British Society for
Cell Biology Symposium 1

ORGAN CULTURE IN BIOMEDICAL RESEARCH

Edited by
Michael Balls and
Marjorie A. Monnickendam

BRITISH SOCIETY FOR CELL BIOLOGY
SYMPOSIUM 1

ORGAN CULTURE IN BIOMEDICAL RESEARCH

FESTSCHRIFT FOR DAME HONOR FELL, FRS

EDITED BY

MICHAEL BALLS
Senior Lecturer in Human Morphology
University of Nottingham

AND

MARJORIE A. MONNICKENDAM
Research Assistant
Institute of Ophthalmology, London

CAMBRIDGE UNIVERSITY PRESS
CAMBRIDGE
LONDON · NEW YORK · MELBOURNE

Published by the Syndics of the Cambridge University Press
The Pitt Building, Trumpington Street, Cambridge CB2 1RP
Bentley House, 200 Euston Road, London NW1 2DB
32 East 57th Street, New York, NY 10022, USA
296 Beaconsfield Parade, Middle Park, Melbourne 3206, Australia

Library of Congress catalogue card number: 75-21034

ISBN: 0 521 21001 1

First published 1976

Printed in Great Britain
at the
University Printing House, Cambridge
(Euan Phillips, University Printer)

PREFACE

BY MICHAEL BALLS AND
MARJORIE A. MONNICKENDAM

This Festschrift on *Organ Culture in Biochemical Research* is the first published symposium of the British Society for Cell Biology. The meeting was held on 8–11 April 1975 at the University of East Anglia and the Norfolk and Norwich Hospital to celebrate the seventy-fifth birthday of Dame Honor B. Fell, FRS. It also coincided with the inauguration of the Society's *Honor Fell Awards*.

In the opening chapter Dame Honor, who pioneered the development of organ culture techniques and recognised their potential value in biomedical research, outlines the history of the subject. The other chapters include papers on general methodology, the analysis of cell interactions in morphogenesis, studies on enzyme and hormone production and release, hormone effects, the elucidation of errors in cell metabolism, carcinogenesis, tumour development and cancer chemotherapy, and on the effects on cultured tissues of drugs, irradiation, viruses and micro-organisms. The book is not intended to be an exhaustive tissue-by-tissue or problem-by-problem catalogue of all aspects of organ culture, but to provide information and inspiration for those using or beginning to use organ culture methods in their research. However, two omissions that should, perhaps, be mentioned are the use of organ culture in studies on the immune response (Globerson & Auerbach, 1965; Auerbach & Ruben, 1970) and on the cardiovascular system (Wildenthal, 1971; Armstrong & Longmore, 1973).

The programme was organised in consultation with Dame Honor Fell, Dr L. M. Franks, Dr Gisele Hodges and Dr J. S. Pryor. We are also very grateful to Flow Laboratories Limited, Irvine, Scotland, for providing Flow Lectureships to enable Professor Etienne Wolff of the *Académie française*, Dr Y. Croisille and Dr R. Dubois to attend the meeting; the Norwich Area Health Authority and the University of East Anglia for their hospitality; and the staff of the Cambridge University Press for an enjoyable collaboration.

REFERENCES

ARMSTRONG, S. R. & LONGMORE, D. B. (1973). The effects of cardiovascular drugs on the performance of cultured foetal hearts. *Nature, London*, **243**, 350–2.

AUERBACH, R. & RUBEN, L. N. (1970). Studies on antibody formation in *Xenopus laevis*. *Journal of Immunology*, **104**, 1242–6.

GLOBERSON, A. & AUERBACH, R. (1965). Primary immune reactions in organ culture. *Science, New York*, **149**, 991–3.

WILDENTHAL, K. (1971). Long-term maintenance of spontaneously beating mouse hearts in organ culture. *Journal of Applied Physiology*, **30**, 153–7.

CONTENTS

The development of organ culture

BY HONOR B. FELL, FRS

University of Cambridge, Department of Pathology,
Division of Immunology, Laboratories Block,
Addenbrooke's Hospital, Hills Road,
Cambridge CB2 2QQ

I am indeed delighted and touched by the Society's decision to make the proceedings of this symposium a Festschrift in my honour, in view of the fact that next month I shall have what the letter to contributors so politely described as a 'very significant birthday'. This charming gesture from the Society will make a very happy landmark in my long scientific career and go far towards consoling me for having virtually completed seventy-five years of what, on the whole, has been a most enjoyable life.

