Thus, for variables $X_2$ and $X_2 - X_1$, in the logistic function the general form will be as follows:

$$K + AX_1 + (A + B)X_2 = k + AX_1 + (A + B)X_2 - AX_2$$

$$= k + (2A + B)X_2 + (-A)(X_2 - X_1)$$

coefficient for $X_2$, $(2A + B)$ is positive

coefficient for $X_2 - X_1$, $(-A)$ is negative

**Appendix B**

Diagnostic Criteria for CBVD

**Definite**

1. Stroke: when all 3 of the following criteria are met:
   1. One or more of the following symptoms and/or signs:
      a) Carotid-cerebral arterial system: weakness or numbness in the contralateral limbs (arm, leg or both), homonymous or monocular visual loss, dysphasia or agnosia.
      b) Vertebral-basilar arterial system: weakness or numbness of one or more limbs; episodes of vertigo and nausea; numbness of the face, particularly about the mouth; diplopia, dysphagia; dysarthrias; homonymous hemianopsia; ataxia; nystagmus or altered consciousness.
   2. The above symptoms or signs for more than 24 hours.
   3. Objective neurological deficits are present.

(Events due to another known cause, for example, trauma, were excluded.)

2. Intermittent Cerebral Ischemic Attacks (ICIA); when all 3 of the following criteria are met:
   1. One or more of the above symptoms and/or signs were present but were equivocal, persisted for more than 24 hours, and equivocal neurological deficits or residua were present.
   2. There were no neurological signs confirmed by the physician’s observations or there were episodes of vertigo or altered consciousness where no attempt has been made to exclude other causes.

**Probable**

1. Stroke — when one or more of the above signs or symptoms were present but were equivocal, persisted for more than 24 hours, and equivocal neurological deficits or residua were present.

2. ICIA — When one or more symptoms were reported by the patient and there were no neurological signs confirmed by the physician’s observations.

**Reported**

Stroke or ICIA reported by physician but no documentation of clinical event available.

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**Effects of Phenobarbital in Cerebral Ischemia**

**Part I: Cerebral Energy Metabolism During Pronounced Incomplete Ischemia**

CARL-HENRIK NORDSTRÖM, M.D., AND BO K. SIESJÖ, M.D.

**SUMMARY**

Changes in cerebral cortex concentrations of high-energy phosphates, glycolytic metabolites, citric acid cycle intermediates, associated amino acids, and ammonia, were studied after 5, 15 and 30 min of incomplete ischemia in rats anesthetized with 70% N₂O or 150 mg·kg⁻¹ of phenobarbital. Previous results have shown that with this type of ischemia (bilateral carotid artery occlusion combined with reduction in blood pressure to 50 mm Hg) cortical blood flow is reduced to below 10% of nitrous oxide control values, whether animals are anesthetized with 70% N₂O or 150 mg·kg⁻¹ of phenobarbital.

In animals under 70% N₂O, changes in tissue concentrations of phosphocreatine, ATP, ADP and AMP were similar to those previously obtained in complete ischemia. However, some glucose remained in the tissue, and the lactate concentrations gradually rose to reach excessive values. Changes occurring in glycolytic and citric acid cycle intermediates were similar to those seen in complete ischemia but, after 30 min, there was some reduction in the pool size of amino acids.

In animals given phenobarbital and which lost all EEG activity during ischemia, changes in cerebral metabolites were virtually identical to those observed in nitrous oxide-anesthetized animals. However, some animals exposed to 5 or 15 min of ischemia had some remaining EEG activity. In these, cerebral energy state was significantly less deranged, and levels of glycogen, glucose and pyruvate were higher.

**Although brain damage has been reported after cardiac arrest of only 4-5 min duration, although the first signs of histological damage may be detected following a 5-10 min period of hypoxia-ischemia, other observations indicate that remarkable functional and biochemical restitution is possible after prolonged periods of complete cerebral ischemia.**

It has been argued that the barbiturate anesthesia used in many of these experiments may have ameliorated the brain damage. However, in a previous communication from this laboratory we described near-complete recovery of cerebral energy metabolism, following 30 min of complete ischemia in rats under 70% N₂O, and the pattern of changes in high-energy phosphates, carbohydrate metabolites and amino acids was the same in animals anesthetized with nitrous oxide and phenobarbital.

