the level of phosphoprotein phosphorus in the whole tissue homogenate. This loss was reflected also in diminished radioactivity in the whole slice. However, the specific radioactivities (counts per min/μg Pi) of the phosphoproteins of both the homogenate and the subcellular fractions were not different from the control values (Table 1). This would suggest that the turnover of phosphoprotein phosphorus in the cerebral tissue was not altered by the treatment with ouabain. It is worth emphasizing that the phosphoprotein content and turnover were determined by measuring the release of inorganic phosphate after prolonged alkaline digestion of the protein. This procedure affords an estimate of the entire phosphoprotein fraction and does not preclude the possibility that some small fraction of the total phosphoprotein molecule may be involved in ionic movements which may, therefore, be susceptible to the action of drugs which affect ionic movements.

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Reversal of mitochondrial inhibition of glycolysis by styrylquinolines

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CERTAIN styrylquinolines have caused a marked regression in the growth of lymphoma 8 tumors in rats, but their successful therapeutic use has been hindered by their toxicity and the limited range of tumors affected.[1–3] The styrylquinolines have little resemblance in either structure or action to any of the known antitumor agents, and the only reports of their effects *in vitro* are those of Dickens[4] and Emmelot and co-workers,[5–7] who found that styrylquinolines inhibit cellular respiration and promote the breakdown of esterified phosphate.

One model system for investigating effects of drugs on energy metabolism is that of Aisenberg and colleagues,[8] in which some of the interactions of oxidative and glycolytic systems can be investigated. The glycolytic system is the supernatant fraction remaining after high speed centrifugation of a rat brain homogenate, and it readily utilizes glucose and produces lactic acid. The oxidative system is supplied by rat liver mitochondria. These mitochondria are not capable of coupling electron transport with oxidative phosphorylation, because they were isolated and incubated in KCl buffer systems. In this type of model system the addition of liver mitochondria to the supernatant fraction of brain depresses glycolysis. Presumably the effects of the mitochondria are related to their ability to carry out electron transport, since inhibitory effects are seen only under aerobic conditions and are reversed by the addition of electron transport inhibitors such as cyanide or antimycin A.[8]

The glycolytic system was prepared by centrifugation of a 30% homogenate of rat brain in 0·154 M KCl containing 0·02 M nicotinamide for 40 min at 76,000 g; 0·67 ml of the clear supernatant fraction was added to each flask. Mitochondria were prepared by differential centrifugation of a

TABLE 1. EFFECT OF STYRYLQUINOLINES ON GLYCOLYSIS BY RAT BRAIN SUPERNATANT FRACTION IN THE PRESENCE AND ABSENCE OF RAT LIVER MITOCHONDRIA

Additions	μMoles of phosphate esterified		μMoles of glucose utilized‡		μMoles of lactate produced§	
	Supernatant*	Supernatant + mitochondria†	Supernatant	Supernatant + mitochondria	Supernatant	Supernatant + mitochondria
Gas phase = 5% CO_2, 95% N_2 None	5·3 ± 0·2	2·8 ± 0·6	7·5 ± 0·6	9·7 ± 1·1	7·2 ± 0·3	11·1 ± 1·3
Gas phase = 5% CO_2, 95% O_2 None	5·4 ± 0·2	2·3 ± 0·4	7·6 ± 0·3	5·9 ± 0·7	7·4 ± 0·5	5·0 ± 0·5
4 M_2O $3·3 \times 10^{-5}$ M	5·5 ± 0·2	2·6 ± 0·7	7·8 ± 0·3	7·8 ± 0·8	7·5 ± 0·4	7·8 ± 0·9
4 NH_2O $3·3 \times 10^{-5}$ M	5·5 ± 0·2	2·7 ± 0·6	7·8 ± 0·3	8·0 ± 0·9	7·4 ± 0·4	7·5 ± 0·6
2 M_2M $3·3 \times 10^{-5}$ M	5·5 ± 0·2	1·2 ± 0·6	7·8 ± 0·5	10·4 ± 0·4	7·6 ± 0·7	12·2 ± 0·4
$3·3 \times 10^{-6}$ M		1·8 ± 0·7	7·8 ± 0·5	7·6 ± 0·7	7·6 ± 0·7	8·1 ± 0·6

