Technique as the basis of experiment in developmental biology

An interview with Denis A.T. New

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Embryology is basically an experimental science. Most of the current achievements have been possible thanks to the use of techniques that have allowed for the morphological, functional, genetical, cytological or molecular analysis of developing organisms. In particular, the existence of reliable, reproducible *in vitro* culture methods becomes crucial when programming any kind of experiment, particularly with amniotes. If we look at the list of scientists who made possible real progress in this area, it is clear that the list is not a long one, and that the name of Denis New stands out for his original contributions to the *in vitro* culture of avian and mammalian embryos, and for the reliability and adaptability of his methods. Use of these methods has become a must in any developmental biology laboratory and in numerous basic and specialized courses on experimental biology the world over. I believe his scientific biography may be the best way to portray how he managed to develop these techniques.

Denis A.T.New was born on April 13, 1929 at Eltham in south east London, England. His father was a scientist and head of the materials laboratories of the Standard Telephones and Cables Company; his mother was a historian and lectured for the Workers' Educational Association and other organizations. His was a happy childhood in which education was liberally interpreted; he himself tells us, "My brother once obtained a rise in pocket money by arguing that the extra was needed as 'educational happiness money'. I kept bees and on one occasion my mother was badly stung and spent several days in bed in great discomfort, her eyes practically closed by an alarmingly swollen face; but there was no word of complaint - bees were *education*. In countless ways, both parents provided a supportive and stimulating environment for us to grow up in. There was only one rule: - 'If a thing is worth doing at all, it's worth doing well'. We did not always follow it, but perhaps it kept us from wasting as much time as we might otherwise have done".

His school years at Eltham College coincided with World War II and the family lived through the German air raids, the V1 and V2 rockets, and other war dangers. But he says of this, "As for the bombs, food rationing and the like, they just became an accepted part of our environment, scarcely more disturbing than examinations, conflicts with rivals and Authority, and all the other hazards of growing up. Sports, hobbies and girls were far more interesting. In retrospect, I am full of admiration for our teachers at Eltham College who, in spite of the many wartime problems of shortages, air raids and interrupted schedules, kept all the usual school activities going and made it possible for us to have such a normal boyhood".

In his university undergraduate years, he studied first at Imperial College of Science and Technology, London, and then, thanks to scholarships, at St. John's College, Oxford, where he was greatly influenced by his Tutor, William Holmes. He obtained his first degree in 1952 and then returned to London, this time to University College, for his PhD under one of the most renowned developmental biologists of the time, Michael Abercrombie. Also here were other distinguished embryologists like Ruth Bellairs and Elizabeth Deuchar. Denis New became a research assistant to Michael Abercrombie and, in all, spent five years in the department. It was a decisive period, since it was at this laboratory

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Fig. 1. Denis A.T. New during the interview.

that he developed his widely known *in vitro* culture technique for the chick embryo.

Next were four years as a Lecturer in Zoology at the University College of the West Indies in Jamaica, followed by a return to England in1961 to continue embryological research as a member of the scientific staff of the Medical Research Council at the Strangeways Research Laboratory, Cambridge. Here he published his book *The Culture of Vertebrate Embryos*, which has become a classic in the methodology of experimental embryology. He was elected a Fellow of Fitzwilliam College, Cambridge, where he taught zoology, and at the same time his research at the Strangeways Laboratory turned towards a particularly challenging problem: the *in vitro* culture of mammalian embryos at post-implantation stages.

In 1967, he moved to the Physiology Department of Cambridge University to join the research group of Colin Austin, who had just arrived as the new Professor of Animal Embryology. Also in the group was Robert Edwards, then at the early stages of his famous work on human IVF, and a number of brilliant young scientists, including Richard Gardner, Martin Johnson, Azim Surani and others. Thus began a very important period in the evolution of mammalian – including human – embryology, which is even known to the general public. Denis New's contribution to this internationally prestigious atmosphere was the development, in collaboration with David Cockroft, Pat Coppola, Stephanie Ellington and Chris Steele, of new culture methods for postimplantation embryos. As a result of his numerous visits over the years as invited professor to research centres, conferences and scientific meetings, and the organisation at his Cambridge laboratory of specialised courses in experimental embryology, his methods have become widely known and are currently used by hundreds of scientists throughout the world.

