on the mating competitiveness of male *C. fatigans*. In a cross where 180 sterilized and normal males in a ratio of 1 : 2 were caged with 180 females, 150 egg rafts were obtained. Of these 57 were sterilized rafts as against an expected number of 50 such rafts.

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Studies on Piericidin. I. Effects of Piericidin A and B on Mitochondrial Electron Transport in Insect Muscle Comparing with Rotenone. Takashi Mitsui*, Jun-ichi Fukami*, Kazuo Fukunaga*, Takao Sagawa**, Nobutaka Takahashi*** and Saburo Tamura*** (* The Institute of Physical and Chemical Research, Saitama. ** Research Laboratories of Chugai Pharmaceutical Co., Ltd. Tokyo. *** Department of Agricultural Chemistry, The University of Tokyo, Tokyo) Received June 23, 1969. *Botyu-Kagaku*, **34**, 126, 1969.

18. ピエリシジンに関する研究 I. ピエリシジンAおよびBのミトコンドリアの電子伝達系に及ぼす影響 満井 喬*, 深見順一*, 福永一夫*, 佐川隆夫**, 高橋信孝***, 田村三郎*** (* 理化学研究所 ** 中外製薬株式会社, 綜合研究所 *** 東京大学農学部) 44. 6. 23 受理

ピエリシジンAおよびBのワモンゴキブリ筋肉ミトコンドリアの電子伝達系に及ぼす影響について，ロテノンと比較検討した．ピエリシジンAおよびBは，NADH 酸化酵素系ではロテノンとほぼ同濃度で呼吸を阻害する．また，その作用性からみて，ロテノンとほぼ同一部位を阻害するものと考えられる．コハク酸酸化酵素系に対しては高濃度で弱い阻害がみられる．また酸化的リン酸化に対しても阻害が認められる．

NADH 酸化酵素系において，ビタミン K_3 による by-pass はロテノンと同様にラット肝臓ミトコンドリアでは認められるが，ワモンゴキブリでは，ほとんど認められない．この by-pass に関与する DT-diaphorase 活性は，ラット肝臓に比してワモンゴキブリでは約1/4に過ぎなかった．

Piericidin A and B have been isolated from *Streptomyces mobaraensis* and their chemical structures were elucidated by Tamura, Takahashi *et al.*[1,2] These compounds were found to have insecticidal activity to certain insects.

Hall *et al.* (1966)[3] found that Piericidin A was a powerful inhibitor of mitochondrial electron transport in beef heart mitochondria and they concluded that insecticidal activity of Piericidin A might be based on its inhibition of mitochondrial function.

The effect of Piericidin A to the aerobic oxidation of substrates linked to pyridine nucleotides was as sensitive as previously described with rotenone.[4~8] In contrast with rotenone, however, Piericidin also inhibited succinoxidation system at considerably higher concentration. The fact that Piericidin resemble Co Q in the chemical structure and respiration was restored in succinoxidation system inhibited by Piericidin A by adding Co Q in this inhibited system suggested Piericidin A act as a competitive inhibitor of Co Q.

More recently, Miji *et al.* (1968)[9,10] further

discussed in detail the activity of Piericidin A and its related compounds to electron transport of beef heart mitochondria.

We previously reported insecticidal activity of Piericidin A and B and concluded these compounds had wide spectrum of insecticidal and miticidal activities, especially their insecticidal and miticidal activities to peach aphid and carmine mite were as excellent as rotenone and the other miticides.[11] In this paper, inhibitory effects of Piericidin A and B on electron transport of insect mitochondria, american cockroach muscle mitochondria, were discussed comparing with their inhibitory effects on rat liver mitochondrial electron transport system.

