EFFECTS OF LINDANE ON THE HAEMOLYMPH OF PAPILIO

YAU-I CHU and TUAN-SHENG MIU

I. INTRODUCTION

Though the insecticidal mechanism of DDT on insects has been reported by many workers, relatively less work has been done on that of lindane (Gamma.-BHC), so the cause of death resulting from the application of lindane to insects is not clearly understood, naturally the previous knowledge about the physiological and biochemical effect of lindane is rather fractional.

On the aspect of the biochemical effect of lindane, inositol theory was first introduced by Slade (1946). Concerning that, Kirkwood & Phillipe reported the antagonism of mesoinositol and lindane on the *Saccharomyces cervisiae*. The similar facts were also proved on the *Nematospora gossypii* etc. (Buston *et al.* 1946, Fuller 1950, Tirunarayan *et al.* 1953). But the attempts to recover insects from the lindane intoxication with the application of inositol were all unsuccessful. (Metcalf 1947, Thorpe & De Meillon 1947, Dresden & Krygsman 1948). According to the work by Doisy & Bocklage (1949), inositol did not show the antidotal action on lindane poisoned mammals. On the other hand, Uedo and Yamamoto (1950) tried the protection of *Brassica sp.* from the insecticidal injury of lindane, but this attempt showed negative results. So inositol theory has not acquired the affirmative conclusion, at least in regard to insects. But inositol shows physiological importance to some insects. For example, *Tribolium confusum* and *Ptinus tectus* grew slightly better when the diet included inositol (Fraenkel and Blewett 1943). Also Forgah (1958) stated that American cockroach, (*Periplaneta americana*) reared on diets efficient in inositol appeared to develop normally for the first 50 days. Thirty days later, however, they weighed only one third as much as those receiving inositol, and at 270 days their weight was less than, one-half that of the controls;

also, they showed 75% mortality and 3% maturation of survivor, as compared with 13% mortality and 78% maturation in colonies receiving inositol. But on the other hand. The following insects have shown that they do not require inositol: *Silvanus surinamensis, Lasioderma sersicorne, Sitodrepa panicea* (Fraenkel and Blewett 1934 a), *Tenebrio molitor*, (Fraenkel *et al.* 1950), *Tineola bisselliella* (Fraenkel and Blewett 1946 a), *Ephestia elutella, Ephestia cantellar*, and *Plodia interpunctella* (Fraenkel and Blewett 1940 a), *Culex molestus* (Lichtenstein 1948), *Drosophila melanogaster* (Schultz *et al.* 1946), *Musca domestica* (House and Barlow).

The correlation between lindane and cholinesterase was studied by Toblas *et al.*, (1946). And they mentioned, lindane acts as a cholinesterase inhibitor in the central nervous system of lindane treated cockroach, and the increase of acetylcholine content was observed, but its increment was not so significant as shown by DDT. On the contrary, Hartley and Brown (1955) reported that the 13 chlorinated hydrocarbons insecticides, including DDT, lindane and chlordane, showed no significant effect on the cholinesterase content from the head of the American cockroach.

Morrison & Browns' work (1945) showed the inhibition of the cytochrome oxidase on the coxal muscle of American cockroach by 26 insecticidal compounds including lindane.

In the lindane treated *Blattella germanica*, the accumulation and aggregation of lipoid granules in fatty cell and nerve ganglion was observed by Yamamoto (1958). Tomizawa and Fukami's work showed negative inhibition of lindane on the oxidative phosphorylation of locust both in vivo and in vitro.

The work on the resistant mechanism of lindane-resistant house fly was carried out by Oppenooth, Bradbury & Stancen, (Yamamoto 1958).

On the physiological aspect, Savit (1946) ascertained the action of lindane, as a nervous poison. Namely, insect ganglion produced violent spontaneous impulse by lindane. If the convulsing leg was separated from the central nervous system the convulsion ceased immediately. Similarly, when lindane injection was made on the separated leg no convulsion was observed. Therefore, lindane seems to act on the central nervous system selectively.

When the haemolymph of BHC treated insects was injected into the body cavity of untreated insects, the same intoxicating appearance was observed. So is suggested that lindane contact with the insect cuticle at

first penetrates the cuticle and dissolves into the haemolymph, then circulates the whole body cavity with haemolymph, and reaches to the suitable point of action, then intoxicated appearance occurs. So if inferior circulation is induced in the insect body, for example, by separation of thorax artificially, the latent period of intoxication is consequently prolonged (Yamazaki & Isii unpublished). But on the other hand, Becht (1958) denied any influence of BHC on the function of the nerve-muscle preparations.

As above mentioned the toxic mechanism of lindane is only partially studied on some aspects, but no work has been carried out in regard to the effect on haemolymph. In addition, as Yamazaki and Isii suggested, if BHC dissolves into the haemolymph before it invades its suitable site, the relation between toxicological mechanism of lindane and change of haemolymph in chemical properties is considerablly important.

II. MATERIAL

For the material of this experiment, matured larvae of *Papilio demoleus L.* were used, which is one of the most important citrus pest on this island. The eggs and caterpillars were directly collected from the school orchard and were reared at laboratory at the room temperature, on citrus leaves, till the matured.

