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# *Influence of Phenobarbital Anesthesia on Carbohydrate and Amino Acid Metabolism in Rat Brain*

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**The present experiments were undertaken to study whether phenobarbital decreases cerebral oxygen utilization and glucose consumption by proportional amounts, or decreases glycolytic rate to such an extent that endogenous substrates must be mobilized to cover the oxidative requirements. Concentrations in brain tissue of glycolytic metabolites, citric acid cycle intermediates, associated amino acids (glutamate, aspartate, alanine, gamma-aminobutyric acid and glutamine), and ammonia were measured 2, 30 or 180 min after administration of phenobarbital, 150 mg/kg, to rats. The values were compared with those obtained from animals anesthetized with nitrous oxide, 70 per cent. In all phenobarbital-treated groups, there was accumulation of glucose-6-phosphate, with depletion of fructose-1,6-diphosphate and subsequent glycolytic metabolites. After 30 min, phenobarbital decreased the concentrations of citric acid cycle intermediates (citrate, α-ketoglutarate, fumarate, malate and [calculated] oxaloacetate), as well as glutamate, and increased aspartate concentration. The results are compatible with the view that phenobarbital retards glycolytic flux at the phosphofructokinase step, and that endogenous substrates are mobilized from existing carbohydrate and amino acid pools. However, the size of the pool of carbohydrate substrates did not decrease further when phenobarbital anesthesia was prolonged from 30 to 180 min; none of the phenobarbital-treated groups showed a decrease in the amino acid pool, and there was no increase in ammonia concentration. There was thus no indication that endogenous substrates are continuously consumed or that phenobarbital leads to oxidative deamination of amino acids. (Key words: Anesthesia, intravenous, phenobarbital; Brain, metabolism; Metabolism, citric acid cycle; Metabolism, glucose; Metabolism, amino acids.)**

EARLY STUDIES, as well as those done more recently, have provided relatively detailed information about the effects of barbiturates on energy and carbohydrate metabolism in the brain. When given in anesthetic concentrations, all barbiturates studied decrease oxygen utilization ($CMR_{O_2}$) and glucose consumption ($CMR_{gl}$) to about 50 per cent of control.[1,2] In spite of the decrease in energy flux, there is only a slight increase in phosphocreatine (PCr) concentration, and no alteration in the tissue concentration of adenosine triphosphate (ATP), adenosine diphosphate (ADP) or adenosine monophosphate (APM).[3–6] In view of the fact that the small increase in PCr concentration can be explained, at least partly, in terms of an influence of alkalosis on creatine kinase equilibrium,[7] barbiturates do not seem to alter the cerebral energy state significantly. Evaluation of changes in glycolytic metabolites indicates that barbiturates inhibit glycolysis at the phosphofructokinase step with accompanying decreases in concentrations of glycolytic metabolites distal to that step.[3,7–9]

There is less information about changes in citric acid cycle intermediates, associated amino acids, and ammonia. Goldberg *et al.*[3] reported that phenobarbital and amytal decrease the tissue concentrations of α-ketoglutarate (α-KG), fumarate and malate in mice, amytal causing decreases in citrate and isocitrate as well. In these experiments, anesthesia was maintained for 60 min, and neither body temperature nor arterial carbon dioxide partial pressure ($Pa_{CO_2}$) were measured. In subsequent experiments in rats, in which these variables were kept constant, it was found that 60 min of anesthesia with phenobarbital[7] or pentobarbital[6] decreased the tissue concentrations of citrate, α-KG and malate. Even less is known about amino acids or ammonia. Published results indicate that barbiturate anesthesia is accompanied by an increase in the tissue concentration of aspartate and by decreases in glutamate and gamma-aminobutyric acid (GABA) concentrations,[9–13] but the results are not consistent.[12,14]

Recent measurements of arteriovenous differences across the perfused dog brain[15] have shown that pentobarbital anesthesia is accompanied by a significant net efflux of amino nitrogen, most of which was contained in urea, glutamate, aspartate, asparagine plus glutamine, and ammonia. The authors tentatively concluded from their results, and from those of others (see above), that pentobarbital anesthesia leads to inhibition of glycolysis in excess of the decrease in $CMR_{O_2}$, necessitating increased metabolism of endogenous substrates, including oxidative degradation of amino acids.

