

TABLE I

Effect of chlormadinone acetate for 40 days on the weight and cholesterol content of the adrenal of the male rat 6/group, mean value \pm S.E.M.

Group	Total weight (mg)	Total cholesterol (mg/gm tissue)
Vehicle control	14.15 \pm 1.09	15.3 \pm 1.59
Chlormadinone acetate:		
0.2 mg/day/rat	13.52 \pm 1.23	19.3 \pm 2.13
0.5 mg/day/rat	10.63 \pm 0.45*	31.6 \pm 2.57**
1.0 mg/day/rat	10.47 \pm 1.01*	37.9 \pm 3.27***

Significantly different compared with control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

to surmise whether the inhibitory action of chlormadinone acetate on the rat adrenal is direct or indirect. It may be mentioned that increasing doses of chlormadinone acetate which were employed in this experiment also caused a complimentary dose-dependent decrease in the weight and gonadotropin content of the pituitary along with gonadal function⁷. A similar type of mediation of action of chlormadinone acetate through the hypophyseal-adrenal axis can be conjectured although on a purely hypothetical basis.

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EXPOSURE OF WHITE MICE TO *BACULOVIRUS AMSACTA* OF GROUNDNUT RED-HAIRY CATERPILLAR, *AMSACTA ALBISTRIGA* (WALKER)

RECENT interest in the use of the nuclear polyhedrosis virus (NPV) against the groundnut red-hairy caterpillar, *Amsacta albistriga*^{1,2} as a possible microbial agent, necessitates to establish whether this virus is pathogenic and/or toxicogenic to other non-target animals. More evidence is being accumulated on the specificity of insect polyhedrosis viruses and their safety to vertebrates³. In India, though the usefulness of NPV of *A. albistriga* has been reported by Jayaraj *et al.*^{1,2} there has been no study on its safety against the other non-target animals except the recent study of Narayanan *et al.*^{4,5} in which the authors have established the innocuous nature of this virus to common carp, *Cyprinus carpio* and to poultry birds. The present report is the result of a similar study of the possible toxicity and/or pathogenicity of NPV of *A. albistriga* to white mice.

The polyhedral inclusion bodies were processed from dead, final instar larvae of *A. albistriga* by differential centrifugation and the final concentration was characterised as to the number of PIBs/ml. To find out the bacterial contamination of the virus preparation, differential bacterial-plate counts were made. The total number of bacterial cells present was estimated by pour plate technique using Difco Nutrient Agar and the presence or absence of coliforms was detected using Mac-Conkey's agar medium. The number of viable spores present was estimated by pasteurization technique. The acute toxicity/pathogenicity evaluation tests were carried out by following standard methods⁶.

Two to four weeks old and randomly mated non-inbred lines of white mice (average weight 26.1 g; range 21.4 to 28.6 g) were used in the study. An oral administration of 0.01 ml of virus suspension was given using a 1 ml tuberculin syringe fitted with 5 cm tiffon sleeve of 0.5 mm dia to each of five male and five female mice. The virus suspension was found to contain 1.9 to 2.5 $\times 10^8$ bacterial cells, 2.1 $\times 10^9$ bacterial spores and no coliforms. The dose fed to each mouse was more than 100 times the average field dose/acre with a conversion ratio of the weight of test animal to weight of man. In addition, ten mice, five in each sex, were used as untreated control, receiving 0.01 ml of sterile distilled water. The animals were maintained in separate cages and standard commercial food material along with sliced carrots and water

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Results of tests in which white mice were exposed to the nuclear polyhedrosis virus of Amsacta albistriga

Observations	Treated		Control	
	Male	Female	Male	Female
Initial mean weight of animals (g)	28.6	27.0	27.6	21.4
Final mean weight of animals (g)	30.8	31.8	30.0	26.2
Mean weight gain	2.2	4.8	2.4	4.8 NS
Body mean temperature	37.6° C		37.4° C	
Blood glucose (mg/100 ml)	140.0		160.0	
Haemoglobin (g/100 ml)	8.2		8.4	
Haematocrit value (%)	49.0		45.0	
Serum alkaline phosphate m. moles/100 ml/min.	9.5		7.5	
Organs wet-weight (mg)				
Heart	323		354	
Lungs	550		471	
Liver	1768		2012	
Kidney	772		645	
Spleen	352		413	
Ovaries	238		240	
Testes	400		420	

NS = Non-significant.

were supplied *ad libitum*. The test was terminated after 21 days.