III. EXPERIMENTATION

1. Toxicity of Lindane on Caterpillar

Preceeding the work of analysis, for the purpose to determine the suitable dosage, method of application, and time of analysis following application, five diffrent concentrations of lindaneacetone solution were applied on various sites of caterpillars either by topical application or by injection.

These insecticidal preparations were prescribed to contain 5, 10, 20, 40, 60 gamma of lindane in each 1 cumm of solution, and 1 cumm of each concentration of lindane solution was used for both topical application and injection, per caterpillar. Also suitable controls were established by pure acetone solution. When the insecticidal preparation was injected into the insect body the toxicity of acetone most be taken into account, and the

second control solution was made, then acetone was substituted by distilled water.

As the average body weight of caterpillar acquired from 50 individuals is approximately 1.6 g., accordingly each 1 cumm of lindane solution contains 3.12, 6.25, 12.5, 25.0, 37.5 ppm of effective ingredient per caterpillar, by weight.

Each test was carried out with 10 caterpillars, and the test was dublicated 5 times.

If there is no note, the caterpillars during the mortality-counting period were fed on citrus leaves. The mortality was obtained from, Abott's formula.

The results obtained are shown in the following tables:

Mortality of Caterpillars by Different Mode of Application of Lindane.
(percent)

I. Intra-digestive canal injection to foregut.

Lindane dosage per caterpillar ppm. (by weight)	time after the application (hr.)											
	2	4	6	8	10	12	14	16	18	20	22	24
3.12	16	22	30	32	32	44	44	44	46	46	48	48
6.25	26	36	44	44	44	46	48	52	52	60	60	60
12.50	76	88	88	88	88	88	88	88	88	88	88	88
25.00	92	92	92	100	—	—	—	—	—	—	—	—
37.50	100	—	—	—	—	—	—	—	—	—	—	—
acetone control	4	16	30	36	40	40	40	40	40	40	40	40
water control	2	2	8	8	8	8	8	8	8	8	8	8

II. Intra-digestive canal injection to hindgut.

Lindane dosage per caterpillar ppm. (by weight)	time after the application (hr.)											
	2	4	6	8	10	12	14	16	18	20	22	24
3.12	32	50	50	50	54	64	82	84	84	92	94	94
6.25	46	80	82	82	90	96	98	100	—	—	—	—
12.50	88	100	—	—	—	—	—	—	—	—	—	—
25.00	92	100	—	—	—	—	—	—	—	—	—	—
37.50	100	—	—	—	—	—	—	—	—	—	—	—
acetone control	32	42	42	60	64	64	68	68	70	72	72	72
water control	20	22	30	32	44	46	46	46	48	48	48	48

III. Topical application on abdominal leg.

Lindane dosage per caterpillar ppm. (by weight)	time after the application (hr.)											
	2	4	6	8	10	12	14	16	18	20	22	24
3.12	0	0	0	4	10	14	20	26	32	40	40	40
6.25	0	0	4	8	10	16	16	22	30	34	40	40
12.50	0	0	2	10	14	40	40	42	46	52	54	56
25.00	10	12	12	16	34	58	72	76	90	92	92	92
37.50	16	48	48	61	66	96	96	100	—	—	—	—
acetone control	0	0	0	0	0	0	0	0	0	0	0	0

IV. Topical application on thoracic notum.

Lindane dosage per caterpillar ppm. (by weight)	time after the application (hr.)											
	2	4	6	8	10	12	14	16	18	20	22	24
3.12	0	0	0	4	10	14	20	26	32	40	40	40
6.25	0	0	4	8	10	16	16	22	30	34	40	40
12.50	0	0	2	10	14	40	40	42	46	52	54	56
25.00	10	12	12	16	34	58	72	76	90	92	92	92
37.50	16	48	48	61	66	96	96	100	—	—	—	—
acetone control	0	0	0	0	0	0	0	0	0	0	0	0

V. Topical application on thoracic notum in starving condition.

Lindane dosage per caterpillar ppm. (by weight)	time after the application (hr.)											
	2	4	6	8	10	12	14	16	18	20	22	24
3.12	0	0	4	6	6	16	24	38	40	42	46	54
3.25	0	10	16	16	20	24	28	36	42	46	50	52
12.50	6	20	20	22	42	46	56	60	62	68	68	68
25.00	30	42	44	50	80	80	82	86	90	100	—	—
37.50	42	50	54	78	96	50	—	—	—	—	—	—
acetone control	0	0	0	0	2	8	12	26	26	26	28	28

VI. Hypodermal injection from thoracic notum.

Lindane dosage per caterpillar ppm. (by weight)	time after the application (hr.)											
	2	4	6	8	10	12	14	16	18	20	22	24
3.12	26	26	26	34	38	46	52	52	54	58	60	60
6.25	42	42	46	46	48	58	72	76	80	82	82	82
12.50	62	62	64	64	70	74	82	84	86	90	92	92
25.00	92	96	100	—	—	—	—	—	—	—	—	—
37.50	50	—	—	—	—	—	—	—	—	—	—	—
acetone control	16	18	18	18	18	18	18	18	18	18	18	18
water control	2	2	4	4	4	4	4	4	4	4	4	4

The effects of topical application and injection of lindane in different sites of caterpillars were tested. As the results most part of rapid death and great mortality was shown by injection, rather than by topical application. In the injection, intradigestive canal injection was more toxic to caterpillar than hypodermal injection.

