Coenzyme Q: Reversal of Inhibition of Succinate Cytochrome c Reductase by Lipophilic Compounds

Abstract. *The activity of a particulate succinate cytochrome* c *reductase is inhibited by antimycin, 2-heptyl-4-hydroxyquinoline-*N*-oxide, 2-(9-cyclohexyl-*n*-nonyl)-3-hydroxy-1,4-naphthoquinone and thenoyltrifluoroacetone. The ratio of antimycin A (required for complete inhibition) to the molar content of the cytochrome* b *of the reductase is approximately 0.5 in contrast to the reported value of 1.0 or higher for succinate oxidase preparations. However, the degree of inhibition by antimycin is dependent on the exogenous coenzyme Q (ubiquinone) present. Indeed, the inhibition from any of these compounds is competitively reversed by exogenous coenzyme Q in the system.*

Recently, we have isolated from the Keilin-Hartree preparation of heart muscle a particulate succinate cytochrome *c* reductase which mediates the electron transfer from succinate to cytochrome *c* (*1*). An interesting property of the reductase is that its ratio of coenzyme Q to cytochrome *b* is less than 0.5 whereas this ratio in the Keilin-Hartree preparation is between 6 and 10 (*1*). Likewise, the ratio of coenzyme Q to cytochrome c_1 decreased from 12 in the heart muscle preparation to approximately 0.9 in the reductase (*1, 2*). Similar results of high ratios have been reported in mitochondrial preparations of succinate oxidase or $NADH_2$ (nicotinamide-adenine dinucleotide) oxidase (*1–4*).

Thus the effect of exogenous coenzyme Q, among others, on the inhibition of the reductase by lipophilic compounds such as antimycin A, thenoyltrifluoroacetone, 2-heptyl-4-hydroxyquinoline-*N*-oxide, and 2-(9-cyclohexyl-*n*-nonyl)-3-hydroxy-1,4-naphthoquinone was tested. This report presents the finding that the inhibition from any of these compounds is competitively reversed by exogenous coenzyme Q in the system.

Table 1. The inhibitory effect of antimycin A on the reductase activity. The system contained 0.1*M* phosphate buffer, *p*H 7.4; 0.3m*M* EDTA; 20m*M* succinate; 0.1m*M* cytochrome *c* or 53μM 2,6-dichlorophenolindophenol; 0.01 percent ethanol; an appropriate amount of reductase; and antimycin A indicated. The temperature was 25°C. Ethanol at the indicated concentration did not affect the reaction rate. The mixture was incubated at 25°C for 2 minutes, and the reaction was started by the addition of succinate.

Antimycin mμmole/mg enzyme	Inhibition of reductase activity (%)	
	Cytochrome *c*	Dichlorophenolindophenol
0	0	0
0.21	5	0
0.44	47	0
0.87	97	3
2.2	100	6
4.4	100	17

Table 2. Effect of coenzyme Q on the inhibition of succinate cytochrome *c* reductase by two concentrations (mμmole per mg of protein) of antimycin A, and 2-heptyl-4-hydroxyquinoline-*N*-oxide (QNO) and two concentrations (μM) of 2-(9-cyclohexyl-*n*-nonyl)-3-hydroxy-1, 4-naphthoquinone (HNHN). The assay system was the same as that described in the legend of Table 1. The mixture with inhibitor was first incubated at 25° for 2 minutes and then coenzyme Q_2 was added. The percentage of inhibition was computed from the control which contained exactly the same amount of coenzyme Q.

CoQ (μM)	Inhibition (%)					
	Antimycin A (mμmole/mg)		QNO (mμmole/mg)		HNHN (μM)	
	0.9	5.5	3.9	287	2.5	63
0	93	100	89	100	94	98
0.048	93	100	47	98	93	98
0.48	91	99	24	98	91	98
4.8	86	97	9	86	80	98
48.0	49	77	0	50	24	96

Succinate cytochrome *c* reductase was prepared with minor modifications of the method described (*1*) up to the last ammonium sulfate fractionation (prior to the treatment at *p*H 9.5). The reductase was then dialyzed against 0.1 *M* phosphate buffer with efficient agitation for approximately 20 hours.