Observations on the general conditions of the test animals, and bio-chemical, necropsy and histopathological studies were made to evaluate the effects of the virus. The mice were examined daily for general appearance and behaviour. Body weight and temperature records were taken at weekly intervals until the test was terminated. At the end of the test period, animals were etherised and blood withdrawn by cardiac puncture. Potassium oxalate was used to prevent coagulation. Blood glucose was estimated according to the methods of Somogyi⁷. Serum alkaline phosphatase enzyme was determined using M/100 disodium

phenyl phosphate as the substrate with carbonate-bicarbonate buffer (pH 9.2)⁸. The packed cell volume or the haematocrit value was obtained using the Wintrobe haematocrit tubes and blood haemoglobin was estimated using Sahlis' haemoglobinometer⁹. At the end of the test period, the animals were dissected. Various organs for gross-pathology, and wet-weight of the important organs like heart, liver, lungs, spleen, kidney and gonads were examined and recorded. Histopathological observations were also conducted on the above organs following the standard microtome procedures.

The above tests yielded the following results. No death occurred in any of the virus-fed or water-fed

animals during the period of the observation. However, one male animal both in treated and water-fed died on 4th and 3rd day respectively. The reason for the death was ascribed to be due to the severe fighting which had occurred when these male animals were put in groups due to the presence of "Bully". The animals were severely mauled and bitten, especially at the back, tail and leg regions. After that these animals were kept in separate cages. All animals were found healthy and there was no change in their bodily appearance, behaviour and feeding activity. Temperature was found normal during the entire test period in both the groups of animals. Body weight gained in each sex of virus treated mice was essentially similar to those of the control. In addition, no difference due to virus treatment could be detected in blood sugar, haemoglobin content and haematocrit value and serum alkaline phosphatase enzyme activity (Table I). Post-mortem examinations indicated no gross pathology in either group. The average weight of each important organ (Table I) did not vary much. Histological examinations of all the tissues examined showed no evidence of tissue damage and they were similar to healthy animals.

Safety of similar insect viruses has been reported earlier in the case of white mice, guinea pigs¹⁰, various mammals, fishes³, chicks¹¹ and human beings¹². Further, the specificity of *A. albistriga* NPV has also been reported when it was tested against 15 species of lepidopteran insects and found to be non-infective¹³. The result of the present study as well as those previously documented for other vertebrates and invertebrates with different insect inclusion viruses, indicate their safety and they are apparently host specific. Hence our study on *Baculovirus amsacta* in mice further corroborates the high specificity and its safety against white mice and its intended use as a viral insecticide.

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ADULT PIGMENTATION AS AN INDEX OF ENDOCRINE ACTIVITY IN THE CRICKET, *GRYLLUS BIMACULATUS*

THE cricket *G. bimaculatus* exhibits orange-yellow/black colour polymorphism. In laboratory population, the females are normally dark with black pigments but males are light coloured with orange-yellow pigments^{1, 2}. Further, pigmentation is found to be influenced by the corpora allata hormone. While allatectomy resulted in dark colouration, implantation of corpora allata (CA) led to lighter cuticle². Following observations were made in this laboratory.

Topical application of a synthetic juvenile hormone analogue (JHA) (RO-20-3600, Hoffman-La Roche) to the last instar nymphs 96 hr prior to metamorphosis resulted in lighter coloured adults. These results, being statistically significant, support the view that more of juvenile hormone (or active CA) will favour lighter colouration. Isolated rearing of nymphs retarded nymphal growth and development as noted for *P. guttiventris*³. Further, male adults were significantly dark coloured as compared with their counterparts reared in groups. On the basis of earlier results, if endocrine activity is to be correlated with pigmentation, CA in group reared nymphs might be secreting higher level of hormone at least during sclerotisation. However, this indirect evidence does not agree with Gona's supposition⁴ that CA might be hyperactive in isolated crickets.

Injection of 1 and 10 μ g of thiotepa into the last instar male nymphs resulted in the appearance