Experimental Procedures

Preparation of mitochondria

Male adults of american cockroach (*Periplaneta americana* L.) were used. Cockroach muscle mitochondria was prepared by following the method of Fukami (1961).[6]

Rat liver mitochondria was prepared with 0.25M sucrose by a modified method of Ernster and Löw (1955).[12]

Preparation of cytoplasmic fractions for DT-diaphorase activity

Cytoplasmic fractions, debris and nuclei, mitochondrial, microsomal and soluble fractions from rat liver and cockroach (whole body, muscle and mid gut) were prepared as described by Ernster and Navazio (1958)[13] by centrifugation a) at 1,600 g for 10min. b) at 10,000 g for 10 min. c) at 105,000 g for 60 min. reffering to the pellets obtained in a, b and c, and the supernatant obtained in c, respectively. Mitochondrial and microsomal fractions were washed with 0.25 M sucrose and resuspended in 0.05M phosphate buffer containing 0.25M sucrose. In the case that whole body and mid gut of american cockroach were used in this experiment, male adults were isolated and fed on glucose solution for 10 days before experiment.

Assay procedures

Respiration was measured by "Warburg method" with air as gas phase and 0.2cc of 20% KOH solution in the center well. Phosphate uptake was determined by measuring inorganic phosphate in medium before and after incubation by following the method of Lowry-Lopez (1946).[14] The composition of the medium for respiration and phosphorylation was as follows; 2.5 mM ATP, 15 mM phosphate buffer (pH 7.4), 7.5mM $MgCl_2$, 5~15mM substrate, 25mM glucose, 30mM KCl, hexokinase in excess and 0.5cc of mito chondrial suspension containing 6.8 mg to 7.2 mg of protein were added to each vessel.

Protein determination

Protein determination was made by the biuret method.[15] In the case that the protein content was less 1 mg/cc, Lowry method (1951)[16] was also adapted.

NADH oxidation

NADH oxidase activity was spectrophotometrically determined by measuring the oxidation of NADH at 340 mμ following the method of Slater (1950)[17] at 22°C. The reagent concentrations were as follows; 33 mM phosphate buffer (pH 7.4), 0.1 mM NADH, and an amount of mitochondria containing 3.4 mg of protein in total volume of 3 cc.

NADH-cytochrome c reductase activity was determined by following the reduction of cytochrome c at 550 mμ in a HITACHI EPU-2 Spectrophotometer at 30°C. The assay system contained 50mM phosphate buffer (pH 7.4), 0.1 mM NADH, 15μg of cytochrome c, 10^{-3}M KCN, and an amount of mitochondria containing 1.36 mg of protein. Total volume of 4 cc.

Absorption spectrum of cytochromes in mitochondrial suspension

Spectrum of cytochromes in cockroach muscle mitochondrial suspension was recorded by the opal-glass transmission method with Cary Recording Spectrophotometer Model 14.[18]

DT-diaphorase activity

This assay was based on the spectrophotometric measurement of a change in absorbance at 600 mμ (the decolorization of the dye, 2,6-dichlorophenol-indophenol) at 25°C by following the method of Ernster and Navazio.[13] The test system contained 0.1 mM NADH or NADPH, 0.04 mM 2,6-dichlorophenol-indophenol (DCPIP), 50 mM phosphate buffer (pH 7.4) and 0.1 to 0.4cc of the fraction to be tested. Final volume of 4cc. In the case of mitochondrial fraction, 0.4 mM

KCN was added to this test system.

Results

Oxidation of pyridine nucleotide-linked substrates

Piericidin A and B were found to strongly inhibit the respiration of cockroach muscle mitochondria with α-ketoglutarate as substrate at the order of 10^{-7}M as shown in Table 1.

I_{50} values (concentration at 50% inhibition) of Piericidin A, B and rotenone were 3.2×10^{-7}M,

Table 1. Effects of Piericidin A and B on respiration and phosphorylation of cockroach muscle mitochondria comparing with rotenone.

