges. Amplitude of the paroxysmal activity is more variable in the seizure-hypoxia animals.

Table 1. *CBF (ml/100 g/min) during hypoxia and during seizures with and without hypoxia (values are mean ± SE; analysis of variance)*

| | Control ($n = 8$) | Hypoxia ($n = 5$) | Seizure–normoxia ($n = 7$) | Seizure–hypoxia ($n = 5$) |
|---|---|---|---|---|
| Gray matter | | | | |
| Frontal cortex | 36 ± 5 | 53 ± 6[a] | 65 ± 5[A] | 48 ± 6[c] |
| Parietal cortex | 36 ± 4 | 50 ± 6[a,c] | 67 ± 5[A] | 46 ± 6[C] |
| Caudate | 34 ± 5 | 59 ± 7[A] | 69 ± 6[A] | 48 ± 8[c] |
| Hippocampus | 35 ± 4 | 46 ±5[C] | 65 ± 4[A] | 46 ±5[C] |
| Thalamus | 50 ± 6 | 66 ± 8[C] | 97 ± 7[A] | 66 ± 8[C] |
| Hypothalamus | 36 ± 5 | 55 ± 6[a,C] | 80 ±5[A] | 64 ± 6[A] |
| Corpora quadrigemina | 41 ± 5 | 54 ± 6[C] | 82 ± 5[A] | 64 ± 6[A,c] |
| Cerebellum | 39 ± 5 | 62 ± 6[A] | 62 ± 5[A] | 46 ± 6[c] |
| Pons | 50 ± 6 | 75 ± 7[A,c] | 98 ± 6[A] | 85 ± 7[A] |
| Medulla | 48 ± 5 | 76 ± 6[A] | 91 ± 5[A] | 80 ± 6[A] |
| Spinal cord | 45 ± 5 | 67 ± 6[A] | 75 ± 5[A] | 73 ± 6[A] |
| White matter | | | | |
| Periventricular white | 10 ± 2 | 16 ± 2[a] | 15 ± 2[a] | 8 ± 2[B,C] |
| Corpus Callosum | 18 ± 3 | 23 ± 3[C] | 36 ± 3[A] | 17 ± 3[C] |

[a] $p < 0.05$ *vs* control.
[b] $p < 0.05$ *vs* hypoxia.
[c] $p < 0.05$ *vs* seizure-normoxia.
[A] $p < 0.01$ *vs* control.
[B] $p < 0.01$ *vs* hypoxia.
[C] $p < 0.01$ *vs* seizure-normoxia.

Table 2. *$^{31}$P-NMR study of cerebral metabolism during hypoxia and during seizures with and without hypoxia (values for PCr, ATP, and inorganic phosphate are percent of total mobile phosphates) (all values are mean ± SE; control values are pooled data)*

| Min | Hypoxia ($n = 6$) | Seizure–normoxia ($n = 6$) | Seizure–hypoxia ($n = 3$) |
|---|---|---|---|
| Phosphocreatine | | | |
| Control | 11 ± 1 | 11 ± 1 | 11 ± 1 |
| 0–15″ | 11 ± 1[C] | 7 ± 1[A] | 8 ± 1[a] |
| 16–30″ | 8 ± 1[a] | 6 ± 1[A] | 8 ± 1[a] |
| 31–45″ | 10 ± 1[c] | 6 ± 1[A] | 6 ± 1[A,b] |
| ATP | | | |
| Control | 22 ± 1 | 22 ± 1 | 22 ± 1 |
| 0–15″ | 23 ± 1[C] | 18 ± 1[A] | 18 ± 2[b] |
| 16–30″ | 23 ± 2[c] | 18 ± 2[a] | 17 ± 2[a,b] |
| 31–45″ | 21 ± 2 | 18 ± 2 | 14 ± 2[A,b] |
| Inorganic phosphate | | | |
| Control | 12 ± 1 | 12 ± 1 | 12 ± 1 |
| 0–15″ | 16 ± 2 | 18 ± 2[A] | 18 ± 2[a] |
| 16–30″ | 17 ± 2[a] | 22 ± 2[A] | 19 ± 3[a] |
| 31–45″ | 18 ± 2[a] | 22 ± 2[A] | 21 ± 3[A] |
| Intracellular pH | | | |
| Control | 7.27 ± 0.04 | 7.27 ± 0.04 | 7.27 ± 0.04 |
| 0–15″ | 7.20 ± 0.06 | 7.10 ± 0.06[a] | 7.06 ± 0.06[A] |
| 16–30″ | 7.21 ± 0.08[C] | 6.89 ± 0.08[A] | 6.81 ± 0.08[A,B] |
| 31–45″ | 6.84 ± 0.09[A] | 6.83 ± 0.09[A] | 6.51 ± 0.09[A,B,c] |