His wife, June, is a geneticist. She and Denis have been married for forty years and they have two daughters: Helen, who is a haematologist, and Laura, who is an epidemiologist.

This interview was held in February 1996 at Denis New's laboratory at Cambridge University and is meant as a personal tribute to him, and as a first-hand portrait of his most significant contributions and of his scientific and human personality.

You showed a very early interest in the study of nature, and in particular of embryology. Was there any family influence or personal experience that made you take on this interest?

Two influences from my early life had a profound effect on me. One was the long holidays spent with my paternal grandparents at their home in a small village in rural Gloucestershire. To me, a small boy from the great city, the countryside was paradise. I roamed the woods and fields, climbed the trees (sometimes the same ones that my father had climbed thirty years before), collected butterflies and flowers, and consumed vast quantities of apples, pears, plums and cherries. I played with the children of the local farmers and was fascinated by the animals and life of the farms. My earliest ambition was to be a farmer, and this was undoubtedly the beginning of a line of interests that ultimately led to my becoming a biologist.

The other major influence on me was music. Both parents were very musical and my brother and I had regular music lessons from private teachers – a luxury in those days. My brother decided to continue it professionally and eventually became a university professor of music. I learnt to play the piano and the cello and in my teens also thought about trying to make music a career. I practised assiduously and performed with friends in many amateur concerts. Those who heard me play were very kind – and strongly advised me to stick to biology. I am sure they were right, and a biologist I became, but music has remained one of my greatest pleasures.

I think my interests in embryology and music may well have been related. The developing organism on its way from egg to adult passes through numerous different forms but, whatever the form at any given moment, essential metabolic processes must continue without interruption. Similarly, in a work of music, the constant changes in sound patterns must always be such that they hold the attention of the audience and carry the work forward. Both embryo and music must function at all stages of their development, in contrast to machines which need only function when completed. I have been particularly interested in this aspect of embryos. Embryology textbooks tend to concentrate on the mechanisms which change the structure of the embryo, and we usually define embryos in terms of the end result: "rat embryo", "mouse embryo", "chick embryo" etc. But it is important to remember, particularly when culturing embryos, that at every stage of its development the embryo is a complete functioning organism and can live only in conditions which allow it to operate as such.

I first became conscious of an interest in embryology during my last year at school. Biology was one of my subjects and among the books we were given was Patten's *The Early Embryology of the Chick*. From the moment I opened this book I found it fascinating, and instead of studying just the first chapter, as we had been instructed, read the whole book from cover to cover like a novel in one weekend. My enthusiasm astonished both my friends and myself. Embryology was regarded as a) difficult, and b) dull, and

was the least popular part of the biology course (I found a similar view of it later among many of my contemporaries at University). Patten's book – a purely descriptive step-by-step account – was generally considered to be particularly tedious. That I found it otherwise convinced me that my future probably lay in embryology.

Tell us about your life in post-war Oxford. What were your Zoology studies like, your teachers and tutors and your first experiences in biological research?

I won two scholarships to St. John's College, Oxford and – after an interlude of National Service in the army – went up to Oxford in the autumn of 1949 to begin three wonderful undergraduate years reading Zoology. My tutor at St. John's throughout this period was Dr. William Holmes. Under the Oxford system, the College tutor reigned supreme. There were of course programmes of lectures and practical classes in the Zoology department, but attendance at these was desirable rather than obligatory. Not so the weekly essay for one's tutor; this took precedence over everything and failure to produce it was excusable only by the most extreme circumstances. Tutors had great freedom of discretion. If they considered it to be in their student's interest, they could, and often did, set whole series of essays that bore little relation to any lectures that might be going on at the time. Students were expected to obtain the information they needed by reading widely in libraries. At each tutorial session, I would begin by reading my essay aloud while William puffed gently at a cigarette through a very long holder. When I had finished, he would make a number of pertinent comments and this might or might not lead to some discussion between us. If it did not, he would pour us both a glass of gin and, after ten minutes, would stand up and I would know that the tutorial for that week was over. The message was always very clear: it was *my* job to interest *him*. He firmly believed that in education it is the student's activity that is important, not the teacher's. This style of teaching is frowned on in some quarters today but it suited me perfectly. William Holmes was a fine zoologist and he gave me the guidance that I needed, but he also gave me the freedom to pursue the subject in my own way.