It was reported by Hossmann and Kleihues that if the cerebral ischemia was incomplete, i.e. if there was a trickling flow during the ischemic period, recovery...
was adversely affected. Since it has been suggested that the restoration of function following transient cerebral ischemia of short duration is mainly limited by impaired recirculation, caused by swelling of endothelial and perivascular cells and by intravascular aggregation of blood corpuscles, it was assumed that incomplete ischemia was complicated by microvascular disturbances (see also Olsson et al. 18). However, in the investigations by Salford et al., and Salford and Siesjo it was shown that widespread neuronal damage, eventually leading to brain infarction, developed after 30 min of pure hypoxia in the absence of signs of impaired recirculation. Thus, it remains a possibility that severe tissue hypoxia (with some remaining perfusion) is more deleterious than complete anoxia, which, in practice, is only achieved by complete ischemia.

It has been demonstrated that barbiturates can ameliorate the effects of complete or incomplete cerebral ischemia. Thus, following total ischemia in the monkey, neurological recovery is improved even when thiopental is administered after recirculation has begun. Furthermore, barbiturates have been reported to protect against focal ischemia in dogs and monkeys.

The present series of experiments was undertaken to study cerebral metabolic changes occurring during severe, incomplete ischemia of maximally 30 min duration, as well as in the recovery period following recirculation, using animals anesthetized with 70% N₂O or 150 mg-kg⁻¹ of phenobarbital. The present article is concerned with metabolic changes occurring during the ischemia. The experiments had 2 objectives: 1) to evaluate the metabolic changes occurring when only a trickling blood flow remains during ischemia, and 2) to define the modulating influence of barbiturate anesthesia. Cerebral metabolic changes occurring in the recovery period are described in Part II.

Materials and Methods

Male Wistar rats (250-350 g) with free access to tap water and rat pellets until operation, were used in the experiments. Control animals and the animals in 1 of the ischemic series were anesthetized with nitrous oxide. In these animals, anesthesia was induced with halothane (2%). After tracheotomy, halothane was withdrawn and the animals were ventilated on a gas mixture containing 70% N₂ and 30% O₂. In the other ischemic series, the animals were given phenobarbital (150 mg-kg⁻¹ i.p.) and, following tracheotomy, they were artificially ventilated on 70% N₂ and 30% O₂. Both femoral arteries and both femoral veins were cannulated. The common carotid arteries were bilaterally visualized and the vagi nerves were carefully separated from the arteries. A skin incision was made over the skull to accomodate a plastic funnel for later freezing of the tissue in situ. Rectal temperature was kept close to 37°C by intermittent heating. An EEG was recorded in all animals from gold-plated copper screws inserted into the skull bone. Anaerobic sampling of blood was performed at regular intervals for measuring arterial PO₂, PCO₂ and pH. Arterial blood pressure was continuously recorded in all animals.

Induction of Ischemia

Following operation, the animals were left undisturbed for 20-30 min. To induce a defined degree of cortical ischemia, rubber-coated clamps were placed on both common carotid arteries, and the systemic arterial blood pressure was rapidly reduced to 50 mm Hg through bleeding of the animals into an automatically controlled syringe. With this technique, the cortical blood flow is reduced to below 10% of N₂O control values without apparent inhomogeneity between different cortical areas. During the ischemic period, a fall in intracranial

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>MAP (mm Hg)</th>
<th>Temp (°C)</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>150 ± 7</td>
<td>37.2 ± 0.2</td>
<td>130 ± 9</td>
<td>37.9 ± 1.2</td>
<td>7.395 ± 0.012</td>
</tr>
<tr>
<td>Ischemia 5 min</td>
<td>50 ± 2</td>
<td>37.2 ± 0.2</td>
<td>129 ± 9</td>
<td>25.9 ± 1.3</td>
<td>7.120 ± 0.038</td>
</tr>
<tr>
<td>Ischemia 15 min</td>
<td>50 ± 2</td>
<td>37.0 ± 0.2</td>
<td>130 ± 6</td>
<td>26.8 ± 1.3</td>
<td>7.053 ± 0.023</td>
</tr>
<tr>
<td>Ischemia 30 min</td>
<td>51 ± 1</td>
<td>36.8 ± 0.1</td>
<td>178 ± 12</td>
<td>24.7 ± 1.6</td>
<td>7.033 ± 0.037</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia 5 min</td>
<td>49 ± 1</td>
<td>37.1 ± 0.2</td>
<td>120 ± 3</td>
<td>33.6 ± 1.5</td>
<td>7.290 ± 0.015</td>
</tr>
<tr>
<td>Ischemia 15 min</td>
<td>50 ± 1</td>
<td>37.2 ± 0.2</td>
<td>126 ± 3</td>
<td>34.3 ± 1.8</td>
<td>7.240 ± 0.017</td>
</tr>
<tr>
<td>Ischemia 30 min</td>
<td>52 ± 1</td>
<td>37.5 ± 0.3</td>
<td>155 ± 10</td>
<td>33.5 ± 2.0</td>
<td>7.217 ± 0.016</td>
</tr>
</tbody>
</table>
temperature was avoided by placing a heating bulb at a predetermined distance from the head. During the ischemic period, the arterial Po2, Pco2 and pH were repeatedly controlled. After 5, 15 or 30 min of ischemia, the brains were frozen by pouring liquid nitrogen into the plastic funnel. The brains were chiseled out under intermittent irrigation with liquid nitrogen and stored at −80°C until extraction.

