On the Mechanism of Action of Antibiotic U-19,718 in Rat Liver Mitochondria*

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ABSTRACT: Antibiotic U-19,718 inhibits respiration and phosphorylation in rat liver mitochondria to varying extents depending on the substrate. Phosphorylation is only marginally affected during glutamate or citrate oxidation but extensively impaired during succinate oxidation. An investigation of individual reaction sequences occurring within the terminal respiratory chain showed that U-19,718 causes substantial inhibition of the dehydrogenase reactions associated with the oxidation of glutamate and citrate but it stimulates the rate of electron transfer at the flavoprotein region past the ferricyanide interacting site and prior to the rotenone inhibition site during the oxidation of reduced nicotinamide–adenine dinucleotide. The reaction rate during succinocytochrome *c* reduction is stimulated or reduced depending on the antibiotic concentration present. Inhibition of cytochrome *c* reductase induced by antimycin A is reversed by U-19,718. These results indicate that the antibiotic has the capability to interact at multiple sites of the respiratory chain.

Antibiotic U-19,718, a new antimicrobial agent, was isolated from the culture broth of *Streptomyces tanashiensis* strain kala. The compound has the empirical formula $C_{16}H_{12}O_6$ (formula weight, 300.0) and crystallizes in the form of bright orange needles which have very limited solubilities in aqueous solutions. Preparation, isolation, characterization, and biological properties are described elsewhere (M. E. Bergy and L. E. Johnson, in preparation). The agent is an extremely active growth inhibitor for a variety of pathogenic fungi, yeasts, and gram-positive bacteria *in vitro*, but is ineffective in the systemic treatment of experimental fungal or bacterial infections in mice when administered parenterally at subtoxic doses. The LD_{50} (intraperitoneally) is 18 mg/kg in mice.

Investigations pertaining to the mode of action of antibiotic U-19,718 indicated that this agent is devoid of any effect on DNA, RNA, and polypeptide synthesis in cell-free *Escherichia coli* systems prepared as described by Reusser (1967). On the other hand, U-19,718 causes severe impairment of mitochondrial energy-generating processes. This paper describes the effects of antibiotic U-19,718 on the terminal respiratory chain in isolated rat liver mitochondria.

Materials and Methods

Rat liver mitochondria were isolated from mixed-sex albino rats (weighing 150–250 g) as described by Lardy and Wellman (1952). Mitochondrial protein was solubilized with deoxycholate and determined with biuret reagent (Jacobs *et al.*, 1956) using crystalline bovine albumin as standard. Mitochondrial respiration was measured manometrically (Lardy and Wellman, 1952). Inorganic phosphate was assayed by the method of Lowry and López (1946).

NADH[1]-linked reduction of ferricyanide catalyzed by rat liver mitochondria was followed spectrophotometrically by reading the decrease in optical density of the reaction mixtures at 420 mμ. Mitochondrial reduction of externally added coenzyme Q_{10} was assessed by the method described by Ramasarma and Lester (1960) and reduction of tetrazolium salts to formazans as described by Lester and Smith (1961). The two tetrazolium salts used were INT and NBT. Cytochrome *c* reductase and oxidase activities in mitochondria were measured spectrophotometrically (Mahler, 1955; Smith, 1955). Cytochrome *c* was reduced as described by Smith (1955) and had an OD_{550}:OD_{565} ratio of 12.6.

Results

Mitochondrial Oxidation of Glutamate, Citrate, β-Hydroxybutyrate, and Succinate. The effects of antibiotic U-19,718 on respiration and phosphorylation associated with the oxidation of the substrates mentioned above were assessed manometrically. During mitochondrial oxidation of glutamate or citrate respiration was inhibited significantly and reasonably strict dose–response relationships were obtained (Table I). An antibiotic concentration of 0.05 μmole/ml was necessary to cause 50% inhibition of respiration during glutamate oxidation and 0.03 μmole/ml to yield 50% inhibition of respiration during the oxidation of citrate.

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[1] Abbreviations used: NADH, reduced nicotinamide–adenine dinucleotide; NAD, oxidized nicotinamide–adenine dinucleotide; INT, 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride; NBT, 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)ditetrazolium chloride; ATP, adenosine triphosphate.