The present study was undertaken to evaluate the effects of phenobarbital anesthesia (of maximally three hours' duration) on concentrations in brain

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tissue of glycolytic metabolites, citric acid cycle intermediates, and ammonia. The main objective of the study was to find out whether these concentrations changed in a direction to suggest that carbohydrate metabolites and amino acids serve as important endogenous substrates during the anesthetic state.

## Methods

The influence of barbiturate anesthesia on cerebral metabolism was evaluated in four groups of rats in which the brains were frozen *in situ*. In one group, studies were made 2 min after intravenous administration of phenobarbital, while in two other groups the studies were made 30 min and three hours after intraperitoneal injection of phenobarbital. The fourth group, a control group, was given nitrous oxide anesthesia for 30 min. It was thus possible to compare, on one hand, animals anesthetized with nitrous oxide or phenobarbital and, on the other hand, animals maintained on phenobarbital for various periods. The 2-min period was chosen because this was the minimal interval required to produce unresponsiveness of the animals to painful stimuli, following intravenous administration. On the basis of such behavioral effects, we assume that anesthetic tissue concentrations of phenobarbital were obtained in all three groups given the drug.

In the two-minute group, catheters were inserted into a tail artery and vein during anesthesia with halothane, 2–3 per cent, and the galea on the convexity of the skull was infiltrated with 0.5 ml of 1 per cent lidocaine. The animals were then allowed to recover. About 30 min later, when the rats were fully awake, phenobarbital, 150 mg/kg, was given intravenously during one minute, a skin incision was made over the skull, the bone was exposed, a plastic funnel was fitted into the incision, and the brain was frozen *in situ* by pouring liquid nitrogen into the funnel.[16] In the 30-min and three-hour groups, anesthesia was induced and maintained with phenobarbital, 150 mg/kg, intraperitoneally. When the animals were unresponsive to painful stimuli (about 10 min), tracheotomy was performed and the lungs were artificially ventilated with nitrogen, 70 per cent, and oxygen, 30 per cent, so as to maintain $Pa_{CO_2}$ values near 40 torr. Immobilization was achieved with tubocurarine chloride, 1 mg/kg, ip. Rectal temperature was maintained close to 37 C by external heating. After 30 min or three hours the brain was frozen *in situ* as described above. For the control group, anesthesia was induced with halothane, 2–3 per cent. Following tracheotomy, insertion of catheters in a femoral artery and vein, and exposure of the skull for subsequent freezing of the brain, halothane was discontinued and the animals were maintained for another 30 min on nitrous oxide, 70 per cent, and oxygen, 30 per cent. The brain was then frozen.

Arterial blood $P_{O_2}$, $P_{CO_2}$ and $p$H were measured using microelectrodes with due corrections for any deviations in body temperature from 37 C. After that, the brain was chiselled out in the frozen state, and cerebral cortical tissue (frontoparietal region) was separated and extracted at −22 C with HCl–methanol. The subsequent handling of the tissue, and the enzymatic, fluorometric analyses of tissue metabolites, were done according to the method of Lowry and Passonneau (1972).[17] Details of the analytic techniques have been reported.[18–21] The energy state of the tissue was calculated (see Appendix) and statistical differences among groups were calculated using Student's t test.

## Results

Phenobarbital anesthesia was associated with a slight decrease in blood pressure compared with control (table 1). Arterial blood $P_{CO_2}$ values were somewhat higher in the 30-min and 3-hour phenobarbital-treated groups than in the control group, but the values were sufficiently close to exclude the possibility that the results were influenced by variation in $Pa_{CO_2}$. Body temperatures were near 37 C, and arterial blood $P_{O_2}$ values were more than 100 torr in all groups.