Analytical Techniques

Arterial Po2, Pco2 and pH were measured with microelectrodes, operating at 37°C.

A fronto-parietal part of the cerebral cortex was taken bilaterally, weighed, and extracted with HCI-methanol at −22°C. All metabolites were analyzed with the specific, enzymatic fluorometric techniques of Lowry and Passonneau2s as previously reported from this laboratory.29,32 All metabolites were expressed as µmol·g−1 of wet tissue. The sum of the adenine nucleotides (Σ Ad) was calculated as Σ Ad = [ATP] + [ADP] + [AMP]. The energy state of the tissue was expressed as the energy charge (E.C.) of the adenine nucleotide pool according to Atkinson;33
\[ E.C. = \frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]} \]

Statistical comparison was performed using Student's t-test, \( P < 0.05 = \ast \), \( P < 0.01 = \ast \ast \) and \( P < 0.001 = \ast \ast \ast \).

Results

Table 1 gives the physiological parameters (Pco2, Po2, pH, arterial blood pressure and body temperature) of the animals in the different groups. It has previously been observed that hypotension is associated with a fall in arterial Pco2.34 During hypotension, Eklof and Siesjo36 also noted a pronounced fall in arterial pH. Both effects can be assumed to reflect peripheral vasconstriction and acid production. In the present series, both the hypocapnia and the decrease in arterial pH were less pronounced in animals anesthetized with phenobarbital.

EEG

Following occlusion of the common carotid arteries the EEG remained unchanged as long as the arterial blood pressure was kept above 140–160 mm Hg. This is in accordance with the report of Eklof and Siesjo36 who also found a perturbation of brain energy metabolism only when occlusion of the carotid arteries was combined with hypotension. When the blood pressure was decreased a progressive slowing of the EEG was observed. The EEG usually disappeared at a blood pressure of 70–80 mm Hg. However, in 6 animals anesthetized with phenobarbital a low amplitude, slow activity persisted during the period of ischemia (5 min in 3 animals, and 15 min in 3 others). The metabolic pattern obtained in these animals is described separately (see below). In all animals kept ischemic for 30 min, the EEG was isoelectric before the blood pressure had reached 50 mm Hg, irrespective of the type of anesthesia used.

Cerebral Energy State

Table 2 gives the concentrations of PCr, ATP, ADP, AMP and lactate, as well as the calculated adenine nucleotide pool, the energy charge (E.C.) and the lactate/pyruvate ratio in control animals (70% N2O) and after 5, 15 and 30 min of ischemia in animals anesthetized with either 70% N2O or phenobarbital (150 mg·kg−1). The Values Are Means ± SEM