Though Brown (1951), denied the toxicity of acetone to insect on injection, in the author's experiment, on both in body cavity and digestive canal, acetone itself displayed considerable toxicity.

Topical application under starving condition exhibited larger mortality than fed condition. This may be caused by mal-nutrition condition which induces the decrement of function to function to the penetrated insecticide. According to Reicer *et al.*, (1953) the increment of insecticide tolerance of boll weevil *Anthonomus grandis Boh*) is directly proportional to its lipid content, namely nutritional advantage. The increment of mortality by starving is also introduced by Perry *et al.* (1958), on heptachlor-treated resistant house flies. Similarly in this experiment, larger mortality was obtained under starving or mal-nutritional condition.

In topical application on abdominal leg, rapid death and large mortality resultes as shown in injection. Accordingly, ventral sclerite consists in thinner cuticule, and insecticidal ingredient is comparatively easy to penetrate from there. In addition, on the abdominal leg there are many secretory glands, and its secretion sometimes promotes the penetration of insecticide. Moreover there exist many chemo-acceptors and terminal nerve ends, which supply active sites of lindane, namely the abundance of sensillar, gland, and nerve end, conducts rapid death and large mortality. Dresden (1948) also recongnized such a special ability of insecticide to penetrate insect integument.

2. Quantitative Analysis of Internal and External Lindane Residue in Papilio Larvae

Preparation of Lindane Extract:

The treated larvae were rinsed out individually with ethyl ether after 5 hours or 15 hours application from the insecticide. This ether-lindane preparation was used for analysis of external lindane residue.

For the analysis of internal residue, each of ether rinsed larvae were minced with dissecting scissors, and internal lindane residue was extracted by ethyl ether with Soxhlet's apparatus about 3 hours, then internal residue preparation was acquired.

The excreta of insect was also rinsed with ethyl ether and the obtained solution was mixed with external lindane residue part.

For this experiment, 30 larvae consisted one test group.

Both external and internal residue preparations were analyzed with polarogram, introduced by the method by Nakazima *et al.* (1949).

Result:

The results introduced are as follows.

The lindane residue analyzed 5 hours after application from papilio larva

lindane weight per larva (mg.)	total lindane weight per test group (mg.)	internal lindane residue per test group (mg.)	external lindane residue (mg.)	difference between analyzed residue and applied weight (mg.)
0.02	0.6	0.251	0.102	0.247
0.04	1.2	0.352	0.231	0.717
0.06	1.8	0.475	0.402	

The lindane residue analyzed 15 hours after application from papilio larva

lindane weight per larva (mg.)	total lindane weight per test group (mg.)	internal lindane residue per test group (mg.)	external lindane residue (mg.)	difference between analyzed residue and applied weight (mg.)
0.02	0.6	0.242	0.085	0.273
0.04	1.2	0.265	0.091	0.844
0.06	1.8	0.623	0.279	0.898

As the above table shows, a large part of lindane applied on the papilio

larva seemed to be metabolized in the insect body, and during first 5 hours almost all the lindane penetrated into the body. During the following 10 hours though penetration still continued its ratio of penetration was comparatively low, and only small part of un-penetrated residue. The proportion of internal/external residue in an appointed period became smaller when the dosage of insecticide was increased.

3. Method of Collection of Haemolymph

For the collection of the haemolymph some-what special care has been taken for avoid under-going peculiar changes called melanosis, when it is taken out of the body and exposed to the air, the body fluid soon begins to exhibit a reddish brown color, which deepens gradually and eventually the whole fluid became blackish brown, and this melanosis is supposed to be due to the presence of tyrosinase, by some researchers (Barker 1938, Bishop 1923, Kuwana 1934).

If the larva was killed by immersion in water at 60°C. for 1 minute before collection, both clotting and darkening could be prevented. Also a visible coagulation of plasma protein has not been observed by this treatment.

The immersed larva was wiped with filter paper thoroughly, and was cut off at osmeteria, and pressed trunk gradually with fingers, then body fluid flowed out from the leakage, the haemolymph was accepted with a vial.

4. Method of Application of Insecticides for Following Work

As the first experiment has shown: the comparative mild action of lindane on papilio larva was obtained by the topical application on the prothoracic shield of caterpillar. For the following physiological and biochemical studies the mild insecticidal condition is preferable than the acute one. So the application of insecticide was carried out by the following method, that is, 1 cumm of insecticidal preparation was dropped on the prothoracic shield of larva with needle of tuberculin syringe.

Both treated and control caterpillars were reared in the breeding cage with citrus leaves.

5. Effect on Body Weight and Water Content

Method:

For the determination of body weight of lindane treated and control

caterpillars, both treated and unterated individuals were weighted after 5 hours and 15 hours of application.