[a] $p < 0.05$ *vs* control.
[b] $p < 0.05$ *vs* hypoxia.
[c] $p < 0.05$ *vs* seizure-normoxia.
[A] $p < 0.01$ *vs* control.
[B] $p < 0.01$ *vs* hypoxia.
[C] $p < 0.01$ *vs* seizure-normoxia.

lactate, ATP, and PCr according to standard enzymatic techniques (10).

*Statistical analyses.* A total of 25 animals were utilized in the blood flow studies (control, eight; hypoxia, five; seizure-normoxia, seven; seizure-hypoxia, five). Values from some of the seizure-normoxia animals have been previously reported data (8). In additiion, 15 animals were utilized for the NMR studies (hypoxia, six; seizure–normoxia, six; seizure-hypoxia, three). For the NMR studies, animals served as their own controls. The brains of some of the animals used in the NMR experiments as well as of 16 additional animals were used for *in vitro* determination of cerebral metabolites (control, seven; hypoxia, two; seizure–normoxia, three; seizure-hypoxia, four). The statistical method employed was analysis of variance utilizing a Tektronix 4051 computer. Differences were deemed significant at the $p < 0.05$ level.

These experiments were carried out with adherence to "Guiding Principles for the Care of Animals" of the American Physiological Society and in accordance with federal regulations and laws.

## RESULTS

*Systemic changes.* Significant mortality occurred in the seizure-hypoxia group (five of 17 animals died), while there was

but one death in the hypoxia group (one of seven died) and no deaths in the control or seizure-normoxia groups ($\chi^2 = 10.8$; DF = 3; $p = 0.01$). Systemic metabolic and physiological measurements from animals surviving the 45-min experimental period were pooled and are presented in Figure 1. It is important to note that animals surviving 45 min of seizure during hypoxia had a trend to lower blood pressure, although this decrease was not statistically significant ($p = 0.07$).

Arterial lactate was significantly raised and arterial pH lowered in both hypoxia and seizure groups. Plasma epinephrine levels were elevated only in the seizure-hypoxia group after 45 min (Fig. 1).

*Cerebral physiological changes.* Scalp recorded EEG showed low voltage fast activity in control animals. There appeared to be little change in background activity in animals subjected to hypoxia alone. Continuous high voltage spike or spike-and-slow wave discharges developed in both the seizure-normoxia and the seizure-hypoxia group, but the amplitude of the paroxysmal activity was more irregular in the seizure-hypoxia group (Fig. 2).

Cerebral blood flow increased to the greatest degree in the seizure–normoxia group. Cerebral blood flow was also elevated in all regions examined in the animals rendered hypoxic. However, in some regions of forebrain (*i.e.* parietal cortex, hippocampus, thalamus), diencephalon, and brainstem, the degree of elevation was not nearly as great as occurred in animals in the seizure-normoxia group (Table 1). CBF in cerebral cortex, basal ganglia, diencephalon, cerebellum, and corpora quadrigemina also failed to increase in the seizure-hypoxia group to the degree which occurred in seizure-normoxia animals. Moreover, CBF in cerebral cortex, caudate, hippocamus, thalamus, and cerebellum in the seizure-hypoxia group was not statistically significantly different from that of normoxic control animals.

