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ALEXIS CARREL AND THE MYSTICISM OF TISSUE CULTURE

by

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SUMMARY

ALEXIS CARREL was one of the pioneers of tissue culture and its chief publicist. He was largely responsible for the early development of the technique, but although he made a number of practical contributions, it was his influence on his contemporaries that was particularly significant. Carrel's tissue culture techniques were based on his surgical expertise and they became increasingly complicated procedures. Contemporary opinion of his work was that the methods were extremely difficult, an opinion enhanced by the emphasis Carrel himself laid on the problems of tissue culture techniques. Because of his flair for publicity, Carrel's views dominated the field and led to a decline in interest in tissue culture which persisted for many years after he ceased tissue culture studies.

INTRODUCTION

In 1907 Ross G. Harrison published a short note entitled 'Observations on the living developing nerve fibre'[1] that described his latest research on the growth and development of the nervous system. He attempted to distinguish between the outgrowth theory of His and the intercellular cytoplasmic bridge theory of Hensen by studying the behaviour of fragments of tadpole spinal cord incubated in a clot of lymph in a hollow-ground glass slide. Harrison found that nerve fibres grew out from the explants by active movements of the nerve fibre tips and he thus resolved one of the major anatomical controversies[2] of the time in favour of His.

However, these experiments aroused much wider interest, for the potential of the tissue culture technique devised by Harrison was immediately recognized, and Abercrombie has described this work as an "astonishing stride forward in the history of biology".[3] Tissue culture is now one of the most widely applied techniques in

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[1] R. G. Harrison, 'Observations on the living developing nerve fiber', *Proc. Soc. exp. Biol., N.Y.*, 1907, 4: 140–143.

[2] For a discussion of this controversy see S. M. Billings, 'Concepts of nerve fiber development, 1839–1930', *J. Hist. Biol.*, 1971, 4: 275–305.

[3] M. Abercrombie, 'Ross Granville Harrison, 1870–1959', *Biogr. Mem. Fellows R. Soc. Lond.*, 1961, 7: 111-126. J. M. Oppenheimer has discussed the emergence of tissue culture technique from earlier experiments in which tissue was transplanted between embryos ('Historical relationships between tissue culture and transplantation experiments', *Trans. Stud. Coll. Physcns Phila.*, 1971, 39: 26–33; and a contemporary discussion of research on isolated organs and tissues and its relationship to tissue culture will be found in R. Legendre, 'Les recherches récentes sur la survie des cellules, des tissus et des organes isolés de l'organisme', *Biologica*, 1911, 1: 357–365. A brief but wide-ranging

biological and medical research with some 14,000 papers indexed in the 1976 edition of the bibliography published by the Tissue Culture Association.[4]

In the years following publication of Harrison's full paper[5] in 1910, the method was enthusiastically adopted by workers throughout the world, but principally by Carrel and Burrows at the Rockefeller Institute, by W. H. and M. R. Lewis at the Carnegie Institute, by Drew and Walton in Britain, Champy in France, and Levi in Italy. A very large number of papers were published, and by 1911 tissue culture was sufficiently well established for the American Association of Anatomists to devote a symposium to it at their annual meeting in Princeton.[6] In the same year an editorial in the *Journal of the American Medical Association* commented on studies by Carrel and Burrows: "It is difficult to estimate the importance of this new work. It lays bare practically a whole new field for experimental attack on many of the most fundamental problems in biology and the medical sciences."[7]

Tissue culture studies continued at a rapid pace throughout the 1910s and in the early 1920s several reviews and books were published that attempted to describe and assess the achievements of the technique. These achievements appeared to be disappointing when compared with the original high expectations held for the method. For example in 1923, twelve years after the optimistic editorial in the *Journal of the American Medical Association*, an editorial in the *Lancet* expressed considerable dissatisfaction with progress in the field: "It was a line of research rich in promise but it must be confessed that its fruits have hitherto been meagre and far from encouraging."[8] Those results that had been obtained seemed to be less than satisfactory, and Fischer commented in 1925 that: "During the short time that this method has been propagated into many different lines of biology many more or less hazardous conclusions have been brought out, mostly built on very imperfect technique."[9]

As Willmer[10] has remarked, tissue culture seemed to become "becalmed in the doldrums", perhaps because it had failed to fulfil its early promise, and also because it had acquired the reputation of being a difficult and esoteric technique. Willmer believes that: "Tissue culture, although a delicate and exacting technique and one in which vigorous asepsis is absolutely essential, gained a spurious and unfortunate reputation for difficulty and almost for mysticism."[11]

For example, Carleton, in a review published in 1923, felt it necessary to warn his readers that "the necessity for elaborate aseptic precautions has been over-empha-

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review of the development of tissue culture can be found in K. Russell, 'Tissue culture—a brief historical review', *Clio Medica*, 1969, **4**: 109–119.

[4] *Index of tissue culture*, New York, Tissue Culture Association, 1976.

[5] R. G. Harrison, 'The outgrowth of the nerve fiber as a mode of protoplasmic extension', *J. exp. Zool.*, 1910, **9**: 787–846.

[6] Symposium on Tissue Culture; American Association of Anatomists, Princeton, 27 December 1911. Papers presented at this symposium were published in *Anat. Rec.*, 1912, **6**.

[7] [Anonymous], 'Growing tissues outside of the body', *J. Amer. med. Assn*, 1911, **56**: 1722–1723.

[8] [Anonymous], 'Tissue culture', *Lancet*, 1923, **i**: 858.

[9] A. Fischer, *Tissue culture*, Copenhagen, Levin & Munksgaard, 1925, p. 29.

[10] E. N. Willmer, 'Introduction', in E. N. Willmer (editor), *Cells and tissues in culture*, New York and London, Academic Press, 1965, pp. 1–17.

[11] Ibid., p. 4.

Figure 1.
Two technicians at work in Carrel's laboratory at the Rockefeller Institute. They are dressed in the full-length, black, hooded gowns adopted by Carrel. Between them is a rack of Carrel flasks that are being filled by the technician on the right while the technician on the left is flaming rubber bungs prior to sealing the flasks. (From R. C. Parker, *Methods of tissue culture,* New York, Paul B. Hoeber, 1938, Figure 23. *Reproduced by kind permission of Harper & Row.*)