William's own speciality was the structure and function of the nervous system, so inevitably I wrote many essays on this and knew more about the nervous system then than at any time since. But he soon realised that I was not to be shifted from my love of embryology and increasingly my essays and projects acquired an embryological flavour. I bought a cheap microtome and during one Spring vacation taught myself how to prepare serial sections of the developing eggs of frogs and toads obtained from a local pond. My father, a Fellow of the Royal Institute of Chemistry and a Member of the Institute of Physics, kindly descended to earth to advise me on such mundane matters as obtaining reagents and on the electronics needed to convert an old coal scuttle into an embedding oven.

In my final undergraduate year, I wanted to carry out a publishable research project to improve my chances of getting a good research studentship. Obviously it had to be something that could be done quickly and with the simplest of apparatus. William suggested that I look at the possibilities of the parasitic nematode worm *Rhabditis pellio*. The larval stages of this nematode infect the nephridia of earthworms, but development to the adult is completed only after the death of the earthworm, the nematodes

Fig. 2. Professor Michael Abercrombie F.R.S. (1912-1979). *Reprinted from Biographical Memoirs of the Royal Society, Vol. 26, December 1980.*

then growing and breeding rapidly in the rotting flesh. It seemed possible that they could be maintained for long enough in very simple watchglass cultures to answer certain questions about their growth, behaviour and curiously variable sex ratio. I was by now living in a room of a house in one of the smarter districts of Oxford and my fastidious landlady would have been horrified to know that I was breeding parasitic worms on her premises. So my cultures were kept hidden in a chest of drawers. Nutrient medium was made by boiling up dead earthworms on her cooker when she was out, but there were problems with the lingering smell and I constantly feared detection. However, all went well and the results were published in my first paper, to which, influenced perhaps by the historical associations of Oxford, I gave the unusually archaic and pompous title *The Reproductive Habits and Sex-determination of the Nematode* Rhabditis pellio *Bütschli, with a Note on its Taxonomy and Nomenclature*. Sadly, I failed to include among the acknowledgements any reference to my landlady "in whose laboratory the work was done".

The time when you were a graduate student at University College, London, was a crucial part of your scientific career. Why did you choose Michael Abercrombie's laboratory for your Ph.D. thesis? What were he and his collaborators like at that time? What technical means did they have in the lab and what was the scientific atmosphere there?

I went to Michael Abercrombie's laboratory at University College, London, because he had a very active research group in

Fig. 3. Denis A.T. New in his laboratory.

areas that interested me. I was very fortunate to have Michael himself as my supervisor. He was a man of great ability combined with an unusual degree of friendliness and modesty. As Peter Medawar later wrote in his biographical memoir for the Royal Society, "Although Michael never did anything to court admiration, he was liked and admired by almost everybody who met him". He was widely-read, and his own writing was beautifully clear and simple, even in the most complex and jargon-laden subjects. The combination of all these qualities made him a superb editor. With his wife, Jane (née M.L. Johnson) and later Gordon Fogg, he edited *New Biology*, a series of small paperback volumes of highly readable articles at sixth-form and undergraduate level that did much to stimulate interest in biology. He wrote a glossary for *New Biology* and then developed it to make *The Penguin Dictionary of Biology*, a very successful work which ran to many editions. But perhaps his most important editorial activity while I was with him was the founding, with David Newth, of the *Journal of Embryology and Experimental Morphology*, which has now become *Development*.

Michael's group were pursuing several lines of research. Elizabeth Deuchar was studying the biochemistry, particularly the amino acid changes, of *Xenopus laevis* embryos, and this work later formed an important part of her book *Biochemical Aspects of Amphibian Development*. Adam Curtis was beginning his well known work on regulation in the early amphibian embryo, and on the role and mechanisms of cell adhesion and aggregation in embryonic development. Michael himself was working with Joyce Santler on peripheral nerve degeneration and regeneration, work that he had begun several years previously with Jane. An important feature of this had been the behaviour of Schwann cells and he had begun to study them in tissue culture. He had extended the investigation to other cells, and had now arrived at what became his most famous work, with Joan Heaysman and Kindi Karthauser, the discovery and analysis of cell contact inhibition. He also had collaborative projects on liver regeneration and wound healing, and was concluding a project with Ruth Bellairs on axis formation and twinning in the chick embryo.

