TABLE II. Effect of Cobalt on Chicks Fed Diets Deficient in Vitamin B_{12}.

Trial	Group	$CoSO_4 \cdot 7H_2O$ /kg of basal ration, mg	Avg wt, g	No. chicks dead	Feed efficiency*
1†	1	0	309	1	2.93
	2	6	308	0	3.01
	3	10	336	1	2.90
	4	14	345	1	2.73
	5	50	356	1	2.91
	6	Pos. control (with B_{12})	415	1	2.56
2‡	1	0	246	1	2.50
	2	10	260	0	2.53
	3	50	279	1	2.88
	4	100	298	1	2.60
	5	Pos. control (with B_{12})	294	0	2.52

* Feed efficiency $= \frac{\text{Total feed consumed}}{\text{Wt gained}}$.

† Carried on exp. 37 days.
‡ " " " 35 " .

volved in this stimulatory effect (the cobalt content of the ration and of the water consumed, was not determined, unfortunately).

Jaffé(12) reported a beneficial action of cobalt on pregnancy and lactation of rats and mice deficient in vit. B_{12}. It appears possible, therefore, that under certain conditions cobalt may improve the nutrition of vit. B_{12}-deficient, non-ruminating animals.

Summary. 1. Chick growth was stimulated by the addition of 10 to 100 mg of cobalt sulfate to each kg of a corn-soybean oil meal diet which was deficient in vit. B_{12}. 2. When similar levels of cobalt sulfate were added to the same diet supplemented with ample vit. B_{12} they produced no observable effect.

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Inhibition of Rat Liver Succinoxidase by Testosterone-17β Diethyl Aminoethyl Carbonate.* (20059)

HAROLD A. LEVEY. (Introduced by Clara M. Szego.)

From the Department of Zoology, University of California, Los Angeles.

Various natural and synthetic estrogens exert an *in vitro* inhibitory action on the succinoxidase activity of many tissues(1). Utilizing synthetic estrogens, McShan and Meyer(2) have localized this inhibition to the cytochrome oxidase component of the system while Case and Dickens(3) demonstrated that, depending upon the estrogen used, the point of action could be succinic dehydrogenase, cytochrome oxidase, or a hypothetical link between these two enzymes. Guidry *et al.*(4) found, however, that estrone and estradiol did not inhibit the oxidation of succinate by rat liver homogenates. Androgens inhibit the oxygen consumption of rat liver,

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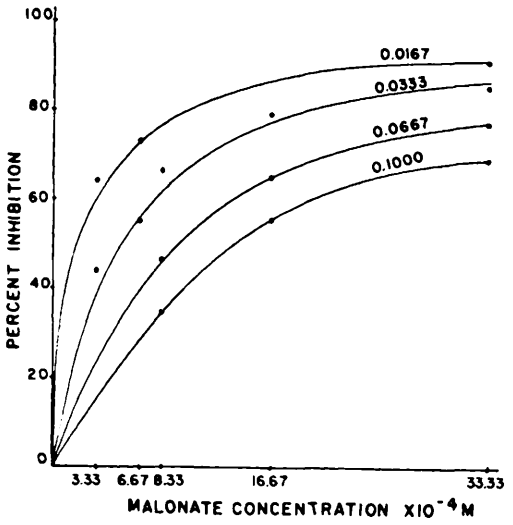

FIG. 1. % inhibition of succinoxidase plotted against malonate concentration. Final molarity of sodium succinate indicated on each curve.

kidney, and brain slices(5), rat brain homogenates(6), and rat striated muscle(7), but the mechanism or significance of these inhibitions is not clearly understood. Kalman(8) has shown that the inhibition of rat liver succinoxidase by testosterone-17β diethyl aminoethyl carbonate hydrochloride, a water-soluble compound of weak androgenic activity, might be by way of succinic dehydrogenase.

In the present report, the nature of the *in vitro* inhibition of rat liver succinoxidase by testosterone-17β diethyl aminoethyl carbonate† is further elucidated.