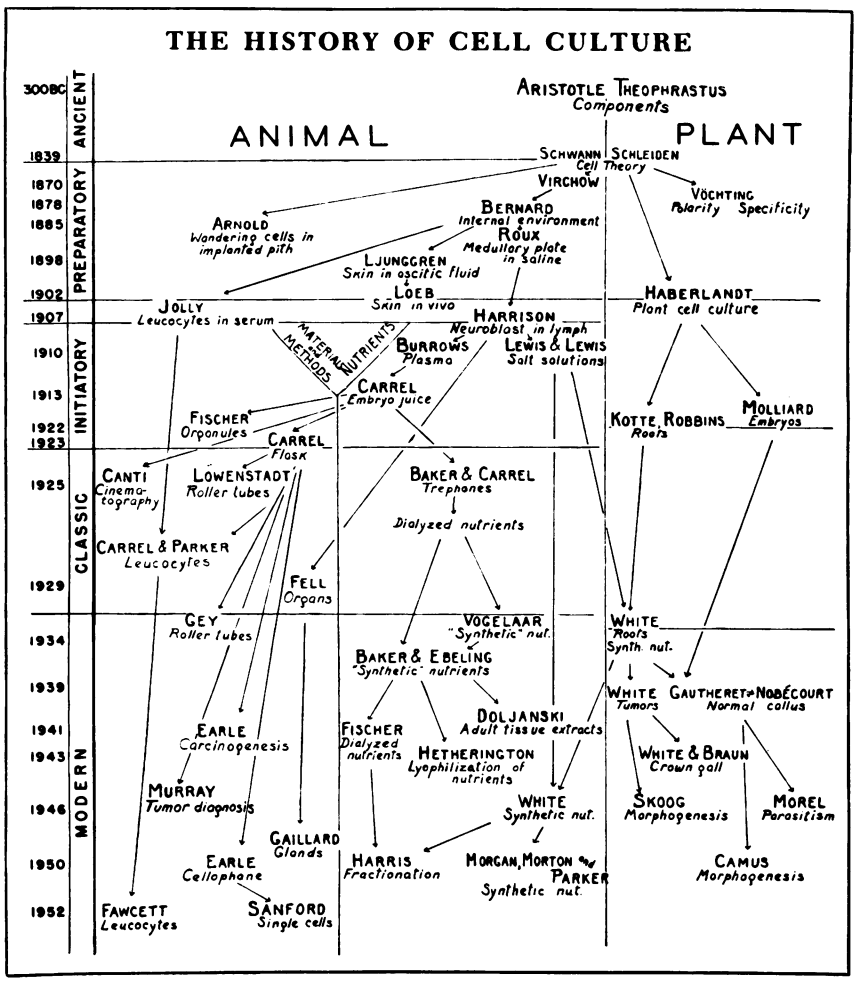

Figure 2.

A diagram drawn up by P. R. White to illustrate the history of tissue culture and the relationships between different workers in the field. It is divided vertically in areas of interest, with "animal" subdivided into "materials and methods" (left) and "nutrients" (right). The central role played by Carrel in the development of animal tissue culture is clearly seen. However, the diagram ignores the substantial contributions of many European workers, e.g., Drew, Strangeways, Waymouth, Willmer, Levi, Ephrussi, Chlopin, Jacoby, and many others. (From P. R. White, *The cultivation of animal and plant cells,* New York, Ronald Press, 1954, Figure 1. *Reproduced by kind permission of John Wiley & Sons.*)

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sised",[12] and as late as 1954, P. R. White declared as one of his purposes in writing his tissue culture manual: "I have sought to strip from the study of this subject its former atmosphere of mystery and complication. The grey walls, black gowns, masks and hoods; the shining twisted glass and pulsating coloured fluids; the gleaming stainless steel, hidden steam jets, enclosed microscopes and huge witches' cauldrons of the 'great' laboratories of 'tissue culture' have led far too many persons to consider cell culture too abstruse, recondite and sacrosanct a field to be invaded by mere *hoi polloi!*"[13]

This is a scarcely veiled reference to the pioneer Alexis Carrel,[14] surgeon and winner of a Nobel Prize in 1912, for it was well known that his tissue culture technicians wore black, full-length, hooded gowns (see Figure 1), and the reference to "shining twisted glass and pulsating coloured fluids" is to the perfusion pump that was devised by Charles A. Lindbergh, the transatlantic flier, in Carrel's laboratory.[15]

Alexis Carrel was principally responsible for the development and elaboration of tissue culture techniques (see Figure 2) and his laboratory at the Rockefeller Institute was the major centre for tissue culture studies. His collaborators included Montrose Burrows and Arthur Ebeling, both of whom made important contributions, but it was Carrel who dominated the group with his ideas and techniques.

Willmer was openly critical of Carrel and declared that it was the work of Carrel and his associates that "caused the method to be wrapped up from the beginning in a considerable cocoon of mumbo-jumbo, derived from the practices that were prevalent at that time in the operating theatres of the world".[16] John Paul, in the introduction to his book *Cell and tissue culture* follows Willmer's criticism and says of Carrel: "Unfortunately the meticulous surgical techniques he employed dissuaded many biologists from using the method and engendered the belief that tissue culture was fantastically difficult."[17]

Is this true, or has Carrel's role become a myth of tissue culture? I intend to review papers published during the first phase of the development of tissue culture to determine as far as possible if Carrel and other early investigators created in their published work the reputation of difficulty described by Willmer and Paul. Such a review must be selective because a large number of papers were published during

[12] H. M. Carleton, 'Tissue culture; a critical summary', *Br. J. exp. Biol.*, 1923, **1**: 131–151.

[13] P. R. White, *The cultivation of animal and plant cells*, New York, Ronald Press, 1954, p. vi.

[14] Alexis Carrel (1873–1944) obtained his degree in medicine at the University of Lyons and began experimental studies on the surgery of blood vessels. In 1904 he left Lyons after medical and political disagreements with the Faculty, and moved to Chicago. He was appointed to the Rockefeller Institute in 1906 and won the Nobel Prize in 1912. He returned to France at the beginning of the First World War and ran a military hospital where he devised a method for cleansing wounds by irrigating them with saline solutions. He retired from the Rockefeller Institute in 1938 and returned to Paris where he established his Institute for Study of Human Problems. During the Second World War he accepted help from the Vichy government and negotiated with the occupying German forces. This led to charges of collaboration, and only his death avoided the humiliation of arrest. For further details see W. S. Edwards and P. D. Edwards, *Alexis Carrel, visionary surgeon*, Springfield, Ill., Charles C Thomas, 1974.

[15] A brief history of Lindbergh's collaboration with Carrel can be found in G. W. Corner, *A history of the Rockefeller Institute, 1901–1953*, New York, Rockefeller Institute Press, 1964, pp. 232–237. See also Edwards and Edwards, op. cit., note 14 above.

[16] Willmer, op. cit., note 10 above, p. 4.

[17] J. Paul, *Cell and tissue culture*, Edinburgh, Livingstone, 1965, p. 2.

this period; Fischer's book[18] published in 1925 listed 542 publications of which some 140 appeared from Carrel's laboratory. A number of reviews and books were published in the 1920s, and I shall take these as signalling the end of the first phase of development, although I shall also look ahead to developments that indicate why there was some justification for the elaborate techniques devised by the pioneers.

TECHNICAL DIFFICULTIES

Harrison published his classic paper[19] in 1910, and in that year Burrows from the Rockefeller Institute visited Harrison's laboratory at Yale[20] to learn his technique.[21] Burrows was particularly interested in growing cells from warm-blooded animals, but it became clear that there were technical problems that Harrison, using amphibian tissue and short incubation times, had not had to face. Burrows found that lymph clots were unsuitable for long-term cultures and used plasma instead.[22] Chick plasma was easily obtained, it could be stored until required and it formed clots that were firm and uniform in consistency. Burrows was able to grow nerves and mesenchymal cells from chick embryos,[23] and on his return to the Rockefeller Institute he and Carrel began to grow a variety of mammalian tissues in plasma clots.[24] In the same paper, Carrel and Burrows described another technical advance that was required for cultivating actively growing cells. It was supposed that the slowing of cell growth after several days in culture was due to the inhibitory effects of metabolic waste products that accumulated in the plasma clot. Carrel and Burrows overcame this by transplanting fragments of the original culture to clean slides and adding fresh plasma.[25] In this way a new vigorous outgrowth was obtained and the procedure could be repeated whenever necessary.

Carrel and Burrows were sufficiently encouraged by these results to state that: "These experiments demonstrate that adult tissues grow very easily outside the body,"[26]

[18] Fischer, op. cit., note 9 above.

[19] Harrison, op. cit., note 5 above.

[20] Harrison began his tissue culture experiments in the Department of Anatomy at the Johns Hopkins University, but in 1907, the year that his first note on tissue culture was published (op. cit., note 1 above), he moved to Yale University as head of the Department of Zoology. A. M. Harvey, 'Johns Hopkins—the birthplace of tissue culture: the story of Ross G. Harrison, Warren H. Lewis, and George O. Grey', *Johns Hopk. med. J.*, 1975, **136**: 142–149.