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control (n = 6)</th>
<th>5 min (n = 6) Ischemia - N2O</th>
<th>15 min (n = 6) Ischemia - N2O</th>
<th>30 min (n = 6) Ischemia - N2O</th>
<th>5 min (n = 3) Ischemia - phenobarbital</th>
<th>15 min (n = 3) Ischemia - phenobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr</td>
<td>4.58 ±0.18</td>
<td>0.21 ±0.08</td>
<td>0.08</td>
<td>0.24</td>
<td>0.12</td>
<td>1.42</td>
</tr>
<tr>
<td>ATP</td>
<td>±0.10</td>
<td>±0.07 ±0.01</td>
<td>±0.01</td>
<td>±0.03</td>
<td>±0.01</td>
<td>±0.23</td>
</tr>
<tr>
<td>ADP</td>
<td>2.57 ±0.10</td>
<td>0.31 ±0.11</td>
<td>0.11</td>
<td>0.46</td>
<td>0.25</td>
<td>0.09</td>
</tr>
<tr>
<td>AMP</td>
<td>0.94 ±0.09</td>
<td>0.96 ±0.04</td>
<td>0.84</td>
<td>1.083</td>
<td>0.94</td>
<td>0.89</td>
</tr>
<tr>
<td>E.C.</td>
<td>±0.007</td>
<td>±0.026 ±0.020</td>
<td>±0.020</td>
<td>±0.045</td>
<td>±0.031</td>
<td>±0.081</td>
</tr>
<tr>
<td>Σ Ad</td>
<td>3.199 ±0.087</td>
<td>3.010 ±0.079</td>
<td>2.87</td>
<td>3.086</td>
<td>2.730</td>
<td>2.065</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.547</td>
<td>1.537 ±0.110</td>
<td>1.021</td>
<td>1.547</td>
<td>1.537</td>
<td>1.128</td>
</tr>
<tr>
<td>Lactate/Pyruvate</td>
<td>±0.004</td>
<td>±0.069 ±0.106</td>
<td>±0.166</td>
<td>±0.103</td>
<td>±0.043</td>
<td>±0.064</td>
</tr>
<tr>
<td>E.C.</td>
<td>±0.002</td>
<td>±0.027 ±0.012</td>
<td>±0.012</td>
<td>±0.028</td>
<td>±0.005</td>
<td>±0.012</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.48 ±0.04</td>
<td>24.19 ±2.730</td>
<td>2.065</td>
<td>14.75</td>
<td>24.44</td>
<td>13.38</td>
</tr>
<tr>
<td>Lact/Pyr</td>
<td>±0.009±1.44</td>
<td>±0.93 ±3.36</td>
<td>±0.85</td>
<td>±0.93</td>
<td>±0.94</td>
<td>±0.94</td>
</tr>
</tbody>
</table>

Downloaded from http://stroke.ahajournals.org/ by guest on December 16, 2017
Complete ischemia  o  Nitrous oxide
Incomplete ischemia  o  Nitrous oxide
Phenobarbital

FIGURE 1. Changes in cerebral lactate concentration after 1 to 30 min of complete and incomplete ischemia. The values for complete ischemia are obtained from Ljunggren et al.15 and Nordström and Siesjö.16

TABLE 3. Glycolytic Metabolites, Citric Acid Cycle Intermediates, Associated Amino Acids, and Ammonia, in Cerebral Cortex of Control Animals (70% N₂O), of Animals Subjected to 5 min of Ischemia during Anesthesia with Either 70% N₂O or 160 mg·kg⁻¹ of Phenobarbital, and of Phenobarbital-Anesthetized Animals Showing Some Remaining EEG Activity During 5 or 15 min of Ischemia. The Values Are Means ± SEM