TABLE I: Effect of U-19,718 on Oxidative Phosphorylation in Rat Liver Mitochondria.[a]

Substrate	U-19,718 (μmoles/ml)	$Q_{O_2(P)}$	% Inhibn of Respiration	P:O Ratio	% Uncoupling
Glutamate	None	35.5	0	2.6	0
	0.12	11.6	67.5	1.1	57.7
	0.06	15.5	56.5	2.7	0
	0.03	22.6	36.6	2.4	7.7
	0.015	26.2	26.5	2.8	0
Citrate	None	19.5	0	2.8	9
	0.12	6.4	67.2	2.3	17.9
	0.06	7.3	62.6	2.1	25.0
	0.03	9.9	49.3	2.8	0
β-Hydroxy-butyrate	None	15.0	0	2.3	0
	0.12	12.3	18.0	2.4	0
	0.06	15.2	0	2.3	0
	0.03	16.4	0	2.2	4.4
Succinate	None	41.3	0	1.5	0
	0.12	24.9	39.8	0.3	80.0
	0.06	23.4	43.3	0.4	73.4
	0.03	23.1	44.1	0	100.0
	0.015	31.4	24.0	0.9	40.0

[a]Each vessel contained in a total volume of 3 ml: 6 μmoles of ATP, 50 μmoles of K-PO_4 buffer (pH 7.4), 15 μmoles of $MgSO_4$, 30 μmoles of sodium L-glutamate, 20 μmoles of sodium succinate, 40 μmoles of sodium β-hydroxybutyrate or 30 μmoles of sodium citrate, respectively, and 0.5 ml of mitochondrial suspension (16 mg of protein/ml) in 0.25 M sucrose. Hexokinase (20 μg) and 50 μmoles of glucose were added from the side arms. Reactions were run for 20 min at 30°. $Q_{O_2(P)}$ = microliters of O_2 uptake per milligram of mitochondrial protein per hour.

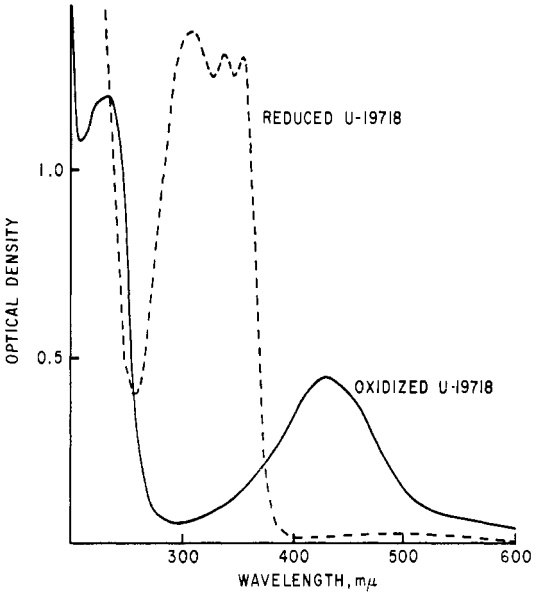

FIGURE 1: Absorption spectra of the oxidized and reduced forms of antibiotic U-19,718. U-19,718 was dissolved in 0.05 M Tris-HCl buffer (pH 7.4) to yield a final concentration of 0.1 μmole/ml. Reduction was done in the cuvet by adding a few crystals of solid $Na_2S_2O_4$. Both spectra were read against a buffer blank.

Phosphorylation was affected less significantly with both these substrates. An antibiotic concentration of 0.12 μmole/ml uncoupled to an extent of 58% during glutamate oxidation while lower drug concentrations induced only small degrees of uncoupling. Similarly phosphorylation associated with the oxidation of citrate was reduced by approximately 20% in the presence of 0.12 or 0.06 μmole per ml of U-19,718. An antibiotic concentration of 0.03 μmole/ml did not interfere with phosphorylation.

During the oxidation of β-hydroxybutyrate respiration was inhibited to an extent of 18% by 0.12 μmole/ml of antibiotic. Lower antibiotic concentrations had no effect on respiration. Phosphorylation remained essentially unaffected at all antibiotic concentrations tested.

