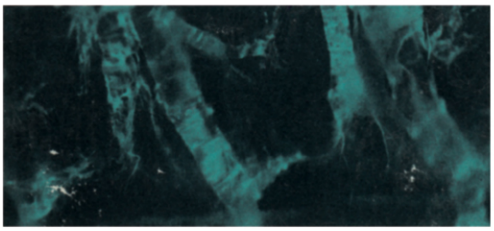
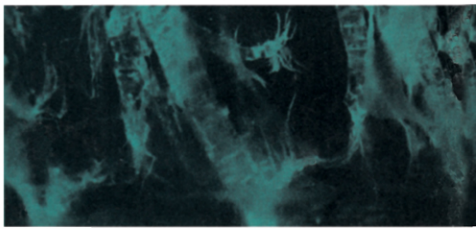
VOLUME TWO

INVERTEBRATE TISSUE CULTURE

EDITED BY C. VAGO





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INVERTEBRATE TISSUE CULTURE

Volume II

ADVISORY BOARD

INVERTEBRATE TISSUE CULTURE

Edited by C. Vago

STATION DE RECHERCHES CYTOPATHOLOGIQUES
INRA-CNRS, SAINT-CHRISTOL
UNIVERSITÉ DES SCIENCES
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LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

GORDON H. BALL, Department of Zoology, University of California, Los Angeles, California (321)

CLAUDIO BARIGOZZI, Istituto di Genetica, Università di Milano, Milan, Italy (163)

JOSETTE BERREUR-BONNENFANT, Laboratoire de Génétique Evolutive, CNRS Gif-sur-Yvette, France (181)

J. DAVID, Service d'Entomologie Expérimentale et de Génétique, Université Lyon, Lyon, France (211)

J. DEMAL, Laboratoire de Morphologie Animale, Université Catholique de Louvain, Louvain, Belgium (3)

L. GOMOT, Laboratoire de Zoologie, Université Besançon, Besançon, France (41)

W. Fred Hink, Department of Entomology, The Ohio State University, Columbus, Ohio (363)

A. M. Leloup, Laboratoire de Morphologie Animale, Université Catholique de Louvain, Louvain, Belgium (3)

Jun Mitsuhashi, Division of Entomology, National Institute of Agricultural Sciences, Nishigahara, Kita-ku Tokyo, Japan (343)

Josef Řeháček, Institute of Virology, Czechoslovak Academy of Sciences, Bratislava, Czechoslovakia (279)

M. Rougier, Service d'Entomologie Expérimentale et de Génétique, Université Lyon, Lyon, France (211)

Takeo Takami, Sericultural Experiment Station, Tokyo, Japan (137)

C. Vago, Station de Recherches Cytopathologiques, INRA-CNRS, Saint-Christol, Université des Sciences, Montpellier, France (245)

PREFACE

The contribution of cell and tissue culture to the development of medical and biological sciences is universally known. Most of the recent progress in cellular biology and pathology has been achieved by *in vitro* investigations. Fifteen years ago this was true for biological experimentation dealing with man and other vertebrates. The use of tissue culture in invertebrate research in physiology and pathology was being considered, but only a few attempts were made to achieve such cultures, and then in only a small number of insect and mollusk species.

The First International Colloquium on Invertebrate Tissue Culture held in 1962 in Montpellier (France) enabled us to evaluate results of previous research and opened new vistas for future research. Since then rapid advances have occurred in cell and organ culture of invertebrates—Arthropoda, Mollusca, Echinoderma, Nematoda, and Coelenterata. Such cultures, often barely developed, were also used in studies involving genetics, physiology, and pathology.

At present, investigations and results are so numerous and diversified that the publication of a treatise to collate this material seemed desirable. With the aid of the foremost specialists in the field of invertebrate cell and organ culture, this two-volume treatise was made possible.

This work was organized so that the techniques utilized and their applications to the various biological disciplines are developed, accompanied by the results and characteristics of the resulting cultures. Some overlap was necessary to ensure linkage of information from chapter to chapter.

Volume I includes general methodology concerning both cell and organ cultures and their preparation from aseptic conditions. Methods for the examination of cultures are also developed, particularly those concerning ultrastructure studies by electron microscopy. Cell cultures obtained from different groups of invertebrates are then discussed with emphasis on peculiarities specific to each group.

Organ cultures of different invertebrates are the subject of several chapters in Volume II, including an important section on the use of cell and organ cultures in physiology, genetics, and pathology. The study of the effect of pathogens is distinguished from that of microorganisms transmitted by vectors.

I wish to thank the members of the advisory board—C. Barigozzi, P. Buchner, J. de Wilde, M. Florkin, E. Hadorn, P. Lepine, K. Maramorosch, N. Oker-Blom, R. C. Parker, G. Ubrizsy, and E. Wolff—for their aid in the preparation of this treatise. The continuous assistance of M. Laporte is gratefully acknowledged.