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control N₂O (n = 6)</th>
<th>N₂O 5 min (n = 6)</th>
<th>5 min isoelectric (n = 3)</th>
<th>Phenobarbital 5 min (n = 3)</th>
<th>15 min EEG (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>2.24 ± 0.11</td>
<td>0.18 ± 0.05</td>
<td>0.26 ± 0.12</td>
<td>1.34 ± 0.05</td>
<td>0.71 ± 0.04</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.15 ± 0.51</td>
<td>0.95 ± 0.15</td>
<td>0.46 ± 0.12</td>
<td>1.52 ± 0.03</td>
<td>2.39 ± 0.03</td>
</tr>
<tr>
<td>G-6-P</td>
<td>0.094 ± 0.003</td>
<td>0.057 ± 0.012</td>
<td>0.037 ± 0.014</td>
<td>0.150 ± 0.016</td>
<td>0.142 ± 0.030</td>
</tr>
<tr>
<td>F-6-P</td>
<td>0.014 ± 0.0001</td>
<td>0.012 ± 0.001</td>
<td>0.014 ± 0.001</td>
<td>0.002 ± 0.002</td>
<td>0.004 ± 0.004</td>
</tr>
<tr>
<td>FDP</td>
<td>0.092 ± 0.003</td>
<td>0.195 ± 0.027</td>
<td>0.263 ± 0.030</td>
<td>0.181 ± 0.049</td>
<td>0.135 ± 0.027</td>
</tr>
<tr>
<td>DHAP</td>
<td>0.023 ± 0.002</td>
<td>0.009 ± 0.013</td>
<td>0.016 ± 0.014</td>
<td>0.025 ± 0.003</td>
<td>0.030 ± 0.004</td>
</tr>
<tr>
<td>3-PG</td>
<td>0.030 ± 0.001</td>
<td>0.021 ± 0.005</td>
<td>0.001 ± 0.001</td>
<td>0.001 ± 0.003</td>
<td>0.004 ± 0.004</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.117 ± 0.001</td>
<td>0.018 ± 0.006</td>
<td>0.016 ± 0.001</td>
<td>0.113 ± 0.006</td>
<td>0.072 ± 0.020</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.339 ± 0.020</td>
<td>0.165 ± 0.009</td>
<td>0.154 ± 0.003</td>
<td>0.199 ± 0.007</td>
<td>0.200 ± 0.005</td>
</tr>
<tr>
<td>α-keto-glutarate</td>
<td>0.152 ± 0.0008</td>
<td>0 ± 0</td>
<td>0 ± 0.008</td>
<td>0.008 ± 0.003</td>
<td>0.026 ± 0.001</td>
</tr>
<tr>
<td>Fumarate</td>
<td>0.065 ± 0.006</td>
<td>0.081 ± 0.005</td>
<td>0.086 ± 0.004</td>
<td>0.081 ± 0.003</td>
<td>0.086 ± 0.004</td>
</tr>
<tr>
<td>Malate</td>
<td>0.404 ± 0.011</td>
<td>0.375 ± 0.021</td>
<td>0.304 ± 0.012</td>
<td>0.426 ± 0.009</td>
<td>0.509 ± 0.007</td>
</tr>
<tr>
<td>Oxaloacetate</td>
<td>6.1 × 10⁻³ ± 0.2 × 10⁻³</td>
<td>0 ± 0</td>
<td>0.4 ± 0.2 × 10⁻³</td>
<td>1.2 ± 0.8 × 10⁻³</td>
<td>0.8 ± 0.8 × 10⁻³</td>
</tr>
<tr>
<td>Glutamate</td>
<td>12.71 ± 0.20</td>
<td>12.23 ± 0.15</td>
<td>11.82 ± 0.20</td>
<td>11.64 ± 0.21</td>
<td>11.32 ± 0.23</td>
</tr>
<tr>
<td>Aspartate</td>
<td>3.43 ± 0.09</td>
<td>3.25 ± 0.06</td>
<td>3.84 ± 0.20</td>
<td>4.11 ± 0.06</td>
<td>3.58 ± 0.04</td>
</tr>
<tr>
<td>Glutamine</td>
<td>6.31 ± 0.30</td>
<td>6.11 ± 0.36</td>
<td>6.93 ± 0.41</td>
<td>6.44 ± 0.19</td>
<td>5.89 ± 0.20</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.545 ± 0.010</td>
<td>0.879 ± 0.037</td>
<td>0.946 ± 0.051</td>
<td>0.859 ± 0.059</td>
<td>1.160 ± 0.018</td>
</tr>
<tr>
<td>GABA</td>
<td>2.24 ± 0.001</td>
<td>2.92 ± 0.10</td>
<td>2.43 ± 0.11</td>
<td>2.50 ± 0.03</td>
<td>2.30 ± 0.16</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.295 ± 0.008</td>
<td>0.867 ± 0.063</td>
<td>0.643 ± 0.040</td>
<td>0.464 ± 0.017</td>
<td>0.569 ± 0.012</td>
</tr>
</tbody>
</table>
Lactate Concentration

Previous results have shown that when cerebral blood flow is completely interrupted in normoglycemic rats, cerebral lactate concentration never increases above 13-14 $\mu$mol·g$^{-1}$, corresponding to the preischemic tissue concentrations of glucose and glycogen.$^{27, 28}$ In the present model, the continuous supply of substrate, though insufficient for the energy demands of the tissue, resulted in an extreme degree of cerebral lactacidosis, similar to that seen in pure hypoxia.$^{17}$ The difference in the amount of lactate accumulated in complete cerebral ischemia$^{37-39}$ and in pronounced, incomplete ischemia is shown in figure 1.