Compound	Concentration 10^{-7}M	Respiration O-μ atom	Respiration Inhibition (%)	Phosphorylation P-μ mol	Phosphorylation P/O
Piericidin A	0	8.76	—	23.88	2.73
	1.0	9.56	−9.1	26.22	2.74
	2.9	7.67	12.4	20.64	2.69
	3.2	3.89	56.3	7.46	1.92
	3.4	2.08	76.3	2.07	1.00
	4.3	0.89	89.8	0.30	
	4.8	0.70	92.0	0.31	
	5.3	0.47	94.6	0	
Piericidin B	0	8.55	—	22.76	2.66
	1.0	9.56	−11.8	22.27	2.33
	1.9	8.66	−1.3	19.46	2.25
	2.8	3.68	57.0	5.05	1.37
	3.7	2.06	75.9	3.71	
	5.6	1.05	87.7	0.99	
	7.4	0.75	91.2	0	
Rotenone	0	8.90	—	23.75	2.67
	1.0	9.44	−6.1	21.35	2.26
	1.3	7.63	14.3	15.75	2.06
	1.5	5.12	42.5	9.61	1.88
	1.7	2.01	77.4	1.65	

Each vessel contained 5mM α-ketoglutarate, 15mM phosphate (pH 7.4), 7.5mM $MgCl_2$, 2.5 mM ATP, 30 mM KCl, 25 mM glucose, 150 units of yeast hexokinase and 0.5 cc of mitochondrial suspension containing 6.8 mg of protein. Final volume, 2 cc. Incubation took place at 30°C for 20 minutes.

Table 2. Effects of Piericidin A and B on respiration and phosphorylation of rat liver mitochondria comparing with rotenone.

Compound	Concentration 10^{-7}M	Respiration O-μ atom	Respiration Inhibition (%)	Phosphorylation P-μ mol	Phosphorylation P/O
Piericidin A	0	8.62	—	24.11	2.80
	1.0	6.89	20.1	16.60	2.41
	1.2	4.56	47.1	10.47	2.30
	1.3	2.63	69.5	4.46	1.70
	1.5	1.97	77.1	4.60	
Piericidin B	0	8.46	—	20.49	2.42
	0.9	5.68	32.9	11.00	1.94
	1.1	4.26	49.6	6.99	1.64
	1.3	4.00	52.7	8.48	2.12
	1.5	3.29	61.1	3.39	
Rotenone	0	8.46	—	22.93	2.71
	1.0	6.00	29.1	14.04	2.34
	1.2	5.71	32.5	14.50	2.54
	1.4	5.84	31.0	14.07	2.41
	1.6	4.58	45.9	13.10	2.86

Test system was same as in Table 1. Each vessel contained 0.5 cc of mitochondrial suspension containing 7.2 mg of protein.

Table 3. Effects of Piericidin A and B on respiration of cockroach muscle mitochondria with glutamate, pyruvate and malate as substrate.

Substrate	Additions	Inhibitor	Respiration O-μ atom	Respiration Inhibition (%)
Glutamate	—	—	7.31	—
	—	Piericidin A	1.19	83.7
	—	Piericidin B	2.16	70.5
Pyruvate+Malate	—	—	7.55	—
	—	Piericidin A	1.43	81.1
	—	Piericidin B	1.47	80.5
α-Ketoglutarate	—	—	6.71	—
	—	Piericidin A	0.97	85.5
	NAD, Cytochrome c	Piericidin A	1.18	82.4

Each vessel contained 5 mM glutamate, 5 mM pyruvate+5 mM malate or 5mM α-ketoglutarate. When indicated, 5×10^{-7}M inhibitor, 5 mM NAD and 1.0×10^{-5}M cytochrome c were added. The other test method was same as in Table 1.

2.8×10^{-7}M and 1.5×10^{-7}M, respectively. They were also active to the respiration of rat liver mitochondria at approximately same concentration as above (Table 2).

Piericidin also inhibited the oxidation of other pyridine nucleotide-linked substrates, such as glutamate, pyruvate and malate. In this case, inhibitory effect of Piericidin was only to a low extent relieved by adding NAD and cytochrome c in the system as was found previously with Amytal (Ernster 1956),[19] as shown in Table 3.

Effects of Piericidin A on NADH oxidation

The effect of Piericidin A to NADH oxidase from american cockroach muscle mitochondria was shown in Table 4 as its activity (μ mol of NADH oxidized/min/g protein) and per cent inhibition of initial activity; 10^{-7}M of Piericidin A showed complete inhibition of NADH oxidase activity. NADH-cytochrome c reductase from cockroach muscle mitochondria was also inhibited by Piericidin A. Fig. 1 showed the change of absorption of reduced cytochrome c with and without 10^{-6} and 10^{-7}M of Piericidin A; 10^{-6} and

Table 4. Inhibition of NADH oxidase activity in cockroach muscle mitochondria by Piericidin A.

