EFFECT OF AMYTAL AND DIETHYLSTILBESTEROL ON THE OXIDATION OF DPNH BY MOUSE TUMOR AND LIVER MITOCHONDRIA EXTRACTS[1]

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Various steroid hormones, and non-steroid compounds possessing estrogenic activity, e.g., diethylstilbesterol, have been shown to inhibit the enzymatic oxidation of reduced DPN, Jensen (1959) and Yielding et al. (1959, 1960), by antagonizing enzymatic activity at some point in the electron chain between DPNH and cytochrome C. Recently, it has been proposed that the locus of inhibition for these compounds is identical to that of the barbituate, sodium amytal [Yielding et al.(1960)]; the observation that DPNH oxidase systems which are insensitive to amytal are also resistant to steroid inhibition, being submitted in part, as evidence supporting this postulate. Studies in this laboratory directed toward a biochemical elucidation of the synergistic antagonism of pyridine nucleotide-dependent enzymatic systems by the simultaneous administration of synthetic estrogens and the niacin antagonist 6-aminonicotinamide, Dietrich et al (1961), have demonstrated that it is possible to extract from liver and tumor mitochondria DPNH - cytochrome C reductase preparations that retain a marked susceptibility to inhibition by diethylstilbesterol, but which are apparently insensitive to sodium amytal.

Experimental. Enzyme systems capable of oxidizing DPNH were isolated from mitochondria of livers of C_{57} mice and the 755 adenocarcinoma grown in C_{57} mice utilizing the procedure of Baltscheffsky (1957) modified by

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employing, as the enzyme source, all material not sedimented by centrifugation at 23,000 x G for 20 minutes. Diethylstilbesterol was dissolved in 95% ethanol and an equivalent amount of ethanol was added to control vessels. No differences were observed when 60% dioxane or propylene glycol were used as the solvent. All reactions were carried out at 25°C in a Beckman DU spectrophotometer and nitrogen values were obtained employing the Biuret procedure, Layne (1957).

Results. The data are presented in Table I. Extracts of mitochondria ground with fine Al_2O_3 exhibit a high rate of DPNH oxidation when cytochrome C is employed as the terminal electron acceptor. Low activity is observed, however, when oxygen is utilized. In the latter enzymatic systems the K_i found for diethylstilbesterol and amytal is around 4.0×10^{-7}M and 1.7×10^{-4}M, respectively. Utilizing cytochrome C as the electron acceptor diethylstilbesterol has a K_i of approximately 2.0×10^{-5}M and sodium amytal at a concentration of 6.7×10^{-3}M is without activity.

Discussion. These studies employing liver and tumor mitochondria extracts confirm the findings of Baltscheffsky (1957), who demonstrated that in heart sarcosomes the oxidation of DPNH employing cytochrome C as the electron acceptor is resistant to amytal, while the oxidation of DPNH using oxygen is sensitive to amytal. As shown in Table I both DPNH oxidation systems are sensitive to diethylstilbesterol. When oxygen is employed the synthetic estrogen is 50 times as sensitive (4.0×10^{-7}M vs. 2.0×10^{-5}M) as when cytochrome C is used as the electron acceptor. The former system appears to be similar to the one studied by Yielding (1960).

The ineffectiveness of amytal against the cytochrome C reductase system in spite of the fact that this system has retained sensitivity to diethylstilbesterol permits one to surmise that amytal and diethylstilbesterol either antagonize at different points in the multi-enzyme complex between

TABLE I

Amytal and Diethylstilbesterol Inhibition of DPNH Oxidation in Extracts of Mouse Liver and Tumor Mitochondria Employing Oxygen and Cytochrome C as the Terminal Electron Acceptor*

Tissue	Inhibitor	DPNH Oxidation micro moles of DPNH removed/5 min/mg protein x 10^3	
		Oxygen	Cytochrome C
Liver	None	38	642
	Amytal+	25	644
	Diethylstilbesterol‡	18	311
755 Tumor	None	9	62
	Amytal+	5	62
	Diethylstilbesterol‡	4	36

* Each reaction vessel contained 0.067 M Phosphate, pH 7.4, DPNH, 1.0×10^{-4}M, Mitochondrial extracts and inhibitor, as indicated, in a total volume of 3 ml. Cytochrome C was added to a final concentration of 1.1×10^{-4}M. Reactions were carried out in duplicate at 25°C.

\+ Amytal added to a final concentration of 1.7×10^{-4}M and 6.7×10^{-3}M in oxygen and Cytochrome C systems, respectively.

‡ Diethylstilbesterol added to a final concentration of 4.0×10^{-7}M and 2.0×10^{-5}M in the oxygen and cytochrome C systems, respectively.

The change in absorbancy observed during the first minute after enzyme addition was discarded in calculating activity. The reaction rates were linear during the time periods reported.

DPNH and cytochrome C or that diethylstilbesterol antagonizes at more than one place on the electron chain. In the latter case, loss of a portion of the electron chain that is highly sensitive to diethylstilbesterol could permit the detection of other points in the electron chain which are less sensitive to the synthetic estrogen.

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