[21] Burrows spent the spring of 1910 with Harrison. At that time Burrows was working with Carrel on problems of wound healing and nerve regeneration, and he intended to apply tissue culture methods to these problems. M. T. Burrows, 'The cultivation of tissues of the chick embryo outside the body', *J. Amer. med. Assn*, 1910, **55**: 2057–2058. For further details of the relationship between Harrison, Burrows, and Carrel, see F. S. Bang, History of tissue culture at Johns Hopkins', *Bull. Hist. Med.*, 1977, **51**: 516-537.

[22] Ibid., p. 2057.

[23] Ibid., p. 2058; M. T. Burrows, 'The growth of tissues of the chick embryo outside the animal body with special reference to the nervous system', *J. exp. Zool.*, 1911, **10**: 63–84.

[24] A. Carrel and M. T. Burrows, 'Cultivation of adult tissues and organs outside of the body', *J. Amer. med. Assn*, 1910, **55**: 1379–1381. These may not have been the first cultures of mammalian cells. In 1908, Margaret Reed (who married Warren H. Lewis in 1910), visited Dr. Max Hartmann's laboratory in Berlin. There she prepared explant cultures of guinea pig marrow that gave rise to cells. G. W. Corner, 'Warren Harmon Lewis', *Biographical memoirs—the National Academy of Sciences of the United States of America*, 1967, **39**: 323–358, see p. 332.

[25] Carrel and Burrows, op. cit., note 24 above, p. 1381.

[26] Ibid., p. 1380.

and that "the cultivation of normal cells would appear to be no more difficult than the cultivation of many microbes."[27] It does not appear that there was yet a preoccupation with method, for Burrows said that: "simple surgical technique, freshly sterilized glassware and instruments are sufficient."[28]

A very different approach was taken in the paper published one year later by Carrel and Burrows.[29] It is perhaps significant that this included the word "technique" in its title, and it is probable that the methods described here formed the basic technique followed by laboratories everywhere.[30] It is particularly unfortunate that in this paper Carrel drew so much on his surgical experience and, while admitting the technique to be theoretically "very simple", Carrel emphasized that a more elaborate, detailed technique was required. The influence of his surgical approach can be seen immediately: "The culture must be made in a warm, humid operating room with the same care and rapidity as a delicate surgical operation . . . the perfect teamwork of well-trained assistants is necessary."[31]

This is a very far cry from Burrows' simple surgical technique and must have been discouraging to researchers who did not have the facilities available in Carrel's surgical unit at the Rockefeller Institute.[32] Many of the precautions and procedures suggested by Carrel and Burrows were valuable, for example guarding against allowing tissue to dry during preparation.[33] Others are now known to be unnecessary: a small electric incubator was recommended for carrying cultures between the operating room and the main incubator, from which cultures could be removed, "for a few seconds without danger to their life."[34] Burrows also recommended carrying out dissections with the tissue maintained at 39°C.[35] These recommendations appear to have been made without any investigation of the effects of cooling on cell viability, for, later in 1912, Carrel recommended cooling cultures to 0°C for one hour at the time of transplanting.[36]

The method devised by Carrel and Burrows for prolonging the growth of cultured

[27] Ibid., p. 1381.

[28] Burrows, op. cit., note 23 above, p. 66.

[29] A. Carrel and M. T. Burrows, 'Cultivation of tissues in vitro and its technique', *J. exp. Med.*, 1911, **13**: 387–396.

[30] G. H. Drew refers specifically to this paper in the introduction to his paper 'On the culture in vitro of some tissues of the adult frog', *J. Path. Bact.*, 1912–1913, **17**: 581–593. He criticizes Carrel and Burrows for not describing the method in sufficient detail, an omission he goes on to correct.

[31] Carrel and Burrows, op. cit., note 29 above, p. 390.

[32] A description of the operating and culture rooms in Carrel's laboratory at the Rockefeller Institute can be found in R. C. Parker, *Methods of tissue culture*, New York, Paul B. Hoeber, 1938. There were two separate suites of rooms, each comprising animal preparation room, scrub-up room, and culture/operating room. The latter rooms had sprays built in so that before use dust in the room could be settled by spraying with water. Other investigators went to great lengths to improvise suitable working conditions. G. H. Drew for example, overcame the problem of not having water sprays by filling the room with steam before beginning work and keeping vessels of water boiling while the culture were being prepared, op. cit., note 30 above, p. 581.

[33] Carrel and Burrows, op. cit., note 29 above, p. 392.

[34] Ibid., p. 393. Carrel and his colleagues checked the temperatures of their incubators several times during the night to make sure that the cells did not fall below 39°C (Carrel quoted in A. Fischer, *Biology of tissue cells*, Cambridge University Press, 1946, see p. 225).

[35] Burrows, op. cit., note 23 above, p. 66.

[36] A. Carrel, 'On the permanent life of tissues outside of the organism', *J. exp. Med.*, 1912, **15**: 516–528.

tissues by repeatedly transplanting them was difficult and tedious.[37] Carrel's first improvement was to form the plasma clot on a piece of silk so that the tissue could be transferred more easily.[38] This does not seem to have been very successful and it was not used in further work in Carrel's laboratory. There was no significant improvement on the transplant method until 1923, when Carrel introduced his flask, which became eponymous.[39] This was a small flask with good optical properties and a long sloping neck designed to prevent particles of dust falling into the flask during handling. Fragments of tissue were attached to the floor of the flask with plasma and a fluid medium of saline solution containing various nutritive substances added. It was a relatively easy matter to remove the fluid medium when necessary and replace it with fresh medium. The development of the Carrel flask was a major advance that enabled more tissue to be handled and reduced the risk of bacterial contamination by reducing the number of manipulations required to set up and maintain cultures.

The practical difficulties of early tissue culture technique do not appear to have been great, although before the introduction of antibiotics experiments were often lost because of infection. Experimental embryologists such as Roux and Spemann had used techniques more demanding in skill than those required for tissue culture, and the apparatus required was simple and could be prepared in most laboratories. However, for whatever reason, papers published at this time by Carrel laid what seems to be undue emphasis on the problems of using tissue culture.

PROBLEMS OF MEASURING CELL GROWTH

Early investigations using tissue culture were concerned with cell morphology or with gross differences in growth, but when the Carrel flask technique was used to study the effects of different media on cell growth, it was realized that a more accurate method of measuring cell growth was required. Although suggestions were made to weigh cultures and methods were devised to do this,[40] it was impractical, and Ebeling devised the method that came into common use;[41] the increase in area of cell outgrowth around an explant was measured and this indicated the growth activity of cells in the culture. There were considerable practical difficulties in standardizing the method and only after "a number of minute details were modified and improved"[42] was a satisfactory procedure attained. The area of outgrowth around an explant was

[37] See, for example, A. Ebeling, 'The permanent life of connective tissue outside of the organism', ibid., 1913, **17**: 273–285. Ebeling lists in detail the subcultures of Carrel's "immortal" line of fibroblasts; between 17 January 1912 and 15 January 1913, it was subcultured on 129 occasions.

[38] Carrel, op. cit., note 36 above, p. 518.

[39] A. Carrel, 'A method for the physiological study of tissues in vitro', *J. exp. Med.*, 1923, **38**: 407–418.