Concentration of inhibitor (10^{-8}M)	Activity	Per cent inhibition of initial activity
0	72.0	0
1.0	52.2	27.5
2.0	57.6	20.0
4.0	45.0	37.5
6.0	39.6	45.0
8.0	7.2	90.0
10.0	0	100

Activity: μmoles of NADH oxidized/min/g protein.

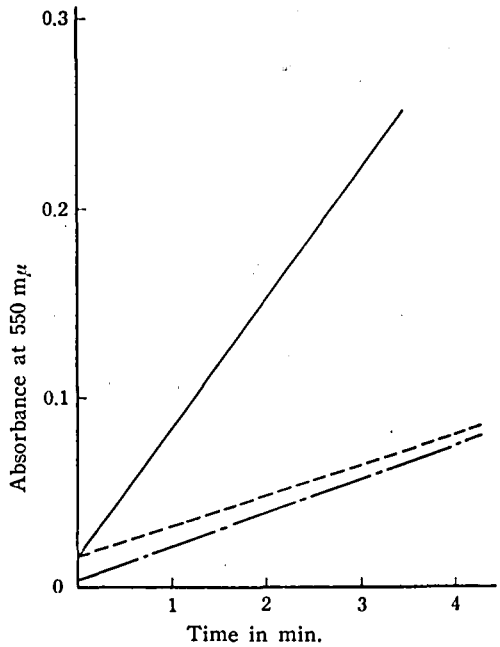


Fig. 1. Effect of Piericidin A on american cockroach muscle mitochondrial NADH-cytochrome c reductase.

-·-·-: 10^{-7}M Piericidin A
-------: 10^{-6}M Piericidin A
————: without Piericidin A

10^{-7}M of Piericidin A inhibited this system 75.9 % and 74.1% respectively.

Absorption spectrum of cytochromes

Absorption spectrum of cytochromes in mitochondrial suspension from cockroach muscle were measured in a Cary Recording Spectrophotometer at room temperature as described in Experimental procedures. Fig. 2 (B) shows the normal reduction of mitochondrial cytochromes when NADH was added to the system without inhibitor. The α-bands of cytochrome components were observed at 603 (cytochrome c), 562 (cytochrome b)

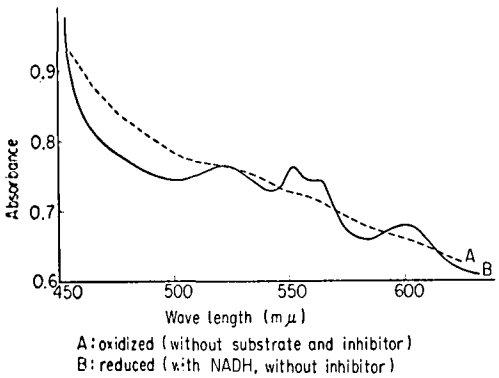


Fig. 2. The absorption spectrum of cockroach muscle mitochondria.

and 551 mμ (cytochrome a) respectively. In the presence of other substrates such as glutamate and succinate, the similar absorption patterns were obtained. Addition of Piericidin A to the system at the concentration of 10^{-6}M made the absorption of cytochromes completely disappear from the spectrum as previously reported with rotenone (Fukami 1961)[6] as shown in Fig. 3 (C). This finding suggested that Piericidin A primarily reacted with the respiratory chain between NADH and cytochrome b as rotenone.

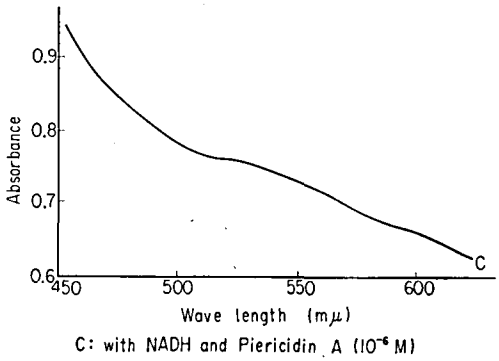


Fig. 3. The absorption spectrum of cockroach muscle mitochondria added with Piericidin A.