*Cerebral metabolic changes.* $^{31}$NMR measurements showed significant reduction in PCr levels in animals subjected to seizures during normoxia or during hypoxia. Animals rendered hypoxic (alone) were less affected (Table 2, Fig. 3). Similarly, ATP concentration was not reduced in animals subjected to hypoxia alone. There was a slight decrease in ATP levels in the seizure-normoxia group, but this decline appeared to plateau after 45 min. In contrast, there was progressive, marked decline in ATP concentration in the seizure-hypoxia group.

*In vitro* measurement of freeze-trapped brain tissue disclosed changes similar to those found with $^{31}$P NMR. The seizure-hypoxia group developed the most severe depletion of ATP and PCr. Brain lactate was highest and brain glucose lowest in the seizure-hypoxia group (Fig. 4).

## DISCUSSION

*Systemic changes.* The cerebral physiological and metabolic effects of hypoxia alone (9, 25, 26) or seizures alone (8, 27) have been well described. To our knowledge, there has been little investigation of how hypoxemia modifies the cerebral physiological and metabolic effects of neonatal seizures.

The present results demonstrate that the singular insult of either hypoxia alone or seizures alone (during normoxemia) does not produce substantial mortality in the neonatal dog. Mortality is excessive only if the seizures are complicated by hypoxemia.

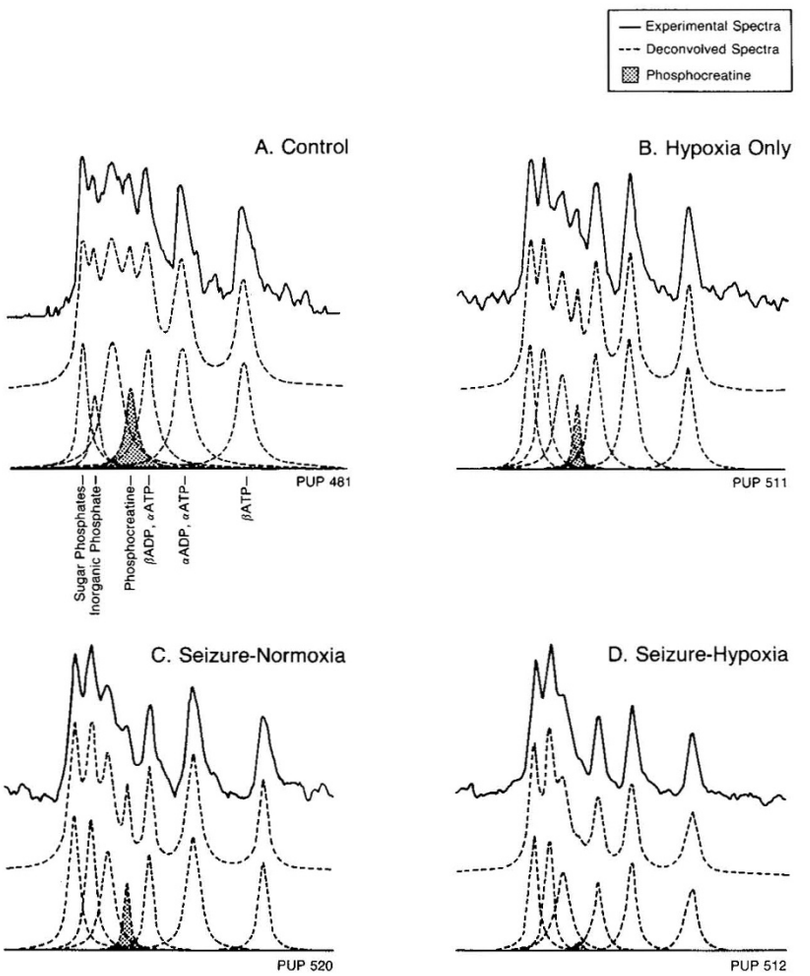


Fig. 3. Representative NMR spectra: levels of inorganic phosphate rise in all three experimental groups. The decline in PCr and ATP is most pronounced in the seizure-hypoxia group.

