FURTHER DESCRIPTION OF A SUBLINE OF GRACE'S MOSQUITO (*AEDES AEGYPTI* L.) CELLS ADAPTED TO HEMOLYMPH-FREE MEDIUM[1]

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When Grace's first established cell line from dipteran tissue was initiated (Grace, 1966) hemolymph seemed to be an essential component of the medium. However, it has since been determined that the cells can be cultured in hemolymph-free medium, containing more easily acquirable vertebrate serum (Hsu *et al.*, 1967). Thus, the potential application of these cells in biological research has been widened, from the practical standpoint. This report describes details of the adaptation process and also presents several properties of the adapted subline.

MATERIALS AND METHODS. The cells used (designated MSQ) were obtained from Dr. T. D. C. Grace shortly after he had begun their culture from larval tissue of *Aedes aegypti*. The subsequent passage history is seen in Table 1. The cells were cultured in 2-ounce prescription bottles, each containing 2.5 ml. of medium at 28° C. Subcultures were carried out at intervals of 3 to 7 days when the cell counts reached approximately 10^5 cells/ml. Since the cells grow in suspension, subcultures were made by transferring a portion of the suspension into fresh medium. They were initially cultured in a medium which Grace developed for *Antheraea* cells (GMA) (Grace, 1962) which was supplemented with hemolymph, treated according to Grace (1962), from the moth *Antheraea eucalypti*. After an initial failure to culture them in the absence of hemolymph, (MSQ–19, Table 1), hemolymph was added to the medium. This hemolymph, however, was from another moth (*Philosamia cynthia pryeri*). At this point it was seen that by adding 10 percent fetal bovine serum (FBS) to the medium, the concentration of the hemolymph could be reduced to 0.5 percent. It was from a cell cultured on such a medium that the cloned line, previously reported (Suitor *et al.*, 1966) was derived.

RESULTS. Several attempts to culture MSQ–22 (see Table 1) in the absence of hemolymph led to only short-term survival of the cells. Therefore, the concentration of the hemolymph was reduced stepwise (see MSQ–48, MSQ–68, Table 1) until the cells were adapted to a medium without hemolymph. It should be pointed out that the number of passages listed in Table 1 to accomplish this are not necessarily minimal, i.e., reduction of hemolymph concentration might well have been possible at an earlier time.

The subline (MSQ–68) consisted primarily of two distinct cell shapes, round and spindle-shaped. Although they did not differ in this respect from the "parent cells" (MSQ–22), the cells of the subline tended to be somewhat smaller. After about a week of culture, small round cells with a distinct outline and somewhat transparent cytoplasm were predominant. Figures 1–4 show typical cell morphology changes of the parent and subline over 7 days of culture.

Figure 5 shows that the growth rate of the adapted subline after many months in culture is very similar to the parent or the intermediate line (MSQ–48), all hav-

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[1] A preliminary report describing this subline was presented at the 60th Annual Meeting of the Formosan Medical Association on 11 November 1967, Taipei, Taiwan, and was abstracted in the Journal of the Formosan Medical Association Vol. 66, No. 12, pp. 645 (1967).

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The opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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TABLE 1.—History of mosquito cell line.

Date	Cell Designation	Medium	No. of Passages	Remarks
9/20/63	MSQ-cells	4% *Antheraea eucalypti* hemolymph in GMA*	8	Rec'd from T. D. C. Grace in 6th subculture of cells from *Aedes aegypti* set September 2, 1963
2/ 1/64	MSQ-19	5% fetal bovine serum (FBS) in GMA; then changed to 10% FBS	1	1 split, 4 medium changes (not counted as passages)
3/18/64	MSQ-22	10% FBS+0.5% *Philosamia cynthia* in GMA	78	78 subcultures
8/27/65	MSQ-48	10% FBS+0.25% *Philosamia cynthia* in GMA	102	102 subcultures
12/20/66	MSQ-68	10% FBS in GMA	108	(to April 2, 1968)

* GMA=Grace's medium for *Antheraea* cells.