Oxidation of succinate

Table 5 showed the effects of Piericidin A and B on the respiration and phosphorylation of cockroach muscle and rat liver mitochondria with succinate as substrate. Both compounds were found to partially inhibit the respiration with succinate at considerably higher concentration, in agreement with earlier reports (Hall 1966).[3] Phosphorylation was also affected by Piericidin. The decrease in the P:O ratio with succinate at

Table 5. Effects of Piericidin A and B on respiration and phosphorylation of mitochondria from cockroach muscle and rat liver with succinate as substrate.

Compound	American cockroach muscle mitochondria				Rat liver mitochondria			
	Respiration		Phosphorylation		Respiration		Phosphorylation	
Concentration	O-μ atom	Inhibition (%)	P-μmol	P/O	O-μ atom	Inhibition (%)	P-μmol	P/O
Piericidin A 0	6.40	—	6.63	1.04	7.59	—	10.65	1.40
1.0×10^{-3}	3.62	43.4	0	0	4.56	39.9	2.78	0.61
1.0×10^{-4}	5.62	12.2	1.67	0.30	7.51	1.1	7.70	1.03
1.0×10^{-5}	6.67	−4.2	3.40	0.51	8.27	−9.0	10.01	1.21
1.0×10^{-6}	6.81	−6.4	2.66	0.39	8.42	−10.9	8.25	1.00
Piericidin B 0	6.40	—	6.63	1.04	7.67	—	11.35	1.48
1.0×10^{-3}	4.74	25.9	0	0	6.36	17.1	6.39	1.00
1.0×10^{-4}	5.63	12.0	2.50	0.44	7.95	−3.7	8.39	1.06
1.0×10^{-5}	6.75	−5.5	3.34	0.49	8.11	−5.7	7.92	0.98
1.0×10^{-6}	6.32	1.2	1.74	0.28	8.01	−4.4	10.99	1.37

Each vessel contained 15mM succinate. Time measured, 15minutes. The other experimental condition as in Table 1.

10^{-5} to 10^{-6}M of Piericidin was found without lowering the respiration of mitochondria.

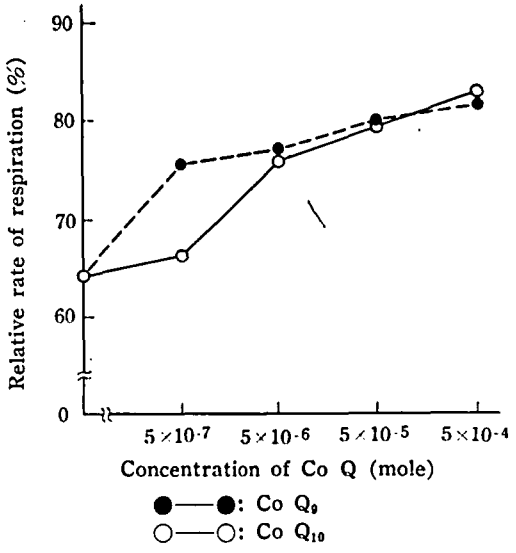
The inhibition of succinoxidation by Piericidin could be partially restored by adding Co Q_9 and Co Q_{10} to the inhibited system as shown in Fig. 4, in which relative rates of respiration were plotted in ordinate as 100 per cent without inhibitor. About 20% of restoration of respiration was found at 5×10^{-4}M of Co Q.

By-pass of the respiratory chain inhibited by Piericidin A

Conover and Ernster (1960)[20] have demonstrated that the inhibition of respiration with pyridine nucleotide-linked substrates in rat liver mitochondria by Amytal and rotenone can be restored by adding vitamin K_3 to the inhibited system. They concluded that vitamin K_3 induced a by-pass of the rotenone-sensitive site of respiratory chain by way of DT-diaphorase.