It was this last which was the starting point for my work. I embarked on a study of cell lineages in the early chick embryo that involved grafting precisely selected groups of radio-labelled cells from one blastoderm to another. The techniques were difficult and laborious and were impossible to carry out on embryos in the egg. Unfortunately, they proved to be scarcely more practicable *in vitro*. The *in vitro* methods then available – growing the embryos on plasma or agar clots – were totally inadequate for the project. I have two dominant memories from this period. The first is of learning the painful lesson that, in research, experiments often fail. The second is of the huge number of eggs that were added to my diet. The war had been over for only a few years and food rationing was still in force. It was a recognised perk of chick embryology that the researcher, having removed the embryo for study, should take the rest of the egg home for the kitchen. When, as in my project, some of the eggs were radioactive, two jars were placed on the bench, one for eatable material, the other for radioactive. It was assumed that the researcher never made a mistake in allocating material between the two jars. Health and safety procedures in the laboratory had not yet been invented.

After several months of trying in vain to extract any useful information from distorted and dead embryos, it became clear that a change of direction was needed. This led to the development of a technique for culturing the chick embryo on vitelline membrane. The technique was born out of frustration and luck. It seemed that a major defect of the clot techniques was that the blastoderm failed to expand over the clot surface, with the result that growth of the embryo was unnaturally restricted. In the egg, the blastoderm rapidly extends over the vitelline membrane surrounding the yolk. I wondered if pieces of vitelline membrane could be used *in vitro*. Luckily, the properties of the vitelline membrane proved more helpful than one might have dared to hope. A few trials showed that (1) the membrane was strong enough for large pieces to be pulled intact off the yolk, (2) it could be supported in a culture dish simply by wrapping the edges round a glass ring, and (3) the membrane slowly contracted during incubation so that initial wrinkles were pulled flat. Such ring-supported membrane provided an excellent substrate for explanted blastoderms and, as a bonus, it turned out that a little albumen from the same egg was all that was needed as a nutrient medium.

The external examiner for my Ph.D. was C.H. Waddington and I felt both flattered and slightly anxious on discovering that the great man had adopted my culture technique in his own laboratory as soon as he had read my thesis – in fact before he had even recommended me for the Ph.D. But with the publication in 1955 of *A New Technique for the Cultivation of the Chick Embryo in vitro*, others were equally quick to find it helpful and very soon it was well

established and in widespread use both in embryological research and in teaching. The technique was based on such a simple and, one might suppose, obvious idea, that I have often thought it surprising that it had not been developed long before. Certainly some younger researchers felt that its origins must be lost in a bygone era. At a conference sometime in the sixties, I met a girl who was using the technique in her own Ph.D. project. She was amazed to find that I really was "New of the New technique" and only ten years older than herself. "I thought you were dead long ago!", she said.

Apart from the initial application of this method in the study of chicken embryo cell lineages, what other applications did you devise for this new culture method?

In fact I never returned to cell lineages. During my early attempts at growing the chick embryo on vitelline membrane, several things had happened which had turned my thoughts in other directions. For example, I found that if a pool of saline was left over the blastoderm, the culture usually failed. This could be explained as an inhibition of respiration and was easily remedied by removing as much of the saline as possible. But I was surprised to find that, having removed the saline, some other fluid gradually appeared and accumulated in its place. At first I thought it might be water that had condensed on, and dripped off, the lid of the Petri dish. But however careful I was to prevent such condensation, the fluid still appeared. Could it have spontaneously seeped through the vitelline membrane from the albumen below? When a culture dish was set up with vitelline membrane and albumen, but without a blastoderm, the vitelline membrane remained dry. On the other hand, when a blastoderm was present, the fluid appeared and increased in volume while the albumen decreased. Only one explanation seemed possible. The blastoderm actively absorbs fluid on its ectoderm (albumen) side and secretes it from its endoderm (yolk) side.