### Cerebral Energy State

Analyses of tissue concentrations of PCr, ATP and AMP confirmed previous findings of an essentially unchanged cerebral energy state during barbiturate anesthesia. The values (mean ± SEM) for PCr for the control group and the combined phenobarbital groups were 4.44 ± 0.04 and 4.80 ± 0.05 $\mu mol \cdot g^{-1}$, respectively, while those for energy charge were 0.946 ± 0.0004 and 0.947 ± 0.0009, respectively.

Previous estimations of $pH_i$ have shown that phenobarbital anesthesia is accompanied by a $p$H increase of 0.05–0.09 units.[7,22] Since information about $pH_i$ is needed to calculate cytoplasmic NADH/$NAD^+$ ratios, we estimated $pH_i$ from the creatine kinase equilibrium (see Appendix). According to these calculations, $pH_i$ increased by 0.04 units in all three phenobarbital-treated groups. Although the creatine kinase reaction may underestimate the increase in $pH_i$,[7] none of the present conclusions would be affected if the true increase in $pH_i$ were twice as

TABLE 1. Effects of Phenobarbital, 150 mg/kg, on Mean Arterial Blood Pressure (MABP), Temperature, and Arterial Blood $P_{O_2}$, $P_{CO_2}$ and *p*H, 2, 30, and 180 min after Administration (Means ± SEM)

| Experimental Group | MABP (torr) | Temperature (C) | $Pa_{O_2}$ (torr) | $Pa_{CO_2}$ (torr) | *p*H |
|---|---|---|---|---|---|
| Control, 70 per cent $N_2O$ (n = 6) | 153 ± 4 | 37.1 ± 0.1 | 126 ± 5 | 38.1 ± 1.1 | 7.386 ± 0.010 |
| Phenobarbital | | | | | |
| 2 min (n = 6) | 91 ± 8 | — | 116 ± 14 | 38.6 ± 1.4 | 7.392 ± 0.005 |
| 30 min (n = 6) | 98 ± 4 | 36.8 ± 0.1 | 158 ± 19 | 42.5 ± 2.4 | 7.333 ± 0.010 |
| 180 min (n = 6) | 113 ± 4 | 36.9 ± 0.2 | 143 ± 11 | 41.3 ± 1.2 | 7.387 ± 0.036 |

large, or if there were a smaller increase in $pH_i$ in the two-minute group.

## GLYCOLYTIC METABOLITES

The glycogen concentration increased significantly from 2.84 ± 0.11 μmol/g at 2 min to 3.82 ± 0.09 μmol/g at three hours. This confirms previous results.[23] In each phenobarbital-treated group, the G-6-P concentration was significantly higher, and fructose diphosphate (FDP), dihydroxyacetone phosphate (DHAP), 3-phosphoglycerate (3-PG) and pyruvate concentrations were significantly lower, than those measured in the control group (table 2). These findings indicate inhibition of phosphofructokinase with depletion of substrates "downstream" (see below). The changes in the concentrations of 3-PG, pyruvate and lactate demonstrate that minimal values were obtained at 30 min, but not at 2 min, with no further decreases thereafter. Two minutes after phenobarbital injection, the lactate:pyruvate ratio was higher than in the control group, but, after three hours, the value had decreased to below the control value, and below the value obtained in the two-minute phenobarbital-treated group.

Little attention should be given to the low glucose concentration in the two-minute phenobarbital-treated group. Animals during anesthesia show a progressive increase in blood glucose concentrations and therefore also in tissue glucose contents.[23,24] Thus, animals studied 2 min after phenobarbital injection should have only a moderate increase in blood and tissue glucose concentrations.