It is hoped that this treatise will not only evaluate the present state of research and the problems in invertebrate tissue culture but will also serve as a guide to those working in this field and as a technical and scientific introduction to those intending to culture invertebrate tissues or to use cultures in pathology, physiology, or other biological disciplines.

C. VAGO

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I

Organ Cultures

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I

ORGAN CULTURE OF INSECTS

J. Demal and A. M. Leloup

I. INTRODUCTION

The *in vitro* technique of explantation, whether applied to vertebrates or to invertebrates, is used in two fairly distinct areas of research. The first, described in the preceding chapters, aims at obtaining from explants of varying size and nature both survival and cell proliferation. More often than not, the cultured cells lose the specific characteristics which they had at the time of explantation, but this is without importance for the aim of the investigation. The second area seeks to maintain in culture the structural and functional integrity of the explant; this calls for survival and growth, as well as for the maintenance, and eventually, an evolution of the object's differentiation. Since Maximov's time (1925), the technique of explantation used in the first area of research has usually been called tissue or histiotypic culture, and that in the second, organ or organotypic culture. Truth to say, this terminology is inadequate. It would seem preferable to speak, in the first case, of cell culture since it is the cell which is the biological unit cultured *in vitro;* this technique could also be termed unorganized culture (Thomson, 1914). In the second instance, that of the method which aims at maintaining the entire explant unimpaired *in vitro,* it would be better to speak of organized culture. This broader term would cover experiments carried out on anatomical bodies lower or higher than the organ, i.e., tissues or fragments of organs, embryos, or whole organisms. Thus, when the term organ culture is used, we consider that it must be given this broader meaning.

II. HISTORICAL SURVEY

A. Preliminary Works

Since the *in vitro* technique was first used in the study of vertebrates, it is not surprising to find that the first attempts at organotypic culture of invertebrates should have been made in laboratories generally studying vertebrates. Goldschmidt (1915, 1916, 1917), working in the Harrison Laboratory at Yale University, was the first to attempt to set up an *in vitro* culture of testicular cysts of *Samia cecropia* pupae. The explants, set in hanging drops of the blood of the insect, showed all the phases of spermatogenesis during a period of three weeks. The death of the sex cells in no way hindered survival and a certain cellular growth

of the follicle membranes and of blood cells during a period lasting more than six months. Goldschmidt immediately grasped the value of the method he had just experimented with, and he was not satisfied with a mere passive observation of the evolution of these cultures. Organ culture presents the advantage of allowing the explant to be observed independently of the organism from which it has been taken and freed from internal correlation and the many influences which it receives from the rest of the animal. The investigator is therefore free to vary systematically the experimental conditions of the culture in order to determine exactly which factors favor or hinder survival, growth, and differentiation of the explant. Once Goldschmidt had become convinced that the evolution of his cultures *in vitro* was analogous to evolution *in vivo,* he began experimental and causal study of spermatogenesis. He varied the chemical composition of the culture medium, and studied the effects of heat, cold, and osmotic conditions on his explants. He was able, in particular, to point out the importance of the action of the follicle membrane in maintaining the physical conditions necessary for the normal evolution of spermatogenesis. Shortly after Goldschmidt's first experiments, other authors, such as Lewis (1916) and Lewis and Robertson (1916), working on *Chorthippus curtipennis,* and Chambers (1925), working on *Dissosteira carolina,* applied this technique to the cytological study of spermatogenesis.

However, the technique, in spite of promising beginnings, showed itself to be of delicate and often fruitless application in the use of the study of insects. This explains why no further publication was devoted to the culture of insect organs until 1928, when Frew (1928) set out to study by *in vitro* culture the formation of the mesoderm in the leg imaginal discs of *Calliphora erythrocephala.* Since the explants showed but very limited differentiation *in vitro,* he realized his aim only partially. But his systematic research for improving culture conditions allowed him to point out a number of important problems besetting the would-be research worker in *in vitro* organ culture.

In the first place, he drew attention to the necessity of severe aseptic conditions in obtaining a good *in vitro* culture; he showed how difficult it is to maintain such conditions in the course of explantation of insect organs and in blood collecting. The majority of types of explants, with the exception of those from the digestive tract and its annexes, may be sterilized by repeated washings. There are various methods for filtration of collected hemolymph, but it always undergoes some modification while passing through the filter. It might be possible to avoid these difficulties by aseptic rearing of experiment animals, wherever this can be done. A second problem confronting the investigator concerns the

knowledge of the composition of the insect's body fluid, and since this composition varies in the course of metamorphosis, it is necessary to have a minimum of knowledge of the physiology of metamorphosis in order to be able to estimate the variations of the hemolymph composition during the development.