Carbohydrate Metabolites, Amino Acids and Ammonia

Table 3 shows the glycolytic metabolites, citric acid cycle intermediates, associated amino acids, and ammonia in control animals, in animals subjected to 5 min of ischemia, and in the phenobarbital-anesthetized animals that showed some remaining EEG activity. In isoelectric animals subjected to 5 min of ischemia, whether anesthetized with 70% $N_2O$ or 150 mg·kg$^{-1}$ of phenobarbital, changes in metabolites were very similar. In general, there were decreases in the concentrations of glycogen, glucose, G-6-P, F-6-P, 3-PG, pyruvate, citrate, $\alpha$-ketoglutarate and oxaloacetate, and increases in the concentrations of FDP, DHAP, pyruvate, citrate, $\alpha$-ketoglutarate and oxaloacetate. These changes are very similar to those previously observed during complete ischemia.$^{25, 42, 43}$ In contrast, phenobarbital-anesthetized animals with some remaining EEG activity showed more moderate reductions in glycogen, glucose, and pyruvate concentrations, increases in G-6-P, F-6-P and FDP concentrations, a fall in pyruvate concentration, and a less pronounced perturbation of citric acid cycle intermediates.

In order to facilitate description of metabolite changes observed after 15 and 30 min of ischemia, all values obtained in ischemia were calculated in percent of control. However, since no phenobarbital controls were performed we used as a basis for this comparison a recent study from the laboratory which describes differences in metabolite concentrations between animals anesthetized with 70% $N_2O$ and 150 mg·kg$^{-1}$ of phenobarbital.$^{41}$ To take one example, if the concentration of a metabolite in these phenobarbital-anesthetized animals was 80% of that measured in animals under 70% $N_2O$, this percentage figure was used to convert the present control values (70% $N_2O$) to a control value for phenobarbital-anesthetized animals.

Percentage changes in glycolytic metabolites and citric acid cycle intermediates are shown in figure 2. In $N_2O$-anesthetized animals, changes observed after 15 min of ischemia were similar to those occurring after 5 min (see table 3). After 30 min, the FDP concentration was reduced to normal levels, and there were excessive increases in the concentrations of DHAP and 3-PG, while little further change occurred in citric acid cycle intermediates. Very similar changes occurred in phenobarbital-anesthetized animals, although the rise in DHAP/FDP ratio was even more pronounced (see Discussion). Another difference was that the fumarate/malate ratio rose in $N_2O$-anesthetized animals, but not in those given phenobarbital. The cause of this difference is not obvious.

Changes in concentrations of amino acids and am-
ischemia, changes in cerebral metabolism are usually considered in the context of cerebral metabolic changes during incomplete ischemia, especially if such a study aims at evaluating effects of barbiturates. The first problem is that, during incomplete ischemia, changes in cerebral metabolism are usually variable, if not unpredictable, and they may also represent inhomogeneous events. Thus, if cerebral perfusion pressure is reduced by e.g. bleeding, there is often a large variability in response, probably due to a corresponding variability in reduction of CBF in spite of a (seemingly) constant cerebral perfusion pressure (see Siesjö et al. and Eklöf et al. In some models, this variability is very pronounced, e.g. when unilateral carotid artery ligation is performed in gerbils. Results appear more consistent if cerebral perfusion pressure is reduced by means of an increase in CSF pressure.

If bilateral carotid artery ligation is combined with hypotension due to bleeding it is possible to achieve pronounced ischemia in cortical regions with partial preservation of flow to brain stem centers. However, in such a situation the possibility of inhomogeneous perfusion must be considered. With the present experimental technique, involving reduction of blood pressure to 50 mm Hg, the cortical blood flow is reduced to less than 10% of normal, and no gross differences in blood flow between different cortical areas are seen. These facts do not exclude the possibility that inhomogeneity of flow existed at the capillary level but, as the present results show, the model reproducibly induces cerebral energy failure in animals maintained on 70% N2O.

The second problem concerns the mode of action of barbiturates in ischemia. Since anesthetic doses of barbiturates reduce metabolic rate by about 50% (see Smith et al. and Harp et al.) their protective action could simply be due to the fact that the energy demands of the brain cells have been reduced to levels that may be covered by a given fall in oxygen supply during ischemia. Previous results have also shown that if blood pressure is reduced to 30 mm Hg in rats, phenobarbital prevents extensive deterioration of cerebral energy state (see Mitchell and Maker). It is clear that a protective effect of barbiturates, unrelated to their ability to reduce metabolic rate, can only be assessed if the model used gives rise to extensive energy depletion whether animals are anesthetized with nitrous oxide or barbiturate. As the present results demonstrate, some animals anesthetized with phenobarbital showed less than complete deterioration of cerebral energy state. However, since all these animals showed some remaining EEG activity they can be easily distinguished from those that develop virtually complete energy failure within the first 5 min.