## CITRIC ACID CYCLE INTERMEDIATES

Of the citric acid cycle intermediates, only the calculated oxaloacetate (OAA) concentrations changed in the two-minute phenobarbital-treated group (table 3). Changes in OAA concentrations were similar whether derived from the aspartate aminotransferase reaction or from the combined LDH–MDH reactions, inspiring confidence in the values obtained. After 30 minutes, all citric acid cycle intermediates were decreased in concentration, with no further decrease in the three-hour group. When the values for glycolytic metabolites and citric acid cycle intermediates obtained in the 30-minute and three-hour phenobarbital-treated groups are plotted as percentages of control, the results illustrate a decrease in glycolytic metabolites distal to phosphofructokinase, and a decrease in the concentrations of citric acid cycle intermediates (fig. 1). The data demonstrate that prolongation of phenobarbital anesthesia from

TABLE 2. Effects of Phenobarbital, 150 mg/kg, on Glycolytic Intermediates (means ± SEM)

| Experimental Group | μmol/g Wet Weight | | | | | | | Lactate/ Pyruvate |
|---|---|---|---|---|---|---|---|---|
| | Glucose | G-6-P | F-D-P | DHAP | 3-PG | Pyruvate | Lactate | |
| Control (70 per cent $N_2O$) (n = 6) | 3.81 ± 0.23 | 0.070 ± 0.003 | 0.122 ± 0.005 | 0.0229 ± 0.0008 | 0.0352 ± 0.0016 | 0.155 ± 0.006 | 1.34 ± 0.05 | 8.7 ± 0.2 |
| Phenobarbital | | | | | | | | |
| 2 min (n = 6) | 2.46*** ± 0.14 | 0.088** ± 0.003 | 0.100** ± 0.004 | 0.0187* ± 0.0012 | 0.0275** ± 0.0017 | 0.117*** ± 0.005 | 1.15 ± 0.07 | 9.8* ± 0.4 |
| 30 min (n = 6) | 4.35 ± 0.16 | 0.091*** ± 0.003 | 0.088*** ± 0.002 | 0.0152*** ± 0.0010 | 0.0183*** ± 0.0015 | 0.076*** ± 0.005 | 0.62*** ± 0.02 | 8.3 ± 0.6 |
| 180 min (n = 6) | 3.94 ± 1.05 | 0.081* ± 0.004 | 0.092*** ± 0.002 | 0.0173** ± 0.0012 | 0.0211*** ± 0.0020 | 0.091*** ± 0.005 | 0.62*** ± 0.03 | 6.8** ± 0.4 |

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

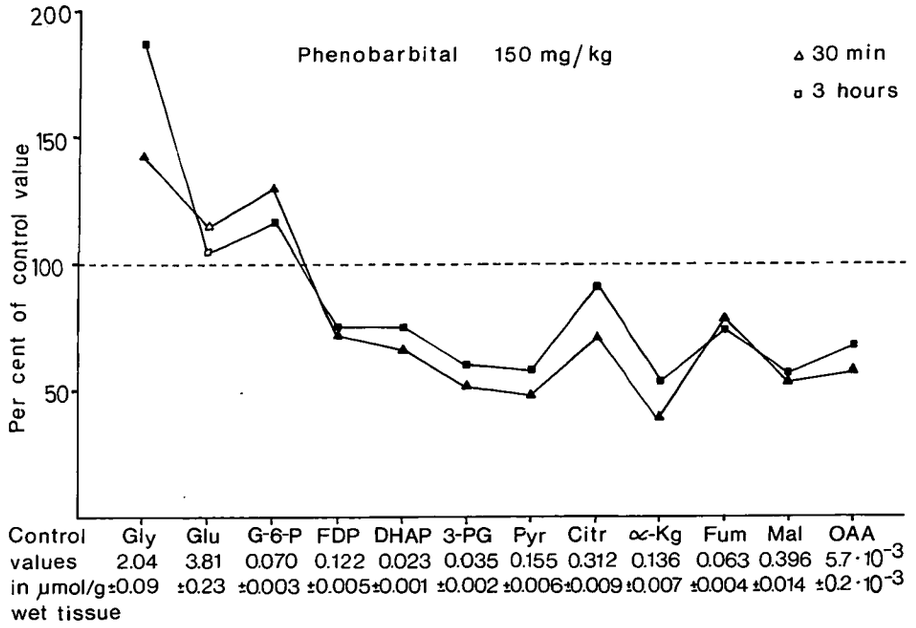


FIG. 1. Changes in glycolytic and citric acid cycle intermediates 30 minutes and three hours following administration of phenobarbital 150 mg/kg, ip. The values are given as percentages of nitrous oxide control values. Filled symbols denote values significantly different from controls ($P < 0.05$).