Mitochondrial respiration associated with the oxidation of succinate was inhibited but to a smaller extent than during glutamate or citrate oxidation and no strict dose–response relationship was apparent when the antibiotic was tested over a concentration range from 0.03 to 0.12 μmole/ml. Uncoupling (80–100%) of oxidative phosphorylation occurred in the presence of these same antibiotic concentrations. Phosphorylation was, therefore, more significantly inhibited during succinate oxidation than during the oxidation of NAD-linked substrates. The effect of U-19,718 on mitochondrial respiration associated with the oxidation of

TABLE II: Effect of U-19,718 on Mitochondrial NADH Oxidation.[a]

Expt	U-19,718 (μmoles/ml)	$Q_{O_2(P)}$	% of Control
1	Control	44.2	100
	0.10	46.2	104
	0.05	50.0	113
2	Control	38.4	100
	0.10	34.4	90
	0.05	35.2	92

[a] Each vessel contained in a total volume of 3 ml: 6 μmoles of ATP, 50 μmoles of K-PO_4 buffer (pH 7.4), 15 μmoles of $MgSO_4$, 20 μmoles of NADH, and 0.5 ml of mitochondrial suspension in 0.25 M sucrose containing 15.4 mg of protein/ml in expt 1 and 21.0 mg of protein/ml in expt 2. Hexokinase (20 μg) and 50 μmoles of glucose were added from the side arms. Reactions were run for 15 min at 30°. $Q_{O_2(P)}$ = microliters of O_2 uptake per milligram of mitochondrial protein per hour.

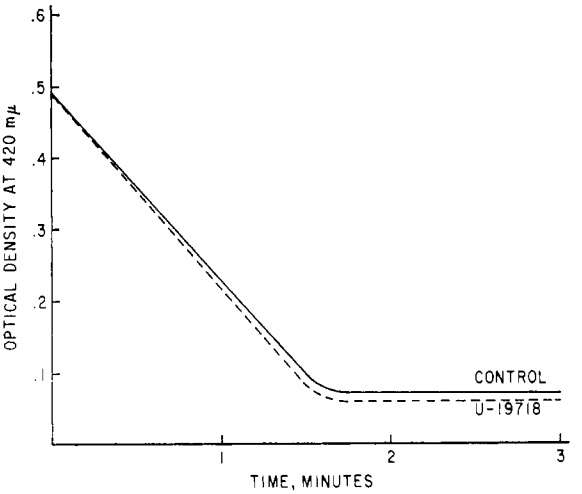

FIGURE 2: NADH-linked reduction of ferricyanide by rat liver mitochondria. The reaction mixtures contained in a total volume of 3 ml: 150 μmoles of Tris-HCl buffer (pH 7.4), 600 μmoles of sucrose, 0.3 μmole of U-19,718, 3 μmoles of KCN, 1.5 μmoles of $KFe(CN)_6$, 5 μmoles of NADH, and 0.77 mg of mitochondrial protein. The reaction was started by adding NADH. The optical density was read at 420 mμ against a blank cuvet containing all the reaction mixture components except NADH.

NADH was also investigated. The results indicate that a slight stimulation of respiration was evident in the presence of antibiotic U-19,718 in expt 1 and a slight inhibition in expt 2 (Table II). Thus, NADH oxidation was not significantly affected by U-19,718 when the reaction was assessed by the uptake of O_2. In a medium deficient in inorganic phosphate, mitochondrial glutamate oxidation was inhibited by U-19,718 (Table III). This inhibition was not reversed by dinitrophenol.

TABLE III: Effect of U-19,718 on Mitochondrial Glutamate Oxidation in a P_i-Deficient Medium.[a]

	$Q_{O_2(P)}$	% of Control
None, control	8.1	100.0
U-19,718, 0.06 μmole/ml	4.8	59.2
DNP	16.3	201.2
DNP + U-19,718, 0.06 μmole/ml	4.3	53.0
DNP + U-19,718, 0.03 μmole/ml	6.7	82.7

[a] Each vessel contained in a total volume of 3 ml: 6 μmoles of ATP, 60 μmoles of Tris-HCl buffer (pH 7.4), 15 μmoles of $MgSO_4$, 30 μmoles of sodium glutamate, 0.45 μmole of dinitrophenol (DNP) when applicable, and 0.5 ml of mitochondrial suspension (~15 mg of protein/ml) in 0.25 M sucrose. Hexokinase (20 μg) and 50 μmoles of glucose were added from the side arms. Reactions were run for 60 min at 30°. $Q_{O_2(P)}$ = microliters of O_2 uptake per milligram of mitochondrial protein per hour.