As with Amytal and rotenone, vitamin K_3 effect was also found in the case of Piericidin A in rat liver mitochondria as shown in Table 6. Perfect restoration was obtained at the concentration of 0.8 to 1.2×10^{-5}M of vitamin K_3 as shown in Fig. 5, which gave the relationship between the concentration of vitamin K_3 and respiratory

Fig. 4. Effect of Co Q on succinoxydation system inhibited by Piericidin A (10^{-5}M) in cockroach muscle mitochondria.



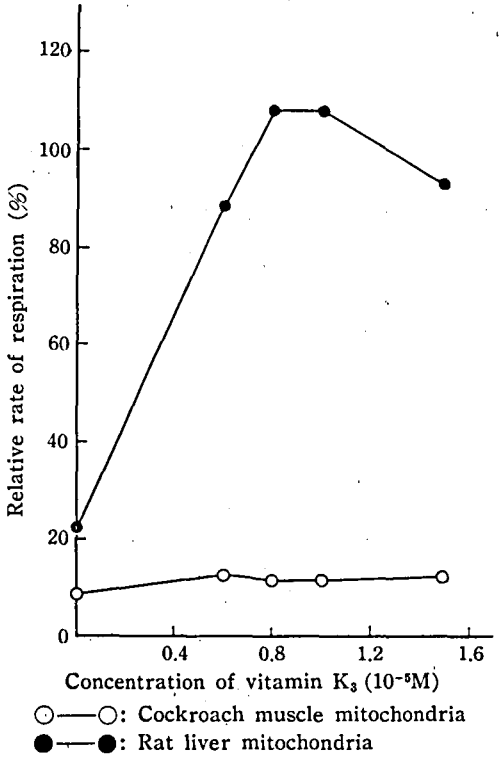
Each vessel contained 15 mM succinate, 15mM phosphate (pH 7.4), 7.5 mM $MgCl_2$, 2.5 mM ATP, 30mM KCl, 0.5 cc of mitochondrial suspension containing 6.8 mg of protein and Co Q indicated above. Final volume of 2 cc. Incubation took place at 30°C for 15 min.

Table 6. By-pass of Piericidin A-sensitive site of respiratory chain by quinones.

Additions	Cockroach muscle Respiration (O-μ atoms)	Rat liver Respiration (O-μ atoms)	P:O
None	8.46	7.59	2.21
Piericidin A	0.65	1.62	0
Vitamin K_1	7.41	—	
Piericidin A, Vitamin K_1	0.76	2.01	
Vitamin K_3	8.64	6.77	2.17
Piericidin A, Vitamin K_3	1.07	8.20	1.39
1,4-Benzoquinone	7.58	8.05	
Piericidin A, 1,4-Benzoquinone	0.68	1.21	
Coenzyme Q_9	8.35		
Piericidin A, Coenzyme Q_9	0.57	1.88	
Coenzyme Q_{10}	8.09		
Piericidin A, Coenzyme Q_{10}	0.52	1.80	
Rotenone	1.08	1.00	0
Rotenone, Vitamin K_3	1.21	7.50	1.40

The substrate was α-ketoglutarate, the final concentration of additions were; Piericidin A, 10^{-6}M or rotenone, 10^{-6}M and each quinone, 10^{-5}M.
The other experimental conditions were as described in Table 1.

Fig. 5. By-pass of Piericidin A-sensitive site of respiratory chain by vitamin K_3.



Each vessel contained 5 mM α-ketoglutarate, 15mM phosphate (pH 7.4), 7.5 mM $MgCl_2$, 2.5 mM ATP, 30 mM KCl, 10^{-6}M Piericidin A, an amount of mitochondria containing 6.8mg of protein and vitamin K_3 indicated above. Final volume of 2 cc. Incubation took place 30°C for 20min.