Cerebral Metabolic Changes

The time course of changes in cerebral metabolites after induction of complete cerebral ischemia has been described in some detail. Thus, extensive information is available for metabolites of the energy reserve, as well as for glycolytic metabolites, citric acid cycle intermediates, associated amino acids, and ammonia.

There is much less information on the time course of changes in cerebral metabolites during severe, incomplete ischemia. The present experiments have given 2 main findings. First, with some exceptions that will be discussed below, the changes observed in cerebral energy reserves, glycolytic metabolites, citric acid...
cycle intermediates, and associated amino acids during severe, incomplete ischemia are similar to those previously reported to occur during complete ischemia.25,27-28,43 Second, in all animals with isoelectric EEG the changes in energy metabolites, carbohydrate intermediates and amino acids were similar irrespective of the type of anesthesia.

In view of the similarity in results the present data can be interpreted in much the same way as those obtained during complete ischemia. First, changes in concentrations of G-6-P, F-6-P, FDP and DHAP are compatible with facilitation of the phosphofructokinase reaction, while depletion of pyruvate should be caused by reduction to lactate via the lactate dehydrogenase reaction and, to some extent, by a shift in the alanine aminotransferase (Ala-AT) reaction. Second, the redox shift (toward increased reduction) may help to explain not only the fall in pyruvate concentration but also that of OAA (via the malate dehydrogenase reaction). Third, depletion of α-ketoglutarate is probably due to a shift in the glutamate dehydrogenase reaction, caused by accumulation of ammonia, H⁺, and NADH. Fourth, alanine accumulation is the expected result of a shift in Ala-AT reaction due to α-ketoglutarate depletion. Fifth, accumulation of GABA probably reflects the fact that GABA production continues under conditions of hypoxia while its removal requires NAD⁺.

There are few previous reports of changes in glycolytic intermediates during ischemia. Although earlier results have shown that complete ischemia is accompanied by increased tissue concentrations of FDP and DHAP the present results demonstrate a very pronounced increase in DHAP concentration even when FDP concentrations remained only moderately increased (or were reduced). It has previously been reported that the concentrations of the components of the aldolase reaction deviate from an equilibrium relationship, possibly because part of the FDP measured is bound within the tissue.24,44 In the present ischemic groups, the changes in DHAP/FDP ratio were in the direction of equilibrium (concentrations of glyceraldehyde-3-phosphate varied in parallel with DHAP concentrations). Possibly, the excessive increase in DHAP concentration could have been due to a reduced degree of binding of FDP. Since the increase in DHAP concentration occurred at reduced 3-PG concentration (after 5 and 15 min of ischemia) it is tempting to assume a block at the glyceraldehyde phosphate (GAP) dehydrogenase reaction due to lack of NAD⁺. However, since 3-PG concentrations increased to high levels after 30 min of ischemia, this explanation seems less plausible. The results leave little support for the view that substrate supply via the GAP dehydrogenase reaction becomes limiting. Thus, depletion of pyruvate probably occurred because the rate of metabolism of pyruvate (mainly via the LDH reaction) exceeded its replenishment via the pyruvate kinase reaction.

In 2 respects, results in incomplete ischemia differ from those in complete ischemia. 1) As mentioned above, during complete cerebral ischemia in normoglycemic rats lactate accumulation never exceeds 13–14 μmol·g⁻¹·h⁻¹. A progressive increase in lactate in the present investigation (fig. 1) proves that some circulatory though insufficient for the energetic demands of the tissue remains during the period of ischemia. Since some animals accumulated more than 35 μmol·g⁻¹ of lactate during the 30 min period of ischemia, the resulting intracellular acidosis must be excessive. 2) After 30 min of incomplete ischemia, there was a significant fall in the size of the amino acid pool. Possibly, this could have been caused by loss of amino acids to the blood, especially since conditions (accumulation of ammonia, NADH and H⁺) should be unfavorable for oxidative deamination of, for instance, glutamate. Whatever the mechanism of this reduction in amino acid pool size is, it cannot be directly related to reversibility of events since it occurred in both nitrous oxide- and phenobarbital-anesthetized animals.

