

PN-AMU-757

5600

~~6.5.75~~
CS-144

Progress Report

US - Israel CDR - Grant: DPE-5544-G-SS-6031-00

by

Marvin Edelman

Period: February - July, 1987

1. Dr. N. Kislav from the Israeli team visited the MBITA field point station laboratory in Kenya in February 1987 to survey facilities and equipments, and to discuss project details with Dr. Otieno and Dr. Odindo. Plans and methods for conducting experiments for the first year in Kenya were decided. These include: a) Survey collection of diseased larvae and blood samples from *S. exempta* and *S. littoralis*. b) Procedures, facilities and technical help for growth and maintenance of healthy stock of larvae (*S. exempta*, *S. littoralis*) for bioassays and for amassing viruses for production. c) Experimental designs for a comparative bioassays of virulence of *S. exempta* NPV and *S. littoralis* NPV against host and heterologous hosts.
2. Dr. Kislav also: (a) initiated with Kenyan workers a healthy *S. exempta* colony from MBITA; (b) initiated field survey collection of diseased larvae and blood samples; (c) explained and demonstrated methods for initiation of insect cell cultures from eggs and ovaries. Ovaries were dissected from *S. exempta* pupae by Dr. M. Odindo, and a few samples of tissue (and egg) particulates in insect cell media were brought to Israel.
3. In Israel, attempts are in progress to keep and grow cell cultures and viruses from the material brought from Kenya. The range of concentration of cystein needed to prevent blackening of samples has been determined.
4. In preparation for cloning of SIMNPV DNA in plasmids, (as well for other bioassays) polyhedra were isolated from frozen, diseased *S. littoralis* larvae and purified on sucrose gradients. Alkali liberated viruses were purified on sucrose gradients, and DNA extracted from a portion of the samples. Cloning experiments in pEMBL and pUC plasmids are now in progress.

5. A research student, Mr. Oduol, is currently receiving training at the MBITA field station in Kenya. After review of his record, he has been provisionally accepted for studies at the Feinberg Graduate School of the Weizmann Institute of Science, Israel. Mr. Oduol will start conducting his advanced studies at FGS and research in my laborarory, in March 1988.

XN-AH2-7/80/19
CS-144

First Year Report - CDR. - February 1988

Dr. N. Kislev from the Israeli team visited the MBITA field point station laboratory in Kenya in February 1987 to survey facilities and equipments, and to discuss project details with Dr. Otieno and Dr. Odindo. Plans and methods for conducting experiments for the first year in Kenya were decided. These include: a) Survey collection of diseased larvae and blood samples from *S. exempta* and *S. littoralis*. b) Procedures, facilities and technical help for growth and maintenance of healthy stock of larvae (*S. exempta*, *S. littoralis*) for bioassays and for amassing viruses for production. c) Experimental designs for a comparative bioassays of virulence of *S. exempta* NPV and *S. littoralis* NPV against host and heterologous hosts.

Dr. Kislev also: (a) initiated with Kenyan workers a healthy *S. exempta* colony from MBITA; (b) initiated field survey collection of diseased larvae and blood samples; (c) explained and demonstrated methods for initiation of insect cell cultures from eggs and ovaries. Ovaries were dissected from *S. exempta* pupae by Dr. M. Odindo, and a few samples of tissue (and egg) particulates in insect cell media were brought to Israel. We have now found that viral material brought from Kenya to Israel can be maintained in *S. frugiperda* cell line (see below).

In preparation for cloning of SIMNPV DNA in plasmids, (as well for other bioassays) polyhedra were isolated in Israel, from frozen, diseased *S. littoralis* larvae and purified on sucrose gradients. Alkali liberated viruses were purified on sucrose gradients, and DNA extracted from a portion of the samples. Cloning experiments in pEMBL and pUC plasmids are now starting,

2. Dr. Kislev had purified polyhedra and viruses from samples brought by her from a field in Kenya, and from *S. exempta* larvae inoculated in the laboratory by diseased

Rec'd in SOI JUN 13 1988

larvae which have been collected from 3 other places in the vicinity of MBITA, by Dr. Odindo. A portion of the alkali liberated viruses was used as inoculum to challenge *S. frugiperda* and *S. littoralis* cell lines; productive infection (production of polyhedra, observed in a phase microscope) was obtained in the first cell line, but only cytopathic changes in the second. In Israel we are trying now to increase the titer of *S. exempta* NPV in *S. frugiperda* cell line.

Dr. Kislev inoculated second and third instar larvae of *S. littoralis* by polyhedra of *S. exempta* NPV. Concentration of polyhedra used per larvae were higher from those reported previously to give 90-100% mortality of *S. exempta* 3rd instar larvae within 4-6 days. No viral mortality was noticed in 3 experiments using more than 600 larvae. We know however (as mentioned above) that these polyhedral preparation yielded an infectious viruses in *S. frugiperda* cell line, but only some cytopathic changes in *S. littoralis* cell line. A few inoculated larvae were homogenized, homogenizates were filter sterilized and inoculated onto cell cultures, polyhedra production was not observed so far. In the same experiment repeated with Pupa homogenates no polyhedra were observed.

3. A research student, Mr. Oduol, has been provisionally accepted for studies at the Feinberg Graduate School of the Weizmann Institute of Science, Israel. Mr. Oduol started conducting his studies at FGS and research in my laboratory, in March 1988. Dr. N. Kislev is training Mr. Oduol in the maintenance of insect cell culture; media preparation; virus bioassays in cell cultures and in larvae; purification of polyhedra and viruses from diseased larvae and cell culture and extraction of DNA from viruses.