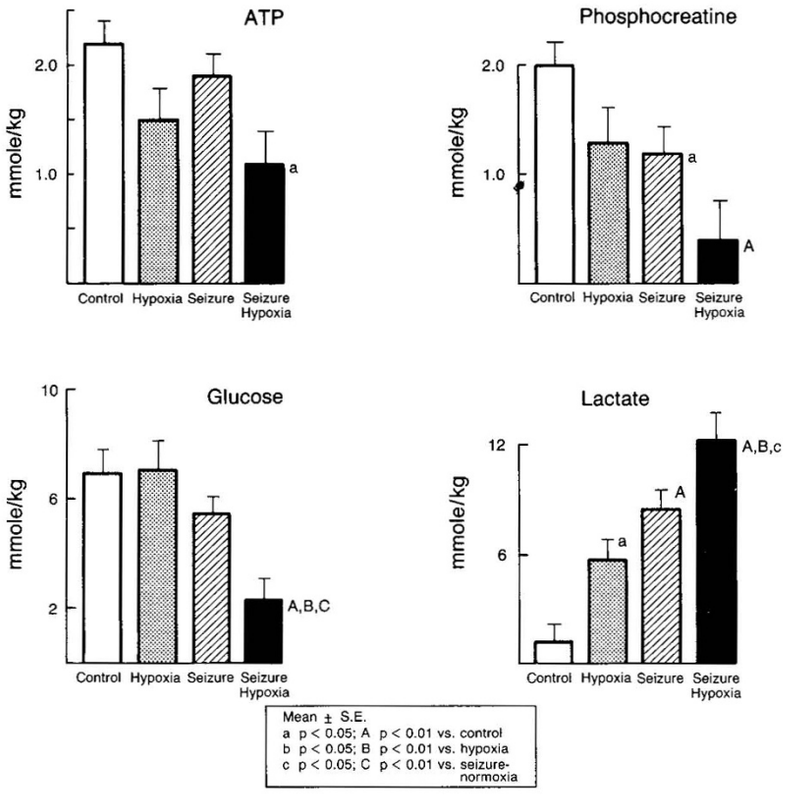


Fig. 4. *In vitro* brain metabolite levels: levels of PCr, ATP, and glucose are the lowest in the seizure-hypoxia animals. Lactate is elevated in all three experimental groups, particularly in the seizure-hypoxia group.

This mortality is probably cardiogenic in origin. The acidosis engendered in animals by the combined insults of both hypoxia and seizures may directly decrease cardiac output and impair ventricular function (28).

*CBF.* Data from the present study are consistent with the view that hypoxemia produces significant increases in CBF in the neonatal dog (29) particularly in brainstem structures (25). It is hypothesized that CBF increases during hypoxemia to maintain oxygen availability and preserve brain energy state (25).

It is also acknowledged that seizures in the paralyzed and ventilated animal are associated with significant increases in CBF, even when the seizures are prolonged (8). It is noteworthy that when seizures occur during hypoxemia, forebrain blood flow fails to increase significantly above control values. Why CBF fails to increase under the combined stress of hypoxia and seizures is uncertain. Hydrogen ion content (believed by some to be a mediator of cerebral vasodilation) was greatly elevated in the seizure-hypoxia group.

Can the failure of CBF to increase be attributed to the mild (nonsignificant) decrease in blood pressure in the seizure-hypoxia group (*i.e.* loss of autoregulation)? Utilizing the adult rat, Blennow *et al.* (30) discovered that autoregulation is abolished during seizures and that CBF becomes pressure passive. However, studies in normoxic neonatal dogs (not undergoing seizures) show that systemic hypotension of a moderate degree (as low as 30 mm Hg) is not associated with a reduction of CBF (31, 32). Preliminary experiments in this laboratory (unpublished results Young RSK, Cowan BE) in bicuculline-injected neonatal dogs indicate that reduction of mean arterial blood pressure by 30 mm Hg does not adversely affect CBF

*Cerebral metabolism.* Although elevated in all three experimental conditions, brain lactate levels were the highest (and brain intracellular pH the lowest) in the seizure-hypoxia group.