It was known that when an egg is incubated, a large mass of watery fluid, usually known as the 'sub-blastodermic fluid', accumulates under the blastoderm. The origins of this fluid were uncertain. Some thought it might arise from the yolk, and indeed had called it 'liquefied yolk'. But the way the fluid was being secreted in my cultures showed that it came not from the yolk but from the albumen (in culture, the structures are inverted compared with their positions in the egg). The amount of sub-blastodermic fluid secreted in the egg is relatively enormous – nearly one third of the total egg volume. Why should the embryo (blastoderm) engage in such a massive transfer? Examination of the distribution of the egg contents during early incubation suggested one reason. The blastoderm expands as a layer just inside the vitelline until it completely surrounds the spherical yolk. At this stage it is separated from the shell by a thick layer of albumen. By transferring fluid from albumen to yolk, the sphere of blastoderm 'blows up' like the inner tube of a bicycle tyre, so that the blastoderm (and later the chorio-allantoic membrane) comes to lie under the shell. Here it can receive oxygen and lose carbon dioxide through the pores of the shell and provide for the respiratory needs of the growing embryo.

The formation of the sub-blastodermic fluid may also have another function. This was suggested by some experiments that I made on the mechanism of blastoderm expansion. In culture, the blastoderm expands over the vitelline membrane just as it would in the egg. Careful examination of the blastoderm showed that it was only the edge that was attached firmly to the vitelline membrane, and the action of this part in trying to creep outwards all the time kept the blastoderm under a tension. I found that if the edge was freed, the tension was removed and expansion was brought to a halt until the edge had attached again. Apparently tension in the blastoderm is a necessary condition for its expansion. In the egg, this tension is first maintained by the pull of the edge creeping over the inner surface of the vitelline membrane round the yolk. But after four days of incubation, the blastoderm has completely surrounded the yolk and its tension is then maintained by the pressure of the accumulating sub-blastodermic fluid. It would seem that there is here a positive-feedback mechanism. Tension stimulates the blastoderm to expand, thereby enabling it to secrete more fluid, which results in more tension and expansion.

I wondered if the formation of sub-blastodermic fluid could also be related to another oddity of chick incubation. It was known that a hen regularly shuffles her eggs while she sits on them, and if eggs are incubated artificially they must similarly be 'turned' each day. There was some evidence that the most important time for turning (i.e. with the greatest effect on development and hatchability) was during the first week, but no explanation was available. At this time, a thin

Fig. 4. Chick embryo (blastoderm) growing on vitelline membrane supported by a glass ring, according Denis New's original method.

Fig. 5. Denis A.T. New in the lecture theater.

membrane, the chorion, develops and expands rapidly just under the shell to keep pace with the respiratory needs of the growing embryo. Between the chorion and the shell is a thin layer of albumen, and I thought it possible that this albumen acted as a 'lubricant' to prevent the chorion from adhering to the shell before it had fully expanded. Withdrawal of fluid from this albumen to form the sub-blastodermic fluid would tend to make it more viscous and less effective as a lubricant. Hence the function of turning the egg might be to cause movement of the contents to replace the layer of viscous albumen with a fresh watery layer. I made some experiments which showed that this explanation is almost certainly correct. First, turning the eggs only between the 4th and 7th day gave a hatchability almost the same as that of eggs turned throughout incubation. Second, this is just the period when the sub-blastodermic fluid is forming most rapidly. Third, rotation of 7-day incubated eggs under transillumination ("candling") showed that the rate of movement of the contents relative to the shell was much faster in eggs that had previously been turned than in those that had not.

I also had various other projects at this time with the vitelline membrane culture technique, including a study of blastoderm phagocytosis with Ruth Bellairs. But it should be emphasised that this technique was only the survivor of a large number of procedures that I had been trying. I have always enjoyed working with my hands and during this period was constantly making bits and pieces of apparatus that I hoped might have some relevance to egg incubation or embryo culture. As a research student, I was definitely of the messy variety and I am grateful to my many close colleagues of the time for their amiable tolerance as well as for much useful advice.