[40] In the discussion following Carrel's presentation at the British Medical Association meeting at Bradford in 1924, the President of the Pathology and Bacteriology section (Professor C. H. Browning) suggested that weighing the cultures would be the most accurate method of measuring growth, but Carrel replied that this was not practicable ('Discussion on tissue culture: its bearing on pathological investigation', *Br. med. J.*, 1924, **ii**: 181). However Parker (op. cit., note 32 above, p. 160) described methods by R. Meier and H. Laser in which the plasma clot was dissolved in ten per cent alcohol or digested with pepsin before drying the tissue and weighing it. See also note 63 below.

[41] A. H. Ebeling, 'Measurement of the growth of tissues in vitro', *J. exp. Med.*, 1921, **34**: 231–243.

[42] Ibid., p. 231.

divided into two parts that were as equal in area as possible (Ebeling warned that a "great deal of practice"[43] was required to do this) and these were transplanted to two separate hollow-ground slides. An identical quantity of plasma was added to each and spread over identical areas outlined around each explant. The capacities of the slides were the same so that the rate of evaporation would be the same in both. Ebeling compared the increases in area to show that using the new method there were no differences between a pair of explants, but that there was significant variation when the normal method was used.

However, the method required great care, and warnings of the difficulties involved were given by various writers. Carleton remarked that "it is unfortunate that quantitation is so difficult,"[44] and Carrel warned that "the technique is delicate and in untrained hands the experimental errors are of such magnitude as to render the results worthless."[45] Such comments could not be expected to encourage other workers and would contribute to the impression that tissue culture was a difficult method beyond the reach of untrained hands.

CULTURE MEDIA

A major source of difficulty lay in the lack of understanding of the nutritional requirements of cells in culture,[46] although it was recognized that some media permitted only cell survival *in vitro* while others stimulated cell growth and division.[47] M. R. and W. H. Lewis prepared hanging drop cultures in simple saline solutions[48] and obtained excellent differentiation of cells;[49] their detailed observations of muscle cells *in vitro* were not equalled for many years.[50] Drew, in England, also advocated the use of saline culture media[51] and formulated one that was recommended by Strangeways.[52] Burrows believed that cells in saline solution survived only because breakdown products were released from degenerating cells in the explant,[53] and it

[43] Ibid., p. 234.

[44] Carleton, op. cit., note 12 above, p. 145.

[45] A. Carrel, 'Tissue culture and cell physiology', *Physiol. Rev.*, 1924, **4**: 1–20, see p. 6.

[46] A lucid contemporary review of the confusion in this area can be found in E. N. Willmer, 'Tissue culture from the standpoint of general physiology', *Biol. Rev.*, 1928, **3**: 271–302.

[47] Fischer, op. cit., note 9 above, p. 24.

[48] M. R. Lewis and W. H. Lewis, 'The cultivation of tissues from chick embryos in solutions of NacCl, $CaCl_2$, KCl and $NaHCO_3$', *Anat. Rec.*, 1911, **5**: 277–293. The Lewises were apparently led to this approach by Margaret Lewis's experience in Dr. Hartmann's laboratory in Berlin where Dr. Erdmann was growing free-living amoebae in a nutrient agar made up with physiological saline. Margaret Lewis's bone marrow cultures grew in similar salines and she continued with this system on her return to the Johns Hopkins Medical School. M. R. Lewis and W. H. Lewis, 'The growth of embryonic chick tissues in artificial media, agar and bouillon', *Bull. Johns Hopk. Hosp.*, 1911, **22**: 126–127.

[49] W. H. Lewis and M. R. Lewis reviewed their studies on cell differentiation in their chapter on 'Behavior of cells in tissue culture', in E. V. Cowdry (editor), *General cytology*, Chicago, University of Chicago Press, 1924, pp. 383–447.

[50] M. R. Lewis and W. H. Lewis, 'Behavior of cross-striated muscle in tissue cultures', *Am. J. Anat.*, 1917, **22**: 169–194.

[51] A. H. Drew, 'Three lectures on the cultivation of tissues and tumours in vitro. Lecture I', *Lancet*, 1923, **i**: 785–787.

[52] T. S. P. Strangeways, *Technique of tissue culture in vitro*, Cambridge, W. Heffer, 1924, p. ix.

[53] M. T. Burrows, 'Some factors regulating growth', *Anat. Rec.*, 1916–1917, **11**: 335–339.

was thought that saline media were unsatisfactory for experimental purposes.[54]

It was considered a most important advance when Carrel found that embryo extracts prepared by homogenization of embyros in saline markedly stimulated cell multiplication,[55] but the situation became more confused as extracts of various adult[56] or malignant[57] tissues were tested on a variety of cell types and found to have stimulatory effects. Attempts were made to isolate and identify the active substances in embryo extracts or find substitutes that could be used in place of a complete extract, but these were unsuccessful.[58]

The situation was further complicated because Ebeling's quantitative method was not a true measure of cell multiplication. As Abercrombie has pointed out, the area of cell outgrowth around an explant depends not on the rate of cell growth, but on the rate of outward migration of cells,[59] a fact recognized by Harrison[60] ten years before publication of Ebeling's paper. However, Ebeling and Carrel immediately used this method to study the nutritional requirements of cells[61] and the effects of serum and plasma from animals of different ages on cell growth.[62] By 1936 the method had fallen from favour and, although Parker discussed it at some length, he emphasized its inaccuracies.[63]

Understanding of the nutritional requirements of cells in culture was very slow to develop, and the papers published by Carrel were confusing and their discussions were abstruse. Carrel's concept of what constituted cell growth in culture is particularly difficult to understand and he was questioned on this in the discussion of his British Medical Association paper.[64] Dr. J. Cruikshank pointed out that if mitotic figures were observed in cultured cells then surely cells were growing. Carrel replied:

[54] M. R. Lewis defended the use of saline media in a footnote in her paper on muscle cells in culture. She said: ". . . most of the failures to obtain growth in tissue cultures in Locke's solution by other observers is probably due to some error in their technique, as I have found it possible to obtain as large a growth in Locke's solution as in plasma." M. R. Lewis, 'Rhythmical contraction of the skeletal muscle tissue observed in tissue cultures', *Am. J. Physiol.*, 1915, **38**: 153–161.

[55] A. Carrel, 'Artificial activation of the growth in vitro of connective tissue., *J. exp. Med.*, 1913, **17**: 14–19.

[56] A. J. Walton, 'The effect of various tissue extracts upon the growth of adult mammalian cells in vitro', *J. exp. Med.*, 1914, **20**: 554–572.

[57] Carrel, op. cit., note 45 above. This subject was also discussed by A. Carrel ('The method of tissue culture and its bearing on pathological problems', *Br. med. J.*, 1924, **ii**: 140–145). and by A. H. Drew ('Three lectures on the cultivation of tissues and tumours in vitro; Lectures II and III', *Lancet*, 1923, **i**: 833–835).

[58] Fischer, op. cit., note 9 above, see p. 44.

[59] M. Abercrombie, 'Concepts in morphogenesis', *Proc. R. Soc., Series B*, 1977, **199**: 337–344.

[60] R. G. Harrison, 'The cultivation of tissues in extraneous media as a method of morphogenetic study', *Anat. Rec.*, 1912, **6**: 181–193.

[61] A. Carrel and A. H. Ebeling, 'The multiplication of fibroblasts in vitro', *J. exp. Med.*, 1921, **34**: 317–337.

[62] A. Carrel and A. H. Ebeling, 'Age and multiplication of fibroblasts', ibid., 1921, **34**: 599–623.

[63] Parker, op. cit., note 32 above, pp. 153–159. Parker pointed out that the method measured cell migration as well as growth and listed ten circumstances that precluded the use of Ebeling's technique. He emphasized that the method was to be used with "extreme caution" and that "surface measurements are useful as a means of recording results; they are of little use as a means of evaluation". Parker later discussed results obtained by Meier and Laser (see note 40 above) and said that these had shown that cell migration was mainly responsible for the area occupied by cells around an explant (p. 161).

