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Biochemical studies on the haemolymph and heart muscle of normal and insecticide treated cockroach *Periplaneta americana* L.

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Abstract. In this paper quantitative estimations of free proteins, total carbohydrates/glycogen and fatty acids from cockroach haemolymph and heart muscle are reported from normal and insecticide treated insects. High content of protein, carbohydrate/glycogen and fatty acids are found in the haemolymph and heart muscle of nymphal insects. Higher amount of carbohydrates/glycogen are found in adult males, while more protein and fatty acids were found in the females. After insecticide treatment, no sex variation was found in the percent depletion of metabolites. The difference in the depletion rates between nymph and adult was also insignificant. High percent depletion of the macro-molecules was found with insecticides which are found more toxic in bioassay studies. A correlation has been made between the rate of depeltion and insecticidal poisoning.

Keywords. Insecticide ; haemolymph ; heart muscle ; carbohydrates ; glycogen proteins ; fatty acids.

1. Introduction

It is generally known that, insecticides interfere with the physiology of insect nervous system, particularly with the nerve conduction mechanism. However, with the lack of sufficient data the ultimate causes of death in insects are usually difficult to prove.

Information on carbohydrate, protein and lipid levels from various tissues of insect is scanty as compared to vertebrates. Only in recent years, insect blood has been studied for both normal and after treatment with some insecticides, especially the chlorinated hydrocarbons (Corrigan and Kearns 1963 ; Hawkins and Sternburg 1964). These studies are limited since the observations were only made on isolated fractions such as amino acids, free sugars or total lipids.

Despite years of research (Jones 1974 ; Florkin and Jeuniaux 1974), limited information is available on the changes in heart-beat and much less on heart muscle due to insecticide action.

The present studies were undertaken to find out the effect of certain insecticides on basic metabolite content of cockroach haemolymph and heart muscle and its significance in the poisoning of the insects. A description of the quantitative variations in the total carbohydrates, proteins and fatty acids from the haemolymph and heart muscle of the last instar nymph and adult cockroach, *Periplaneta americana* L. are given. The effects of a plant extract 'Morindin' reported to be toxic to insects (Surender Reddy *et al* 1978) is also mentioned.

2. Material and methods

The test insects *Periplaneta* were collected and developed at room temperature in glass cages with a wire mesh lid at the top. A layer of sawdust was laid on the floor of the cage for the deposition of oothecae. Once in a week the oothecae deposited were separated to another cage for hatching. The insects were fed on glucose biscuits mixed with yeast powder and potato peels. The following insecticides were used.

Fenitrothion : O,O-dimethyl-o-(3-methyl-4-nitrophenyl)-thionophosphate (Baeyer India Limited, Bombay) ; Carbofuran : 2,3-dihydro-2,2-dimethyl-7-Benzofuranyl methyl carbamate (Rallis India Limited, Bangalore) ; Ekalux : 25% (w/w) Quinolphos (O,o-diethyl-o (quinoxalinyl-(2) Thionophosphate) and 75% (w/w) stabilizers, emulsifiers and other adjuvants (Sandoz India Limited, Bombay) ; Morindin : The glycoside morindin 6-primeveroside of morindone ($C_{26}H_{28}O_{14}$, 1,5,6-trihydroxy 2-methyl anthraquinone) has been extracted and purified from the root bark of *Morinda tinctoria* var. *tomentosa* Hook. as described by Rao and Reddy (1977) ; Nicotine : Manufactured by E Merck, Dermstadt, Germany. The insecticides were dissolved in ethyl alcohol and insects were treated intraperitoneally (Menusan 1948) with the help of an Agla micrometer syringe. Insects to be treated were weighed individually and the dosage was calculated per gram (5 μl/g) of the body weight. Last instar nymphs of developing wing base were selected to maintain uniformity of age. Adults belong to the age group of 1–4 days after molt. After determining the lethal dosages, one producing 50% kill (LC_{50}) was selected for the present experiments and the insects were taken for biochemical estimations 4–6 hours after treatment. This particular time lag was chosen because the initial symptoms of poisoning such as hyperactivity and convulsions were complete in less than 4 hr, thus the insects were with complete knock down effect. Besides the normal insects, control insects were taken 4–6 hr, after treatment with ethyl alcohol (5 μl/g).

Haemolymph was collected according to the method described by Sternburg and Corrigan (1959) and the haemocytes were not allowed to sediment. For cardiac muscle, insect heart was fully exposed with specially made needles and carefully separated from the alary muscles throughout the length, then it was gradually lifted on to a cover slip to weigh it gently before transferring into the test tube. Care was taken to eliminate all foreign tissue associated with heart, including the alary muscles and fat content.