The pH determined by NMR for control animals in this study (7.27, Table 2) is somewhat higher than the values obtained by non-NMR methods (33), but is consistent with intracellular brain pH noted by other investigators using NMR, viz., 7.2 (34–36)–7.33 (14). Absolute pH measurement by $^{31}$P NMR, being dependent on titration curves based on intracellular environment and compartmentation is uncertain but measurement of pH changes is quite reliable (22, 23).

The cerebral metabolic consequences of hypoxemia on brain high energy phosphate metabolism have been well documented. *In vitro* analytic methods in adult rats (37) and in the neonatal dog (26, 38) disclose that cerebral ATP levels are maintained during nonfatal hypoxia. More recent *in vivo* $^{31}$P NMR studies by Gonzalez-Mendez *et al.* (34) and Prichard *et al.* (7) show that rabbits maintain normal levels of ATP during hypoxia and metabolic acidosis. Severe (fatal) hypoxia will produce changes in ATP levels. In a study of graded hypoxia in lambs, Younkin *et al.* (9) observed that ATP concentration did not decrease until PCr was nondetectable. Hilberman *et al.* (35) noted that during hypoxia brain ATP declined when PCr fell to approximately one-half of that of control values.

ATP levels are also minimally reduced during seizures if the animal is normoxic. *In vitro* studies by Chapman *et al.* (39) disclose that although PCr declined by approximately two-thirds during prolonged seizures in ventilated (normoxic) rats, ATP levels were close to normal. $^{31}$P NMR studies by Petroff *et al.* (6) in adult rabbits and from this laboratory in neonatal dogs (8) show little change in ATP levels during bicuculline induced seizures when animals are oxygenated.

Blennow *et al.* (30) recently reported that cerebral metabolic changes are more pronounced in adult rats who seized during hypoxia compared to those undergoing during normoxia. Similarly, the animals in the seizure-hypoxia group in the present study showed significant perturbation of both PCr and ATP levels.

The neuropathological consequences of seizures complicated by hypoxia are uncertain. Hypoxemia frequently accompanies prolonged seizures (40) and may be an important mechanism underlying neuronal damage (3). However, Blennow *et al.* (41) observed that ischemic cell change was more pronounced in male Wistar rats subjected to bicuculline-induced seizures during normoxia than when the seizures occurred during hypoxia. Lactate levels were also highest in their seizure-hypoxia group, yet none of the animals showed definite evidence of ischemic cell change. Future neuropathological experiments will be necessary to determine whether seizures complicated by hypoxia cause neuropathological alterations as well as metabolic perturbation.

*Acknowledgments.* The authors gratefully acknowledge the advice of Drs. H. Chugani and R. P. Simon and the secretarial assistance of Betty Riccio.