30 minutes to three hours did not decrease further the pool sizes of carbohydrate metabolites.

### AMINO ACIDS AND AMMONIA

The mean values for ammonia concentration were lower in the phenobarbital-treated groups than in the control group, but the differences were not statistically significant. The amino acid changes in the phenobarbital-treated animals were dominated by decreases in glutamate and increases in aspartate concentration at 30 minutes and three hours (table 4). There was no significant change in glutamine; there were slight decreases in aspartate at 2 min and in GABA at three hours, and a highly significant decrease in alanine concentration at 2 min. At no time was there a change in the sum of amino acids during phenobarbital anesthesia. Neither the apparent Asp-AT nor the Ala-AT equilibrium constants were influenced by phenobarbital.

### REDOX RATIOS

Cytoplasmic NADH/$NAD^+$ ratios were calculated from the LDH and the MDH reactions, using $pH_i$ values derived from the creatine kinase reaction (see above). The derived NADH/$NAD^+$ ratios were higher in the two-minute phenobarbital-treated group than in the control animals, suggesting reduction of $NAD^+$ (fig. 2). The ratios subsequently decreased, with the ratio derived from LDH being significantly below control in the three-hour group. When a compari-

TABLE 3. Effects of Phenobarbital, 150 mg/kg, on Intermediates of the Citric Acid Cycle (means ± SEM)

| Experimental Group | μmol/g Wet Weight | | | | |
|---|---|---|---|---|---|
| | Citrate | α-KG | Fumarate | Malate | OAA† |
| Control (70 per cent $N_2O$) (n = 6) | 0.312 ± 0.009 | 0.136 ± 0.007 | 0.063 ± 0.004 | 0.396 ± 0.014 | 0.0057 ± 0.0002 |
| Phenobarbital | | | | | |
| 2 min (n = 6) | 0.290 ± 0.009 | 0.119 ± 0.007 | 0.066 ± 0.002 | 0.385 ± 0.016 | 0.0046** ± 0.0003 |
| 30 min (n = 6) | 0.220*** ± 0.005 | 0.054*** ± 0.004 | 0.050* ± 0.003 | 0.215*** ± 0.006 | 0.0033*** ± 0.0002 |
| 180 min (n = 6) | 0.284* ± 0.007 | 0.072*** ± 0.003 | 0.047** ± 0.002 | 0.223*** ± 0.007 | 0.0039*** ± 0.0002 |

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.
† OAA calculated from the Asp-aminotransferase reaction.

son is made with the two-minute phenobarbital-treated group, there was a significant decrease in NADH/$NAD^+$ ratio both at 30 minutes and three hours.

## Discussion

Before discussing the main results of the present study, a few comments on methodology seem warranted. In this study, results obtained during phenobarbital anesthesia were compared with those obtained in animals given nitrous oxide. It might be argued that nitrous oxide itself alters the metabolic pattern of the tissue and that, accordingly, the true effects of phenobarbital are not obtained. The following facts indicate that this is not so. First, two previous animal studies demonstrated that nitrous oxide, 70 per cent, does not depress $CMR_{O_2}$ to values lower than those recorded in unanesthetized animals.[25,26] Second, withdrawal of the nitrous oxide supply in adrenalectomized or non-adrenalectomized animals does not induce significant changes in glycolytic metabolites, citric acid cycle intermediates, associated amino acids, or ammonia.[27] Third, administration of thiopental to animals maintained on nitrous oxide, 70 per cent, induces changes in glycolytic metabolites and amino acids similar to those obtained presently, although the period of observation in the previous study (15 minutes) was too short to reveal maximal effects on citric acid cycle intermediates and amino acids. For all these reasons, we conclude that most of the differences in metabolite concentrations between the phenobarbital-treated and control groups reflect true barbiturate effects.

