(TFFPS, which include free amino acids) estimated in TCA supernatants using Folin-Ciocalteu reagent in conjugation with cobalt reagent[9] showed increases in weight specific as well as holo-histontic levels in the 'molted' muscle. The granular and more fluid consistency of the molted muscle, the decrease of total protein content and increase of TFFPS (which include free amino acids) at the holo-histontic level are reasonable indications of atrophy of the muscle associated with molting.

The tissues predominantly concerned during crustacean ecdysis are blood, hepatopancreas and integument[10]. Nevertheless the muscle also undergoes certain changes. Extensive histolysis of the large chela muscle of *Gecarcinus lateralis* has been reported. This involves considerable loss of muscle protein[12]. The data presented here for *H. fulvipes* muscle involving decrease of total protein at holo-histontic level may be due to such histolytic process.

The degradation of the chela muscle tissue in *G. lateralis* has been suggested to aid in the removal of large muscle mass through narrow basal joint during ecdysis. It could be the same in scorpion.

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A COMPARISON OF THE EFFECT OF CYCLOHEXIMIDE TREATMENT ON THE ANTIVIRAL ACTIVITY OF DIFFERENT TYPES OF INTERFERON INDUCERS IN MICE

INTERFERON induction and antiviral activity of Newcastle disease virus (NDV)[1,2] have already been reported. Tests with 6–MFA, an antiviral agent from *Aspergillus flavus*. DU/KR/162 *b* (now re-identified by Dr. D. I. Fennell, NRRL, Peoria, Illinois, U.S.A. as *Aspergillus ochraceus*, Personal communication) showed that mice treated with 6–MFA produce serum and tissue interferon (unpublished data) and also develop anti-Semliki Forest virus resistance[3-5]. A subculture of the fungus has been deposited with the American Type Culture Collection (ATCC 28706).

Evidence over the past few years has accumulated to show that interferon production stimulated by viral or nonviral inducers can significantly be enhanced by cycloheximide treatment in animals[6] as well as in cultured tissue cells[7-8]. Because of a possible clinical significance of this, we have compared the inducing capacity of antiviral resistance of 6–MFA in mice with that of NDV in the presence of cycloheximide and have noticed important differences.

Thirty-five day old Swiss-CDRI mice of either sex weighing 16–18 g were used in the present experiments. Semliki Forest Viruh (SFV) was procured from the ATCC and passaged intracerebrally (i.c.) in the 35 day old mice. A 10% brain homogenate was prepared in Hanks' balanced salt solution, which formed the stock pool and maintained in aliquots at $-20°$ C. The strain of neurovaccinia virus was procured from the ATCC and maintained similarly. The LD_{50} titres of both viruses were calculated by the Reed and Muench method[9], on the basis of ten-fold dilutions of inocula.

The egg-adapted vaccine (allantoic fluid) strain of NDV was obtained from the Animal Husbandry Department, Lucknow. 6–MFA was prepared by acetone treatment of the crude filtrate of *A. ochraceus* as described earlier[3]. Cycloheximide was

TABLE I

Proportion of mice surviving vaccinia/SFV challenge, following injection with two inducers (6–MFA or NDV) in presence of cycloheximide

Treatment	Interferon Inducers							
	NDV (410 HA units/0·2 ml)				6–MFA			
	IHD CHALLENGE		SFV CHALLENGE		IHD CHALLENGE		SFV CHALLENGE	
	Survivors*	Survival (%)	Survivors	Survival (%)	Survivors	Survival (%)	Survivors	Survival (%)
Cycloheximide** 1 *hr before* inducer	0/10	0	9/18	50	2/8	2	1/10	10
Cycloheximide *along with* inducer	3/9	33	0/18	0	0/10	0	2/9	22
Cycloheximide 1 *hr after* inducer	0/10	0	6/18	33	1/10	10	4/10	40
Only Cycloheximide) Controls	0/10	0	2/10	20	0/10	0	0/10	0
Only Inducer (Controls)	0/10	0	5/10	5/10	2/8	25****	0/10***	0
Only PBS+ (Controls)	0/10	0	5/10	50	0/10	0	0/10	0

* Mice surviving/total number.
** Cycloheximide injected at the rate of 1 mg/mouse.
*** 6–MFA injected at the rate of 0·1 mg/mouse.
**** 6–MFA injected at the rate of 1·0 mg/mouse.
\+ Phosphate buffer saline.

purchased from the Upjohn Company, Kalamazoo, Michigan, U.S.A. It was given at the rate of 1 mg/mouse i.p.

Mice were injected with sub-protective doses of 6–MFA or NDV (R_2B strain) by the intraperitoneal (i.p.) route. Cycloheximide was given 1 h. *before,* simultaneously, or 1 h. *after* the inducer. Twenty-four hours following the administration of the inducer, all the animals were challenged with SFV, subcutaneously (s.c.), or with IHD vaccinia virus by the i.c. route. Animals were observed for 14 days for specific symptoms of encephalitis and death. The percentage of mice surviving fatal virus challenge was calculated in terms of the control.