* μMoles of phosphate esterified by 200 mg equivalents of rat brain supernatant fraction during 30 min incubation at 37·5 °C. In all cases the values given express the mean ± standard error of three experiments.
† 300 Mg equivalents of rat liver mitchondria were present in addition to the supernatant fraction.
‡ μMoles of glucose taken up from the medium during the incubation period.
§ μMoles of lactate produced during the incubation period.

10% homogenate of rat liver in isotonic sucrose, and the fraction sedimenting between 600 and 1650 **g** (10 min centrifugation) was used. The mitochondria were resuspended and centrifuged twice in isotonic KCl. Each incubation flask contained the following additions in a volume of 3 ml: 20 μmoles glucose, 12 μmoles $MgCl_2$, 2 μmoles adenosine triphosphate, 0·3 μmole diphosphopyridine nucleotide, 120 μmoles nicotinamide, 75 μmoles $KHCO_3$, and 4 μmoles potassium phosphate (pH 7·4). The styrylquinolines were dissolved in ethanol, and the required amount was added in a volume of 0·02 ml of ethanol; the addition of 0·02 ml of ethanol to control flasks did not affect glycolysis. At the concentrations used, all compounds appeared to be in solution throughout the experiments. Phosphate was determined by the method of Allen,[9] glucose by the coupled glucose oxidase procedure,[10] and lactate by the method of Barker and Summerson.[11]

The supernatant fraction utilized glucose and produced lactate when incubated in the medium used; this glycolysis was not affected by the oxygen concentration of the gas phase (Table 1). Rat liver mitochondria neither utilized glucose nor produced lactate when incubated alone (unpublished experiments), and therefore no mitochondrial controls are included. However, the addition of these mitochondria to the supernatant fraction brought about an inhibition of glycolysis under aerobic, but not anaerobic, conditions. In the presence of styrylquinolines glycolysis by the supernatant fraction alone was not affected. The addition of mitochondria to the supernatant in the presence of styrylquinolines failed to depress glycolysis. Adding the styrylquinolines under aerobic conditions to the mitochondria plus supernatant produced the same effect as did anaerobic conditions in the absence of the styrylquinolines.

The styrylquinolines 4 M_2O and 4 NH_2O reversed the effect of liver mitochondria at concentrations that did not affect the breakdown of esterified phosphate. The compound 2 M_2M, however, did increase the net breakdown of esterified phosphate.

2 M_2M was approximately as effective at $3{\cdot}3 \times 10^{-6}$ M, as were 4 NH_2O and 4 M_2O at $3{\cdot}3 \times 10^{-5}$ M, and the latter compounds were without effect at a concentration of $3{\cdot}3 \times 10^{-6}$ M; 2 M_2M was ineffective at a concentration of $3{\cdot}3 \times 10^{-7}$ M. The approximately tenfold greater activity of the quaternary salt of a styrylquinoline (2 M_2M) *in vitro* is of interest, because the quaternary salts are much more toxic than are the free bases *in vivo* if administered intraperitoneally.[2, 3] Since styrylquinolines in which the styryl group is attached to the number two carbon of quinoline (2 M_2M) are very weak antitumor drugs as compared with styryl groups attached to the number four carbon (4 M_2O, 4 NH_2O), the effects seen are probably not related to the antitumor properties of styrylquinolines.[12]

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Abbreviations used: 2 M_2M = 2-(4-dimethylaminostyryl) quinoline methiodide; 4 NH_2O = 4-(4-aminostyryl) quinoline; 4 M_2O = 4-(4-dimethylaminostyryl) quinoline. These compounds were a gift from Dr. C. T. Bahner, Carson-Newman College, Jefferson City, Tenn.

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