Materials and methods. Adult Sprague-Dawley rats were killed by decapitation and 5% liver homogenates were made up in ice-cold distilled water using an all-glass homogenizer. The reaction mixture was prepared in Warburg flasks as described by Umbreit *et al.* (9), and contained 1.0 ml of 0.1 M phosphate buffer (pH 7.4), 0.2 ml of 5% homogenate, 0.3 ml of 4×10^{-3} M $CaCl_2 \cdot 2H_2O$, 0.3 ml of 4×10^{-3} M $AlCl_3 \cdot 6H_2O$, 0.4 ml of 10^{-4} M cytochrome C (standardized colorimetrically according to the method described by Umbreit *et al.*(9)), sodium succinate in varying concentrations, inhibitors in varying concentrations, and sufficient distilled water to make 3 ml. The center wells contained 0.2 ml of 10% KOH. After introduction into the bath (37°C), the flasks were gassed with 100% O_2 for 10 minutes and manometer

† This compound was generously contributed by Dr. Ernst Oppenheimer of the Ciba Corp.

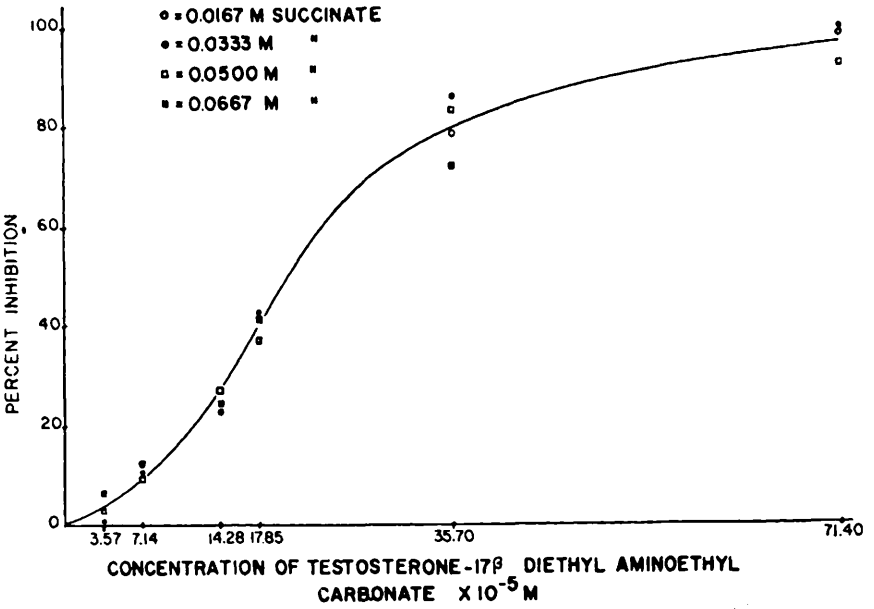

FIG. 2. % inhibition of succinoxidase plotted against concentration of testosterone-17β diethyl aminoethyl carbonate.

TABLE I. Fractional Activity of Rat Liver Succinoxidase in the Presence of Various Concentrations of Testosterone-17β Diethyl Aminoethyl Carbonate.

Final succinate conc.	v_i at indicated final molarity of testosterone-17β diethyl aminoethyl carbonate			
	3.57×10^{-5}	7.14×10^{-5}	14.28×10^{-5}	17.85×10^{-5}
.0333 M	100	89.6	77.3	57.7
.0500	97.1	89.6	72.8	62.7
.0667	93.5	87.6	75.7	57.7

v_i is expressed as % of non-inhibited succinoxidase activity.

readings were taken every 10 minutes for the next 40 minutes. The inhibitors used were sodium malonate and testosterone-17β diethyl aminoethyl carbonate. The former is a known competitive inhibitor of succinic dehydrogenase(10) and was employed in the present studies for comparison with the inhibitory effects of the androgen. The androgen was received as the hydrochloride and preliminary experiments indicated that it was sufficiently acid, even in very low concentrations, to cause extensive tissue damage. Therefore, it was titrated with dilute NaOH to a point at which it was barely soluble before addition to the flasks.

Results. In Fig. 1-2 all experiments are summarized graphically by plotting % inhibition against concentration of inhibitor at several levels of substrate. With malonate as the inhibitor (Fig. 1), the degree of inhibition is dependent upon substrate concentration, indicating competitive inhibition. In the presence of the androgen (Fig. 2), the degree of inhibition is independent of substrate concentration, suggesting non-competitive inhibition. When the 1/v:1/s plot described by Lineweaver and Burk(11) is applied to the same data, confirmation is obtained for the competitive inhibition with malonate and the non-competitive inhibition with testosterone-17β diethyl aminoethyl carbonate.

