PB-225 790

CELL CULTURES DERIVED FROM MOSQUITO TISSUES, 'ANOPHELES STEPHENSI,' LISTON

Paul H. Silverman, et al

Illinois University

Prepared for:

Agency for International Development

1973

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

BIBLIOGRAPHIC DATA SHEET	1. Report No. 614-4623-R773	3.	PB 225 790

4. Title and Subtitle Cell Cultures Derived From Mosquito Tissues, ANOPHELES STEPHENSI, Liston*	5. Report Date 1973
7. Author(s) Paul H. Silverman, et al	8. Performing Organization Rept. No.
9. Performing Organization Name and Address University of Illinois Dept. of Zoology Urbana, Ill. 61801	10. Project/Task/Work Unit No. 93-19-511-522 11. Contract/Grant No. AID/csd-1432
12. Sponsoring Organization Name and Address Department of State Agency for International Development Washington, D.C. 20523	13. Type of Report & Period Covered Research Bulletin 14.

15. Supplementary Notes

16. Abstracts

This communication dealt with the attempt to maintain long-term primary culture consisting of cells, tissues and organs of mosquitoes for as long as 60 days without change of nutrient medium. Newly hatched larvae of Anopheles stephensi were used to initiate the primary culture. The growth patterns of the various outgrowths could be distinguished morphologically as four different types of monolayers of cells, cellular and non-cellular vesicles, fatty tissues and what appeared to be two types of fiber-like elements. Phase contrast cinemicrographic studies showed activities of large epithelial cells and fibroblast-like cells (both types of cells exhibit oscillatory movement) and individual or aggregates of myoblast-like cells (exhibit pulsatile activity typical of gut peristalsis). Demonstrable activity and growth of isolated tissues and fragmented and intact organs was shown. Genesis of the cellular vesicle, types of cells present and change occurring as the culture ages was discussed.

17. Key Words and Document Analysis. 17a. Descriptors

17b. Identifiers/Open-Ended Terms

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

17c. COSATI Field/Group 614

18. Availability Statement	19. Security Class (This Report) UNCLASSIFIED	21. No. of Pages 10
	20. Security Class (This Page) UNCLASSIFIED	22. Price 3.00/1.45

339057

PROCEEDINGS THIRD INTERNATIONAL COLLOQUIUM ON INVERTEBRATE TISSUE CULTURE

SMOLENICE NEAR BRATISLAVA, JUNE 22-25, 1971

EDITED BY

J. ŘEHÁČEK
Institute of Virology Slovak Academy of Sciences
Mlynská dolina 1 Bratislava, Czechoslovakia

D. BLAŠKOVIČ
Institute of Virology Slovak Academy of Sciences
Mlynská dolina 1 Bratislava, Czechoslovakia

W. F. HINK
The Ohio State University Department of Entomology
Invertebrate Pathology Laboratory Columbus, Ohio U.S.A.

PUBLISHING HOUSE OF THE SLOVAK ACADEMY OF SCIENCES
BRATISLAVA 1973

CELL CULTURES DERIVED FROM MOSQUITO TISSUES, *ANOPHELES STEPHENSI*, LISTON*

MARIA C. ROSALES ROMOUILLO, ROBERT W. SIMONS and PAUL H. SILVERMAN

Department of Zoology, University of Illinois, Champaign - Urbana, Illinois 61801, U. S. A.

The need for an insect-oriented environment that will support the growth and development of the sporogonic form of the malarial parasites has been demonstrated by several workers (Ball, 1954; Ball and Chao, 1957, 1964; Schneider, 1968a, 1968b; and Walliker and Robertson, 1970). This present investigation which is in progress is designed to develop an *in vitro* system that will serve as a "living host" for the full development of the mosquito-borne phase of *Plasmodium berghei*.

The primary culture (in which fragments of newly hatched larvae of *Anopheles stephensi* serve as the sources) consists of nine different types of outgrowths which appear at different times during the cultivation period. The outgrowths could be morphologically distinguished as large and small epithelial cells, fibroblast-like cells, neuron-like cells, two types of myoblast-like cells, polygonal cells, and non-cellular and cellular vesicles.

* Supported by a contract from the Agency for International Development, The United States Department of State.

A point of interest in this study is the successful establishment of primary cultures which show similar growth patterns and viability of outgrowths within a temperature range of 21° C to 27° C and pH range of 6.5, 7, 8, and 8.5.

Large epithelial-like cells. These cells vary in shape from round, ovoid forms to star-like configurations (Figs. 1 and 2). Each cell has a large nucleus which is spherical or irregular in shape, a granular cytoplasm and a thin membrane at the periphery. This membrane which is either slightly curled-up or folded, is hardly visible in the more compact, roundish type of cells but is very distinct in flat disc type of cells.