Results presented in Table I showed that with NDV (inducer) given to mice at a subeffective dose (410 HA units/0·2 ml) that confers *no* protection to challenge fatal neurovaccinia virus (100 LD_{50}, 0·03 ml, i.c.), greater protection (33%) of the animals was obtained when cycloheximide was given *along with* the inducer. However, with 6–MFA as the inducer, a *depressive effect* (lowered protection) of cycloheximide was noticed : 6–MFA, at a dose of 1 mg/mouse, protected only 25% of the animals challenged with a lethal dose (100 LD_{50}, 0·03 ml, i.c.) of IHD virus ; the protection rate fell to zero, if cycloheximide was administered along with 6–MFA.

NDV given at a rate of 410 HA units/0·2 ml per mouse did not protect any animal challenged with a low dose (1 LD_{50}, 0·5 ml s.c.) of SFV which itself caused only 50% mortality in the untreated control. If cycloheximide was given *along with* NDV, the protection rate fell to zero. However, with 6–MFA as the inducer, injection of cycloheximide *enhanced* the antiviral activity (10 to 40%) of a dose of 6–MFA (0·1 mg/mouse), which only increased the mean survival time of the mice against a lethal challenge with SFV (100 LD_{50}, 0·5 ml, s.c.).

Thus, the effect of cycloheximide in increasing or decreasing the antiviral activity of interferon-inducing agents is here seen to vary not only with the inducer used, but also with the challenge virus employed. The time of administration of cycloheximide also plays an important role in connection with the protection level.

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SUSCEPTIBILITY OF MOUSE PERITONEAL MACROPHAGES TO INFECTION WITH ARBOVIRUSES

MACROPHAGE cultures from normal mice support the growth of mouse hepatitis viruses[2-4] and peritoneal macrophages (PM) from ectromelia-immune[1] mice support the growth of ectromelia viruses. Two arboviruses, *viz.*, West Nile (WN) and Yellow Fever (YF) have been shown to grow in mouse PM[5]. We report the results of susceptibility studies of mouse PM to thirteen different arboviruses isolated in India.

In the preliminary experiments, two ml of 2% starch[5] (in normal saline) was injected into mice by the intraperitoneal (IP) route (on the previous day) to activate the macrophages. In further experiments no such activator was used, because the yield of PM was adequate without its use. Three ml of sterile Hanks' balanced salt solution[6] was injected IP into 8–10 week old male Swiss albino mice and after kneading the abdomen gently, the IP fluid from each mouse was collected in a culture tube. After two hours of incubation at 37° C, saline was removed and the tubes fed with medium M 199 (Earle's), supplemented with 20% goat serum, loosely stoppered and incubated at 37° C in 5% CO_2 atmosphere[6]. After 24 hours, tubes with well-spread macrophages were selected and washed with the medium, tightly stoppered and incubated at 37° C for 48 hours. For the experiment, twenty tubes were selected and taken for inoculation of one virus. The viruses employed (Table I) were diluted in M 199 to contain approximately 3–4 dex[7] of infant mouse LD_{50} or $TCID_{50}$ virus and the tubes were inoculated with 0·1 ml of virus suspension per tube.

TABLE I

Antigenic group	Name of the virus, abbreviation strain number and passage history
Group A	Sindbis (SIN) (AR 339) M_5V_2; Chikungunya (CHIK) (634029) $M_{11}V_1$
Group B	Japanese encephalitis (JE) (P 20778) M_9; Kyasanur Forest disease virus (KFD) (P 9605) M_{16}; West Nile (WN)* (E 101) M_5V_2
VSV	Chandipura (CHP) (653514) $M_{22}V_1$
Kaisodi	Kaisodi (KSO) (G 14132) M_{43}
Bunyamwera	Batai (BAT) (G 20217) $M_{12}V_1$
Nairobi Sheep Disease	Ganjam (GAN)+ (G 619) M_5BH_1
Simbu	Ingwavuma (ING) (633970) M_7BH_1; Sathuperi (SAT) (G 11155) M_{13}
Ungrouped	Wanowrie (WAN) (G 700) M_{19}; Bhanja (BHA) (G 690) M_5BH_1

* Not isolated at Virus Research Centre.
+Renamed Nairobi Sheep Disease (NSD) since 1975.
M = Suckling mouse brain passage.
V = Vero passage.
BH = Baby hamster kidney (BHK-21) passage.

After adsorbing the virus for one hour at 37° C, the tubes were washed five times with neutralized Hanks' BSS and fed with M 199 with 10% goat serum and incubated at 37° C.

The tubes were observed daily for cytopathic effect (CPE) upto ten days. On the zero, third, seventh and tenth days of post-inoculation (PI) about 4–5 tubes were taken out and stored at − 50° C for a maximum period of one week. The tubes were subjected to three cycles of freezing and thawing and centrifuged at 250 g for 10 mins. The supernatant fluid was assayed in 2–3 day old infant mice by the intracerebral (IC) route. The sick mice were harvested and 10% brain suspension prepared in normal saline was tested by the quick complement fixation test[8] for identification of the viruses.