**Influence of Barbiturate Anesthesia**

As demonstrated above, the changes in energy state, glycolytic metabolites, citric acid cycle intermediates and associated amino acids were similar in all isoelectric animals irrespective of the type of anesthesia used. However, in 6 of the animals given phenobarbital, a low-voltage EEG activity persisted during the period of ischemia (5 and 15 min of ischemia). In these animals, the energy state was significantly less deranged and the pattern of glycolytic intermediates was somewhat different. Thus, considerable amounts of glycogen and glucose remained, the concentrations of G-6-P and F-6-P increased above control and the concentrations of pyruvate, α-KG and OAA were higher than in isoelectric animals. The results demonstrate that a reduction of cortical blood flow to 0.1 ml·g⁻¹·min⁻¹, or lower,37 may not induce complete energy failure when cerebral metabolic rate has been reduced by barbiturate anesthesia. Since barbiturate anesthesia has been shown to ameliorate brain damage during ischemia (see above) it must be asked whether any effect observed is related to a reduction in cerebral metabolic rate, which allows preservation of cerebral energy state during ischemia, or if barbiturates have other actions as well. Since our objective was to explore the latter type of effects, the following communication concerns recovery of cerebral energy metabolism in animals that had no traces of remaining EEG activity during the ischemia.

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**References**


Effects of Phenobarbital in Cerebral Ischemia

Part II: Restitution of Cerebral Energy State, as well as of Glycolytic Metabolites, Citric Acid Cycle Intermediates and Associated Amino Acids After Pronounced Incomplete Ischemia

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SUMMARY Recovery of cerebral energy metabolism, following 15 or 30 min of pronounced, incomplete ischemia, was studied after 90 min of recirculation in rats that were either anesthetized with 70% N₂O or 150 mg • kg⁻¹ of phenobarbital. In all animals arterial blood pressure, P₂O₂ and Pₐ₃O₂ were close to normal during recirculation. In nitrous oxide-anesthetized animals kept ischemic for 30 min, but not in those given phenobarbital, a gradual rise in intracranial cerebrospinal fluid (CSF) pressure (to about 20–25 mm Hg) occurred during the last 20–30 min of recirculation.

Following 15 min of ischemia, all phenobarbital-anesthetized animals, and 20 out of 24 animals anesthetized with 70% N₂O, showed extensive restitution of cerebral energy metabolism, including normalization of phosphocreatine concentration, return of adenosine energy charge to about 99% of control, and disappearance of virtually all of the lactate accumulated during the ischemia. These changes, and the pattern of changes in glycolytic and citric acid cycle intermediates, indicated that a near-normal mitochondrial metabolism returned. Following 30 min of ischemia in phenobarbital-anesthetized animals, a similar degree of recovery was observed. However, no animal maintained on 70% N₂O showed such signs of metabolic recovery. The present results, and those previously reported from this laboratory, demonstrate that complete ischemia is followed by a significantly better recovery of cerebral energy metabolism than is a corresponding period of incomplete ischemia. Furthermore, the results demonstrate that phenobarbital protects under conditions of incomplete ischemia even when it does not prevent energy depletion from rapidly occurring during ischemia.

This series of experiments was designed to study cerebral metabolic changes during incomplete ischemia, as well as in the recovery period following recirculation, and to assess the modulating influence of phenobarbital anesthesia. This second communication deals with restitution of brain energy metabolism following 15 and 30 min of severe, incomplete ischemia. It will be shown that although none of the superficially anesthetized animals (70% N₂O) recovered a normal cerebral metabolic state following 30 min of ischemia, all animals given phenobarbital (150 mg • kg⁻¹ i.p.) demonstrated a near-complete normalization of the energy state. The possible explanations for the protective effects of barbiturate anesthesia in the present model of transient cerebral ischemia will be discussed. Furthermore, we will discuss possible biochemical mechanisms leading to irreversible neuronal damage, taking into account that a 30 min period of complete ischemia appears to be better tolerated than a similar period of incomplete ischemia.

Materials and Methods

Most of the experimental and analytical techniques used presently were described in the previous communication. In general, fed male Wistar rats
Effects of phenobarbital in cerebral ischemia. Part I: cerebral energy metabolism during pronounced incomplete ischemia.

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