In the water content experiment, Ludwing (1946) introduced two methods. One uses drying oven and the other decicate the sample with anhydrous calcium chloride and he acquired identical results.

The present author used the later method, this method was also applied by Ingam (1955) etc. That is, the individuals were dessicated over anhydrous calcium chloride until constant weights were obtained.

Results:

The results obtained are shown in the following table.

The body weight and water content loss of lindane treated papilio larvae

lindane dosage per caterpillar ppm. (by weight)	time after application (hr.)	average original weight (gram)	weight after treatment (gram)	change of body weight (gram)	weight of excreta during treating period	weight loss per original body weight gram/gram	dry water water content weight content per body weight		
							(gram)	(gram)	(gram/gram)
12.5	5	1.62	1.73	+0.11	0.21	+0.647	0.25	1.48	0.855
25.0	5	1.50	1.47	−0.03	0.55	−0.220	0.23	1.24	0.843
37.5	5	1.61	1.57	−0.04	0.15	−0.248	0.15	1.42	0.905
acetone control	5	1.55	1.61	+0.06	0.18	+0.387	0.21	1.39	0.863
12.5	15	1.72	1.60	−0.12	0.32	−0.069	0.21	1.39	0.869
25.0	15	1.82	1.50	−0.32	0.33	−0.179	0.22	1.28	0.854
37.5	15	1.55	0.89	−0.66	0.91	+0.426	0.13	0.76	0.855
acetone control	15	1.69	1.69	0.00	0.31	0	0.17	1.52	0.899

As shown in the above table the body weight is increased in low dosage-treated caterpillar, in the first 5 hours but after 15 hours from application, the loss of body weight was observed in each dosage of insecticides. And the level of the weight loss was directly proportional to the dosage of applied insecticides.

The total weight of excreta during appointed period was also increased as the dosage in creased. This was almost due to the violent diarrhoea, which is one of the sympton of lindane intoxication. Though a little level, loss of water content was also observed in the treated caterpillar.

Discussion.

It was observed that the decrement of body weight and also the increment of the excreta were directly proportional to the given dosage of lindane.

Water content loss of insects by insecticides were also reported by many researches, such as, Mori (1955), Ludwing (1952), Hewlett & Gostick (1955), Ingram (1955), with pyrethrin, and Ludwing (1952), Buck & Keister (1949), Buck, Keister & Posner (1952), with DDT.

As the cause of water content loss, less and Browing (1952) suggested to be the prevention of the function of water absorption from the integument. The effects of DNOC and pyrethrin on the hypodermal cells in certain insects were studied by Stelling (1931) and Kruger (1931) respectively, and they found that the hypodermal cells were destroyed by those chemicals. So the increment of the level of body weight loss by insecticides were presumedly due to the compulsive water evaporation from the destruction of hypodermal cells by insecticides.

In the author's observation, the body surface of intoxicated caterpillars exhibits the wet-apperance, namely, excess water evaporation was presumably carried out from the integument, though the cause of such wet-apperance is not clear yet, it is undoubtful that the water content loss was promoted by the occurrance of the wet-appearance. Moreover though in the healthy caterpillar the excreta was almost dry and granular shaped, the excreta from the lindane treated caterpillar did not show dry granule and excreted the amount of diarrhoea. So the water content loss of the lindane-treated caterpillar was partly attributed to such a abnormal water excretion.

Concerning the body weight loss of the insecticides treated insects, DDT-treated Japanese beetle lost approximately one-third of their original weight, (Ludwig 1946), Also Ingram (1955), and Kitaoka (1958), observed similar phenomena on cockroach with pyrethrum, and on tick with lindane respectively.

And Ludwig (1946) also concluded his results that, the decrement of body weight was attributed to two causes, one is the hyper-consumption of storage nutrient, and the other is the reduction of water content. In the author's experiment, both water content loss and excess consumption of glucose were also observed (See p. 24 of this paper.). Moreover the excess excretion of solid particles by the diarrhoea seemed to be an important factor.

6. Effect on Number of Haemocytes

For this experiment, haemolymph was directly poured on the Thomas-Zeiss haemocyte counter and counting was carried out. For one experimental series 15 individuals were used, and its average value was taken.

The results obtained are as follows:

Lindane dosage per insect ppm. by weight	time following application (hr.)	No. of Haemocytes per mm^3
acetone control	5	18,000
12.50	5	15,500
25.00	5	13,700
37.50	5	11,800
acetone control	15	19,000
12.50	15	12,300
25.00	15	8,100
37.50	15	5,200

As the above table shows, treatment with lindane reduced the number of haemocytes, and at the treatment of 37.5 ppm. lindane after 15 hours of application its number is reduced to about $\frac{2}{3}$ of acetone control.

The reduction of haemocytes by insecticides was also reported by Lepesme (1935) with arsenite. According to him, when the insect contacts with the arsenite, it penetrates into the body cavity, and then haemocytes may be destroyed by arsenite, or carried by the haemolymph to all the tissues and deposited.

By the microscopic observation, the author also found miscellaneous particles which seem to be destroyed haemocytes in the haemolymph of treated caterpillar.