As shown in Table 1, the reductase in mediation of electron transfer from succinate to cytochrome *c* was sensitive to low concentrations of antimycin A; however, when 2,6-dichlorophenolindophenol was used as the acceptor, the reductase was insensitive to the inhibitor. The concentration of antimycin A required for complete inhibition was less than 1.0 mμmole per mg protein, approximately half of the molar cytochrome *b* content of the reductase (that is, the ratio of antimycin to cytochrome *b* is 0.5 for 100 percent inhibition). Since the literature (5) shows values much higher than the 0.5 ratio we obtained, the effect of exogenous coenzyme Q was tested. It was found that the degree of inhibition by antimycin was dependent on the coenzyme Q concentration (Table 2). Coenzyme Q_2 was used because it was almost impossible to obtain a homogenous suspension of Q_{10} without addition of a detergent. The results are summarized in Table 2. Of special significance is the fact that the inhibition was reversed as much as 50 percent by 48μM coenzyme Q. In the presence of 480μM coenzyme Q, 0.9 mμmole of antimycin per milligram of reductase protein showed less than 5 percent inhibition. In other words, coenzyme Q is a parameter in the titration of the reductase (activity) by antimycin; the ratio of the inhibitor to the reductase protein increased with the increase of coenzyme Q concentration. It must be mentioned that high concentrations of antimycin A still completely inhibited the enzymatic activity, even the system with excessive coenzyme Q. Here we recall vitamin E as the "antimycin factor" suggested by Nason and Lehman (*6*) who observed the reversal of antimycin inhibition by the vitamin.

However, coenzyme Q could reverse the inhibition on succinate cytochrome *c* reductase caused not only by antimycin A but also by 2-heptyl-4-hydroxyquinoline-*N*-oxide, 2-(9-cyclohexyl-*n*-nonyl)-3-hydroxy-1,4-naphthoquinone and thenoyltrifluoroacetone as shown in Tables 2 and 3. Thenoyltrifluoroacetone in contrast to other inhibitors also affected the reductase activity when 2,6-dichlorophenolindophenol

Table 3. Effect of coenzyme Q on thenoyltrifluoroacetone (TTFA) inhibition of succinate cytochrome *c* reductase measured by cytochrome *c* and 2,6-dichlorophenolindophenol (DCIP). The system was the same as that described in Table 2.

CoQ (μM)	Inhibition (%) in the presence of TTFA	
	70 μM	700 μM
	Cytochrome c	
0	82	96
0.048	61	96
0.48	53	92
4.8	39	85
48	30	76
	DCIP	
0	84	93
0.048	70	93
0.48	69	88
4.8	48	76
48	28	70

was used as the acceptor. This inhibition was also reversed by coenzyme Q.

Tappel (*7*) suggested that the inhibition by naphthoquinone derivatives appears to be related to their lipophilic nature. The results presented here may suggest that the reversal effect of coenzyme Q on the inhibition of the reductase by lipophilic compounds may be due to the lipophilicity of coenzyme Q. Recently, Green and co-workers (*4*, *8*) have considered coenzyme Q as a "floating" component of the respiratory chain. Indeed, Redfearn (*2*) and Chance (*9*) have reported that only a fraction of the coenzyme Q in mitochondrial preparations undergoes oxidation-reduction commensurate to the overall rate of electron transfer. The reversal effect of coenzyme Q on these inhibitions, in a certain sense, is in accord with the floating concept.