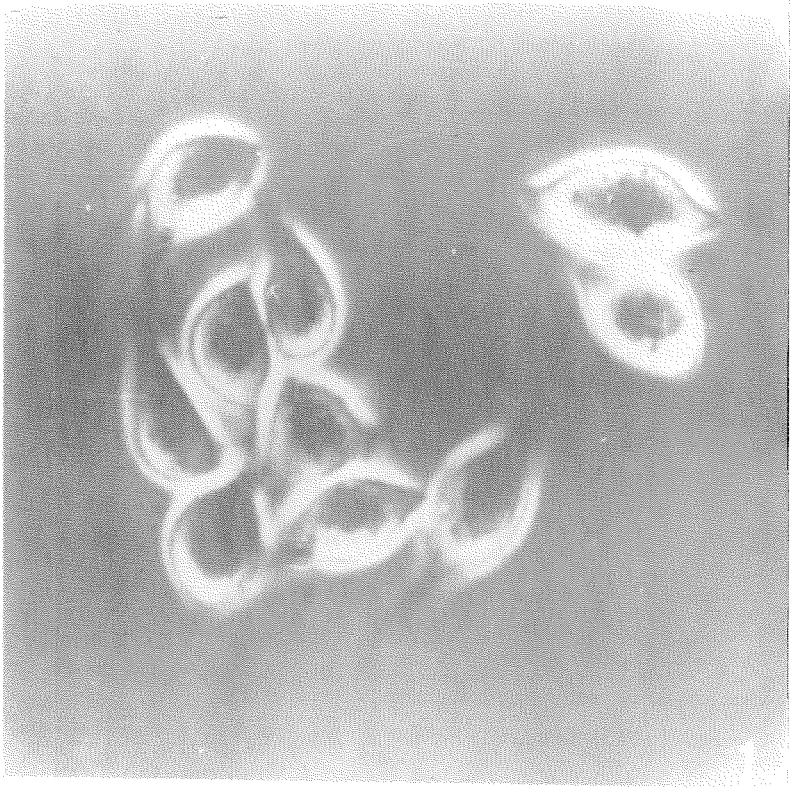

FIG. 1.—Subline (MSQ-68), 4 days in culture, phase contrast, 1480 X.

ing a doubling time of approximately 38 hrs. The maximum cell number of the parent tends in general to be greater.

Since the initial concentration of 10 percent FBS was arbitrarily chosen, tests were next performed with different levels of FBS in the medium. In Fig. 6, it can be seen that, while doubling the FBS concentration (20 percent) of the medium did not improve growth of the adapted subline, reducing it one-half (5 percent) did not adversely affect it either. As little as 2 percent FBS in the medium was sufficient to promote cell multiplication, although to a decidedly lesser extent.

Converse and Nagle (1967) have reported on a medium for culture of Grace's *A. aegypti* cells which is less complex than GMA. We have tested this medium and found it to be as suitable for the culture of our subline as GMA.

In order to roughly characterize that portion of the FBS responsible for supporting cell growth, the effect of separating large from small molecules by dialysis was tested. Fetal bovine serum was dialyzed through a membrane (average pore radius of 24 Angstroms) against 10 volumes of GMA, with constant mixing, for 48 hr. at 4° C. Comparisons of growth of MSQ–68 cells in the dialysate (that which diffused through the membrane), in the portion of the FBS which did not diffuse (adjusted to 10 percent in GMA), and in whole, untreated FBS (10 percent in GMA) were made. No cell multiplication occurred in

FIG. 2.—Parent line (MSQ–22), 4 days in culture, phase contrast, 1480 X.

the medium containing the dialysate, whereas multiplication of cells in medium containing nondialysable or whole FBS was equivalent and at the same rate. The results indicate that the cell growth-supporting portion of FBS resides in the nondiffusable portion of FBS, presumably of relatively high molecular weight.

We have also recently adapted by a similar method the recloned line (Suitor *et al.*, 1966) of Grace's *A. aegypti* cells to a medium free of hemolymph (10 percent FBS in GMA). It grows well and has been subcultured 13 times to May 8, 1968.

DISCUSSION. It is not possible to speculate on the mechanism whereby the adapted subline acquired its independence from hemolymph in the medium, but of prime importance is the fact that it was able to do so. However, this fact is not surprising in light of the adaptation of both Grace's *Antheraea* line by Yunker, Vaughn, and Cory (1967) and Grace's *Bombyx* line (1967) by Suitor and Paul (unpublished data, 1968) to a hemolymph-free medium. Also, neither of the two other existing continuously cultured lines of insect cells (leafhopper: 2; mosquito: 8) require hemolymph in their media, but rather have as supplementation 20 percent FBS. Such adaptation is probably no more difficult an accomplishment than is adaptation to heterologous hemolymphs (Suitor *et al.*, 1965).