Efforts were made in many laboratories to realize all the conditions favorable to a good *in vitro* culture of insect explants. However, it was a good ten years after the work carried out by Frew before any satisfactory results were obtained, but from that time onward, the aim of the investigations carried out by means of this technique, as well as the forms which it took, varied. We shall therefore now attempt to describe briefly the principal types of work done. The results will be commented upon at greater length in later paragraphs or in other chapters. Nevertheless, it must be remembered that any such systematization is relative, because on the one hand, certain works correspond to several fields of research, and on the other hand, results obtained in one field may prove to be very precious for the progress of other studies.

B. Cellular Substrata by Organ Culture

A certain number of animal and plant parasites live part, or even the whole, of their life cycle within vector insects. The study of these parasites is of great importance, and the method of *in vitro* culture of insect organs, tissues, or cells has raised the hope of being able to analyze better the factors conditioning the development and the transmission of certain viruses or pathogenic protozoa. It was with this end in view that Trager (1937, 1938, 1959a,b) undertook the culture of the walls of the ovaric tubules of *Bombyx mori* and of different organs of *Aedes aegypti* and *Glossina palpalis*. Gavrilov and Cowez (1941) assured *in vitro* survival of organs of *Stegomyia fasciata* and *Anopheles maculipennis,* and Nauck and Weyer (1941) obtained the same result with different organs of *Cimex lectularius, Ctenopsyllus segnis,* and *Pediculus humanus*. Ball (1947, 1948, 1954) and Ball and Chao (1960) maintained an *in vitro* culture of the digestive organs of *Culiseta incidens, Culex tarsalis,* and *Culex stigmatosoma*. Pursuing a similar aim, Ragab (1949) cultivated in hanging drops or by perfusion the digestive tract of *Anopheles maculipennis* and *Aedes aegypti*. Vago and Chastang (1960) maintained in *in vitro* culture for several weeks ovaries and fragments of ovaries of *Antheraea pernyi, Bombyx mori, Galleria mellonella,* and *Lymantria dispar*. Similar works were carried out by Hirumi and Maramorosch (1963) on different organs of *Agallia constricta* and *Macrosteles fasci-*

frons. Lastly, Peleg and Trager (1963) obtained cell survival from larval imaginal discs of *Aedes aegypti* and *Culex molesus*.

The different authors we have just mentioned have, for the most part obtained *in vitro* cultures of insect organs. However, their aim was not to obtain an organized culture but rather a cellular substratum which would favor the development of protozoa or viruses.

C. Morphological Studies

Experimenting with the *in vitro* technique, many investigators have tried to obtain a real organized culture for studies in histogenesis or morphogenesis. They met with the greatest of difficulties for they expected the explant to show not only *in vitro* survival but also growth and differentiation. Fischer and Gottschewski (1939) and Gottschewski and Fischer (1939) explanted wing, leg, and eye imaginal discs of *Drosophila melanogaster,* and they were able to observe a certain *in vitro* differentiation of these organs without, however, proceeding with histological control. Similarly, Stern (1940), limited himself to the external morphological aspect, reproduced *in vitro* a stage of the differentiation of pupal testes of *D. melanogaster*. Goodchild (1954) had very little success with *in vitro* culture of the epiderm, heart, and testis of *Rhodnius prolixus* and of *Blatta orientalis*. Demal (1955, 1956), using first Wolff and Haffen's culture medium (1952), then a liquid medium, obtained *in vitro* a restricted differentiation, histologically controlled, of imaginal buds of *Calliphora erythrocephala* legs and of *D. melanogaster* eyes. Adult ovaries of *Aedes hexodontus, A. aegypti,* and *A. communis* were kept in survival by Beckel (1956), but the eggs did not mature and no mitosis was observed. Pursuing his research on the development of the *D. melanogaster* eye, Gottschewski (1960) was able to observe *in vitro,* in different strains, distinct bud growth and differentiation. In the years 1956–1960 the Japanese school made several important contributions in favor of organized *in vitro* culture of insect organs. Their investigations were chiefly of the genetical order and will therefore be mentioned under that heading. One important study of morphogenesis must, however, be mentioned here, i.e., that of Horikawa and Sugahara (1960) on the effects of *in vivo* irradiation on the behavior of larval organs of *Drosophila melanogaster,* Oregon strain, cultured *in vitro*. Hanly (1961) signaled a good *in vitro* survival of third stage larval brains of *D. melanogaster*. Demal (1961) obtained *in vitro* survival and continued contraction of the aorta of *C. erythrocephala* as well as limited differentiation of the pronymphal ovaries and testes of the same insect