I think that the introduction of this symposium should begin with a definition of organ culture. It might be described quite simply as the maintenance of tissues in a differentiated functional state in a nutrient medium *in vitro*. I am sometimes asked: 'but do differentiated tissues really grow in organ culture?'. The answer is that although cell division takes place, often quite actively, in a healthy explant of organised tissue, the study of growth, in the sense of cell multiplication, is seldom the primary object of the organ culture technique; rather, the method is designed to provide an environment that will permit differentiated tissues to exercise their normal functions under the closely controlled conditions obtainable in an in-vitro system. Once this has been achieved for a given tissue, all kinds of experiments become possible which could not be done *in vivo*; this is clearly illustrated by the programme of the present meeting.

EARLY HISTORY

There were many early investigations that might be regarded as precursors of organ culture. These mainly concerned the self-differentiating capacity of pieces of early amphibian embryos isolated in non-nutrient fluid, but such preparations were not organ cultures according to our definition. If we regard the cultivation of entire avian and mammalian embryos in blood plasma as organ culture, we can go as far back as 1912, when McWhorter & Whipple published their observations on the progressive development of chick blastoderms in

hanging drop cultures. This investigation was followed by a number of other similar studies. Maximow (1925) has given an excellent review of all this early work.

In my view, the first true organ culture experiments were those of David Thomson which were published in 1914. Using the original hanging drop method, he explanted toes, feather germs, the tip of the tongue, optic lens and tail bud from embryonic chicks of different ages, embedding them in a plasma: embryo extract clot. To quote his own words (Thomson, 1914*b*): 'I showed that when definite uninjured masses of a chick embryo were placed in the medium, then these masses increased in size while retaining their original shape; in other words they showed a controlled increase in size or growth... It would appear to me that uncontrolled growth can only take place from injured surfaces where there is no basement membrane'. Surprisingly, Thomson never examined the histological structure of his explants to see if they had continued to differentiate, as well as to enlarge, *in vitro*.

At this point the 1914–18 war broke out and the only paper that I can find during this period is by Shipley (1916) who explanted pieces of chick blastoderm in plasma, and reported the formation of beating heart muscle and rather abnormal erythropoiesis in his cultures.

In 1922, however, three papers appeared which deserve mention. Maximow's pupil, Chlopin, cultured the gut and other tissues from rabbit and guinea-pig embryos and noted an advance in differentiation. Fischer cultivated pieces of intestine from a 21-day embryonic chick in plasma and tissue juice, and obtained mucus secretion and muscular contraction even after a month *in vitro*. The most interesting paper, however, was by Ebeling & Fischer; this reported the first example of an experiment on cell interaction in culture, a subject that later was to be developed in such a dramatic way by Moscona, Grobstein and others. Ebeling & Fischer mixed a 10-year-old strain of fibroblasts with a 2-month-old strain of epithelium and cultivated them together, with several subdivisions, for seven passages. When the cultures were sectioned and examined histologically, the epithelium was found to have formed tubules and the fibroblasts had differentiated into fibrous connective tissue. These changes were not seen in controls in which the two cell types were grown near but not in contact with each other.

The next landmark in the development of organ culture was a monograph by Maximow in 1925, on the differentiation of pieces of rabbit embryo in plasma; the cultures were made by Maximow's double-coverslip method which is still in active use and will be mentioned again later. Maximow described and convincingly illus-

trated a variable degree of differentiation in a wide variety of tissues; this paper is particularly noteworthy, however, for the terminology introduced to describe the main forms of growth *in vitro*. Maximow substituted the term *histiotypic* for Thomson's 'uncontrolled' growth: 'The characters of the single tissues remain unaltered and independent and the disintegration of the individuality of the former organism does not go beyond this limit.' In *cytotypic* growth: 'the various tissues *in vitro*, remaining independent from each other, become dissolved into irregular crowds of single cells'. Finally he replaced Thomson's 'controlled' or 'somatic' by '*organotypic*' growth: 'The cells and tissues of the explant are combined in the form of a new, artificially created unit of a higher degree, corresponding to an organ or even an organism.'

I do not know, or have forgotten, who invented the term 'organ culture', but I suspect that it was derived from Maximow's word 'organotypic'. It is transatlantic in origin and I fancy that it came into use after the tissue culture conference at Hershey (more famous for its chocolate bars), which in 1947 initiated the formation of the now large and powerful American Tissue Culture Association.