7. Effect on The Oxygen Consumption

Respiratory readings were made with the caterpillars which were poisoned with lindane on their prothoracic shield topically, and modified Krogh's manometer was used, the oxygen consumption was determined at 5 hours and 15 hours after the application of the insecticide.

The results obtained are as follows.

Oxygen consumption of lindane treated caterpillar 5 hours after the application

Lindane dosage per caterpillar ppm. by weight	No. of caterpillars	Av. Wt.	MM^3O_2/caterpillar/min.	MM^3O_2/gm. caterpillar/min.
12.5	10	1.68	2.95	1.76
25.0	10	1.53	2.99	1.95
37.5	10	1.51	3.31	2.19
acetone control	10	1.70	2.72	1.60

Oxygen consumption of lindane treated caterpillar
15 hours after the application

Lindane dosage per caterpillar ppm. by weight	No. of caterpillars	Av. Wt.	MM^3O_2/caterpillar/min.	MM^3O_2/gm. caterpillar/min.
12.5	10	1.65	3.11	1.88
25.0	10	1.52	3.52	2.32
37.5	10	1.46	3.88	2.66
acetone control	10	1.71	2.75	1.61

Lindane poisoning results in an increased oxygen consumption which was already evident about 5 hours after application, and was at least kept its increment for 15 hours after application.

The period of increased O_2 consumption corresponed with the period during the caterpillar was under going charactristic tremors in which the metabolism was increased.

The increment of oxygen consumption of insecticide-poisoned insect, was reported by many researches, especially with DDT. (Yamamoto 1958, Ludwig 1946, Buck and Keister 1952), with pyrethrum (Ingram 1955), and with heptachlor (Havey & Brown 1931), and these researches also concluded that the increased oxygen uptake was attributed to the hyperactivity caused by the toxicity of insecticides.

8. Effect on pH Value of Haemolymph

Effect of lindane on the pH value of haemolymph of papilio larva was tested with the Tōyō pH test paper Methly Red.

The results obtained by the test are as follows.

Lindane dosage per caterpillar ppm. by weight	time following application (hr.)	pH value of haemolymph
12.5	5	6.8
25.0	5	6.8
37.0	5	6.8
acetone control	5	6.8
12.5	15	6.8
25.0	15	6.8
37.0	15	6.8
acetone control	15	6.8

As the above table shows, with this method, there existed no variation in pH value of haemolymph between the treated and untreated individuals.

9. Effect on Calcium Content in Haemolymph

Calcium contents of haemolymph both in lindane treated and untreated caterpillar were as follows:

Calcium content of haemolymph in lindane treated Papilio larvae

Lindane dosage per caterpillar ppm. by weight	time after application (hr.)	calcium content wet weight m Eq/1
12.5	5	30.5
25.0	5	31.3
37.5	5	30.2
acetone control	5	31.1
12.5	15	30.8
25.0	15	30.6
37.5	15	31.2
acetone control	15	30.4

The effect of calcium in insect body was reported by a number of investigators, above all, its effect on nervous system and muscle seemed to have some correlation to the lindane intoxication. accordingly, Loeb (1909) found that lowering the calcium level causes nerves to become more excitable and fire spontaneously. Also, Welsh and Gordon (1947) and Gordon and Welsh (1948) found that, DDT and other organic compounds brought about a toxic action on the nerve, which was similar to that caused by lowering the concentration of calcium and magnesium ions, they also postulated that these neurotoxins are absorbed on the surface of the nerve axons, and that the repetitive firing of the nerve following an initial exciting impulse which breaks the calcium iōn-axon surface linkage is caused by a delay in the stabilizing restoration of calcium ions to the axon surface comples.

Calcium is also released during muscle contraction. It may be released in one part of the muscle and immediately bound elsewhere in the same or another muscle (Weiss 1934), or it may be set free as the calcium ion in the blood (Wacker 1929). Also the initiation of shorting of muscle fiber by calcium was also reported by Heilbrun (1934), Overton (1904), Hukuda and Morija (1936). And calcium can also initiate a chemical reaction of

primary importance in muscle metabolism, namely, the adenosine triphosphate breakdown, making available a large amounts of energy when calcium activates adenosine triphosphatase; although other ions may activate adenosine triphosphtase, the muscle will not contract unless calcium is persent (Bailey 1942).

Though the effect of calcium on the nerve and muscle were studied by many researches, no study was made on the haemolymph. In the author's experiment the calcium contents of haemolymph was done and the result is shown in the preceeding table. But there was no significant variation between the calcium content of haemolymph and lindane intoxication on *Papilio* larvae.

10. Quantitative Analysis of Glucose in Haemolymph

The results introduced are as follows

Lindane dosage per caterpillar ppm. (by weight)	time following application (hr.)	glucose content (mg/ml)
12.5	5	1.42
25.0	5	1.04
37.5	5	1.04
acetone control	5	1.58
12.5	15	0.87
25.0	15	0.82
37.5	15	0.78
acetone control	15	1.47

Loss of glucose contents of haemolymph in lindane treated caterpillar was observed, when lindane dosage was increased, or time following the application was prolonged. In other words, the greater decrement of glucose content was shown in the case of the higher level of intoxication. Glucose, fat and glycogen are compounds which furnish energy for muscular activity and their loss was associated with the violent muscular tremor, and diarhoea which occureed accompaning lindane poisoning.