[64] A. Carrel, 'Tissue culture', *Lancet*, 1924, **ii**: 507–508, see p. 508.

"With regard to his [Carrel's] definition of growth, the presence of mitotic figures and cell multiplication did not mean growth in his sense . . . .By growth he meant the fabrication of new cells wholely and solely from the constituents of the culture medium."

It is not at all clear what Carrel meant by this for he seems to be ignoring Virchow's dictum that all cells arise from pre-existing cells, but it is unlikely that he was searching for a culture medium in which cells would spontaneously arise. There was considerable discussion of what constituted "growth" *in vitro*, and Legendre[65] attempted to distinguish "conservation", in which tissues were kept at low temperatures and recovered their function on transplanting back into the body; "survival", in which tissues were kept at body temperature but lost the ability to resume normal functioning on transplanting; "culture" was considered to be "the development and multiplication of cells". The problem of cell nutrition was clearly of great practical importance, and research workers were unlikely to enter a field where it appeared that even the most experienced investigators were uncertain of what cells required to function normally *in vitro*.

CONTEMPORARY VIEWS

In the period between 1923 and 1925 a summing-up of progress in tissue culture was under way and a number of reviews and books were published. These were not restricted to specialist journals and both the *British Medical Journal* and the *Lancet* published articles and editorials on tissue culture. Tissue culture was considered of sufficient interest for the British Medical Association to invite Carrel to address the Pathology and Bacteriology Section at the annual meeting at Bradford in 1924, and his address was printed in both the *British Medical Journal* and the *Lancet*.[66] It was concerned with developments in technique and its application to pathology and it began: "The attempts made during the past years to apply the method of tissue culture to pathological studies did not meet generally with great success".[67]

The admission that culture studies had yielded disappointing results is a recurring theme in the articles of this period. The editorial commenting on Carrel's talk opened bravely: "Dr. Alexis Carrel may be perhaps considered the leader of the small band of workers who have given much time to a line of inquiry which is not only of obvious importance to biologists in general and to followers of medicine and pathology in particular. . . . That the cells of complex animals can be persuaded to live and multiply under a cover-glass . . . is astonishing".[68] But having reviewed Carrel's results the editorial closed rather quietly, referring only to "hopeful" possibilities rather than already achieved successes.

Drew's lectures[69] at University College, London, in 1923, were concerned with technique and some of his experiments with malignant cells. An editorial commented that "in this line of research technique is indeed, everything", but Drew's account is

[65] Legendre, op. cit., note 3 above, p. 364.
[66] Carrel, op. cit., notes 57 and 64 above.
[67] Carrel, op. cit., note 57 above, p. 140.
[68] [Anonymous], 'Tissue culture', *Br. med. J.*, 1924, ii: 152–154.
[69] Drew, op. cit., note 57 above.

straightforward and free from the surgical complexities characterizing papers from Carrel's laboratory. The disparaging comments of this editorial[70] have already been referred to, but the writer was optimistic for the future, commenting that Drew's experiments were of "extreme importance".

In addition to the article published in the *British Medical Journal,* Carrel also published a review article in *Physiological Reviews*[71] but this paper was a reworking of that in the *British Medical Journal* and there are many passages common to both articles. Carrel began by saying that the application of tissue culture to physiological problems had long been delayed, "because the technique was not adapted to the requirements of such investigations".[72] Although "important technical improvements" had taken place, Carrel's remarks on the latest methods were not encouraging: for example, the original method of subculturing by transplanting tissue fragments was described as "laborious",[73] and the new methods "must be used with great care to yield accurate results."[74]

Strangeways' laboratory manual *The technique of tissue culture in vitro*[75] was published in 1924, and presented forty-seven step-by-step accounts of tissue culture procedures ranging from setting up a laboratory (including specifying imitation oak linoleum for the floor) to vital staining of mitochondria. He made few comments on the status of tissue culture research except that the results already obtained were of "extreme interest"[76] and, prophetically, that tissue culture would become "one of the most valuable methods of biological research".[77] Strangeways also recommended his readers not to attempt to improve the methods detailed in the manual until they had first succeeded in growing cells by following these methods. This insistence on adhering to prescribed procedures was emphasized by early workers and may have contributed to the mysticism of the technique. The methods became formulae that assumed the role of spells in magic; rigid procedures that had to be performed according to tradition, unquestioningly, with failure the result of inattention to detail. Carrel and Burrows warned: "When the technique is applied in all its details, the results of the cultures are practically uniformly positive. If some of the details are neglected, the tissues do not grow or their growth is altered."[78]

Among the research workers who trained in Carrel's laboratory at the Rockefeller Institute was A. J. Walton from the London Hospital Medical School and he found it necessary to give a detailed account of Carrel's methods in his first paper: "The technique used is practised by Carrel in the Rockefeller Institute, but since this has presented many difficulties to those commencing this line of investigation, it will be described in detail".[79]

[70] [Anonymous], op. cit., note 8 above.
[71] Carrel, op. cit., note 45 above.
[72] Ibid., p. 1.
[73] Ibid., p. 4.
[74] Ibid., p. 17.
[75] Strangeways, op. cit., note 52 above.
[76] Ibid., p. ix.
[77] Ibid., p. xii.
[78] Carrel and Burrows, op. cit., note 29 above, p. 395.
[79] A. J. Walton, 'The technique of cultivating adult tissues in vitro and the characteristics of such cultivations', *J. Path. Bact.*, 1913–1914, **18**: 319–324.

It is instructive to examine the paper published by H. F. Smyth in 1914.[80] Smyth does not appear to have been associated with the Rockefeller Institute group and makes no acknowledgement in this paper of help or instruction from any other group. Smyth described tissue culture technique following Carrel's methods and then discussed applications in the fields of morphology, radiobiology, cancer research, bacteriology, and virology. Although he did not comment on the difficulties of the method, he can have left his readers in no doubt; he listed five reasons why cultures may fail to grow and no less than fifteen factors causing variability in growth.

The most interesting review of this period is the book published in 1925 by Albert Fischer.[81] Fischer was a prolific tissue culturist[82] who learned the technique in Carrel's laboratory between 1920 and 1922. His book was published after his return to Denmark, but because of his close association with Carrel his views are particularly informative. Despite the apparent interest in tissue culture at the time, Fischer commented that: ". . . today there seems to be only a relatively limited understanding of the importance and significance of the tissue cultivation as a method for making elementary and fundamental studies of the physiology and pathology of higher organisms."[83]

After paying tribute to the technical contributions of Carrel and Burrows, Fischer went on in a revealing passage to describe the attitude of research workers elsewhere to the activities of Carrel's group: "The result of these experiments [by Carrel and Burrows] were looked upon by the scientific world with great expectations. The experiments were repeated by the majority of biologists, morphologists and pathologists, without however much success and rather disappointing results. Consequently many investigators became sceptic and pessimistic in regard to the employment of the method."[84]

The solution of these difficulties again emphasized the necessity of following the methods of the pioneers exactly: "It was necessary accurately to follow up the technique if good results were to be expected and this particular work requires much care and patience if success is to be expected."[85] It is hardly surprising in the light of these comments that Fischer found that: ". . . only very few people work with tissue cultivations in a confident manner, whatever may be the cause; lack of technical difficulties [*sic*] or understanding."[86]

Direct criticism of Carrel is rare in English-language journals, but Carleton, while acknowledging Carrel's technical skill and contribution, seems to have been particularly disappointed by the lack of critical morphological studies of cells in culture and repeatedly deplored the absence of any histological approach.[87] Of Carrel's studies Carleton said: "His earlier work especially abounds in technical innovation

[80] H. F. Smyth, 'The cultivation of tissues in vitro and its practical application', *J. Amer. med. Assn.*, 1914, **62**: 1377–1381.
[81] Fischer, op. cit., note 9 above.
[82] Fischer published some 175 papers on tissue culture between 1921 and 1949.
[83] Fischer, op. cit., note 9 above, p. 17.
[84] Ibid., p. 21.
[85] Ibid.
[86] Ibid., p. 24.
[87] Carleton, op. cit., note 12 above, p. 143.

but the interpretation of the histology of the growth-changes is often lacking."[88] This criticism of Carrel is rather unfair in that most of his studies had as their aim development of technique rather than its application to particular problems. Carrel's pre-occupation with long-term cultures of actively growing cells diverted attention away from the study of cell differentiation in culture. This became the province of the Lewises in America, Champy in France, and Carleton and Honor Fell in England.