Reduction of Antibiotic U-19,718 by Reducing Agents. Antibiotic U-19,718 can undergo oxidoreduction reactions. The absorption spectra of the oxidized and reduced forms (reduced with solid $Na_2S_2O_4$) are given in Figure 1. The oxidized form of U-19,718 shows maxima at approximately 265 and 430 mμ. Upon reduction with $Na_2S_2O_4$ the yellow U-19,718 solution bleaches to almost colorless (loss of absorptivity at 430 mμ) and a new three-fingered maximum in the range from 320 to 360 mμ emerges. A visual change can be observed during the reduction of U-19,718 in solution when very limited amounts of solid $Na_2S_2O_4$ or KBH_4 are added. A red color (absorption maximum at 530 mμ) appears before the yellow color is bleached in a manner similar as described for coenzyme Q_{10} (Lester *et al.*, 1959). These observations suggest that some transient states are assumed by the molecule during reduction. The reduced form of the antibiotic is subject to autoxidation and is only stable in the presence of excess reducing agent. In order to study further the mode of action of this antibiotic, individual oxidoreduction reactions occurring within the respiratory chain were investigated in detail in whole rat liver mitochondria.

Reduction of Ferricyanide by NADH. Ferricyanide accepts electrons from the flavoprotein during the oxidation of NADH in mitochondria without the participation of coenzyme Q or cytochromes (Yielding *et al.*, 1960). In the presence of 0.1 μmole/ml of antibiotic U-19,718, NADH oxidation proceeded at a similar rate

TABLE IV: Effect of U-19,718 on NADH–Tetrazolium Salt Reductase in Mitochondria.[a]

Sample	Reaction Time (min)	ΔOD_{490} or ΔOD_{530}	% of Control
NADH + NBT, control	2	0.163	100
NADH + NBT + U-19,718 (0.12 μmole/ml)	2	0.585	360
NADH + NBT + coenzyme Q (0.2 mg/ml)	2	0.177	108
NADH + NBT + coenzyme Q + U-19,718	2	0.595	364
NADH + INT, control	2	0.523	100
NADH + INT + U-19,718 (0.12 μmole/ml)	2	1.140	218
NADH + INT + coenzyme Q (0.2 mg/ml)	2	0.571	109
NADH + INT + coenzyme Q + U-19,718	2	1.210	230
NADH + NBT, control	5	0.314	100
NADH + NBT + U-19,718 (0.1 μmole/ml)	5	0.677	216
NADH + NBT + rotenone (0.2 μmole/ml)	5	0.267	85
NADH + NBT + rotenone + U-19,718	5	0.644	205
NADH + INT, control	2	1.02	100
NADH + INT + U-19,718 (0.1 μmole/ml)	2	1.61	158
NADH + INT + rotenone (0.4 μmole/ml)	2	0.845	83
NADH + INT + rotenone + U-19,718	2	1.60	157

[a] The reaction mixtures contained in a total volume of 1 ml: 40 μmoles of Tris-HCl buffer (pH 7.5), 1 μmole of EDTA, 3 μmoles of tetrazolium salt, 1 μmole of KCN, 3 μmoles of NADH, and ~0.5 mg of mitochondrial protein. The reactions were run at 30° and stopped by adding 4.7 ml of Triton–formate buffer as described by Lester and Smith (1961). The optical densities of the samples containing INT were read at 490 mμ, those containing NBT were read at 530 mμ.

as in the absence of antibiotic (Figure 2). Therefore, U-19,718 does not seem to interfere with the oxidation of NADH coupled to ferricyanide.

NADH or Succinate–Coenzyme Q Reduction. The absorption spectra of the reduced and oxidized forms of U-19,718 indicate that this agent might interfere with the assay for reduced coenzyme Q. This assumption proved to be correct. The results showed a high degree of variability in the presence of antibiotic and they are therefore only mentioned briefly. The reduction of externally added coenzyme Q was generally stimulated in the presence of antibiotic U-19,718 when NADH was used as the reducing agent. The extent of stimulation varied between zero and sevenfold in several experiments. Succinate-linked coenzyme Q reduction was usually inhibited but the same high degree of variability pertained between experiments as mentioned for the NADH-linked reaction.