The same phenomena with DDT were also observed by Merril and Ludwig (1946) in american cookroach and Japanese beetle respectively.

The results of Ludwing's experiment which showed the data at the time of death to DDT poisoning was as follows. Japanese beetle larvae had used 85.5 percent of the original supply of the glycogen, 40.4 percent

of glucose, and 32.5 percent of the fat. Comparable figures obtained by Newton (1954), during the starvation of this insect were 80.0, 28.6 and 71.7 percent respectively. Therefore, it is clear that more of the available carbohydrate reserve is utilized following exposure to DDT than during starvation. But contrary results were also introduced by Joseph (1958). By his biochemical determination on the blood of the mealworm (*Tenebrio molitor*), showed 90 percent increase in reducing compound with DDT exposure.

As both Joseph and Ludwig used Hagedorn and Jensen technique for this analysis, different results between their experiments might be due to the difference of insect species.

In the author's experiment with lindane also showed the decreased about 40.5–46.6 percent during the 15 hours intoxication. This results almost coincided with that of Ludwig's.

11. Paper Chromatographic Analysis of Monosaccharides and Certain Ninhydrin-Positive Amino Acid in Lindane Treated Papilio Larvae

(a) Monosaccharide.

In this experiment, glucose and 2 unknown substances were identified. The paper chromatographical appearances are as follows:

Monosaccharide identified	Rglucose	Color of spot	Color intensity
glucose	1.00	Brown	++
unknown (A)	0.32	Brown	++
unknown (B)	0.28	Cherry red	+

The distribution of identified monosaccharide in each treated group is as follows:

Monosaccharide identified	After 5 hr. following application lindane dosage per caterpillar				After 15 hr. following lindane dosage per caterpillar ppm.			
	0	12.5	25.0	37.5	0	12.5	25.0	37.5
Glucose	+	+	+	+	+	+	+	+
unknown (A)	+	+	+	+	+	+	+	−
unknown (B)	−	−	−	−	−	−	−	+

The spots of glucose were quite identified with that of standard glucose in the spot position and color appearance. Judging from the R-glucose of other two unknown substances, they could not be any free monosaccharide, and suggested to the phosphated glucose and fructose 1-6 diphosphate, but due to the lacking of standard sample, further identification of those substances were not carried out.

(b) Amino acid.

In this experiment comparing with 20 standard amino acids, 8 amino acids were certainly identified in every sample. They were leusine, phenylalanine, proline, valine, alanine, glycine, glutamic acid, and aspartic acid. Serine was also observed but its existence was doubtful, also 7 unknown nin- hyrin-positive substances were recognized. All the ninhydrin-postive substances identified chromatographically are shown in the following tables.

Amino acids chromatographically identified in the haemolymph of lindane treated papilie Larvae

(5 hours after topical application on prothracic shield)

Amine acid identified	Rf value to phenol	Rf value to butanol	Lindane desage per a caterpillar ppm. (by weight)			
			12.5	25.0	37.5	acetone control
leusine	0.690	0.691	+	+	+	+
phenylalanine	0.553	0.631	+	+	+	+
proline	0.652	0.557	+	+	+	+
valine	0.516	0.575	+	+	+	+
alanine	0.492	0.487	+	+	+	+
glycine	0.412	0.393	+	+	+	+
glutamic acid	0.314	0.407	+	+	+	+
aspartic acid	0.275	0.317	+	+	+	+
Serine (?)	0.309	0.291	+	+	+	—
arginine	0.586	0.411	—	—	+	—
tyrosine	0.354	0.641	—	—	+	—
unknown 1.	0.447	0.427	—	—	+	+
unknown 2.	0.786	0.874	—	—	—	+
unknown 3.	0.416	0.546	—	—	—	+

Amino acids chromatographically identified in the haemolymph of lindane treated papilio Larvae

(15 hours after topical application on prothoracic shield)

Amine acid identified	Rf value to phenol	Rf value to butanol	Lindane dosage per a caterpillar ppm. (by weight)			
			12.5	25.0	37.5	acetone control
leusine	0.690	0.681	+	+	+	+
phenylalanine	0.553	0.631	+	+	+	+
preline	0.652	0.557	+	+	+	+
valine	0.516	0.575	+	+	+	+
alanine	0.492	0.487	+	+	+	+
glycine	0.412	0.393	+	+	+	+
glutamic acid	0.314	0.407	+	+	+	+
sapartic acid	0.275	0.317	+	+	+	+
serine (?)	0.309	0 391	+	+	+	+
arginine	0.586	0.411	−	−	+	−
tyrosine	0.354	0.641	+	−	−	+(?)
histidine	0.553	0.342	+(?)	−	−	−
unknown 4.	0.317	0.635	−	+	+	−
unknown 5.	0.110	0.225	−	−	+	−
unknown 6.	0.718	0.134	−	−	−	+
unknown 7.	0.040	0.429	−	−	−	+

(c) Discussion.