I became involved in organ culture in 1924 when, having just finished my PhD at the University of Edinburgh, I joined the late Dr T. S. P. Strangeways as his assistant, at what was then the Cambridge Research Hospital and is now the Strangeways Research Laboratory. Dr Strangeways was Huddersfield Lecturer in Special Pathology at Cambridge University and he was also a clinician specialising in rheumatoid arthritis and related diseases; in addition he was the chief pioneer of tissue culture in this country. When I joined him, he happened to be interested in the loose cartilaginous bodies that sometimes cause trouble in joints and which he regarded – correctly I think – as tissue cultures *in vivo*. He was making some experiments with fragments of embryonic chicken cartilage which he had explanted on the surface of a plasma: embryo extract clot at the bottom of a small centrifuge tube. He found that the isolated piece of cartilage survived and enlarged under these conditions, thus lending support to his interpretation of the loose bodies *in vivo*. Being interested in developmental problems, I wondered whether cartilage would differentiate *de novó* in this in-vitro system. Always delighted to try anything new, Strangeways agreed that we should look into the matter. So we cut off the undifferentiated limb buds of 3-day chick embryos and explanted them on the clots in the little tubes; every 2 days we sucked them into a pipette and transferred them to new medium. It was obvious that they were enlarging quite rapidly; finally we fixed and sectioned them and I

well remember our delight when we found that each bud had formed a nodule of cartilage, some nodules being several millimetres in length. We decided to investigate a more complex organ in the same way and chose the eye, again using the 3-day chick embryo. We were astonished at the degree of development that took place in the simple epithelial rudiment during 10 days' cultivation; lens fibres were formed, pigment epithelium developed and the retinal epithelium acquired the characteristic structure of the adult retina in all its complexity. The gross anatomy, however, was much distorted.

These studies were published in 1926 (Strangeways & Fell, 1926*a*, *b*). Sadly enough, in December of that year Dr Strangeways died. I presented the work as a demonstration at the Tenth International Congress of Zoology at Buda-Pesth in a new section devoted to experimental cell biology. I mention this, because this section later became independent and was the primordium from which the International Society for Cell Biology eventually developed.

For a good many years very little organ culture was done outside the Strangeways Laboratory, and those wishing to practise tissue culture turned their attention to what is now known as cell culture; this was due mainly to the powerful influence of Carrel and his school. Then in 1933 a young Dutch scientist, Pieter Gaillard, became interested in organ culture, the possibilities of which he clearly understood. He came to work with me for a short time, after which he returned to Leiden and set up an organ culture unit in his University. As you all know, he became the pioneer in applying the technique to physiological studies.

In 1939 the progress of organ culture was again arrested by war. When this was over and we had thankfully resumed our normal activities, the select band of organ culturists was joined by a distinguished recruit in the person of Professor Etienne Wolff, already an eminent experimental embryologist. He was impressed by the potential value of the technique as an embryological tool, and successfully exploited it for the investigation of an enormously wide range of developmental problems. Some of you may have visited his large and very active laboratory near Paris, and we look forward to hearing some of the 'hot news' from there later in this programme.

I think this marks the end of the first chapter in the development of organ culture. The three laboratories in England, Holland and France metastasised all over the scientific world, and recently the American Tissue Culture Association established a special division for organ culture and related topics.

TECHNICAL DEVELOPMENT

The scientific potentialities of organ culture obviously depended on the methodology available. Before 1924 only the hanging drop method was in use and the medium consisted of clotted plasma usually with the addition of embryo extract. For some types of small explants this may give quite good results, and W. Jacobson and I (1941) used it successfully to analyse the developmental potencies of different regions of the mandible of 3- and 4-day chick embryos. For this work minute pieces of mesenchyme were excised from different parts of the jaw rudiment. When cultivated in hanging drop preparations, explants from some areas produced only bone, from others only cartilage and from others again only soft connective tissue or muscle. In general, however, the hanging drop method is unsatisfactory for organ culture. An exception is Maximow's double-coverslip method, in which the tissue and medium are placed on a small coverslip held by a tiny droplet of saline to a larger coverslip which is sealed over a hollow ground slide; the preparation is often incubated as a sitting rather than a hanging drop. At subculture the tissue is not disturbed, but the little coverslip is detached, washed, the tissue re-fed with fresh medium and the small coverslip is again attached by surface tension to a large one. In the hands of Margaret Murray and Edith Petersen (Petersen & Murray, 1955) this method has proved excellent for the cultivation of ganglion cells which form myelinated nerve fibres and, to judge by electron micrographs, the cells remain in a perfectly normal state during several months *in vitro*.

Strangeways's tube method was a great improvement on the hanging drop technique. This was probably due to the fact that the tissues were grown on the top of a relatively large volume of medium and had a big air space above them; thus they received both an ample food supply and good oxygenation which permitted the early rudiments of such complex organs as the eye (Strangeways & Fell, 1926*b*) and ear (Fell, 1928) to attain an advanced stage of differentiation *in vitro* with little or no necrosis.