Reduction of Tetrazolium Salts. The somewhat ambiguous results obtained with the coenzyme Q reduction reactions were verified *via* the reduction of tetrazolium salts. The reduction of the salts was shown to take place at the flavoprotein site when NADH serves as an electron donor and after the coenzyme Q reduction site when succinate functions as an electron donor (Lester and Smith, 1961).

Antibiotic U-19,718 stimulated NADH-linked tetrazolium salt reduction significantly and reproducibly with both INT or NBT (Table IV). These reactions were independent of coenzyme Q. Rotenone inhibits the reoxidation of NADH by the flavoproteins. Under our experimental conditions NADH-linked reduction of tetrazolium salts was only partially but reproducibly inhibited by rotenone (Table IV). The rotenone-induced inhibition of these reactions was reversed by U-19,718 and the extents of stimulation caused by the antibiotic as compared to the control samples were of the same magnitude regardless of the presence of rotenone.

The succinate-linked reduction of tetrazolium salts was coenzyme Q dependent. This dependence was more pronounced with NBT than with INT (Table V). In the absence of coenzyme Q, succinate-driven NBT reduction was slightly stimulated by antibiotic concentrations of 0.2 and 0.1 μmole per ml. An antibiotic concentration of 0.01 μmole/ml might cause some inhibition of this reaction. In the presence of coenzyme Q, NBT reduction was slightly inhibited by the antibiotic at all concentrations tested (0.2, 0.1, and 0.01 μmole per ml). In the absence of coenzyme Q, INT reduction was slightly stimulated by the antibiotic. In the presence of coenzyme Q succinate–INT reductase remained unaffected by the antibiotic at an antibiotic concentration of 0.2 μmole/ml and was slightly inhibited at 0.1 μmole/ml and possibly somewhat stimulated at 0.01 μmole/ml.

TABLE V: Effect of Antibiotic U-19,718 on Succinate-Tetrazolium Salt Reductase in Mitochondria.[a]

Sample	ΔOD_{490} or ΔOD_{530}	% of Control
Succinate + INT, control	0.246	100
Succinate + INT + U-19,718 (0.2 μmole/ml)	0.313	127
Succinate + INT + U-19,718 (0.1 μmole/ml)	0.326	132
Succinate + INT + U-19,718 (0.01 μmole/ml)	0.271	110
Succinate + INT, control	0.252	100
Succinate + INT + coenzyme Q (0.2 mg/ml)	0.329	130
Succinate + INT + coenzyme Q + U-19,718 (0.2 μmole/ml)	0.329	130
Succinate + INT + coenzyme Q + U-19,718 (0.1 μmole/ml)	0.209	83
Succinate + INT + coenzyme Q + U-19,718 (0.01 μmole/ml)	0.400	158
Succinate + NBT, control	0.180	100
Succinate + NBT + U-19,718 (0.2 μmole/ml)	0.216	120
Succinate + NBT + U-19,718 (0.1 μmole/ml)	0.216	120
Succinate + NBT + U-19,718 (0.01 μmole/ml)	0.167	93
Succinate + NBT, control	0.108	100
Succinate + NBT + coenzyme Q (0.2 mg/ml)	0.233	216
Succinate + NBT + coenzyme Q + U-19,718 (0.2 μmole/ml)	0.204	189
Succinate + NBT + coenzyme Q + U-19,718 (0.1 μmole/ml)	0.158	146
Succinate + NBT + coenzyme Q + U-19,718 (0.01 μmole/ml)	0.182	168

[a] The experimental details were as described under Table IV except that 5 μmoles of sodium succinate was added/ml of sample instead of NADH. The reaction time was 5 min.

Cytochrome c Reductase. The NADH-linked reduction of cytochrome *c* was stimulated in the presence of U-19,718 (Figure 3). Antimycin inhibited this reaction only moderately in our system. The antimycin-induced inhibition was abolished in the presence of antibiotic U-19,718 and the extent of stimulation was identical with the one observed in the absence of antimycin. Lower concentrations of U-19,718 than the ones used in the experiments shown in Figure 3 caused proportionally less stimulation of cytochrome *c* reduction but no inhibition was apparent as observed during succinate-linked cytochrome *c* reduction (see below).