It has been clearly shown that two conditions, hypoglycemia and hypercapnia, are associated with decreased delivery of carbohydrate substrate from exogenous glucose. Since the delivery of pyruvate is insufficient to cover the oxidative metabolism, oxidation of endogenous carbohydrate and amino acid substrates occurs. In hypoglycemia, there is a larger decrease in $CMR_{gl}$ than in $CMR_{O_2}$[28] and depletion of glycolytic metabolites, citric acid cycle intermediates, and associated amino acids.[3,29,30] In hypercapnia, a similar pattern is observed, in all probability secondary to inhibition of phosphofructokinase.[31–33] In both conditions, carbon skeletons are mobilized from amino acids by a shift in the Asp-AT equilibrium in the direction of aspiratate formation. This shift may be triggered by relative accumulation of OAA, secondary to decreased availability of acetyl CoA for condensation to citrate[11] or to a decrease in malate/OAA ratio.[29] However, since the size of the amino acid pool is decreased, and since there is accumulation of ammonia, there is also oxidative deamination of amino acids. In all probability, this occurs by oxidative deamination of glutamate. The mechanism has not been defined, but may involve depletion of NADH. Thus, experiments with insulin-induced hypoglycemia suggest oxidation of cytoplasmic and mitochondrial redox systems.[29] In hypercapnia, the cytoplasmic NADH/$NAD^+$ system is either unchanged or oxidized,[19,31] and direct measurements of mitochondrial redox states indicate oxidation of cyt $a_3$.[34]

The present experiments have confirmed previous results in showing that barbiturate anesthesia leads to accumulation of G-6-P, and to depletion of glycolytic metabolites distal to phosphofructokinase. In view of the fact that substrate flux is decreased, this pattern is compatible with inhibition of phosphofructokinase (Krebs theorem).[35] The effects on citric acid cycle intermediates corroborate those found by

TABLE 4. Effects of Phenobarbital, 150 mg/kg, on Levels of Amino Acids and Ammonia, and on "Apparent Equilibrium Constant" ($K_{app}$) for Aspartate and Alanine Aminotransferase Reactions (means ± SEM)

| Experimental Group | μmol/g Wet Weight | | | | | | | $K_{app}$ Asp-AT | $K_{app}$ Ala-AT $\cdot 10^{-2}$ |
|---|---|---|---|---|---|---|---|---|---|
| | Glutamate | Asp | Glutamine | GABA | Ala | $NH_4^+$ | ε-Amino Acids | | |
| Control (70 percent $N_2O$) (n = 6) | 13.46 ± 0.20 | 3.77 ± 0.08 | 5.75 ± 0.25 | 2.03 ± 0.09 | 0.519 ± 0.013 | 0.269 ± 0.054 | 25.52 ± 0.20 | 9.92 ± 0.54 | 3.38 |
| Phenobarbital | | | | | | | | | |
| 2 min (n= 6) | 13.29 ± 0.13 | 3.46* ± 0.08 | 5.31 ± 0.26 | 2.15 ± 0.13 | 0.444** ± 0.013 | 0.226 ± 0.022 | 24.64 ± 0.29 | 9.89 ± 0.99 | 3.40 |
| 30 min (n = 6) | 12.42** ± 0.19 | 5.06*** ± 0.11 | 5.82 ± 0.32 | 1.91 ± 0.07 | 0.525 ± 0.014 | 0.186 ± 0.029 | 25.73 ± 0.22 | 9.48 ± 1.06 | 3.00 |
| 180 min (n = 6) | 12.14** ± 0.33 | 4.34*** ± 0.10 | 6.49 ± 0.17 | 1.72* ± 0.10 | 0.473 ± 0.021 | 0.196 ± 0.035 | 25.16 ± 0.56 | 9.04 ± 0.78 | 3.08 |