The tube method, however, was rather inconvenient, and although it allowed the cultivation of larger pieces of tissue than would survive in a hanging drop culture, the explants were still rather small and only one could be grown in each tube. Some months after I came to the Research Hospital, Strangeways began some experiments on the early development of isolated chick embryos *in vitro*. He incubated the embryos on a plasma:embryo extract clot contained in a large watch-glass. To

provide a moist chamber the watch-glass was enclosed in a pot with water at the bottom. The work was never published. After Strangeways's death it occurred to me that the method might be adapted for ordinary organ culture and in particular for the cultivation of large explants. This was the origin of the watch-glass method which, in various forms, was to become the standard organ culture technique.

The earliest watch-glass culture (Fell & Robison, 1929) consisted of a watch-glass with its convex surface painted black to facilitate macroscopic observation, placed on a layer of absorbent cotton wool at the bottom of a Petri dish. After sterilisation 30–40 ml of sterile distilled water was pipetted into the dish where it was absorbed by the cotton wool. Equal parts of fowl plasma and chick embryo extract were introduced into the watch glass and allowed to clot. Three or four explants were placed on the surface of the clot in each watch-glass, and the preparation was then incubated. The medium maintained a neutral pH without gassing. Every 2 days the explants were removed from the clot, washed and replanted on fresh medium, as in the tube cultures.

This culture method was designed for some experiments with the late Professor Robert Robison (Fell & Robison, 1929) on the development and alkaline phosphatase activity *in vitro* of the femur rudiments of 5½–6-day chick embryos. The rudiments enlarged rapidly to several times their original size, and not only differentiated histologically with the formation of periosteal bone, but also acquired much of their normal anatomical shape. At that time Robison, who was a distinguished biochemist, was interested in the fact that ossifying cartilage *in vivo* actively synthesised alkaline phosphatase, an enzyme that he believed to be concerned in calcification; examination of the explants at different stages of cultivation showed that they produced increasing amounts of this enzyme. In subsequent experiments (Fell & Robison 1930) we found that alkaline phosphatase was not produced by explants of the non-ossifying cartilage, Meckel's rod, from the embryonic mandible. To the best of my knowledge these are the first biochemical experiments on organ cultures. The data, however, referred only to the explants and did not include the culture medium; as we shall see later, this was a serious limitation of the early watch-glass technique and was imposed by the plasma: embryo extract clot which was quite unsuitable for biochemical study.

Although many tissues thrived on a plasma: embryo extract clot, some did not. James Chen (1954) working in our laboratory, wished to grow the embryonic rat pancreas in organ culture, but found that when explanted on a clot the tissue was soon killed by the accumulation

around it of its own secretory products. A liquid medium, which would allow these materials to diffuse away, seemed desirable, but most explants degenerate when lying at the bottom of a pool of fluid medium. Chen produced an ingenious solution to the problem. He found that the particular lens paper used at the Strangeways would float when placed on a fluid medium composed of serum and embryo extract; so he placed the pancreas rudiments on pieces of this paper which served as rafts to keep the tissue on the surface of the medium. This arrangement was a great success, and after 10 days in culture the rudiment, which showed little or no necrosis, had formed secretory cells, ducts and islets of Langerhans.

The method had its disadvantages, however. Not all lens paper floated, and even that which did float was not very seaworthy; if overloaded or if the upper surface became too extensively wetted, the paper sank with all on board. Moreover, it could not be used for very invasive tissue which incorporated the paper into the substance of the explant so that the latter became impossible to section for histological study.

Organ culture owes much to a simple technical innovation introduced by the late Dr O. A. Trowell (1959). For the cultivation of adult organs he supported the explants on a shallow table of tantalum gauze standing in a flat-bottomed culture dish and covered with a sheet of lens paper; since mature organs are not very invasive, the lens paper was quite suitable for his purpose. In various forms this system is the most widely used at the present day, but the mesh tables are now made of a much cheaper, non-toxic, stainless steel gauze and when soft tissues are to be cultivated, the lens paper is replaced by a Millipore membrane with a large pore size which presents no histological difficulties. Trowell also demonstrated that mature organs thrive better in an atmosphere containing raised oxygen than they do in air. The type of chamber that he devised for gassing his cultures is not very satisfactory, and in our experience a modified Fildes–MacIntosh jar, normally used for the culture of anaerobic bacteria, is much simpler and more reliable.