The qualitative analysis of amino acid of haemolymph in lindane treated Papilio larva was carried out. In this experiment, though some miscellaneous changes existed, no significent variation was observed. As the pathological change in the amino acid in Bombyx larvae have been reported by Drilhon, Busnel and Vage (1951). For example, in flecherie the concentration of all the amino acids were said to decrease. In the lysis produced by the Grasserie virus, howerver, tryptophane was particularly abundant. In regard to this observation Drilholnetal claimed that tryptophane is never found in the normal Bombyx larvae. In the blood of DDT-treated Tenebrio molitor as Joseph (1958) menstioned, 70 percent increase of amino nitrogen is observed, and also eighteen amino acids were separated, and identified chromatographically with phenol butanolacetic acid. They were alanine, aspartic acid, cysteine, glycine, glutamic acid, histidine, isoleucine, lysine, methionine, norleucine, ornithine, phenylalanine, proline, serine, taurine threonine,

tyrosine, and valine, Most of the compounds increased as a result of DDT poisoning, but norleucine and taurine decreased. But in his experiment no qualitative change of amino acid was observed.

In regard to the quantitative determination of amino acid in lindane-treated insect further studies were remained to be done.

IV. REFERENCES CITED

BARBER, F. H. 1938.: An analysis of the blood of the sixth-instar southern army worm (Predenia). Jour. Agric. Res. 57:697-706.

BAILEY, K. 1942.: Myosin and adenssinetriphosphatase. Biochem. Jour. 36:121.

BEAHT, G. 1958.: Insect toxicologists information service N. I. Utrecht Netherland.

BISHOP, G. H. 1983.: Blood of anoney bee larvae. Jour. Bio. Chern. 58:543.

BROWING. T. O. 1954.: The evaporation of water from spiders. Jour. Exp. Biol. 31:331.

BUCK. J. B. and M. L. KEISTER, 1949.: Respiration and water less in the adult blowfly. Phermia Regina. and their relation to the Physilogical action of DDT. Biol. Bull. 97:64-81.

BUCK, J. B., M. L. KEISTER and I. POSNER, 1952.: Physiological effects of DDT on Phormia larvae. Ann. Ento. Soci. Ame. 45:369.

BUSTON, H., JACOBS. S., and GOLDSTEIN A. 1946.: Nature 154, 22. (In Yamamoto 1958).

CHANG, K. Y. (張廖貴祥) ○ 1953. 鷺尾鳳蝶生活史之研究 (Unpublished).

CLARK, E. W. 1958.: A review of literature on calcium and magnesium in insects. Ann. Ente. Soci. Ame. 51:142.

Doisy & Booklege, 1949.: in Yamazaki 1951.

Demeiller. 1947.: in Yamazaki 1951.

BREADEN, D., and B. J. Krigsman. 1948.: Surface and internal lethal dose of ensecticides. Bull. Ento. Res. 38:575.

DRILHON, A., and R. G. BUSNEL 1951.: Free aminsacids and fluerescent substances, blood and Malpighian tubes, silkwerm larvae. Comp. Rend. Acad. Sci. 232, 182.

FURGASH, A. 1958.: The effect of inositol on growth. Survival, and naturation in Periphaneta, americane (L). Ann. Ento. Soci. Ami. 51:406.

FRAENKEL, G. and M. BLEWETT. 1943.: a. Intracellular synbionts of insects as a source of vitamins. Nature. 152:506.

FRAENKEL, G. and M. BLEWETT. 1943.: b. The vitamin B. Complex requirements of several insects. Biochem. Jour. 37:686.

FRAENKEL, G. and M. BLEWETT. 1946.: The dietetico of the caterpillar of three Ephestia species, E. Knehniella, E. elutella, and E. cautella, and of a closely related spscies. Pledia interpunetella. Jour. Exptl. Biol. 22:162.

FRAENKEL, G. and BLEWETT 1946.: The dietetics of the clethes moth. Ibid. 22:156.

FRAENKEL, G. M. BLEWETT, and M. COLES, 1950.: The untrition of the meal worm. Tenebrio Melitov L. Physiol. Zool. 23:92.

FULLOR, R. C., BORRATT, R., TATUM, L., (1950).: J. Biol. Chem. 186, 823.

GORDON, H. T., and J. H. WELSH, 1948.: The role of ions in axon surface reactions to toxic organic compocmds. Jour. Cell. Comp. Physiol. 31:395-419.

HARTLEY, J. B. and A. W. A. BROWN 1955.: The effects of certain insecticides on the cholinesterase of the american occkroach. Jour. Econ. Ento.

HEWLETT, P. S. (1947). Ann. Appl. Biol. 34:357. (In Kitaoka. 1958).

HEILBRUNN, L. V. 1943.: An ontline of general physiology. W. B. Saunders Co. Phyladelphia.

HOUSE, H. L. and J. S. BARLOW. 1958.: Vitamin requirement of the house fly Musca domestica L. Ann. Ento. Soci. Ame. 51:299.

HUKADA, K., and K. MOVIYA, 1936.: On the tension development of calcium contracture in frog's muxole. Nagoya Jour. Med. Sci. 10:285-297.