Small epithelial-like cells. Small ovoid and spindle-shaped epithelial cells were observed in the second week of the cultivation period (Fig. 3). Each cell is characterized by a round nucleus and a thin cytoplasm filled with minute droplets and fine granules.

Fibroblast-like cells. These types of cells become apparent 16 days into the culture period migrating independently of each other from tissue fragments. The cells are irregular in size and shape (Fig. 4). Each cell has either an oval or elongated nucleus, and a cytoplasm which is drawn out into one or more processes of variable length.

Polygonal Cells. These cells are large, flat, polygonal in shape, and form a contiguous sheet around the explant (Fig. 5). Each cell has a well-defined, centrally placed spherical nucleus, a very prominent nucleolus, and a clear, nearly granule-free cytoplasm.

Myoblast-like cells. One type consists of cells that are conspicuously long and very slender (Fig. 6). Each cell has a prominent central body containing an ovoid nucleus and a cytoplasm

which is drawn out into long thread-like processes. The second type (Fig. 7) appears thin, membrane-like and extends a short distance from their origin (gut fragment).

Neuron-like cells. Cells of this type arise from fragments of the thorax and abdomen during the second week of cultivation (Fig. 8). Characteristically, each cell consists of a cell body with very fine fibrillar processes. Initially, the processes are short but as growth proceeds, each process lengthens into a very thin fibril, either as an unbranched, T-shaped, or multibranched form. No evidence of pulsatile activity is observed among these outgrowths.

Vesicles. Two types of vesicles are apparent from cut edges of the thoracic and abdominal fragments during the culture period: cellular (Fig. 9) and non-cellular (Fig. 10). A mature cellular vesicle consists of a single layer of either squamous-like cells or small epithelial cells interspersed with fibroblast-like cells (Figs. 9 and 12). As the culture ages, the cells tend to aggregate, giving rise to a wrinkled, non-transparent cellular wall (Fig. 11). In spite of the morphological change that does occur, the vesicles remain hollow and show no evidence of collapse throughout the cultivation period. Non-cellular vesicles remain small and persist as such throughout the cultivation period.

The survival and viability of the different outgrowths for a prolonged period of time (60 days without change of nutrient medium) demonstrate their adaptability to the changing conditions of the milieu during the cultivation period. Long-term maintenance of germ-free adult organs in this cell-cultured system has been successfully accomplished (Simons et al., elsewhere in this colloquium). Preliminary attempts to cultivate the sporogonic forms of the malarial

parasite (Plasmodium berghei) in this substrate suggested that it is a receptive system for the cultivation of the parasite.

SUMMARY

This communication dealt with the attempt to maintain long-term primary culture consisting of cells, tissues and organs of mosquitoes for as long as 60 days without change of nutrient medium. Newly hatched larvae of Anopheles stephensi were used to initiate the primary culture. The growth patterns of the various outgrowths could be distinguished morphologically as four different types of monolayers of cells, cellular and non-cellular vesicles, fatty tissues and what appeared to be two types of fiber-like elements. Phase contrast cinemicrographic studies showed activities of large epithelial cells and fibroblast-like cells (both types of cells exhibit non-oriented gliding movement), small epithelial cells (exhibit oscillatory movement) and individual or aggregates of myoblast-like cells (exhibit pulsatile activity typical of gut peristalsis). Demonstrable activity and growth of isolated tissues and fragmented and intact organs was shown. Genesis of the cellular vesicle, types of cells present and changes occurring as the culture ages was discussed.

REFERENCES

BALL, G. 1954. Prolonged contraction of mosquito digestive tract *in vitro* with partial development of oocysts of *Plasmodium relictum*. Expl. Parasit. 3:358-367.

BALL, G., and CHAO, J. 1957. Development *in vitro* of isolated oocysts of *Plasmodium relictum*. J. Parasit. 43:409-412.

CHAO, J., and BALL, G. 1964. Cultivation of the insect cycle of Plasmodia. Am. J. Trop. Med. Hyg. 13:181-192.

WALLIKER, D., and ROBERTSON, E. 1970. Cultivation *in vitro* of sporozoites of *Plasmodium berghei*. Trans. R. Soc. Trop. Med. Hyg. 64:5.