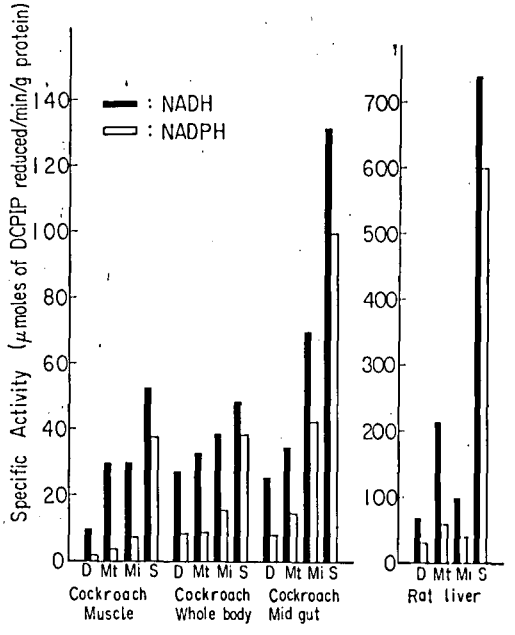
restoration. Phosphorylation coupled with this vitamin K_3-mediated respiratory system was lower at 1 P:O unit than that of the original system. The other quinones, such as vitamin K_1, benzoquinone, Co Q_9 and Co Q_{10}, have no effect in rat liver mitochondria. These findings were in agreement with earlier report with rotenone by Ernster (1963).[8]

On the other hand, only a slight restoration of respiration was found by adding vitamin K_3 to the inhibited respiratory chain by Piericidin A in american cockroach muscle mitochondria as shown in Fig. 5, in which the relative rates of respiration were also plotted in ordinate as 100 per cent without inhibitor as in Fig. 4. The lack of the vitamin K_3-mediated respiratory chain was also observed with rotenone in cockroach muscle mitochondria as shown in Table 6.

DT-diaphorase activity

Fig. 6 and 7 show the comparative data concerning the specific and total DT-diaphorase activity in cockroach and rat liver mitochondria. In this experiment, the enzyme was activated by adding 0.07% of bovin serum albumin as activator and DCPIP was used as an electron acceptor as shown in Experimental procedures.

Fig. 6. Cytoplasmic distribution of DT-diaphorase activity in rat liver and american cockroach.

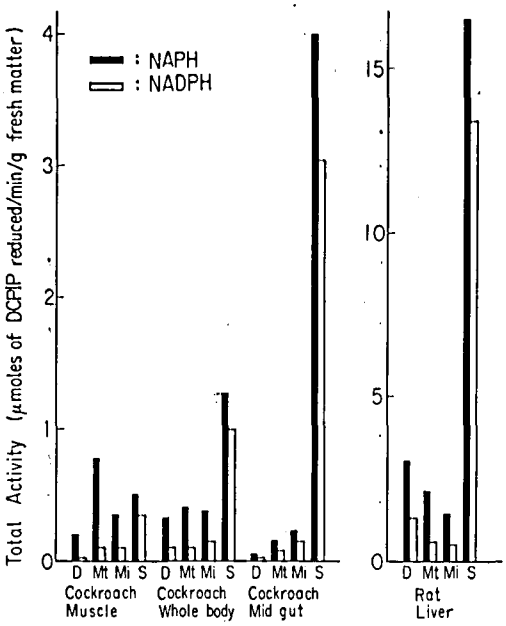


D: debris and nuclei
Mt: mitochondrial fraction
Mi: microsomal fraction
S: soluble fraction

Specific and total activities of both enzymes (DPNH(NADH)-and TPNH(NADPH)-diaphorase) in rat liver were highest in soluble fraction. Furthermore, DPNH-diaphorase activity was generally higher than NADPH-diaphorase activity in each fraction except for soluble fraction which exhibited only a little higher activity with NADH than with NADPH. These data have an approximately same tendency as shown by Ernster (1962).[21]

On the other hand, the activities of both enzymes from american cockroach were found to be a little different in each enzyme source, but

Fig. 7. Comparison of total DT-diaphorase activity in rat liver and cockroach.