The succinate-linked reduction of cytochrome *c* was

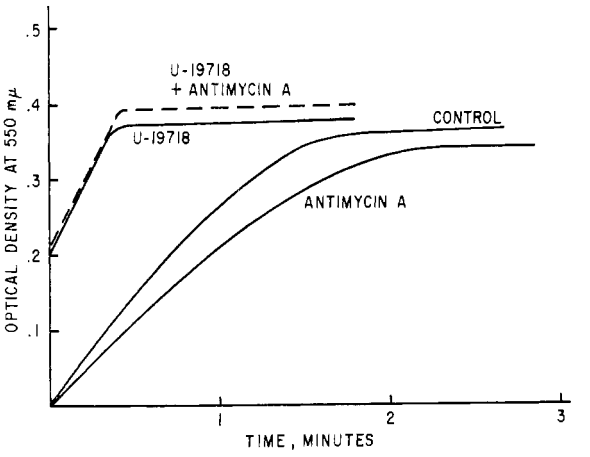

FIGURE 3: NADH-linked reduction of cytochrome c by rat liver mitochondria. The reaction mixtures contained in a total volume of 3 ml: 150 μmoles of Tris-HCl (pH 7.4), 575 μmoles of sucrose, 0.3 μmole of antibiotic U-19,718, 15 μg of antimycin A, 3 μmoles of KCN, 1.5 mg of cytochrome *c* (type III, Sigma), 15 μmoles of NADH, and 0.23 mg of mitochondrial protein. The reactions were started by the addition of NADH. The blanks contained all the reaction mixture components above except NADH.

stimulated in the presence of high concentrations of antibiotic U-19,718 (0.2 and 0.15 μmole per ml) and inhibited at low antibiotic concentrations (0.1 and 0.05 μmole per ml) (Figure 4). Succinocytochrome *c* reductase was almost completely blocked by antimycin (Figure 5).

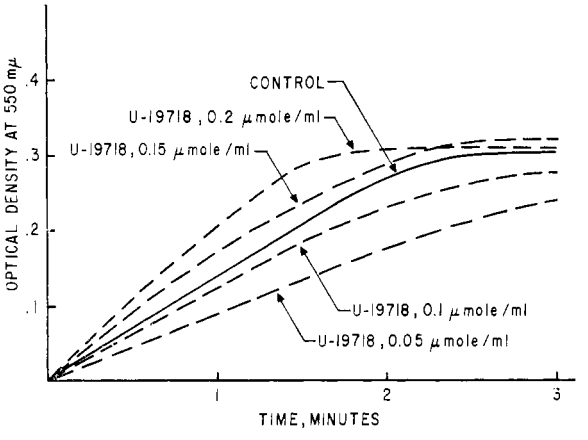

FIGURE 4: Succinate-linked reduction of cytochrome *c* by rat liver mitochondria. The reaction mixtures contained in a total volume of 3 ml: 150 μmoles of Tris-HCl (pH 7.4), 600 μmoles of sucrose, 3 μmoles of KCN, 1.5 mg of cytochrome *c*, 50 μmoles of succinate, and 0.9 mg to mitochondrial protein. The reactions were started by the addition of succinate. The reaction mixtures were read against blank cuvets containing all the reaction mixture components except substrate.

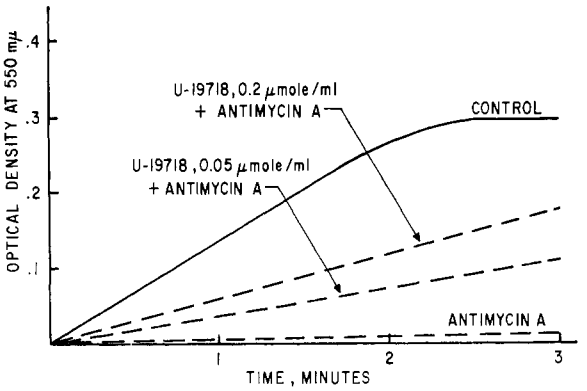

FIGURE 5: Experimental details were identical with those described under Figure 5. Antimycin A was added to yield a concentration of 20 μg/ml.