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

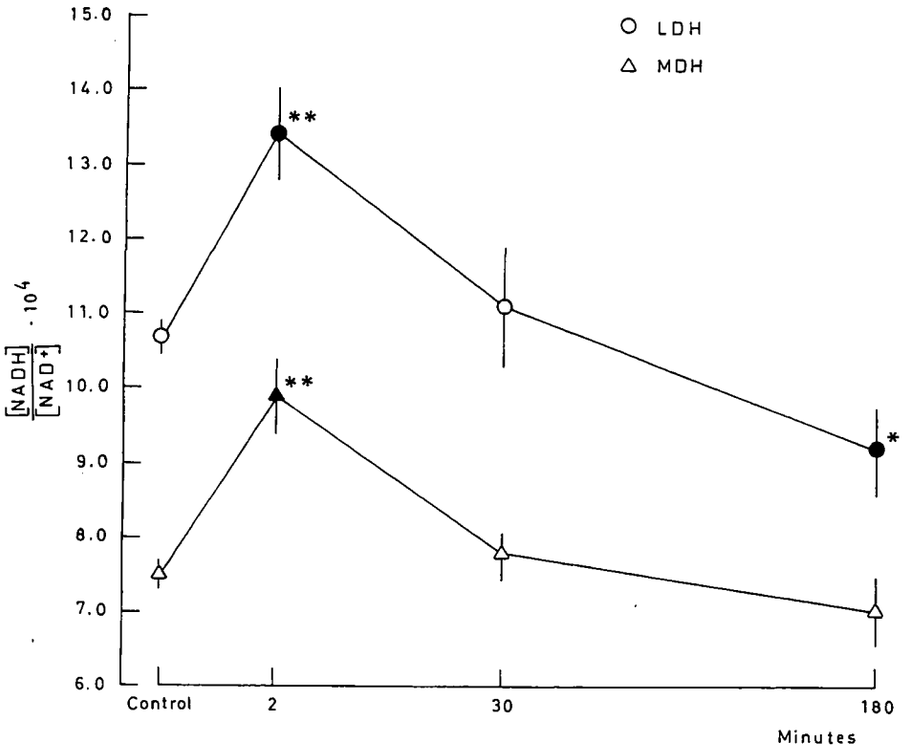


FIG. 2. Cytoplasmic redox state in control animals during administration of nitrous oxide, 70 per cent, and 2, 30, and 180 minutes after administration of phenobarbital, 150 mg/kg. The [NADH]/[NAD+] ratios were calculated from either the lactate dehydrogenase (LDH) or the malate dehydrogenase (MDH) reactions using $pH_i$ calculated from the creatine kinase reaction. The values are means ± SEM. Filled symbols denote values significantly different from controls (* = $P < 0.05$, ** = $P < 0.01$).

Goldberg *et al.*[3] after amytal, although these authors did not observe a decrease in OAA concentration. In the present study, OAA was calculated either from the Asp-AT reaction or from the combined LDH–MDH reactions. Since these calculations are designed to give the cytoplasmic concentrations, it is conceivable that cytoplasmic and whole-tissue concentrations behave differently.

The present pattern is analogous to that observed in hypercapnia, in that there is depletion of glycolytic metabolites and citric acid cycle intermediates, and an indication of a shift in Asp-AT equilibrium. All these changes are compatible with partial substrate depletion due to phosphofructokinase inhibition and with delivery of alternative substrate from endogenous stores. However, the results fail to support the view that there is severe impediment of substrate delivery from exogenous glucose, or that amino acids are oxidized. Thus, prolongation of phenobarbital anesthesia from 30 minutes to three hours did not cause further changes in concentrations of carbohydrate substrates or amino acids, and there was no decrease in the size of the amino acid pool during the two hours of phenobarbital anesthesia. Furthermore, the ammonia concentration was not increased to above normal in any of the phenobarbital-treated groups. We conclude from our results that a steady state was reached 30 minutes after phenobarbital administration.