There was also criticism of Carrel in the European journals, but this was directed against his interpretation of his observations rather than the technique itself. Fischer singled out research workers in France and Germany who claimed that Carrel had not observed cell multiplication, but simply active cell migration or passive outfloating of cells from the explants.[89] These criticisms require some consideration here, even though they do not directly concern the difficulties, real or otherwise, of the method. There was no doubt in Fischer's mind that these mistaken criticisms of Carrel's work were the result of poor experimentation due to inadequate technique: "The reason why so much opposition was encountered was primarily that the organisation of the culture work made by others than the pioneers was very poor and that from few unsuccessful experiments unreliable conclusions were usually drawn."[90]

Carrel's principal critic was the eminent French biologist, Dr. J. Jolly. It is not perhaps surprising that Carrel's work was particularly badly received in his native country. He had left Lyons after a conflict with its medical faculty,[91] and in later years he lost no opportunity of castigating French medical research as conservative and behind the times.[92] Carrel's flair for publicity led to difficulties throughout his time at the Rockefeller Institute,[93] and his choice of a meeting of the Société de Biologie in Paris in November 1910 as a suitable venue for presenting some highly controversial results was unfortunate.[94]

This paper was immediately attacked by Jolly who, in a paper[95] published in the following week on 26 November, challenged Carrel's claims that he was able to culture cells and not simply to preserve them, and that he had grown organized cultures of epithelial cells. Jolly wrote that he realized that tissue culture did not conflict with any established principle of biology and he hoped that it would soon be achieved. "Mais M. Carrel a-t-il obtenu de véritables cultures? Voilà la question." Jolly's answer to his rhetorical question was an unequivocal "no". Jolly himself had been doing similar work and on the basis of this experience he objected that Carrel and Burrows had mistaken changes associated with cell death for signs of cell growth,

[88] Ibid., p. 136.
[89] Fischer, op. cit., note 9 above, p. 21.
[90] Ibid.
[91] Edwards and Edwards, op. cit., note 14 above, pp. 15, 56–57.
[92] Ibid., p. 61.
[93] Corner, op. cit., note 15 above, p. 158.
[94] Carrel and Burrows presented no fewer than seven communications to the Société de Biologie between 22 October 1910 and 12 November of the same year. It was the presentations of 5 and 12 November that proved controversial. A. Carrel and M. T. Burrows, 'Cultures primaires, secondaires et tertiaires de glande thyroide et culture de peritoine', *C.r. Soc. Biol., Paris*, 1910, **69**: 328–331. A. Carrel and M. T. Burrows, 'Seconde generation de cellules thyroidiennes', ibid., 1910, **69**: 365–366.
[95] J. Jolly, 'A propos des communications de M. M. Alexis Carrel et Montrose T. Burrows sur la "culture des tissus",' ibid., 1910, **69**: 470–473.

although it is not clear what he meant by this: "Jusqu'à présent M. M. Carrel et Burrows ne semblent avoir démontré que des phénomènes de survie. Certaines de leurs descriptions semblent même se rapporter à des phénomènes de nécrobiose".[96]

Jolly thought it was an abuse of language for Carrel and Burrows to describe their results as "cultures", and that there was an "abyss" between their system and true cultures that might one day be filled.

Carrel and Burrows counterattacked in a communication[97] to the Society on 7 January 1911, when they described the appearance of mammalian cells *in vitro*. They presented photographs illustrating the state of the cells, and were scornful of Jolly's interpretation of the granularity of the cells being a sign of cell death. Jolly's reply[98] followed, and he laid emphasis on the migration of cells from an explant as an explanation of the increasing area of cells around an explant. He demanded evidence of active division of these cells, and declared that the photographs exhibited by Carrel and Burrows were inadequate. Jolly was finally silenced by Carrel's demonstration of mitotic figures in cultured cells in a paper[99] published later in 1912. However Carrel had not grown epithelial cells and his claims to have obtained organized cultures of kidney and thyroid epithelium were greatly exaggerated. These justifiable criticisms by Jolly seem to have been lost in the enthusiasm for the technique; references to Jolly's papers were infrequent and only appeared in Fischer's book among the early reviews.

This type of criticism of Carrel is exemplified by a paper published by R. Legendre that reviewed the historical background to the development of *in vitro* techniques.[100] Legendre set out to show that Carrel had not achieved anything, and if he had, it was not original. He complained, with some justification, that such a novel technique required better documentation; Carrel and Burrows had written only short notes that gave insufficient detail with illustrations too few in number and at too low a magnification to be interpreted properly. Legendre also criticized the manner in which Carrel presented his results: "La mannière dont elles se présentent pourait faire penser à une réclame indiscrète; je ne crois pas cependant qu'elles ne soient que cela."[101]

A few months before, a newspaper had described such work as "the greatest discovery made since Claude Bernard", but Legendre pointed out that no medical or biological review had commented on this astonishing discovery and asked acidly, "A quoi attribuer ce silence et que faut-il penser de ces nouvelles sans écho?"[102] In the following year, Carrel was awarded the Nobel Prize, and Legendre, in a second article, carefully pointed out that Carrel was awarded the prize for his surgical

[96] Ibid., p. 473.
[97] A. Carrel and M. T. Burrows, 'A propos des cultures "in vitro" des tissus mammifères', ibid., 1911, **70**: 3–4.
[98] J. Jolly, ibid., 1911, **70**: 4.
[99] Carrel and Burrows, op. cit., note 29 above, p. 396. Carrel described the mitotic figures as "beautiful".
[100] Legendre, op. cit., note 3 above.
[101] Ibid., p. 357.
[102] Ibid.

work and not for his tissue culture studies![103]

In the early stages of tissue culture development, it was not even clear if tissue culture was theoretically possible and Legendre was able to write: "Quant à la 'culture' telle que l'extendant Carrel et Burrows c'est-à-dire la développement et la multiplication des cellules in vitro, si elle n'est pas théoriquement impossible, il ne semble pas cependant qu'elle ait été déjà réalisée."[104]

In a short while it became clear that cells did grow and divide in culture and Jolly's criticisms became irrelevant. Although other controversies, for example about "dedifferentiation",[105] arose, tissue culture was accepted as a technique and became popular throughout Europe. In 1927 the Tenth International Zoological Congress[106] in Budapest was devoted to tissue culture and was attended by Harrison, W. H. Lewis, Burrows, and leading tissue culturists from all over Europe. Carrel was unable to attend, but the President of the Congress, Professor M. von Lenhossek, in his opening address referred to Carrel as the "genius" who had developed the method fully.[107] G. Levi and C. Olivo in their contribution to the Congress spoke of Carrel: "Infatti col perfezionarsi della tecnica, è divenuto possibile, specialmente per merito di Carrel, di analizzare le transformazioni qualitative e quantitative dei tessuti in condizioni di esperienza ben determinate."[108]

By 1932 Carrel's pre-eminence in the field was recognized even in his native country. B. Ephrussi in his book *La culture des tissus* acknowledged Harrison's original contribution but went on: ". . . le mérite d'avoir, le premier, compris l'énorme portée de ce que nous appelons maintenant la *culture in vitro* revient a Alexis Carrel, que nous devons de ce fait considérer désormais comme le véritable fondateur de la méthode."[109]

But these were the opinions of established experts in the field, and it is clear from the earlier sources that the techniques devised by Carrel and his colleagues were generally considered to be difficult.