Once one has adapted cells to a new medium, it is important then to char-

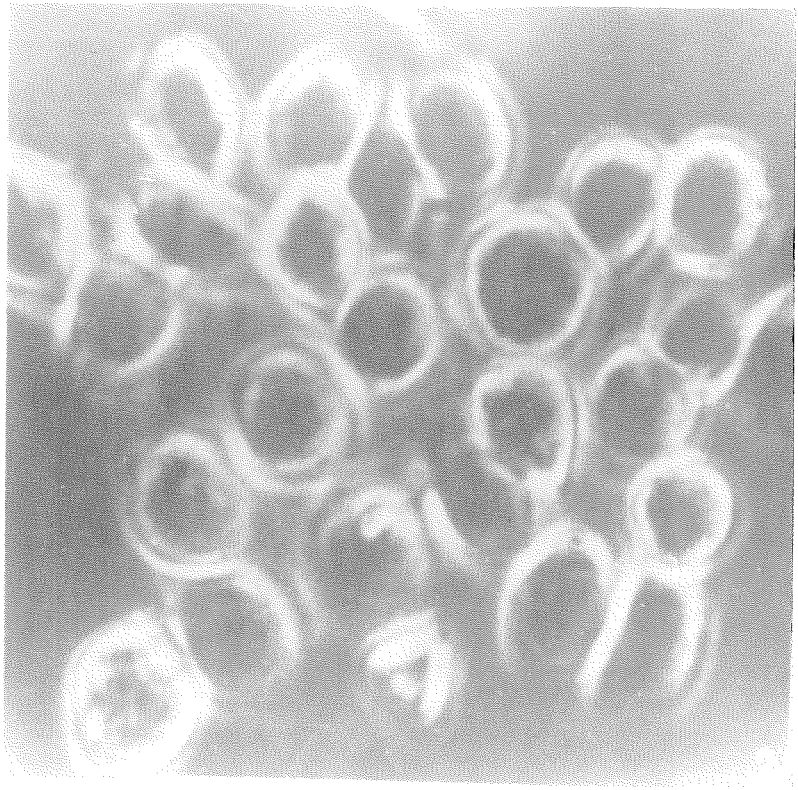

FIG. 3.—Subline, 7 days in culture, phase contrast, 1480 X.

acterize them, particularly if they are to be used in experiments which have already been initiated with the "parent" cell type. In general, the adapted line here described does not grow quite as well, in terms of final numbers or cell-size, as does the parent. However, the concentration of the FBS added did not seem to be the limiting factor in this respect: in fact, the medium of Converse and Nagle (1967) seemed to improve the condition of the adapted cells to a point where, subjectively speaking, they were comparable in every respect to the parent cells. This impression, of course, requires experimental backing.

It was surprising that the concentration of the FBS could be varied so greatly (5–20 percent) without changing its growth-promoting potential for the subline. Obviously, anything in excess of 5 percent FBS is superfluous, but not (at least to 20 percent) deleterious.

The results of the dialysis experiments on the FBS are similar to those by Aizawa, Sato and Murakami (1961) using hemolymph in a silkworm cell primary culture system, and Suitor and Liu (1965) using hemolymph in the medium for Grace's *Antheraea* cell line. In both cases, the growth-promoting portion of the hemolymph was not diffusable through the dialysis membrane. Further studies to characterize the essential portion of FBS are underway.

SUMMARY. A subline of Grace's *Aedes*

FIG. 4.—Parent, 7 days in culture, phase contrast, 1480 X.