Representative data are presented in Table I and treated in Fig. 3 according to the method described by Ebersole *et al.*(12). In plotting v/v_i:(I) (v = reaction velocity in absence of inhibitor, v_i = reaction velocity in presence of inhibitor, and (I) = concentration of inhibitor) for several concentrations of substrate, competitive inhibition is demonstrated by a series of straight lines with unit intercept on the ordinate and with slope dependent upon substrate concentration. The malonate-inhibited system satisfies these conditions. Non-competitive inhibition is demonstrated when the plots form lines that coincide (Fig. 3), indicating that slope is independent of substrate concentration. The lines will not coincide if the substrate concentration is limiting.

The plots in Fig. 3 were derived from the equation, $v/v_i = 1 + (I)/K_i$, representative of a straight line. Since the graph in Fig. 3 is concave upward it will not satisfy this equation. The appropriate form then becomes $v/v_i = 1 + (I)^r/K_i$, in which r equals the number of molecules of inhibitor which combine with one molecule of enzyme(12). By plotting log $(v/v_i - 1)$:log(I), a straight line is obtained with slope equal to r(12). This procedure is carried out in Fig. 4 and it is found that r equals 2.

Discussion. While many estrogenic compounds act as enzyme inhibitors *in vitro* they do not appear to do so *in vivo*(1). It seems, therefore, that the hormones may possess chemical properties which, while readily de-

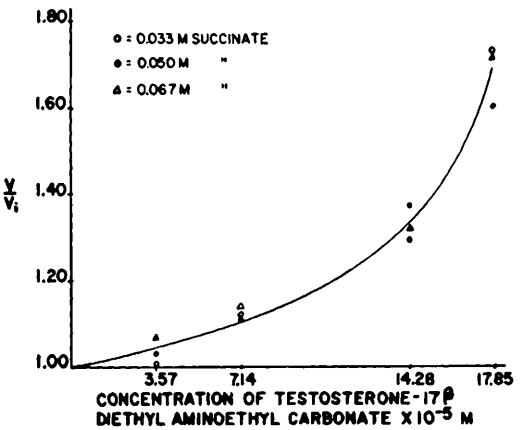

FIG. 3. Alternative plot demonstrating non-competitive inhibition of rat liver succinoxidase by testosterone-17β diethyl aminoethyl carbonate. Method described by Ebersole *et al.*(12).

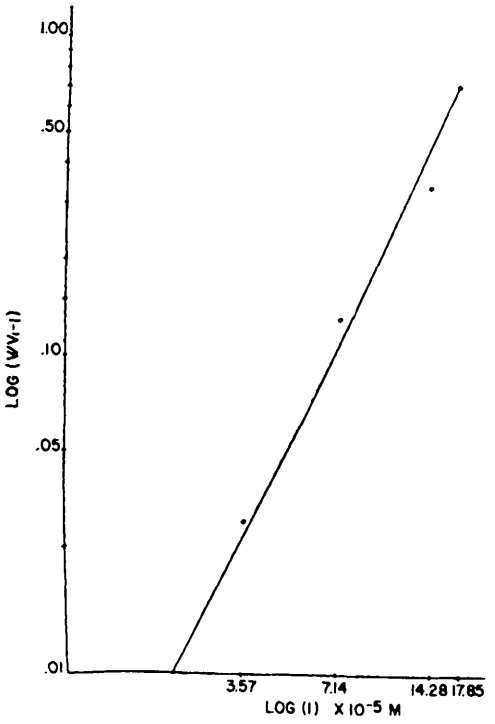

FIG. 4. Double log plot showing slope of line equal to 2. Method described by Ebersole *et al.* (12).

tectable *in vitro*, have little or nothing to do with their physiological effects when injected. The androgens have not been as thoroughly studied as the estrogens in this regard. The inhibitory property of testosterone-17β diethyl aminoethyl carbonate might reside, for the most part, in the C-17 substituted grouping, as has been previously suggested(5). In spite of this, the compound was used in the present study because of the technical advantage of its water-solubility.

Summary. It has been demonstrated that the *in vitro* inhibition of rat liver succinoxidase activity by testosterone-17β diethyl aminoethyl carbonate is non-competitive, with 2 molecules of inhibitor apparently interacting with one molecule of enzyme.

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Interference Phenomenon in Animal Infections with Rickettsiae of Rocky Mountain Spotted Fever.* (20060)

WINSTON H. PRICE.† (Introduced by Bacon F. Chow.)

From the Departments of Biochemistry and Epidemiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

The interference phenomenon has been

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