[103] R. Legendre, 'La survie des organes et la "culture" des tissus vivants', *Nature, Paris,* 1912, **40**: 359–363. The rejection of Carrel by his native country aroused comment in the country that adopted him. His Nobel Prize was only the third to be awarded to a resident of the United States, and the publicity aroused by the award in the popular press contrasted markedly with the lack of comment in France. The *New York Times* ran a short article: 'France neglects Carrel. Honors to famous scientist everywhere except in his own country'. (Paris dateline, 21 December 1912.)

[104] Legendre, op. cit., note 3 above, p. 364.

[105] Champy pointed out that differentiated cells in tissues *in vivo* did not divide and he believed that such cells dedifferentiated in culture, reverting to an embryonic type that was able to multiply. (C. Champy, 'Résultats de la méthode de culture des tissus en dehors de l'organism', *Presse méd.*, 1914, **22**: 87–89). This was clearly not the case for epithelial cells that retained their characteristic morphology and growth pattern, and Champy's extreme views were generally rejected, see, for example, Fischer, op. cit., note 34 above, chapter 8, 'Differentiation and organization'. The "dedifferentiated" ubiquitous fibroblast in culture is believed to be derived from endothelial cells and vascular pericytes, L. M. Franks and T. W. Cooper, *Int. J. Cancer,* 1972, **9**: 19–29. For a general discussion of the origin and form of cells in culture, see E. N. Willmer, 'Morphological problems of cell type, shape and identification', in Willmer (editor), op. cit., note 10 above, pp. 143–176.

[106] The proceedings of the congress were edited by R. Erdmann and published as *Arch. exper. Zellforsch.*, 1928, **6**.

[107] Ibid., see p. 45.

[108] G. Levi and C. Olivo, 'Le Proprietà structurali delle cellule e dei tissuti coltivati "in vitro",' ibid., pp. 46–69.

[109] B. Ephrussi, *La culture des tissus*, Paris, Gauthier-Villars, 1932, see p. 1.

THE EFFECTS OF SUBSEQUENT TECHNICAL DEVELOPMENTS

Although it was because of Carrel's surgical experience that his tissue culture procedures became complex, it should not be thought that all the measures taken by the Rockefeller group were unjustified. It is difficult to appreciate the conditions and intellectual background of early scientific research and this is particularly true of fields in which technical advances have since altered radically the patterns of work. I should now like briefly to consider three such advances in tissue culture methods.

The overriding consideration in all tissue culture technique is the need to avoid bacterial contamination of the cultures, and early writers repeatedly warned that aseptic techniques were essential. Although the insistence on a rigid aseptic method may have deterred potential users, the consequences of contamination were very serious as can be judged by Carrel's statement that: "Nearly all the cultures made in the latter part of 1911 died in the same manner [contaminated] after one or two months."[110] Carrel was often able to subculture the non-infected portions of cultures but "many cultures died of sepsis".[111] He described a case in which a flourishing culture of embryonic chick portal vein was carried through fifteen subcultures (fifty-two days) before a massive infection by bacteria destroyed the cultures. The situation was revolutionized by the introduction of antibiotics in the 1940s.[112] It is possible that tissue cultures were initially used to assay the toxicity of antibiotics on mammalian cells,[113] but it became clear that antibiotics could also be used to maintain the sterility of tissue cultures. A strict aseptic technique was still essential but the addition of low levels of antibiotics to culture media or their use at high concentrations to control an infection lessened one of the main hazards of tissue culture studies. Over-elaborate aseptic precautions were clearly superfluous and laboratories that did not have access to the operating suites advocated by Carrel may have been encouraged to undertake studies using cultures.

A much more fundamental change in procedure was the use of cell cultures rather than tissue cultures. In the former cells are grown as individuals in an unorganized fashion in monolayer or suspension cultures while in the latter there is repeated outgrowth of cells from an explant. Cell cultures depend on the use of trypsin or other enzymes to dissociate cells growing out from an explant or directly by treatment of embryonic or adult tissues.[114] The method was devised by Rous and Jones in 1916 and used by them to obtain suspensions of cells from the outgrowth around an explant[115] which were then plated in new culture vessels. This technique is rapid and

[110] Carrel, op. cit., note 36 above, p. 527. [111] Ibid., p. 527.

[112] Early tissue culture studies on antibiotics were reviewed by J. F. Metzger, M. H. Fusillo, I. Cornman, and D. M. Kuhns, 'Antibiotics in tissue culture', *Exp. Cell Res.*, 1954, **6**: 337–344.

[113] For example P. B. Medawar carried out cytotoxic assays for penicillin in an early experimental study of penicillin activity. He determined the minimum concentration of penicillin required to inhibit outgrowth of fibroblasts from explants of embryonic chick heart or epithelial cells from embryonic chick intestine or lung, or rat embryo kidney. Medawar found that a concentration 1/800 inhibited all cell growth but the effect was reversible. (E. P. Abraham, E. Chain, C. M. Fletcher, A. D. Gardner, N. G. Heatley, M. A. Jennings, and H. W. Florey, 'Further observations on penicillin', *Lancet*, 1941, **ii**: 177–189, see p. 182).

[114] The development of this technique was reviewed by C. Waymouth 'To disaggregate or not to disaggregate. Injury and cell disaggregation, transient or permanent?', *In Vitro*, 1974, **10**: 97–111.

[115] P. Rous and F. S. Jones, 'A method for obtaining suspensions of living cells from fixed tissues and for the plating out of individual cells', *J. exp. Med.*, 1916, **23**: 549–555.

simple and reduces the chance of contamination because it involves fewer manipulations than the original method of transferring fragments of tissue. However, it was not generally adopted and, although Fischer[116] referred to it briefly, it was not discussed by Strangeways,[117] Willmer,[118] or Parker[119] in their books. Willmer later prepared cell suspensions from monolayer cultures using trypsin and the method came into common use through the work of Moscona and later Rinaldini,[120] Trypsinization is now the standard method of subculturing and it enables large numbers of cultures to be handled rapidly. It is not clear why the method was not adopted earlier, for the paper by Rous and Jones is admirable for its clarity. It may have been thought that cells would be severely damaged by enzymic treatment, a problem that still causes concern.[121]

Increased knowledge of cell metabolism has brought about significant changes in the formulation of culture media.[122] It had long been recognized that biochemical and physiological studies of cells in culture would be hampered until cells could be grown in defined media, the composition of which was known exactly and could be controlled. W. H. and M. R. Lewis, who used simple saline solutions supplemented with amino acids,[123] attempted this, but it was Vogelaar and Erlichman[124] and Baker[125] who took the first substantial steps towards devising fully defined media. Their pioneering studies were followed up by Fischer and White, and work in many laboratories has since led to production of numerous media, some of which are very complex (for example NCTC 135 contains sixty-two components in addition to inorganic salts[126]) and are able to support the growth of some cells in the absence of any biological supplement. But for many cells it is still necessary to add natural supplements, such as serum in quantities of up to twenty per cent, and for certain cells such as muscle, chick embryo extract is still required.[127] The use of defined media

[116] Fischer, op. cit., note 9 above, see p. 156.