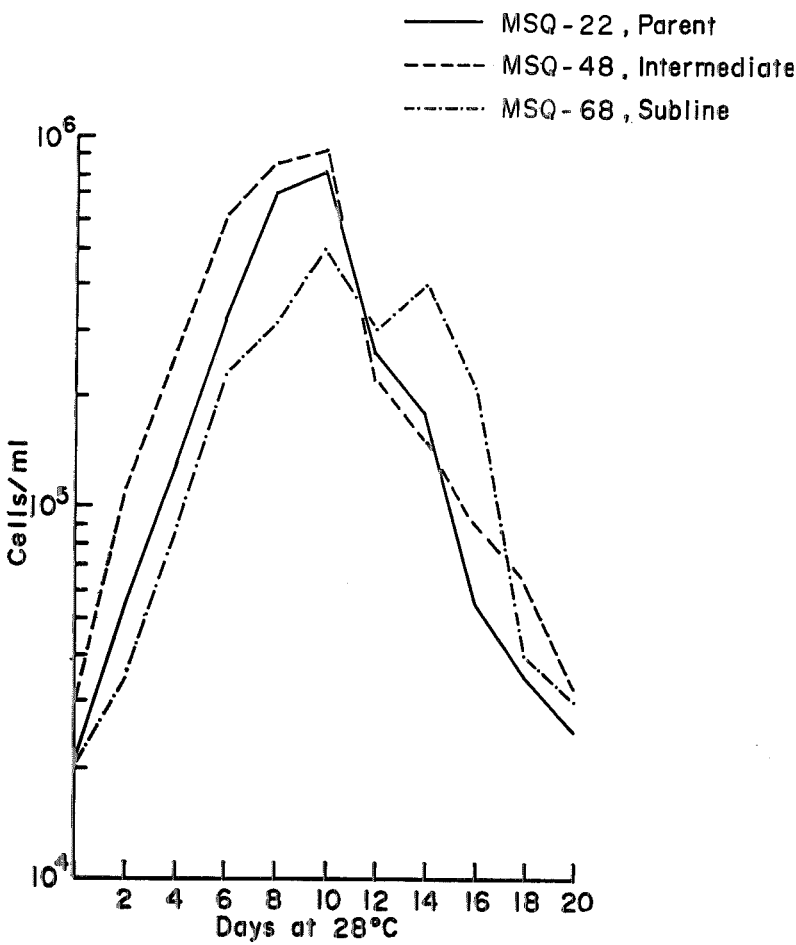

FIG. 5.—Growth of parent and adapted subline mosquito cells.

aegypti line has been adapted to a medium, without insect hemolymph, containing 10 percent fetal bovine serum in Grace's medium. The cells of the subline appear somewhat smaller. While the maximum cell numbers tend to be less than the parent cells, the doubling times are nearly identical. The growth rate was not affected by halving or doubling the above concentration of FBS, and even as little as 2 percent was able to promote some cell multiplication. The growth-promoting factors reside in the non-dialyzable portion of the FBS.

References Cited

AIZAWA, K., SATO, F., and MURAKAMI. 1961. Role of hemolymph in tissue culture of the silkworm, *Bombyx mori*. Sericult. Sci. Jap. 30:445–450.

CHIU, R. L., and BLACK, L. M. 1967. Monolayer cultures of insect cell lines and their inoculation with a plant virus. Nature 215:1076–1078.