REFERENCES

1. Bergman I, Painter MJ, Hirsch RP, Crumrine PK, David R 1983 Outcome in neonates with convulsions treated in an intensive care unit. Ann Neurol 14:642–647
2. Hill A, Volpe JJ 1981 Seizures, hypoxic-ischemic brain injury and intraventricular hemorrhage in the newborn. Ann Neurol 10:109–121
3. Kreisman NR, Rosenthal M, LaManna JC, Sick TJ 1983 Cerebral oxygenation during recurrent seizures. In: Delgado-Escueta AV, Wasterlain CG, Treiman DM, Porter RJ (eds) Status Epilepticus. Raven, New York, pp 231–239.
4. Meldrum BS, Corsellis JAN 1984 Epilepsy. In: Adams JH, Corsellis JAN, Duchen LW (eds) Greenfield's Neuropathology, 4th ed. Wiley, New York, pp 921–950
5. Howse DC 1979 Metabolic responses to status epilepticus in the rat, cat and mouse. Can J Physiol Pharmacol 57:205–212
6. Petroff OAC, Prichard JW, Behar KL, Alger JR, Shulman RG 1984 *In vivo* phosphorous nuclear magnetic resonance spectroscopy in status epilepticus. Ann Neurol 16:169–177
7. Prichard JW, Alger JR, Behar KL, Petroff OAC, Shulman RG 1983 Cerebral metabolic studies *in vivo* by $^{31}$P NMR. Proc Natl Acad Sci USA 80:2748–2751
8. Young RSK, Osbakken MD, Briggs RW, Yagel SK, Rice DW, Goldberg S 1985 $^{31}$P NMR study of cerebral metabolism during prolonged seizures in the neonatal dog. Ann Neurol 18:14–20
9. Younkin DP, Wagerle LC, Delivoria-Papadopoulos M, Maris J, Chance B 1984 The effects of graded hypoxia on cerebral phosphorus metabolites and intracellular pH in newborn lambs. Ann Neurol 16:383(abstr)
10. Bergmeyer HU 1974 Methods of Enzymatic Analysis. Academic, New York
11. Peuler JD, Johnson GA 1975 A sensitive radio-enzymatic assay of plasma catecholamines. Clin Res 23:474A
12. Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff L 1978 Measurement of local cerebral blood flow with iodo($^{14}$C) antipyrine. Am J Physiol 234:H59–H66
13. Evelhoch JL, Crowley MG, Ackerman JJH 1984 Signal to noise optimization and observed volume localization with circular surface coils. J Magn Reson 56:110–124
14. Delpy DT, Gordon RE, Hope PL, Parker D, Reynolds EOR, Shaw D, Whitehead MD 1982 Noninvasive investigation of cerebral ischemia by phosphorus nuclear magnetic resonance. Pediatrics 70:310–313
15. Deuel RK, Yue GM, Sherman WR, Schickner DJ, Ackerman JJH 1985 Monitoring the time course of cerebral deoxyglucose metabolism by $^{31}$P nuclear magnetic resonance spectroscopy. Science 228:1329–1331
16. Naruse S, Takada S, Koizuka I, Watari H 1983 *In vivo* $^{31}$P NMR studies on experimental cerebral infarction. Jap J Physiol 33:19–28
17. Evelhoch JL, Ewy CS, Siegfried BA, Ackerman JJH, Rice DW, Briggs RW 1985 $^{31}$P spin lattice relaxation times and resonance linewidths of rat tissue *in vivo*: dependence upon the static magnetic field strength. Magn Reson Med 2:410–417
18. Shoubridge EA, Briggs RW, Radda GK 1982 $^{31}$P NMR saturation transfer measurements of the steady state rates of creatine kinase and ATP synthetase in the rat brain. FEBS Lett 140:289–292
19. Ackerman JJH, Grove TH, Wong GG, Gadian DG, Radda GK 1980 Mapping of metabolites in whole animals by $^{31}$P NMR using surface coils. Nature 283:167–170
20. Veloso D, Guynn RW, Oskarsson M, Veech RL 1973 The concentrations of free and bound magnesium in rat tissues: relative constancy of free $Mg^{2+}$ concentrations. J Biol Chem 248:4811–4819
21. Gadian DG, Radda GK, Richards RE, Seeley PJ 1979 $^{31}$P NMR in living tissue: the road from a promising to an important tool in biology. In: Schulman RG (ed) Biological Applications of Magnetic Resonance. Academic Press, New York, pp 463–535