The technique now used by the Strangeways group and myself is a hybrid between the original watch-glass method and Trowell's modification of this method; several people have been concerned in its development, but it owes most to T. Fainstat (1968), an American visitor at the Strangeways Laboratory. A pair of flat-bottomed culture vessels, each containing a table of stainless steel mesh, are enclosed in a Petri dish carpeted with filter paper in which two large round holes have been bored to accommodate the culture vessels. The filter paper is

saturated with isotonic saline containing 0.005 % potassium permanganate. The height of the mesh table is so adjusted that the fluid culture medium just reaches the top of the grid. Eight Petri dishes are stacked in a special rack, enclosed in a Fildes–Macintosh jar and gassed with whatever gas mixture is required. At 2-day intervals the used medium is removed with an angled pipette and replaced by fresh. There are, of course, disposable plastic dishes of a similar type, but for various reasons I prefer the glass vessels that I have described.

Nowadays there is a very wide variety of organ culture methods which have been developed for different purposes, and I know that Dr Hodges is going to review them for us in the next paper.

As the potentialities of organ culture increased, it became more and more desirable to be able to investigate both tissues and medium biochemically. For this there were two requirements: a sufficiently simple fluid culture medium and microchemical methods that could be applied to the small quantities of material that these cultures provide. From the biochemist's point of view one of the great disadvantages of pre-war culture methods was the use of embryo extract in the culture medium, as this hopelessly complicated component precluded any significant analysis being made. The advent of chemically defined media was a godsend, since when mixed with a certain percentage of serum, some of these media were almost or quite as good as a mixture of serum and tissue extract. Of course ideally we should use a chemically defined medium without the addition of serum, but in practice this is seldom satisfactory because few tissues remain normal in chemically defined medium alone. Recently, however, Sylvia Fitton-Jackson has produced a medium that permits various types of skeletal cells and tissues derived from embryonic chicks to differentiate and to survive for many weeks in culture without serum, so perhaps we may hope for better things in the future.

Since the Second World War, microchemistry has developed to a remarkable degree, and we are now in a position to investigate a fairly wide range of biochemical phenomena even in medium containing serum.

THE USES OF ORGAN CULTURE

The progress of organ culture has been marked by the increasing sophistication of the questions that the cultures have been required to answer. At first only one, quite simple question was asked. Thomson (1914*a*, *b*) probably said to himself: 'I wonder what will happen if I make cultures of these intact bits of embryo'. Chlopin (1922) and

Maximow (1925) were more specific in asking: will bits of embryo differentiate in culture? Strangeways and I (Strangeways & Fell, 1926*a*, *b*; Fell, 1928) asked: will isolated organ rudiments develop *in vitro*? During this initial period the most subtle question was that posed by Ebeling & Fischer in 1922: will dedifferentiated fibroblasts and epithelium grown separately as cell cultures, redifferentiate if mixed together?

With the advent of the watch-glass method and its various modifications, the potentialities of organ culture expanded. In 1938 Gaillard & de Jongh published the first paper on the effect of a hormone on an organ *in vitro*. They wished to know whether the mouse uterus would respond to the direct action of oestrone. The answer was 'yes'; a dose of 1/5000 of an IU caused a great increase in muscle, including the formation of a second longitudinal muscle layer.

Instead of being confined to one simple question, we soon found ourselves in a position to ask a long series of related questions. To give you an example: in the early 1930s one of the problems in which I was interested was the developmental mechanics of early joint formation in the embryonic chick (Fell & Canti, 1934). For this work I used the skeletal blastema dissected from the leg buds of 4-day embryos. First, would the knee-joint develop in such explants? The articular surfaces developed and separated, but no joint cavity was formed. Would the isolated knee-joint region give rise to a joint? No. Would it form a joint if grafted into the tibio-fibular region of another explant? Yes, provided the graft became neatly incorporated into the host blastema. Did excision of the presumptive articular tissue inhibit joint formation in the blastema? Not if the blastema was still unchondrified, but the shape of the articular surfaces was abnormal. If chondrification had begun, a joint formed only if a very small part of the articular region was removed; if too large a portion was taken no joint appeared and the femur developed in direct continuity with the tibia and fibula. From these and other data we proposed an hypothesis to explain the separation of the articular surfaces.

In Professor Gaillard's laboratory, a long series of questions began in 1949 (Gaillard, 1955) and is still in progress. It concerns the action of the parathyroid gland on skeletal tissues. As you know, *in vivo* excessive activity of the gland causes bone resorption; if placed in contact with bone in organ culture, would the parathyroid cause the resorption of bone matrix *in vitro*? The answer was 'yes'. Would purified parathyroid extract produce the same effect? Yes (Gaillard, 1959). Is cartilage also affected? Yes, but it is less sensitive than bone and responds