A Specific Method for the Determination of Glycosides Containing Desoxymethylpentose

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The methods proposed for the chemical estimation of cardiac glycosides depend upon (1) the unsaturated lacton ring, or (2) the steroid skeleton, or (3) the sugar moiety. These tests, however, can scarcely be regarded as specific for cardiac glycosides. The reactions based on the lacton ring or those based on the steroid skeleton codetermine not only steroid derivatives other than cardiac glycosides but also other fairly common compounds which have an active H. The reactions based on the sugar moiety also proved to be unsatisfactory: in some cases they were given even by other non-specific sugars being present in the sample analyzed, in other cases they were not strictly quantitative.

Since the sugar moiety of the cardiac glycosides consists of highly specific desoxysugars, a possibility is afforded of detecting glycosides by utilizing the specificity of the sugar component. The data recorded show that desoxymethylpentoses, *i. e.* the specific sugar compound occurring only in the cardiac glycosides, give a reaction with orcinol which may be used for the quantitative determination of these glycosides. Although a great variety of carbohydrates react with orcinol, the test, in this modified form, seems to be highly specific for desoxymethylpentoses. This permits the detection of minute amounts of cardiac glycosides even in several thousandfold excess of common sugars. All the primary and secondary cardiac glycosides of digitalis give the reaction, as do k-strophantin and oleandrin. Ouabain and the squill glycosides, both containing a methylpentose, do not react, nor do other methylpentoses or desoxypentoses. Aglycones, saponins and other steroid derivatives do not interfere with the reaction. A further advantage of the test is that all the glycosides react in relation to their theoretical sugar content. The method may be used for the quantitative determination of desoxymethylpentose in amounts of 0.5—60 μg; this amount corresponds to about 1—100 μg of glycosides. The experimental error of a double determination is $\pm$ 1.51 %.

On the Effect of Substituted Barbiturates * on Mitochondrial Respiration

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Substituted barbiturates have long been known to affect the cellular respiratory system. In 1949, Eiler and McEwen [1], working with brain homogenates, showed that the oxidation of pyruvate and fumarate could be blocked by pentobarbital while that of succinate was unaffected. Later investigations, performed on isolated mitochondria, did not lead, however, to a generally valid interpretation of these findings as to the mode of barbiturate action. Brody and Bain [2] reported a decrease of the phosphorylation/oxidation ratio and attempted to interpret the barbiturate action in terms of an uncoupling effect rather than a respiratory inhibition. This view, however, could not be shared by Johnson and Quastel [3]. These authors found, as an effect of barbiturates, a decreased capacity of brain mitochondria to activate acetate, but came to the conclusion that this effect might be a secondary one.

From the above mentioned results of Eiler and McEwen it may be envisaged that the effect of barbiturates must be of a rather fundamental character and may therefore also be studied in a system metabolically better defined than brain, *e. g.* liver mitochondria. In experiments with this system we have found that amytal, in a concentration of $1.8 \cdot 10^{-3}$ *M* totally inhibits the oxidation of a series of substrates (Table 1); a clearly marked exception is formed by succinate, the oxidation of which is not at all influenced by amytal. This finding — which is consistent with, and an extension of, the early results by Eiler and McEwen — indicates that the action of barbiturates is concerned with the transfer of hydrogen *via* the pyridine nucleotides.

In some preliminary experiments we were also able to obtain an indication of a correlation between narcotic power and inhibition of respiration. This is shown in Table 2 where

* pentobarbital = 5-ethyl-5-(1-methylbutyl)barbiturate; amytal = 5-ethyl-5-*iso*amylbarbiturate; hydroxy-amytal = 5-ethyl-5(3-hydroxy-*iso*amyl)barbiturate.

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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.