22. Jacobson L, Cohen JS 1982 Intracellular pH measurements by NMR methods. In: Cohen JS (ed) Noninvasive Probes of Tissue Metabolism. John Wiley and Sons, New York, pp 5–24
23. Roberts JKM, Wade-Jardetzky N, Jardetzky O 1981 Intracellular pH measurements by $^{31}$P nuclear magnetic resonance. Influence of factors other than pH on $^{31}$P chemical shifts. Biochemistry 20:5389–5394
24. Ratcheson RA 1980 Funnel freezing for preservation of intermediary metabolites in rats. In: Passonneau JV, Hawkins RA, Lust WD, Welsh FA (eds) Cerebral Metabolism and Neural Function. Williams & Wilkins, Baltimore, pp 20–28
25. Cavazzuti M, Duffy TE 1982 Regulation of local cerebral blood flow in normal and hypoxic newborn dogs. Ann Neurol 11:247–257
26. Vannucci RC, Nardis EE, Vannucci SJ, Campbell PA 1980 Tolerance of the perinatal brain to graded hypoxemia. Neurology 30:443–444(abstr)
27. Wasterlain CG, Dwyer BE 1983 Brain metabolism during prolonged seizures in neonates. In: Delgado-Escueta AV, Wasterlain CG, Treiman DM, Porter RJ (eds) Status Epilepticus. Raven, New York, pp 241–260
28. Young RSK, Fripp RR, Yagel SK, Werner JC, McGrath G, Schuler HG 1985 Cardiac dysfunction during status epilepticus in the neonatal pig. Ann Neurol 18:291–297
29. Camp D, Kotagal UR, Kleinman LI 1982 Preservation of cerebral autoregulation in the unanesthetized hypoxemic newborn dog. Brain Res 241:207–213
30. Blennow G, Nilsson B, Siesjo BK 1985 Influence of reduced oxygen availability on cerebral metabolic changes during bicuculline-induced seizures in rats. J Cereb Blood Flow Metab 5:439–445
31. Hernandez MJ, Brennan RW, Bowman GS 1980 Autoregulation of CBF in the newborn dog. Brain Res 184:199–202
32. Young RSK, Hernandez MJ, Yagel SK 1982 Selective reduction of blood flow to white matter during hypotension in newborn dogs: a possible mechanism of periventricular leukomalacia. Ann Neurol 12:445–448
33. Siesjo BK, von Hanwehr R, Nergelius G, Nevander G, Ingvar M 1985 Extra and intracellular pH in the brain during seizures and in the recovery period following the arrest of seizure activity. J Cereb Blood Flow Metabol 5:47–57
34. Gonzalez-Mendez R, McNeill AM, Gregory GA, Wall S, Gooding CA, Lih L, James T 1985. Effects of hypoxic hypoxia on cerebral phosphate metabolites and pH in the anesthetized infant rabbit J Cer Blood Flow Metab 5:512–516
35. Hilberman M, Subramanian VH, Haselgrove J, Cone JB, Egan JW, Gyulai L, Chance B 1984 *In vivo* time-resolved brain phosphorus nuclear magnetic resonance. J Cereb Blood Flow Metabol 4:334–342
36. Thulborn KR, du Boulay GH, Duchen LW, Radda GK 1982 A $^{31}$P nuclear magnetic resonance *in vivo* study of cerebral ischemia in the gerbil. J Cereb Blood Flow Metabol 2:299–306
37. Blass JP, Gibson GE 1979 Consequences of mild, graded hypoxia. In: Fahn S, Davis JN, Rowland LP (eds) Advances in Neurology, Vol 26. Raven, New York, pp 229–250
38. Duffy TE, Cavazzuti M, Cruz NF, Sokoloff L 1982 Local cerebral glucose metabolism in newborn dogs: effects of hypoxia and halothane anesthesia. Ann Neurol 11:233–246
39. Chapman AG, Meldrum BS, Siesjo BK 1977 Cerebral metabolic changes during prolonged epileptic seizures in rats. J Neurochem 28:1025–1035
40. Glaser GH 1983 Medical complications of status epilepticus. In: Delgado-Escueta AV (eds) Status Epilepticus. Raven, New York, pp 395–398
41. Blennow G, Brierly JB, Meldrum BS, Siesjo BK 1978 Epileptic brain damage: the role of systemic factors that modify cerebral energy metabolism. Brain 101:687–700