Between 1957 and 1961, you stayed in the Zoology Department of the University College of the West Indies in Jamaica. Why did you apply for that job and what were your scientific activities during those years?

After five years in London I wanted to see something of the wider world. I had recently married a research student from the Botany Department at University College who was completing her Ph.D., and my new wife shared my desire to travel. But travel at that time was extremely expensive and only Royalty and the super-rich could afford to take holidays further afield than Europe. For most people, and certainly for junior academics, the only way to reach really distant places was to emigrate, and June and I began to look for possible destinations abroad where we could both get a job. Eventually we settled for the University College of the West Indies in Jamaica, where there was a lectureship in the Zoology Department available immediately for me and a lectureship the following year in the Botany Department for June.

The University was very new and very small, still with less than a thousand students, and the academic staff were mostly expatriates from Great Britain. Housing for the staff was good and was conveniently located on the University campus. In the departments, however, there were many problems, particularly for biologists. Technical support was limited and poorly trained. All but the most basic supplies had to be ordered from abroad, involving expense and long delays. The only biology text books were British or American and these often did not match the local flora and fauna. This affected also the biology teaching in schools and many of our students had learnt about the English frog and buttercup but had never looked at what lived in the school yard!

But despite all the difficulties, it was an immensely stimulating environment to work in. I was extremely lucky in my zoological colleagues, who were full of ideas and pursuing them enthusiastically. David Steven, a marine biologist, was head of the department. With him, besides myself, were three young zoologists who have all since had distinguished careers in their own fields: Ivan Goodbody, a marine biologist; Michael Locke, an entomologist; and Garth Underwood, a herpetologist. The five of us had to cover the entire animal kingdom in our teaching. David and Ivan were also building up the University marine station at Port Royal and developing research programmes there. Michael, as well as developing the entomology side of the department, was actively researching on pattern formation in insects; and Garth, the longest serving of us, had already become a world authority on West Indian reptiles.

What was my contribution to be? My arrival coincided with that of Arthur Hughes, a distinguished embryologist who was on sabbatical leave from Cambridge, and my first research project in Jamaica was a collaborative study with him on lizard tail regeneration. The small *Sphaerodactylus* lizards that were abundant on the island proved ideal for the purpose. They could be maintained easily in the laboratory, and when held by the tail would readily release it, and, over the course of a few weeks, would regenerate a new one. Garth Underwood taught us how to find and catch the lizards by sorting through the dead 'trash' under palm trees. This operation was simple in principle but hazardous in practice because the trash was also the home of numerous scorpions and Black Widow spiders. The locals told us enthusiastically that,

whereas the sting of the former was just exceedingly painful, the bite of the latter meant a spell in hospital – or worse. But fortunately we never put either statement to the test.

By now I had decided not to try to continue my former lines of work with chick embryos. The local conditions created too many difficulties, and in any case I wanted to pursue something more appropriate to a tropical setting. So I turned to honeybees. I had set up some observation hives of bees in order to demonstrate to the students the bees 'dance' mechanism of communication. It was still only a few years since the great Austrian researcher, Karl von Frisch, had made the remarkable discovery that bees can communicate the direction and distance of a food source by performing a dance on the vertical comb. In this dance, the angle between the line of the dance and the direction of gravity represents the angle between the food source and the compass direction of the sun (and the speed of the dance indicates distance). The students followed all this easily enough but one day a student asked me, "What do the bees do when the sun is overhead?". It was an excellent question. In the tropics, during one or two periods each year, the noon sun passes close to the zenith and loses any usable compass direction. Von Frisch, working in European latitudes, had never had this problem.

I wanted to find the answer. With the help of two students, Fay Burrowes and Arthur Edgar, watch was kept at some feeding dishes during the critical days in August and the frequency of arrival of new bees at different times of day was recorded. We found that the frequency fell around noon – but never reached zero however close the sun passed to the zenith. Control experiments ruled out the possibility of bees finding the dishes by random searching. It seemed that the bees had a means of communication which went beyond the dance mechanism described by Von Frisch.