Previous results with hypoglycemia and hypercapnia, as well as in hypoxia,[36,37] indicate that many of the changes affecting citric acid cycle intermediates and associated amino acids can be explained in terms of a transient mismatch between glycolytic and citric acid cycle flux, and in terms of a redox change. If phenobarbital retards glycolytic flux at the phosphofructokinase step, the subsequent decrease in pyruvate concentration may explain both the decrease in the size of the citric acid cycle pool (succinate was not measured but previous results[3] demonstrate that its concentration is not affected by barbiturates) and the transient decrease in alanine concentration. The decrease in citric acid pool size is explained if one assumes that the decrease in pyruvate (and phosphoenolpyruvate) concentration reverses the normal flux in the reactions leading to $CO_2$ fixation,[38,39] and induces a shift in the Ala-AT reaction. It is more difficult to relate any of the changes observed to redox shifts, since the calculated cytoplasmic NADH/$NAD^+$ ratio is dependent on a number of assumptions. The present results indicate that, shortly after phenobarbital injection, there is a moderate decrease of cytoplasmic $NAD^+$. The results agree with those reported by Chance *et al.*,[40] who found a decrease in mitochondrial $NAD^+$, as measured with noninvasive microfluorometric techniques, following administration of amytal. It is tempting to assume that an initial $NAD^+$ reduction, by causing a relative decrease in OAA, is responsible for the moderate decrease in aspartate at 2 min. It is more difficult to

relate the subsequent reversal of flux in the Asp-AT reaction to redox changes. Thus, the calculated cytoplasmic NADH/$NAD^+$ ratios gradually decreased.

In conclusion, the present results confirm the proposal by Betz and Gilboe[15] that barbiturates, by inhibiting glycolytic flux, induce oxidation of endogenous substrates and mobilization of carbon skeletons from amino acids (by transamination). However, our results indicate that this is a transient phenomenon, and that no oxidative deamination of amino acids occurs.

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## Appendix

The energy state of the tissue was calculated in terms of energy charge of the adenine nucleotide pool[42] as

$$\text{E.C.} = \frac{[\text{ATP}] + 0.5[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$$

The oxaloacetate (OAA) concentration was calculated from the aspartate aminotransferase reaction ($K'_{eq} = 6.7$)[43]

$$[\text{aspartate}] \cdot [\alpha\text{-ketoglutarate}] = K_{eq} \cdot [\text{glutamate}]\,[\text{oxaloacetate}]$$

or by assuming the lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) to be in equilibrium with the cytoplasmic $NADH–NAD^+$ pool according to the equation

$$\frac{[\text{OAA}]}{[\text{malate}]} = \frac{K'_{eq}[\text{MDH}]}{K'_{eq}[\text{LDH}]} \cdot \frac{[\text{pyruvate}]}{[\text{lactate}]}$$

The equilibrium constants for the MDH and LDH reactions were $0.98 \cdot 10^{-12}$ and $0.53 \cdot 10^{-11}$, respectively.[44,45]

Changes in intracellular *p*H ($p\text{H}_i$) were tentatively calculated using the creatine kinase reaction:

$$[\text{H}^+] \cdot K'_{eq} = \frac{[\text{Cr}]\,[\text{ATP}]}{[\text{PCr}]\,[\text{ADP}]}$$

using a control $p\text{H}_i$ of 7.044.[41] A provisional $K'_{eq}$ was derived by inserting into the equation a $p\text{H}_i$ of 7.044 and the measured concentrations of PCr, Cr, ATP and ADP during control conditions. This $K'_{eq}$ was then used to derive changes in $p\text{H}_i$ by inserting measured values for PCr, Cr, ATP and ADP.

The cytoplasmic $NADH/NAD^+$ ratio was estimated from the assumed equilibria of the LDH or MDH reaction

$$\frac{[\text{NADH}]}{[\text{NAD}^+]} = \frac{K'_{eq}}{[\text{H}^+]} \cdot \frac{[\text{reduced substrate}]}{[\text{oxidized substrate}]}$$

Total tissue concentrations of lactate, pyruvate and malate were used.