the inhibitory effects of identical concentrations of a narcotic (amytal), a sedative (pentobarbital) and a pharmacologically inactive barbiturate (hydroxyamytal) are compared.

The results suggest that pharmacologically active barbiturates exert an inhibitory effect on hydrogen transfer *via* pyridine nucleotides with a general inhibition of cell respiration as a consequence. The fact that this effect is restricted to the central nervous system *in vivo* may be interpreted as indicating that a limitation of the respiratory capacity is expressed more primarily in this tissue than in other organs.

*Table 1. Effect of amytal on the oxidation of different substrates in rat liver mitochondria.*

| Substrate | Respiration (microatoms oxygen) | |
|---|---|---|
| | *without* amytal | *with* 1.8 m*M* amytal |
| L-glutamate | 26.2 | 0 |
| pyruvate | 9.5 | 0 |
| citrate | 11.7 | 0 |
| α-ketoglutarate | 22.0 | 3.5 |
| succinate | 26.2 | 23.4 |
| fumarate | 9.4 | 0 |
| L-malate | 8.5 | 0 |
| DL-β-hydroxybutyrate | 8.5 | 0 |

Each Warburg vessel contained: rat liver mitochondria (prepared in 0.25 *M* sucrose — 0.01 *M* versene), 1/12 liver; substrate, 30 micromoles; adenylic acid, 4.3 micromoles; orthophosphate, 40 micromoles; glucose, 47 micromoles; KCl, 270 micromoles; $Mg^{++}$, 7.5 micromoles; yeast hexokinase (prepared according to Berger *et al.*, "Step 5"), 0.05 ml. pH 7.5. Final volume, 2.0 ml. Temp. 30° C. Gas phase, air. Time of incubation, 25 min.

*Table 2. Inhibition of mitochondrial respiration by different derivatives of barbiturate*

| Barbiturate $M \cdot 10^3$ | amytal | pentobarbital | hydroxyamytal |
|---|---|---|---|
| | percent inhibition of respiration | | |
| 0.9 | 67 | 16 | 0 |
| 1.8 | 100 | 44 | 7 |

Substrate, glutamate. Experimental conditions as in Table 1.

1. Eiler, J. J. and McEwen, W. K. *Arch. Biochem.* **20** (1949) 163.
2. Brody, T. M. and Bain, I. A. *Proc. Soc. Exptl. Biol. Med.* **77** (1951) 50.
3. Johnson, W. J. and Quastel, J. H. *J. Biol. Chem.* **205** (1953) 163.

## Yield of Oxidative Phosphorylation in the Succinic Oxidase System

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We have recently shown[1] that $Ca^{++}$-treated rat liver mitochondria catalyze the one-step oxidation of succinate to fumarate. The addition of adenosine triphosphate and $Mn^{++}$ to this system results in a coupled phosphorylation. At the optimal concentration of $Mn^{++}$, and with increasing amounts of adenosine triphosphate, the phosphorylation / oxidation (P/O) ratio asymptotically approaches the value of 1.1.

Another way of obtaining a single-step oxidation of succinate is by means of substituted barbiturates which, as shown previously (Jalling *et al.*, *cf.* p. 198), are able to block further oxidation of the fumarate formed. Eiler and McEwen [2], using 5-ethyl-5-(1-methylbutyl)barbiturate as a blocking agent, obtained a P/O ratio of about 1.4 for the one-step oxidation of succinate in rat brain homogenates. In rat liver mitochondria treated with $1.8 \cdot 10^{-3}$ *M* 5-ethyl-5-*iso*amylbarbiturate (amytal) we have obtained about the same value, 1.42 (Table 1). There is thus a significant difference in the P/O ratio between the succinic oxidase systems obtained in the two different ways. Obviously, the $Ca^{++}$-treated system does not yield a maximum P/O ratio.

On the other hand the data in Table 1 show that the value obtained in the $Ca^{++}$-treated system can be lowered further by amytal. This indicates that amytal may be able to lower the P/O ratio in the succinic oxidase system, *i. e.* that not even the value of 1.42 may be regarded as uninfluenced. The possible effect of amytal on the phosphorylation coupled with succinate oxidation is a subject of further study.