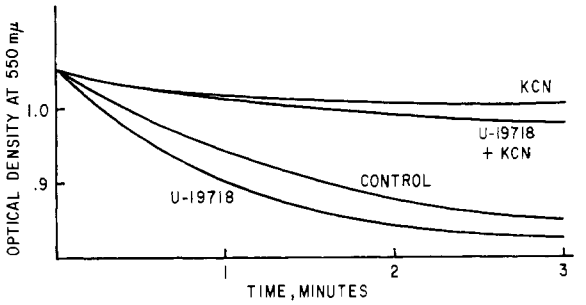

FIGURE 6: Oxidation of reduced cytochrome *c* by rat liver mitochondria. The reaction mixtures contained in a total volume of 3 ml: 150 μmoles of Tris-HCl (pH 7.4), 550 μmoles of sucrose, 0.3 μmole of antibiotic U-19,718, 0.5 mg of reduced cytochrome *c*, 3 μmoles of KCN, and 0.4 mg of mitochondrial protein. The reactions were started by the addition of mitochondria. The reaction mixtures were read against blank cuvets containing all the reaction mixture components except mitochondria.

Antibiotic U-19,718 caused substantial reversal of the antimycin inhibition. This reversion was directly proportional to the antibiotic concentration and was also functional at drug concentrations which proved inhibitory to the system in the absence of antimycin.

Cytochrome c Oxidase. In the absence of KCN, this reaction was very moderately stimulated in the presence of antibiotic U-19,718 (Figure 6). The reaction was blocked in the presence of 0.001 M KCN but slightly reversed by U-19,718.

Discussion

Inhibition of respiration during glutamate oxidation in rat liver mitochondria by antibiotic U-19,718 occurred under both phosphorylating and nonphosphorylating conditions. Substantial inhibition of respiration was also apparent during citrate oxidation but only marginal inhibition occurred during the oxidation of β-hydroxybutyrate. Respiration remained unaffected during NADH oxidation. These results indicate that antibiotic U-19,718 severely inhibits the glutamate- and citrate-linked reduction of NAD (Figure 7). Oxidation of β-hydroxybutyrate, which does not proceed *via* the Krebs cycle, remains practically unaffected by the drug. Uncoupling of oxidative phosphorylation during glutamate and citrate oxidation was less severely inhibited than during succinate oxidation (see below) and no uncoupling occurred during the oxidation of β-hydroxybutyrate.

Although U-19,718 inhibited respiration during mitochondrial succinate oxidation no distinct dose–response relationship was apparent. The uncoupling of oxidative phosphorylation was more severely affected than was respiration. This relative specific uncoupling effect exhibited by the antibiotic during succinate oxidation cannot be explained at this time.

Mitochondrial NADH–ferricyanide reduction remained unaffected by U-19,718. NADH-linked reduction of tetrazolium salts and NADH–cytochrome *c* reduction were considerably stimulated in the presence of antibiotic U-19,718. Rotenone-induced partial inhibition of NADH-linked cytrochrome *c* reduction was completely abolished by antibiotic U-19,718. In these two reactions, the extent of stimulation induced by antibiotic U-19,718 remained unchanged regardless of the presence of rotenone or antimycin. These findings provide strong evidence that antibiotic U-19,718 stimulated electron transfer at the NADH–flavoprotein oxidoreductase site past the ferricyanide region and prior to the rotenone-sensitive site of the respiratory chain (Figure 7). The observation that U-19,718 *per se* undergoes oxidoreduction in the presence of reducing agents suggests that the antibiotic might divert electrons from the respiratory chain at the flavoprotein level. These electrons are then fed back into the chain at one or possibly several sites past the flavoprotein site and might account for the bypass of the antimycin-sensitive region located at the reduced site of cytochrome *b* (Lee *et al.*, 1965).

In the absence of externally added coenzyme Q, the succinate-driven tetrazolium salt reductase was generally slightly stimulated by U-19,718. In the presence of coenzyme Q the antibiotic did not interfere with the reduction of INT and caused only insignificant inhibition of NBT reduction. The succinate-linked cytochrome *c* reductase was stimulated by high antibiotic concentrations (*e.g.*, 0.2 μmole/ml) and inhibited at low concentrations (*e.g.*, 0.05 μmole/ml) but was never blocked completely. The antimycin-induced inhibition of succinocytochrome *c* reductase was partially reversed even at U-19,718 concentrations which would cause inhibition of this reaction in the absence of antimycin. These observations provide strong evidence that antibiotic U-19,718 interferes with the transport of electrons somewhere after coenzyme Q and before the antimycin-sensitive site when the terminal respiratory chain is driven by succinate (Figure 7). The concen-