To estimate the total carbohydrates from haemolymph and glycogen from heart muscle, the modified anthrone method of Klicpera *et al* (1957) was adopted. For proteins the procedure of Lowery *et al* (1951) and for total/esterified fatty acids, the methods of Stern and Shapiro (1953) were followed.

3. Results

The values of total carbohydrates/glycogen, total proteins and fatty acids recorded from the normal nymphs and adult cockroaches of both sexes are mentioned in tables 1 and 2. It may be seen that, the three constituents of haemolymph of adult insects are relatively lower than those of nymphs. Haemolymph from nymphal cockroach shows about 15–20% more of carbohydrates, proteins and fatty acids. Among the adults, males show 15% more carbohydrates than females, while females possess 18% more proteins and 27% more fatty acids than males. In the heart muscle also it is observed that, nymphs of both the sexes possess relatively higher values of glycogen (25–30%), proteins (13–20%), and fatty acids (17–23%) as compared with adults. In adult cockroaches, glycogen content of heart is about 20% more in males while proteins and fatty acids are about 18% and 20% higher in females. In the control insects treated with ethyl alcohol slight decrease in the total content was seen as compared to normal.

In haemolymph, the percent depletion caused due to fenitrothion treatment in relation to control values were 33–38% in proteins, 22–24% carbohydrates and 20–39% fatty acids. With carbofuran, 52–60% proteins, 23–40% carbohydrates and 33–42% fatty acids, which is considered to be highly significant. After ekalux treatment, 20–30% of proteins, 14–42% of carbohydrates and 11–17% of fatty acids were found to be depleted. After morindin application, 15–26% proteins, 11–28% carbohydrates and 8–28% fatty acids were found to be depleted. Similarly, nicotine caused 17–20%, 10–15% and 5–14% depletion in proteins, carbohydrates and fatty acids respectively (table 1). The concentration of insecticides employed are given in the table.

In heart muscle, the percent depletion observed after fenitrothion treatment in the nymphal and adult cockroaches of both sexes were glycogen 24–33%, proteins 18–21% and fatty acids 17–38%. After carbofuran treatment, glycogen 29–35%, proteins 24–47% and fatty acids 24–40%. After ekalux, glycogen 20–26%, proteins 11–17% and fatty acies 10–18% were found to be depleted. With nicotine treatment, glycogen 10–16%, proteins 2–6% and fatty acids 4–8%, while with morindin, glycogen 12–18%, proteins 4–10% and fatty acids 5–16% were depleted (table 2).

4. Discussion

Quantitative studies on the total proteins, carbohydrates and fatty acids from the haemolymph of normal cockroach give a general indication of higher content in the nymphs than adults. It is suggested that the initial high values of protein and aminoacids in the young ones and the rapid fall during adult stage was associated with the completion of maturation processes involving protein synthesis during the first few days after the final moult (Nowosielski and Patton 1965). Relatively lower content of fatty acids and carbohydrates in adults, as found in the present study, can be attributed to the higher rate of metabolism during metamorphosis (Weis-Fogh 1952 ; Guthrie and Tindall 1968). High amounts of total proteins and fatty acids found in female cockroaches are in agreement with the findings of Nath *et al* (1958) and Anderson (1964).

As in case of haemolymph, the normal values of glycogen, total proteins and fatty acid contents from the cardiac muscle of *P. americana* (alary muscles

Table 1. Effect of different insecticides on carbohydrates, proteins and fatty acids of cockroach haemolymph.

Content		Normal std. error	Control	Fenitro-thion	Carbo-furan	Ekalux	Nicotine	Morindin
				*N 0·2 A 1·0	0·05 0·06	0·2 0·4	0·25 0·75	0·15 0·25
Total carbohydrates	N	1070±39	1020±50	780±38	700±38	840±32	910±21	900±23
	A	910±47	850±70	620±62	650±62	570±78	720±40	610±50
	N	920±32	870±25	680±29	630±36	740±16	760±29	730±26
	A	770±65	750±40	510±49	440±18	430±19	700±25	537±24
Proteins	N	1000±39	900±50	600±26	360±19	720±26	740±24	760±17
	A	840±53	800±60	500±60	320±77	560±77	640±112	600±77
	N	1200±33	1100±30	700±29	520±27	820±30	880±16	900±29
	A	1020±98	980±60	600±50	440±20	700±77	780±101	720±76
Fatty acids	N	300±19	280±25	190±17	160±26	230±27	240±26	200±26
	A	240±34	210±30	160±20	140±22	185±18	195±19	180±21
	N	400±23	380±15	280±24	230±28	320±21	360±18	340±20
	A	330±32	300±25	240±19	200±21	260±20	285±28	275±44

Values represent the average of 20 individuals, expressed in mg per 100 ml haemolymph.
N : nymph ; A adult ; * concentration of insecticides expressed in μg/insect.