D: debris and nuclei
Mt: mitochondrial fraction
Mi: microsomal fraction
S: soluble fraction

generally they were lower than that of rat liver, with about 1/7 and 1/3 to 1/10 in specific and total activity in each mitochondrial fraction, respectively. The debris and nuclei of homogenates also exhibited the considerably high diaphorase activity as shown in Fig. 6 and 7, but it might be attributed to the contamination of the other fractions because no efforts were made to further purify this fraction.

Discussion

As reported in beef heart mitochondria by Hall *et al.* (1966)[3], Piericidin A and B were also proved to inhibit the mitochondrial electron transport system in cockroach muscle mitochondria. In NADH oxidation system, Piericidin A, B and rotenone exhibit the powerful inhibition at the similar concentration, at the order of 10^{-7}M, and identical pattern of action of Piericidin suggests that they react with this electron transport system at the common site with rotenone in cockroach muscle mitochondria. In contrast with rotenone, Piericidin A and B also inhibit the succinoxidation system at considerably higher concentration and this inhibited system can be partially overcome by addition of Co Q.

Piericidin also differs from rotenone in that they seem to have a slight decreasing effect on oxidative phosphorylation at relatively higher concentration; 10^{-6} to 10^{-5}M of Piericidin A inhibited the phosphorylation by approximately one half to two-thirds without appreciably inhibiting the respiration.

In sharp difference from rat liver mitochondria, cockroach muscle mitochondria has no effect on restoration of respiration by addition of vitamin K_3 to the system inhibited by Piericidin A and rotenone. As shown in Fig. 5, complete restoration was made in rat liver mitochondria by adding 10^{-5}M of vitamin K_3 to NADH oxidation system inhibited by Piericidin A or rotenone. On the other hand, only partial restoration was found in cockroach muscle mitochondria. Similar phenomena were found in another mitochondria from cockroach mid gut and honey bee (*Apis mellifera* L.) muscle, but these data were not shown in this paper and they will be reported forthcoming papers in this series.

The enzyme catalizing the oxidation of NADH and NADPH by artificial electron acceptor was first found in soluble fraction of mammalian tissues by Ernster and Navazio (1958.)[13] This enzyme, called DT-diaphorase, was first isolated and its properties were reported by them (Ernster *et al.* 1962.)[21] Furthermore, occurence of this enzyme in mitochondria, the relation of the enzyme to mitochondrial electron transport system and the similarity in properties with vitamin K reductase described by Martius and Märki (1957)[22] were investigated in detail by them.[23,24,25]

Since vitamin K reductase or DT-diaphorase is strongly inhibited by dicoumarol, which is a powerful uncoupler of oxidative phosphorylation, Martius *et al.* suggested that the enzyme might have an important function in coupling of respiration to phosphorylation. The possibility was eliminated, however, on the evidence that pigeon liver mitochondria, which lacks DT-diaphorase,[26] exhibits a normal phosphorylation which is uncoupled by dicoumarol.

From our data described above, DT-diaphorase

activities were directly measured by DCPIP as an electron acceptor in various fractions from cockroach comparing that from rat liver as shown in Fig. 6 and 7.

As the results, it is apparent that the enzyme in each fraction from cockroach is less active than that in corresponding fraction from rat liver. It will be further studied whether this enzyme is generally less active in insects than that of mammals and whether it has a relation to selective toxicity of this pesticide.

Summary

The effects of Piericidin A and B on electron transport system of muscle mitochondria from american cockroach, *Periplaneta americana* L., were studied comparing with rotenone. These compounds strongly inhibit NADH oxidation system at approximately same concentration as rotenone and identical pattern of action of Piericidin A suggests that they react with this system at the common site with rotenone.

In contrast with rotenone, they also inhibit succinoxidation system at considerably higher concentration and they have a slight decreasing effect on oxidative phosphorylation.

Respiration in NADH oxidation system inhibited by Piericidin A is restored by adding vitamin K_3 to this system in rat liver mitochondria as with rotenone. In difference with rat liver mitochondria, cockroach mitochondria has no vitamin K_3-effect. DT-diaphorase activity in each cytoplasmic fraction from rat liver and cockroach is directly measured. The enzymes in cockroach are less active than that in rat liver, with about 1/7 in mitochondrial fraction.

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