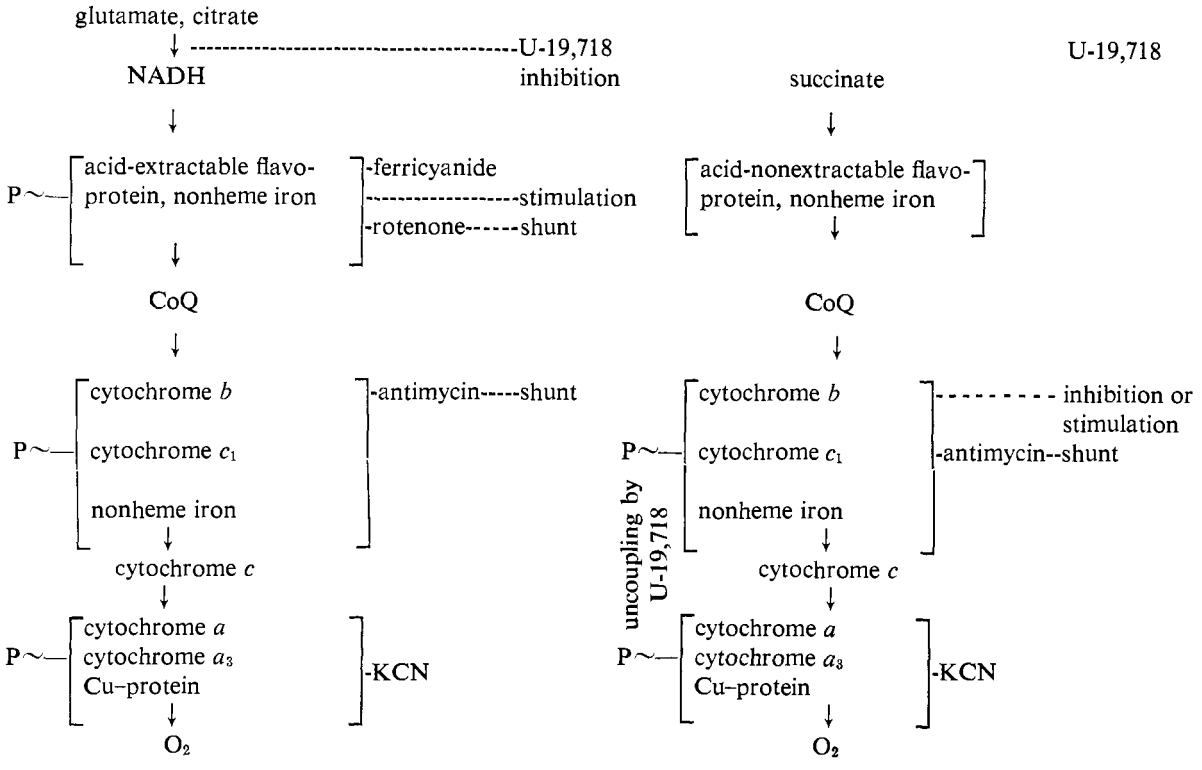

FIGURE 7: Sites of interaction of antibiotic U-19,718 in the terminal respiratory chain in rat liver mitochondria.

tration-dependent stimulation–inhibition effect of antibiotic U-19,718 on succinocytochrome *c* reductase might be explained by postulating that the antibiotic changes the rates of one or several enzymatic steps occurring in the cytochrome *b*,*c* region of the terminal respiratory chain.

The primary sites of inhibition causing severe impairment of the energy-generating processes by U-19,718 in mitochondria are located at the dehydrogenase regions associated with the oxidation of glutamate or citrate (and probably most Krebs cycle intermediates) and at the coupling sites of respiration-linked phosphorylation during succinate oxidation. Stimulation of electron flow at the NADH–flavoprotein reduction site was observed on isolated segments of the respiratory chain. Considering that the glutamate and citrate dehydrogenase functions are located proximal from the flavoprotein region in mitochondria, one can assume that the stimulatory effect observed at the flavoprotein site is minute or absent and of a secondary nature within the fully functioning respiratory chain due to the primary curtailment of electron flow at the dehydrogenase sites. The ultimate significance of the concencentration–dependent stimulation–inhibition effect of U-19,718 observed during succinate oxidation which was shown to take place prior to the antimycin-sensitive site is difficult to assess. This phenomenon is probably interrelated with the substantial uncoupling effect induced by U-19,718 during succinate oxidation and the concurrent, somewhat less severe impairment of respiration.

Acknowledgments

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