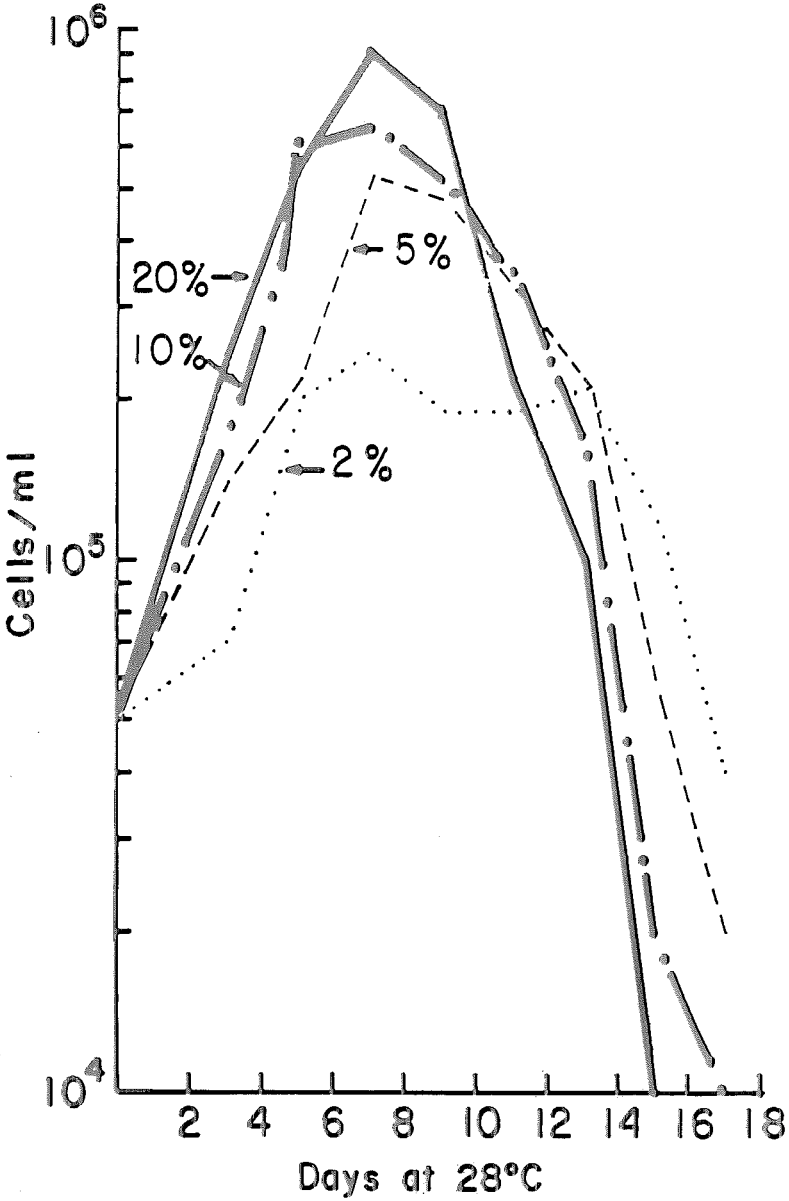

FIG. 6.—Comparison of subline growth in various concentrations of fetal bovine serum (FBS).

CONVERSE, J. L., and NAGLE, S. C., JR. 1967. Multiplication of yellow fever virus in insect tissue cell cultures. J. Virol. 1:1096–1097.

GRACE, T. D. C. 1962. Establishment of four strains of cells from insect tissues grown *in vitro*. Nature 195:788–789.

GRACE, T. D. C. 1966. Establishment of a line of mosquito (*Aedes aegypti* L.) cells grown *in vitro*. Nature 211:366–367.

GRACE, T. D. C. 1967. Establishment of a line of cells from the silkworm *Bombyx mori*. Nature 216:613.

HSU, S. H., LIU, H. H., and SUITOR, E. C., JR. 1967. Adaptation of Grace's continuous line of mosquito (*Aedes aegypti* L.) cells to hemolymph-free medium. J. Formosan Med. Ass. 66(12):645.

SINGH, K. R. P. 1967. Cell cultures derived from larvae of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.). Curr. Sci. 36:506–508.

SUITOR, E. C., JR., CHANG, L. L., and LIU, H. H. 1966. Establishment and characterization of a clone from Grace's *in vitro* cultured mosquito (*Aedes aegypti* L.) cells. Exp. Cell Res. 44:572–578.

SUITOR, E. C., JR., and LIU, H. H. 1965. Adaptation of Grace's continuous lines of moth and mosquito cells to heterologous hemolymph. Proc. 12th Regional Meeting Formosan Med. Ass.

YUNKER, C. E., VAUGHN, J. L., and CORY, J. 1967. Adaptation of an insect cell line (Grace's *Antheraea* cells) to medium free of insect hemolymph. Science 155:1565–1566.

AN EVALUATION OF THE MOSQUITO CONTROL PROGRAM IN A SMALL MIDWESTERN URBAN COMMUNITY [1]

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Mosquito control programs for small urban communities in the midwestern United States are often modifications of programs designed for other areas such as coastal, swamp, or irrigated lands where mosquitoes pose acute problems necessitating costly measures. The use of these programs in the Midwest, where mosquitoes occur in lower densities, is often ineffective and wasteful.

This report deals with a 2-year study (1967–1968) of the mosquito control program in a small midwestern urban community located in the lower Missouri River Valley bordering the Ozark Highlands to the south and the prairie regions to the north and west. Approximately 40,000 people live in the 22.4 square miles of the city limits. The depth of the city lots averages 150 feet per lot. There are few, if any, alleyways between the properties.

In this mosquito control program, approximately $10,000 per year was allocated to the following; (1) operation of four New Jersey mosquito light traps with weekly tabulation of catch by species; (2) application of fenthion in diesel oil as a larvicide to suspected mosquito breeding sites; (3) fogging with fenthion for adult control.

MATERIALS AND METHODS. The City Health Department was responsible for the mosquito control program; however, the Department of Public Works carried out most of the actual mosquito control measures. Larval control was based on survey records of breeding sites from 1966 made by an untrained inspector. Approximately 20 percent of the money spent annually for mosquito control was used in attempted larval control. This included the larviciding crew which worked an average of 2 days per week from April 1

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