Table 4. Effect of Mixed Solutions of Mercuric Chloride and Sulfhydryl Compounds on the Diapause Eggs of *A. menestriesi*

Chemicals mixed with mercuric chloride	Concentration (M)	Diapause* breaking (%)	Surviving** eggs (%)
Glutathione (reduced)	0.01	0	65
Cysteine hydrochloride	0.01	2	77
α-Lipoic acid	0.005	0	82
β-Mercaptoethanol	0.04	9	60
Control (distilled water)	—	91	—

One hundred eggs were examined each experiment.

* Represented by hatched eggs and dead eggs reached the eye-spot stage.

** This was confirmed by hatching after re-treatment with mercuric chloride.

REFERENCES

Hogan, T. W. (1961) *Aust. J. Biol. Sci.* **14** : 538-542.

Hogan, T. W. (1962) *Aust. J. Biol. Sci.* **15** : 419-426.

Maki, T. and Y. Ando (1967) *J. Fac. Agr. Iwate Univ.* **8** : 209-221.

Maki, T., M. Kurihara and Y. Ando (1966) *J. Fac. Agr. Iwate Univ.* **7** : 299-306.

Miya, K. (1965) *J. Fac. Agr. Iwate Univ.* **7** : 155-166.

Slifer, E. H. (1964) *J. Exp. Biol.* **102** : 333-356.

Morihisa Kurihara
Yoshikazu Ando

to Prof. K. Miya for his constant guidance in the course of the work and for reading the manuscript. The authors also greatly indebted to Dr. T. Maki, Prof. T. Oya and Dr. S. Masaki for their helpful advices and encouragement.

Laboratory of Applied Entomology,
Faculty of Agriculture,
Iwate University,
Ueda, Morioka, Japan

Received May 19, 1969

Preliminary Report on the Primary Culture of Smaller Brown Planthopper Cells *in Vitro* (Hemiptera : Delphacidae)[1]

Since Mitsuhashi and Maramorosch (1964) established the method for the cultivation of leafhopper cells *in vitro*, the cells from several species of leafhoppers have been cultivated successfully in primary cultures. The culture medium NCM-2B (Mitsuhashi, 1969) and the like supported well the outgrowth of the embryonic cells from *Macrosteles fascifrons, Agallia constricta, Dalbulus maidis, Nephotettix cincticeps, N. apicalis,* and *Inazuma dorsalis* (Mitsuhashi and Maramorosch, 1964; Mitsuhashi, 1965, 1969; Chiu et al., 1966). All the above leafhoppers are vectors of plant pathogenic microbes and the cultures of their cells had been done for the purpose of investigating those pathogenic microbes. With the same aim the present author has attempted to cultivate the cells of the smaller brown planthopper, *Laodelphax striatellus* Fallén, which is the vector of rice stripe virus, rice black streaked virus, and northern mosaic virus of barley, wheat and oat, and the results hitherto obtained are reported here.

For the cultivation of *L. striatellus* embryonic cells, the same technique and the same culture medium as those used in the leafhopper cell cultures were employed (Mitsuhashi and Maramorosch, 1964). But almost no outgrowth of cells was obtained by this method. Then each process of setting up cultures and the composition of the medium were examined for their adequacy for *L. striatellus* cell culture.

Firstly, the stages of embryos to be used as explants were examined. The embryos around blastokinetic stage have been reported to be the most suitable explants in the case of leafhopper

[1] Appl. Ent. Zool. **4** (3) : 151—153 (1969)

cell cultures, not only because of their ability for proliferation but also because of the easiness for handling (MITSUHASHI and MARAMOROSCH, 1964). The same was true in the case of *L. striatellus* embryos. But *L. striatellus* embryos became hard to handle shortly after the completion of blastokinesis, whereas leafhopper embryos even after blastokinetic stage could sometimes be handled in the same manner as those before and under blastokinesis. The main difficulty to handle the *L. striatellus* embryos after blastokinetic stage seemed to be due to the deposition of a waxy substance on the surface of the embryo. Dorsal closure which encloses the yolk hindered handling also. The appearance of these obstacles for handling may be due to rapid development of the embryos after blastokinesis in *L. striatellus*. It was ascertained, therefore, that the selection of the embryos of not later than completion of blastokinesis is important for providing the suitable explants of *L. striatellus*.

Secondly, the effect of surface-sterilization of eggs was examined. The treatment in 70 % ethanol for 1 min, which had been used in the case of *N. cincticeps* (MITSUHASHI, 1965) gave no deleterious effect on the *L. striatellus* eggs. The eggs developed normally after such treatment.

Thirdly, the effect of trypsinization was examined. The treatment with 0.1 % trypsin for about 5 min at 25°C had no harmful effect. By this treatment adherance of tissue fragments to glass was enhanced, although without trypsinization tissues adhered to glasses when they were released from RINALDINI's solution into RINGER-TYRODE's solution (see MITSUHASHI and MARAMOROSCH, 1964).

Fourthly, the size of explants was considered. The embryos of *L. striatellus* were very small compared with the embryos of the leafhoppers mentioned previously. When the embryos of *L. striatellus* were explanted as a whole, cell migration was scarcely observed, while the embryogenesis proceeded to some extent. If the embryos were cut into several pieces and then explanted, cell migration from the explants occurred when the medium was adequate. The number of explants per culture vessel seemed to have little effect on the cell migration and multiplication.