Table 2. Effect of different insecticides on glycogen, proteins and fatty acids of cockroach heart muscle.

Content		Normal std. error	Control	Fenitro-thion	Carbo-furan	Ekalux	Nicotine	Morindin
				*N 0·2 A 1·0	0·05 0·06	0·2 0·4	0·25 0·75	0·15 0·25
Glycogen	N	2580±104	2500±95	1720±89	1630±92	1840±93	2100±78	2050±96
	A	1918±80	1900±80	1470±92	1350±101	1520±98	1700±98	1658±101
	N	2270±128	2220±100	1640±82	1560±86	1720±89	1917±89	1888±89
	A	1520±87	1470±100	1060±78	980±72	1140±51	1305±49	1250±82
Proteins	N	2826±97	2760±90	2268±101	2089±98	2448±78	2695±79	2640±106
	A	2250±110	2200±100	1726±89	1608±90	1833±104	2149±48	2043±89
	N	3168±118	3090±115	2452±87	2286±119	2620±87	2896±112	2768±122
	A	2742±120	2675±110	2160±88	1920±92	2280±86	2509±88	2390±124
Fatty acids	N	680±78	610±72	505±42	463±46	562±32	584±40	575±38
	A	520±50	480±65	388±36	320±39	409±29	440±20	428±30
	N	780±70	750±60	564±72	502±42	610±84	706±88	628±92
	A	648±82	600±62	450±42	390±40	504±42	560±46	520±49

Values represent the average of 20 individuals, expressed in μg per 100 mg w.w. of muscle.
N : nymph ; A : adult ; * concentration of insecticides expressed in μg/insect.

excluded) also show an increase in nymphs. A similar decrease in the glycogen content of adult locust muscles was observed by Chari (1970). A decrease in the content of metabolites of adult cockroach heart muscle (observed in the present studies) appear to be due to their utilization during metamorphosis from young to adult, as it was emphasized by Rockstein (1964).

It has been observed that sexual variation is higher than the influence of age on the concentration of the basic constituents. Males have high amount of carbohydrate/glycogen while females have higher amount of protein and fatty acids. This is true for the heamolymph as well as for the heart muscle. Such similarity in the metabolite ratios between the insect haemolymph and heart muscle reveals, perhaps, their physiological association. It is well known that the heamolymph, having a number of reserve transport material, constantly circulates between the dorsal tubular heart and body cavity. The heart is a connective tissue, pulsating and pumping the blood which enters it through the ostia and is emptied through the dorsal aorta.

In the insects treated with insecticides, haemolymph proteins were depleted the most, followed by carbohydrates and fatty acids. While in heart muscle, the difference in the percent depletion of three metabolites was however not significant. This is applicable for nymphs and adults of both sexes. More percent depletion was observed with carbofuran followed by fenitrothion > ekalux > morindin > nicotine. In general, the degree of percent depletion found in the three metabolites do not vary much between one another. However, the percent depletion noted in blood proteins is found to be significantly higher.

As in case of vertebrates, binding of insecticides both to cellular components and soluble proteins in insects is suggested by Olson (1973). The small and insignificant difference found in macromolecule content of control insects may be attributed to the dilution of heamolymph after solvent treatment.

Relatively low depletion of proteins, carbohydrates/glycogen and fatty acids found with nicotine is in agreement with its low toxicity in the bio-assay studies (Surender Reddy 1979). This may be attributed to quick metabolism and excretion of nicotine from the insect body. Extensive metabolism of nicotine when fed to grasshoppers or applied topically to house-flies was observed by Self *et al* (1964). With tobacco hornworm it is reported that 90% of oral dose of nicotine was excreted in about 4 hr while 83% of nicotine injected into the body cavity was seen in feces in about 15 min (Self *et al* 1964).

From the present studies it appears that, besides the target tissue like central nervous system, susceptibility of insects to an insecticide will also be necessarily accompanied by biochemical variations in other vital tissues, proportionate to the toxicity of the substance. A similar conclusion was drawn by Mansingh (1965) in his studies with *Blatella germanica*. It is also corroborated by the opinion of other workers (Hollingworth 1976), that besides acetylcholinesterase there exist other targets in the insecticide poisoning of insects.

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