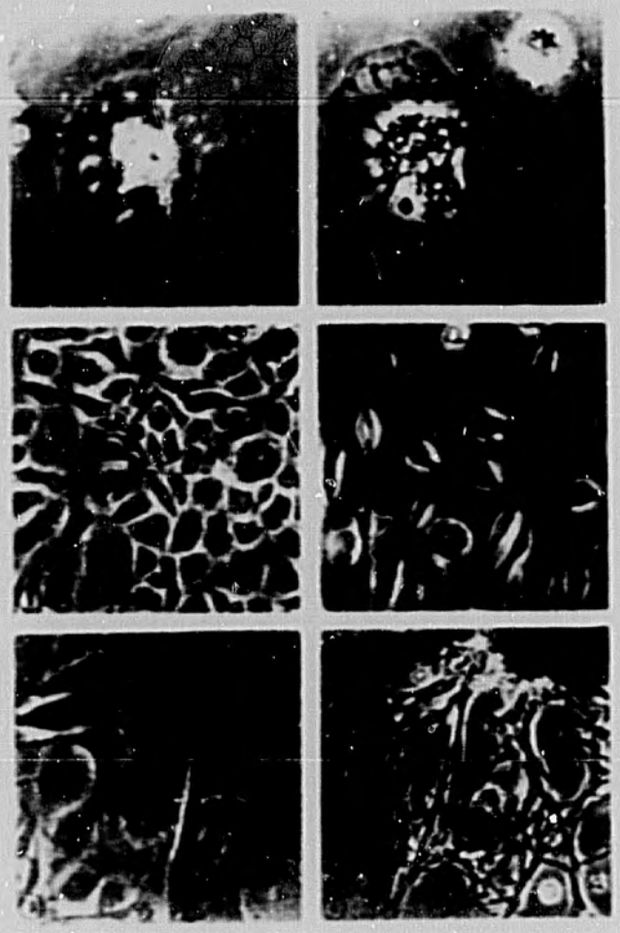

Fig. 1. A large spheri[illegible]-shaped epithelial cell (at 7 days old) X 700.

Fig. 2. A large epithelial cell (39 days old) X 700.

Fig. 3. Monolayer of small epithelial cells (14 days old) X 700.

Fig. 4. Fibroblast-like cells (32 days old) X 700.

Fig. 5. A contiguous layer of polygonal cells (17 days old) X 435.

Fig. 6. Myoblast-like cells (28 days old) X 775.

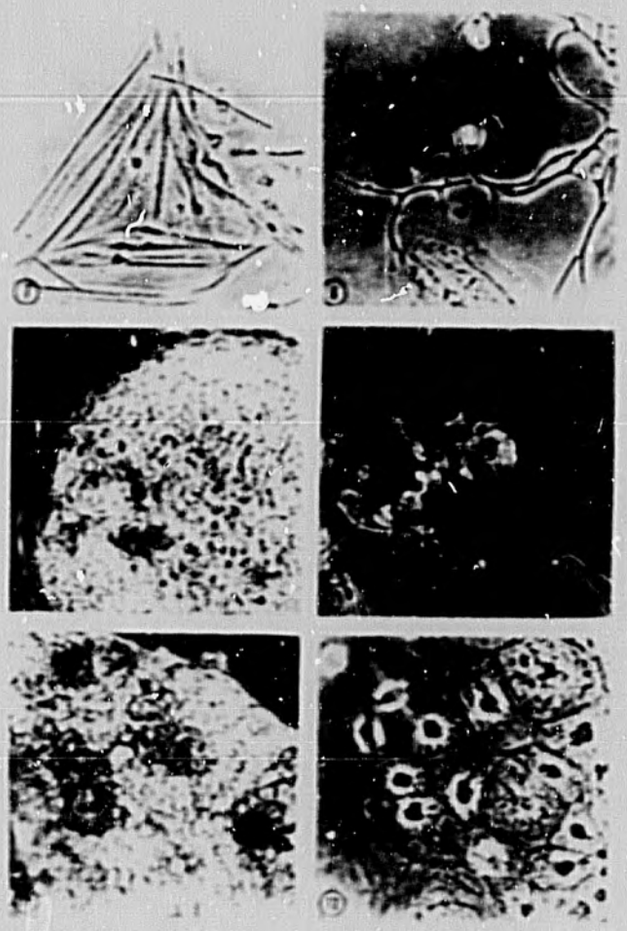

Fig. 1. Fibroblast-like cells (19 days old) X 350.

Fig. 2. Neuron-like cells (26 days old) X 350.

Fig. 3. A portion of a cellular vesicle (32 days old) X 350.

Fig. 10. Non-cellular vesicles (13 days old) X 140.

Fig. 11. A portion of a cellular vesicle (19 days old) X 130.

Fig. 12. Squamous-like cells of a cellular vesicle (37 days old) X 350.

All photomicrographs are of live cells. Figure 1 is illuminated by optical shadow casting. Figures 2 - 12 are illuminated by phase contrast.