Finally, the composition of the culture medium was modified in various ways. In NCM-2B medium, occasionally the cells migrated to form hollow spherical vesicles, but very few cells migrated out of the explants, and usually the migrated cells deteriorated within a few days. When the fetal bovine serum contained in NCM-2B medium was substituted with fetal bovine serum inactivated by heating at 56°C for 30 min, new born calf serum, calf serum, or bovine serum, the results of the cultures were the same as that with NCM-2B medium. The chicken serum, in place of the fetal bovine serum, was found to be rather deleterious. If a half volume of the fetal bovine serum in NCM-2B medium was substituted with CEE_{50}, the results of the cultivation were not better than that with NCM-2B medium. The complete substitution of the fetal bovine serum with the same volume of 0.02 % fetuin did not improve the outgrowth in the culture. GRACE's medium (GRACE, 1962) supplemented with 20 % fetal bovine serum instead of 5 % insect serum gave better cell migration from the explant than NCM-2B medium, but the migrated cells were again short-lived. The incorporation of the extract of *L. striatellus* eggs into NCM-2B and GRACE's medium with fetal bovine serum at the final concentration of 1 % apparently improved the culture. The extract of *L. striatellus* eggs was prepared in the following manner; eggs within 48 hr after oviposition were homogenized with 9 times in volume of RINGER-TYRODE's solution, the homogenate was heated at 60°C for 5 min and then frozen at −20°C, the thawing and freezing repeated 3 times, and finally the homogenate was centrifuged and the supernatant was sterilized by passing it through a 0.45 μ pore size Millipore filter. The resulting extract was designated as 10 % extract.

The best outgrowth was obtained with the GRACE's medium supplemented with 20 % fetal bovine serum and 1 % *L. striatellus* egg extract. Cell migration started within 24 hr after the culture was set up. Most of the cells at this stage were fibroblast-like cells (Fig. 1), and a few epithelial-like cells were observed in the proximity of the explants. The development of compact cell sheets which were common in the cultivation of leafhopper cells (MITSUHASHI and MARAMOROSCH, 1964; MITSUHASHI, 1965) were not observed so far. The hollow spherical vesicles which consisted of mostly epithelial-like cells were often observed (Fig. 2A). The epithelial-like cells in the vesicles contained bacteroid intracellular symbiotes which were moving actively in the cells (Fig. 2B). Some

explants started contraction movement within a week after the culture was set up. Pigmentation of compound eyes in the embryo fragments markedly progressed during the cultivation. This pigmentation was more prominent in GRACE's medium than in NCM-2B medium.

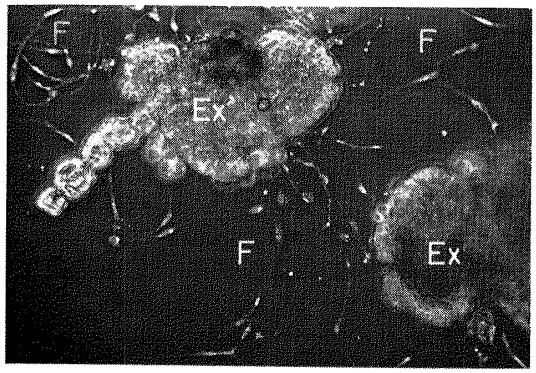

Fig. 1. Migration of fibroblast-like cells (F) from the explanted embryo fragments of *Laodelphax striatellus* (Ex). (Bright contrast ×120).

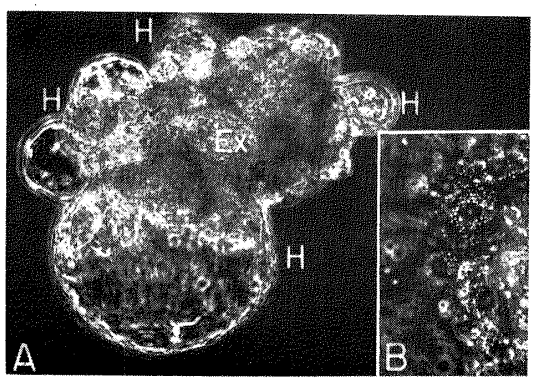

Fig. 2. A : Hollow spherical vesicles (H) formed on the explanted embryo fragment of *Laodelphax striatellus* (Ex). (Bright contrast ×130). B : High magnification of a part of the hollow spherical vesicle. Bacteroid intracellular symbiotes are visible as small white spots in the cytoplasm. (Bright contrast ×400).

Mitoses were observed in the cells which constituted hollow spherical vesicles, but not in the cells which migrated on the glass.

The migrated cells and the explants deteriorated after about 40 days of cultivation.

Yeast symbiotes which were brought into the culture with the explants, somewhat elongated. But all of them were granulated within a week and no further change was observed.

The cells of *L. striatellus* seems to have less adhesiveness to glass compared with those of leafhopper cells. This nature might cause the frequent formation of hollow spherical vesicles. Although outgrowth of cells were obtained with *L. striatellus* embryonic cells the growth was poorer than those of leafhopper cells. In order to improve cell growth of *L. striatellus in vitro* further modification of the culture medium is under way.

REFERENCES

CHIU, R.-J., D. V. R. REDDY and L. M. BLACK (1966) *Virology* **30** : 562-566.

GRACE, T.D.C. (1962) *Nature* **195** : 788-789.

MITSUHASHI, J. (1965) *Jap. J. appl. Ent. Zool.* **9** : 107-114.

MITSUHASHI, J. (1969) *Viruses, Vectors, and Vegetation* (Ed. by K. MARAMOROSCH) Interscience Publishers, New York, 475-503.

MITSUHASHI, J. and K. MARAMOROSCH (1964) *Contrib. Boyce Thompson Inst.* **22** : 435-460.

Jun MITSUHASHI

Division of Entomology,
National Institute of Agricultural Sciences,
Nishigahara, Kita-ku, Tokyo, Japan

Received May 19, 1969