SHIGEKI TAKEMORI
TSOO E. KING
Oregon State University, Corvallis

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Immunological Tolerance Induced in Animals Previously Sensitized to Simple Chemical Compounds

Abstract. *Adult guinea pigs develop a delayed hypersensitivity to intradermally injected neoarsphenamine. If the animals are treated intravenously with high doses of this compound before sensitization, a specific and permanent immunological tolerance develops. Permanent tolerance is also obtained in previously sensitized animals when an intravenous injection of neoarsphenamine is followed 6 hours later by an intradermal one. When, however, the interval between the two injections is extended to 7 or 14 days, no tolerance is observed.*

In the "Sulzberger-Chase" type of tolerance as defined by Medawar (*1*) a specific and long-lasting state of unresponsiveness to simple chemicals is induced in adult guinea pigs by administering the compounds before sensitization. The experiments initiated by Frei (*2*) and Sulzberger (*3*) with neoarsphenamine have not been reproduced on a broad scale (*4*) because of the irregular responses obtained when different strains of guinea pigs or different batches of the sensitizing compound were used (*5*).

Tolerance studies with simple chemical compounds that provoke allergic contact dermatitis have therefore been pursued by "feeding" adult animals with compounds like dinitrochlorobenzene and picryl chloride before sensitization (*6*), by feeding or injecting these substances intraperitoneally to pregnant animals in order to produce tolerance in their offspring (*7*), or by intravenous injection to adult animals (*8*, *9*). But up to now the state of permanent tolerance has never been achieved if previously sensitized animals were used instead of unsensitized animals (*10*).

We have reproduced and confirmed Sulzberger's experiments and have obtained quite regularly a state of cutaneous hypersensitivity manifested by a tuberculin-type response to neoarsphenamine injected intradermally. Furthermore, we have been able to induce tolerance in untreated animals as well as in previously sensitized animals. Our experiments were performed as follows.

Sensitization of untreated animals was studied in an experiment in which 22 spotted Himalayan guinea pigs of both sexes weighing 450 to 500 g and derived from a closed colony were sensitized by intradermally injecting on the shaved flank 0.35 mg of neoarsphenamine (*11*) per kilogram of body weight. The neoarsphenamine was dissolved in 0.1 ml normal saline. This injection provoked a pale reddish papule, 5 to 7 mm in diameter, which persisted for 24 to 36 hours (primary toxic reaction). Seven to 14 days later, 14 animals spontaneously developed a "flare-up" at the site of injection characterized by a larger, strongly red, infiltrated papule, 9 to 13 mm in diameter, which persisted 4 to 8 days. Twenty-one days after the first injection, the animals received an identical dose of neoarsphenamine (test) in the other flank, and 12 to 24 hours later 18 of the 22 developed a large, strongly red, infiltrated papule, 12 to 16 mm in diameter, which persisted for 3 to 5 days leaving some desquamation (tuberculin-type reaction). Histologically, these lesions are indistinguishable from the tuberculin reaction observed in animals we infected with BCG (Bacille Calmette-Guérin) and tested with purified protein derivative, and they correspond morphologically to those described by Waksman (*12*) and Arnason and Waksman (*13*).

Subsequent intradermal injections (tests) always provoked the same tuberculin-type reactions which contrasted with the small primary toxic reactions seen in these animals after the first injection or in unsensitized control animals. This state of cutaneous delayed hypersensitivity persisted for 21 weeks, as demonstrated by subsequent tests performed first at intervals of 1 week, and then at intervals of 1 and 2 months. The hypersensitivity was further evidenced by the fact that subsequent intradermal tests provoked a generalized dermatitis in 5 out of 18 animals. Four additional groups of eight animals each were sensitized in the same manner but were tested weekly for only 2 to 3 months; these showed a similar proportion of flare-ups and sensitization.

In animals so sensitized, no contact dermatitis could be provoked by epicutaneous application of neoarsphenamine, nor could an anaphylactic shock be elicited by intravenous injection of this haptene.

Induction of tolerance in untreated animals was studied in a second series of experiments in which 60 mg of neoarsphenamine per kilogram of body weight in a 3-percent aqueous solution was injected into a vein of the hind leg of 12 animals. As in Sulzberger's experiment, these animals had received an intradermal injection of 0.35 mg of this substance per kilogram of body weight 24 hours earlier. Twenty-one