Table 1. Radioactivities recovered from "outside" and "inside" of the American cockroaches after topical application of BHC-1-C¹⁴

After applica- tions hours	α -isomer			β -isomer			γ -isomer		
	outside	inside	remainder	outside	inside	remainder	outside	inside	remainder
0		781 cpm			616 cpm			551 cpm	
1.5	378.5cpm (89.9%)	35.8 cpm (8.5%)	6.3 cpm	4948.9cpm (90.2%)	50.5cpm (9.9%)	0cpm	136.6cpm (57.8%)	74.1cmp (31.3%)	25.8cpm
6	319.0 (78.1)	82.6 (20.3)	6.3	396.0 (92.5)	25.2 (5.9)	6.7	107.0 (39.1)	139.2 (50.9)	27.3
24	176.0 (62.1)	101.2 (35.7)	6.2	272.0 (68.6)	96.4 (24.3)	28.0	41.1 (19.9)	157.0 (76.0)	8.4

Solubility of the isomers in organic solvents coincide with the velocity of penetration of respective isomers.

ARMSTRONG, BRADBURY & STANDEN (1951) have pointed out that the amount of each isomer taken in by the grain weevil is in an approximate proportion to its solubility in hydrocarbon solvents, and the γ -isomer penetrates through the outer layer of the insect integument much more rapidly than other isomers. OPPENOORTH (1956) has also observed that the absorption of the isomers in this house fly decreases in the following order: γ -, δ -, ζ -isomers.

Although insecticidal potencies of these isomers are different according to the insects tested and also to application methods, the γ -isomer is several hundred to thousand times higher in its toxicity than the other isomers (METCALF, 1955). It is noteworthy that, in the present result, the amount of the γ -isomer penetrated through the cuticle is only several times that of α - or of the β -isomer, after 6 to 24 hours from the treatment. Anyhow, the γ -isomer penetrates the cuticle more easily than the other two isomers, and this property is believed to be an important factor concerning its insecticidal potency. However, as it has been pointed out by DRESDEN & KRIJGSMAN (1948), that some biochemical aspects of the isomers at a site of action due to differences of the molecular configuration of these isomers are more important to solve the mode of action of BHC isomers.

SUMMARY

The penetration of α -, β - and γ -isomers of BHC through insect cuticle has been studied in order to find why only γ -isomer has a high insecticidal potency among the isomers. BHC-1-C¹⁴ isomers were topically applied on the male American cockroaches *Periplaneta americana*, and radioactivities recovered from "outside" and "inside" were determined at 1.5, 6 and 24 hours after the treatment. The γ -isomer penetrates more easily than the other isomers, α - and β -isomers following in decreasing order. The rapid and easy penetration of the γ -isomer through the insect cuticle is believed to be an important factor for its insecticidal potency. It is considered, however, differences of certain biochemical changes among isomers which will be taken place after the penetration through insect cuticle are more importance to solve the mode of action of BHC isomers.

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On the Role of Diphosphopyridine Nucleotide (DPN) in Xanthine-Oxidation by the Xanthine Dehydrogenase of Silkworm, *Bombyx mori*¹

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NAWA et al. (1958) showed that pteridine dehy-

drogenase (or xanthine dehydrogenase) of *Drosophila melanogaster*, in the presence of its substrate such as 2-amino-4-hydroxypteridine (AHP), needs DPN as electron acceptor in aerobic conditions, suggesting the possibility that DPN plays a role of electron acceptor in AHP-oxidation *in vivo*. On the other hand, IRZYKIEWICZ (1955) also pointed out in cloth moth and some other insects that DPN operates as active part in xanthine oxidations.

Previous studies have demonstrated the occurrence

of xanthine dehydrogenase in the silkworm (*Bombyx mori*), and the enzyme which converts xanthine to uric acid reacts more efficiently with DPN and flavin adenine dinucleotide (FAD) than with molecular oxygen (HAYASHI, 1957, 1960, 1961).

The present paper deals with a further study of oxidation of xanthine by the xanthine dehydrogenase under both aerobic and anaerobic conditions using DPN as electron acceptor.

By KIELLEY's methods (1955), the enzyme of fat body of the silkworm larvae was prepared about 100 times as potent as the original activity.

The enzyme activity was determined by a differential spectrometry (KALCKAR, 1947), and the formation rate of uric acid followed at 285 m μ in Beckman model spectrophotometer. The experiments of aerobic and anaerobic conditions were carried out with WARBURG apparatus and THUNBERG procedure respectively. The reaction was started by adding the xanthine after 10 minutes pre-incubation. The reaction mixtures, in all the aerobic and anaerobic experiment, consisted of 1 ml (2 mg-Alb.) of enzyme solution in 0.1 M pyrophosphate buffer at pH 8.3, 0.5 ml. of 2×10^{-2} M xanthine, and 0.3 ml of 10^{-3} M DPN or water. After 60 minutes incubation at 37°C, the reaction mixture was deproteinized with 0.7 ml of 5 per cent perchloric acid.

Table 1 shows the effect of each condition of the uric acid formation by the xanthine dehydrogenase using xanthine as substrate. Uric acid formation by the xanthine dehydrogenase, when measured without adding DPN as electron acceptor, was not detectable, but in the case of having DPN, the acid formation was increased markedly in the aerobic condition. Furthermore, uric acid was amply formed under the anaerobic condition with decreasing oxygen tension, i. e., the acid formation level in aerobic experiment was equal to about 50 per cent of that of anaerobic one.

It was found that the rate of uric acid formation by the xanthine dehydrogenase in anaerobic condition was greater than that of aerobic one with DPN as electron acceptor.

REMY et al. (1955) have shown, however, that the xanthine dehydrogenase in the chicken liver could not react with DPN as electron acceptor in aerobic procedure. However, the present work has been studied with addition of DPN as electron acceptor in the xanthine-oxidation reaction, and the possibility that xanthine dehydrogenase is not

Table 1. Uric acid formation by xanthine dehydrogenase of the silkworm under the aerobic and anaerobic conditions using DPN as an electron acceptor

Exptl. No.	Aerobic				Anaerobic	
	Without DPN ΔE_{285}	Uric acid formation* ΔE_{285}	With DPN ΔE_{285}	Uric acid formation* ΔE_{285}	With DPN ΔE_{285}	Uric acid formation* ΔE_{285}
1	0.020	0.30 μ g	0.134	2.03 μ g	0.312	4.73 μ g
2	0.035	0.53	0.134	2.03	0.266	4.03

* Calculated from optical constant of uric acid in ultraviolet region at 285 m μ

activated directly by molecular oxygen in silkworm tissues was suggested.

It was of interest in the present experiment, therefore, to compare the ability of the xanthine dehydrogenase of chicken liver. And it may be indicated that the properties of xanthine enzyme vary in different source.

The author indebted to Prof. Dr. K. HASEGAWA and Dr. S. KAWASE for their valuable advices in the course of the present study.

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¹ 家蚕のキサンテン脱水素酵素によるキサンテンの酸化におけるジホスホピリジンダレオチッドの役割
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Japanese Journal of Applied Entomology and Zoology, Vol. 5. No. 4, 1961
 (Received for Publication October 10, 1961)