INGRAM, R. L. 1955.: Water loss from insect treated with Pyrethrum. Ann. Ento. Soci. Ame. 48:481.
ISIBASHI, M., and T. FUZINAGA. (石橋雅義 藤永太一郎) 1956.: p. 314-316. Maruzen Inc.
JCSEPH, M. T. 1958.: Effects of DDT on the blood of Tenebrio Moliter. Ann. Ento. Soci. Ame. 51:554.
Kirkwood & Phillips.
KITAOKA, S. and A. YASHIMA. 1958.: マダニ類に對する殺蟲劑の效果及日本應用動物昆蟲學會誌 2:11
KUWANA Z. 1937.: Effedts of pyrethrins on many insects Zeit. angew. Ent. 18:344-352. (In Brown 1951).
LEPESME, P. 1937.: Penetration of culticle by arsenites comp. Rend. acad. Sci. 204:714.
LECHTENSTEIN, E. P. 1948.: Growth of culex molestus under sterile conditions. Nature. 162:227.
LOEB, J. 1901.: On an apparently new form of umsculer, irritability produced by solution of salts. Ame. Jour. Physiol. 5:362-373.
LUDWIG, D. 1946.: Effect of DDT on the metabolism of the Japanese bectle. Popillia japenica Newman. Ann. Ento. Soci. Ame. 38:496.
METCALF R. 1947.: J. Econ. Ento. 40:522. (In Yamamoto 1958).
MORI, H. 1941.: J. Coll Agr. Tokyo. 15:33. (In kitaoka. 1958).
HORRICON, P. E. and A. W. A. BROWN, 1954.: The effects of insecticides on cytochrome exidase obtained from the american cockreach. Jour. Econ. Ente. 47:723.
NAKAZIMA, KIEKA. KATSUMURA. 1949.: 防蟲科學 13, 14. (In Isibashi 1956).
NEWTON, O. J. 1954.: Effect of starvation on the composition of Japanese beetle larvae. Physiol. Aool. 27:248.
NOLS, S. J. L., J. H. LILLY, and C. A. BANMANN. 1949.: Vitamin requirements of the cockroach Blatella germanica (L). Ann. Ento. Soci. Ame. 42:154.
OVERTON. 1904.: Beitrage zur allegemeinen muskel und nervenphysiologie. Pfliigeis Arch. Ges. Physiol. 105; 179-290. (In chark 1958).
REISER, R., CHADBOURNE, D. S., KNIBEN, K. A., RAINWATEN, C. F., and E. E. Ivy. 1953.: Variation in lipid content of the boll weevil and seasonal variation in its resistance to insecticides. Jour. Econ. Ento. 46:337.
ROEDER, K. D. 1953. Insect physiology John. wiley. & Sons. INC.
SCHALTZ, J. P., ST LAWRENCE and D. NEW MEYER. 1946.: A. chemically defined medium for the growth of Drosophila melanogaster. Anat. Rec. 96:540.
SLADE 1946.: (IN Yamazaki 1951).
STELLWAAWG, F., and T. STANDENMAYER 1940.: Cuticular penetration of DNOC. Anz. Schiidlingski, 16; 37-39.
THORPE & DE MEILLON 1947.
TIRNNARAYANAN, M. SARMA, P. S., 1953.: Jour. Sci. Ind. Research. 123, 251. (In Yamamoto 1958).
UEDO, and YAMAMOTO. 1950.
WACKER, L. 1929.: Zur kenntnis der Vorgange bei der Arbeit und Ermiding des Muskels. (Zunahne der Mg und Ca un blute). Klin. Wockenschr 8:244. (In chark 1958).
WEISE, E. 1934.: Untersuchungen zur Frage der Verteilung und der Bindungsart des Calcium in Muskel. Arch. F. Exptl. Path. Pharm. 176:367-377. (In chark 1958).
WELSH, J. H., and H. T. GSRDON 1947.: The mode of action of certain insecticide on the arthroped nerve axon. Jour. Cell. Comp. Physiol. 50:147.
WIGGLEAWORTH, V. B. 1941.: Effect of pyrethrum on the spiaacular mechanism of insects. Proc. Roy. Ent. Soci. Lond. 16:11.
YAMAMOTO, L. 1956.: 新農藥研究法 (南江堂) p. 236-238.
YAMAZAI ISII. (unpublished).
YAMAZAKI, T. 1951.: 26. 1. 119-23.
YANAGISAWA, F., 1955.: New colorimetric determination of calcium and magnesium. Jour. Biocheme. 42:3-10.

BHC對鳳蝶幼蟲血淋巴之影響

繆端生　朱耀沂

中文摘要

BHC 爲神經性藥劑，一般試驗其對於神經系統之影響，對於循環系統則尚少研究。現以 BHC 處理鳳蝶幼蟲，然後採取其血淋巴，加以分析，藉以明白血淋巴有何變化。本研究之重點爲：血淋巴之含水量，血球數，氧消費量，pH 值，血鈣，血糖，及氨基酸等。其中血球及血糖顯見減少，氧氣消費量顯見增加，pH 值無變化，血鈣無顯著變化，含水量略減少，氨基酸之種類稍有消長。