[117] Strangeways, op. cit., note 52 above.

[118] E. N. Willmer, *Tissue culture*, London, Methuen, 1935.

[119] Parker, op. cit., note 32 above.

[120] E. N. Willmer, 'Growth and form in tissue culture', in W. E. Le Gros Clark and P. B. Medawar (editors), *Essays on growth and form*, Oxford University Press, 1945, pp. 264–294, see p. 273. A. Moscona, 'Cell suspensions from organ rudiments of the early chick embryo', *Exp. Cell Res.*, 1952, **3**: 535–539; L. M. Rinaldini, 'The isolation of living cells from animal tissues', *Int. Rev. Cytol.*, 1958, **7**: 587–647.

[121] Waymouth, op. cit., note 114 above.

[122] For a detailed discussion of the development of defined media, see C. Waymouth, 'Construction and use of synthetic media', in Willmer, (editor) op. cit., note 10 above, pp. 99–142.

[123] W. H. Lewis and M. R. Lewis, 'The cultivation of chick tissues in media of known composition', *Anat. Rec.*, 1912, **6**: 207–211.

[124] J. P. M. Vogelaar and E. Erlichman, 'A feeding solution for cultures of human fibroblasts', *Am. J. Cancer*, 1933, **18**: 28–38.

[125] R. E. Baker, 'Artificial media for the cultivation of fibroblasts, epithelial cells and monocytes', *Science*, 1936, **83**: 605–606.

[126] NCTC 135 is one of a series of fully defined media devised by Evans and Earle and their co-workers and intended to support cell growth in the absence of any biological supplement such as serum. (See, for example, V. J. Evans, J. C. Bryant, H. C. Kerr, and E. C. Schilling, 'Chemically defined media for cultivation of long term cell strains from four mammalian species', *Exp. Cell Res.*, 1964, **36**: 439–474.)

[127] C. R. Slater, 'Control of myogenesis in vitro by chick embryo extract', *Dev. Biol.*, 1976, **501**: 264–284.

has greatly simplified tissue culture work because it has reduced the variability of preparations of natural media such as plasma. The media can be prepared in large quantities and stored easily until needed.

Despite technical advances such as subculturing using enzymes or the use of defined media, tissue culture is still a time-consuming technique and it has been estimated that up to to fifty per cent of the researcher's time can be spent maintaining cultures.[128] However, because of the current importance and popularity of tissue culture there is now a large industry supplying materials for tissue culture on a scale undreamed of fifty years ago when each laboratory prepared its own materials. One firm offers seventeen different types of serum and seventy-three varieties of defined media, together with culture flasks and pipettes, enzyme solutions, and even cultures of cells. As Willmer has remarked, this ready supply of materials has "greatly eased the rather tiresome technical problems which used to face the solitary worker in the field of Tissue Culture".[129]

Paul remarked in the introduction to his book that "the belief that tissue culture was 'fantastically difficult' was only being dispelled when the first edition (1959) of this book was written."[130] It was technical developments of the type I have just discussed that contributed to this changing opinion of the usefulness of tissue culture.

CONCLUSION

In the years following Harrison's seminal paper, Carrel became recognized as the leading figure in the tissue culture field[131] and the work of his group at the Rockefeller Institute dominated the field. He received considerable publicity, particularly over his "immortal" cell strain,[132] and other investigators such as the Lewises appear to have been overshadowed by him. Carrel's domination may also be due to his preoccupa-

[128] G. D. Fischbach, D. Fambrough, and P. G. Nelson, 'A discussion of neuron and muscle cell cultures', *Fed. Proc.*, 1973, **32**: 1636–1642, see p. 1637.

[129] Willmer, op. cit., note 10 above, p. 12.

[130] Paul, op. cit., note 17 above, p. 2.

[131] See, for example, the editorial in the *British Medical Journal*, op. cit., note 68 above. In the discussion of Carrel's paper at the British Medical Association meeting, Dr. S. Ricart of Barcelona, who learned the technique from Carrel, commented on the importance of the American workers in the tissue culture field, Carrel, op. cit., note 64 above.

[132] Carrel's "immortal" cells were derived from cultures of chick embryo heart established by Carrel on 17 January 1912, and later maintained by Ebeling (op. cit., note 37 above). Papers were published occasionally by Ebeling describing progress of the culture; for example A. H. Ebeling, 'A strain of connective tissue seven years old', *J. exp. Med.*, 1919, **30**: 531–537; ibid., 'A ten year old strain of fibroblasts', 1922, **35**: 755–759. Ebeling wrote a popular account of the culture after it had been growing for thirty years; 'Dr. Carrel's immortal chicken heart. Present authentic facts about this oft-falsified scientific celebrity', *Scientific American*, 1942, **166**: 22–24. More recent studies on cell ageing in culture have established conclusively that normal (i.e. non-transformed) cells have a finite lifespan in culture. Normal cells are able to undergo only a limited number of divisions before they die; for example human embryonic lung cells will divide between fifty and sixty times, a maximum lifespan of about thirty weeks before dying. (See L. Hayflick, 'The biology of human ageing', *Am. J. med. Sci.*, 1973, **265**: 433–445.) It is not clear how Carrel was able to maintain his strains of cells for over thirty years. It has been suggested that the cultures were periodically contaminated by cells present in the chick embryo extract used to feed the cultures (L. Hayflick, 'The limited in vitro lifetime of human diploid cell strains', *Exp. Cell Res.*, 1965, **37**: 614–636, see p. 628). This seems unlikely in view of the methods used to prepare chick embryo extract. See also B. Strehler, *Time, cells and aging*, New York, Academic Press, 1977, chapter 3.

tion with developing the technique rather than its application. In his preface to Parker's book, Carrel said that: "From the technique of Harrison to the elaborate procedures described by Dr. Raymond Parker in this book, the road has been very long. But such an effort toward technical perfection was indispensable, for the progress of experimental sciences depends entirely upon that of techniques."[133]

Refinements and improvements in techniques are alway to be welcomed when they lead to better and more interesting experiments, and while tissue culture methods were undoubtedly in great need of improvement it is unfortunate that Carrel's approach led to increasingly complex and elaborate techniques. It is impossible to know from published sources how difficult Carrel and his colleagues found tissue culture techniques to be in practice, but Carrel's papers undoubtedly emphasized the difficulties of the method. The difficulties may have been exaggerated to increase admiration of their work, but it partly was on these public accounts that Carrel's contemporaries formed their opinion of the technique. People who attempted culture techniques and did not succeed at first, were unlikely to be encouraged to persist in their efforts by Carrel's writings, particularly when the papers were written in a style that was difficult to understand. It is perhaps significant that the Lewises did not dwell unduly on the technical difficulties of tissue culture, but applied it successfully to specific problems.

The criticisms of Carrel made by Willmer and Paul certainly appear to be supported by this examination of Carrel's work, but it is too easy to lose sight of Carrel's contribution to the field (Fig. 2). Tissue culture was a technique fraught with frustrations, but Carrel persisted in attempting to develop and improve it. That he was able to grow cells without antibiotics for many years was in itself a considerable technical feat and by his example he demonstrated to his contemporaries that tissue culture was a method of practical value for experimental studies. It is ironical that the methods by which he achieved his success may have deterred others from following his example.

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[133] Carrel, in Parker, op. cit., note 32 above, p. xi.