*Table 1. The action of different agents on the P/O ratio in the succinic oxidase system.*

| Agent blocking fumarate oxidation | Additions after 5 min of pre-incubation with blocking agent | Respiration (μatoms oxygen) | Phosphorylation (μmoles phosphate) | P/O |
|---|---|---|---|---|
| — | succinate | 16.6 | 27.6 | 1.66 |
| amytal | succinate | 16.2 | 23.0 | 1.42 |
| $Ca^{++}$ | succinate, ATP, $Mn^{++}$ | 16.9 | 19.0 | 1.12 |
| amytal + $Ca^{++}$ | succinate, ATP, $Mn^{++}$ | 16.8 | 15.3 | 0.91 |

Each Warburg vessel contained: rat liver mitochondria (prepared in 0.25 *M* sucrose — 0.01 *M* versene), 1/12 liver; adenylic acid, 4.3 μmoles; orthophosphate, 40 μmoles; glucose, 47 μmoles; KCl, 270 μmoles; $Mg^{++}$, 7.5 μmoles; yeast hexokinase (prepared according to Berger *et al.*, "Step 5"), 0.1 ml. Additions (where indicated): succinate, 30 μmoles; amytal, 3.6 μmoles; $Ca^{++}$, 1.15 μmoles; ATP, 1.5 μmoles; $Mn^{++}$, 1.5 μmoles. pH 7.5. Final volume, 2.0 ml. Temp 30° C. Gas phase, air. Time of incubation, 20 min.

1. Ernster, L., Lindberg, O. and Löw, H. *Nature (In press)*.
2. Eiler, J. J. and McEwen, W. K. *Arch. Biochem.* **20** (1949) 163.

## The Action of 5-Ethyl-5-*iso*amylbarbiturate (Amytal) on the Oxidation of Reduced Diphosphopyridine Nucleotide (DPNH) in Rat Liver Mitochondria

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Previously (Jalling *et al.*, *cf.* p. 198) it has been shown that $1.8 \cdot 10^{-3}$ *M* amytal completely blocks those mitochondrial oxidations which proceed *via* pyridine nucleotides. To study further the localization of this effect within the respiratory chain, DPNH, as generated by added alcohol dehydrogenase [1] and ethanol was used as a substrate for mitochondria. As seen in Table 1, the respiration in this system is, in contrast to the case of DPNH generated by the mitochondrial dehydrogenases, but partially — about 33 % — inhibited by $1.8 \cdot 10^{-3}$ *M* amytal. The accompanying phosphorylation is, on the other hand, almost completely inhibited.

This indicates that DPNH generated by "external" dehydrogenases is probably oxidized by two pathways, only one of which is sensitive to amytal. Both pathways differ markedly with respect to the extent of phosphorylation coupled to them. In the system studied, the amytal-insensitive pathway seems to be inaccessible to DPNH generated by mitochondrial dehydrogenases.

These findings suggest a possible similarity between the action of amytal and that of antimycin A on mitochondrial DPNH- and TPNH-cytochrome *c* reductase systems, as recently studied by Reif and Potter [2] and by Pressman and de Duve [3].

*Table 1. The effect of amytal on the oxidation of DPNH in rat liver mitochondria.*

| | *Without* amytal | *With* 1.8 m*M* amytal |
|---|---|---|
| Respiration (μatoms oxygen) | 9.6 | 6.4 |
| Phosphorylation (μmoles phosphate) | 13.1 | 2.2 |
| P / O | 1.37 | 0.34 |

Each Warburg vessel contained: rat liver mitochondria (prepared in 0.25 *M* sucrose — 0.01 *M* versene), 1/12 liver; adenylic acid, 4.3 μmoles; orthophosphate, 40 μmoles; glucose, 47 μmoles; cytochrome *c*, 0.0002 μmoles; KCl, 270 μmoles; $Mg^{++}$, 7.5 μmoles; yeast hexo-