To investigate this further, it was necessary to observe and analyse many dances at the critical times. By now June had joined me in the project but there were only a very few days in the year in Jamaica when the sun passed close enough to the zenith for relevant observations to be made. So to augment the data from Jamaica (18°N) we also made observations on bees in Trinidad (11°N) and Guyana (5°N). (The setting at this last locality was spectacular. Our bees were located at a government rest hut deep in the forest, close to the foot of the Kaiteur Falls. It was a world of giant trees, howler monkeys, leaf-cutter ants and brilliantly coloured birds and butterflies. And dominating all was the Kaiteur Falls itself, five times as high as Niagara, its thunder reverberating for miles around. Friends were later persuaded only with difficulty that we had really gone there for the bees).

When we had analysed all the results, it became clear that the bees were indeed communicating by dances even when the sun was almost directly overhead. They did so by a remarkable subtlety of the dance mechanism. When the sun was too close to the zenith to determine its compass direction by direct observation, they based their dance angles on an extrapolation of the sun path observed at other times of day. This implied that the bees had a sense of time, as well as memory, and could use it in determining the angles of their dances. There has since been much further evidence from other sources that bees can use time as well as direct observation in their dance communication, but for us the idea was new and exciting. We published the work in *The Journal of Experimental Biology*.

In the summer of 1961, you went back to England and joined the scientific staff of one of the most prestigious centres of the time, the Strangeways Research Laboratory in Cambridge, whose director was (Dame) Honor Fell. What was this laboratory like and what were its main lines of research when you were there?

To answer the first part of your question, I must first tell you a little about the remarkable history of the Strangeways Research Laboratory. In 1905, Dr. T.S.P. Strangeways, a Lecturer in Pathology and a man of extraordinary dedication and philanthropy, converted a house in Cambridge into a small hospital for research into rheumatoid arthritis and allied diseases. Strangeways contributed a third of his own small income to the project and was supported by a number of medical friends. The staff were a retired hospital matron and a nurse, who both worked unpaid. Then, after a very successful public appeal, a larger building was erected on a new site and opened in 1912 as the Cambridge Research Hospital, which in 1928 became the Strangeways Research Laboratory. Further large extensions have been made over the years, funded particularly by the Medical Research Council, the Rockefeller Foundation and the Wellcome Trust. But even now, visitors enter through what appears to be the doorway and hall of an Edwardian country house, part of the original Research Hospital, so constructed because the initial uncertainties about its future demanded that, if necessary, it be saleable as a gentleman's residence.

A few years before his death in 1926, Strangeways decided that further research on rheumatoid arthritis could best be carried out by studies *in vitro* on the cells and tissues of joints. He therefore ended the clinical work, closed the wards, and became a pioneer of tissue culture. In this he was followed by Honor Fell, a young research worker who was appointed Director of the Laboratory in 1929 and continued to hold the post with enormous success for the next forty one years. The reputation of the Strangeways Research Laboratory grew rapidly and for many years was particularly associated with the development of tissue and organ culture. Among the many famous biologists who worked at the Laboratory during this period were M. Abercrombie, B. Balinsky, A. d'A. Bellairs, G. de Beer, J.D. Biggers, E. Borghese, F.H.C. Crick, H. Florey, A.F.W. Hughes, P.B. Medawar, A. Moscona, G. Pincus, R.J. Pumphrey, A.K. Tarkowski, C.H. Waddington and E.N. Willmer.

At the time I joined the Laboratory, the staff included over forty research scientists, including visitors, and a large number of different investigations were being pursued. If I mention just a few of them, it may give some idea of the range covered. In line with the original purposes of the laboratory, various aspects of cartilage and bone development were being studied by Honor Fell, Tony Barrett, John Dingle, Sylvia Fitton Jackson, Duncan O'Dell, John Reynolds and others. Carcinogenesis research was also well represented and included work on folic acid and its derivatives in relation to leukaemia by Werner Jacobson, hormonal modifications of carcinogenesis by Alfred Glücksmann, the carcinogenic effects of metals by John Heath, and the growth of malignant cells and organs in culture by Geoff Clarke, Mary Daniel and Ilse Laznitski. Jim Dodson was studying the differentiation of embryonic epidermis, Kirstie Lawson tissue interactions in a number of systems. Geoff Cooke and Leonard Weiss were examining the surface properties of cells, Ross Munro and Frederick Spear problems of

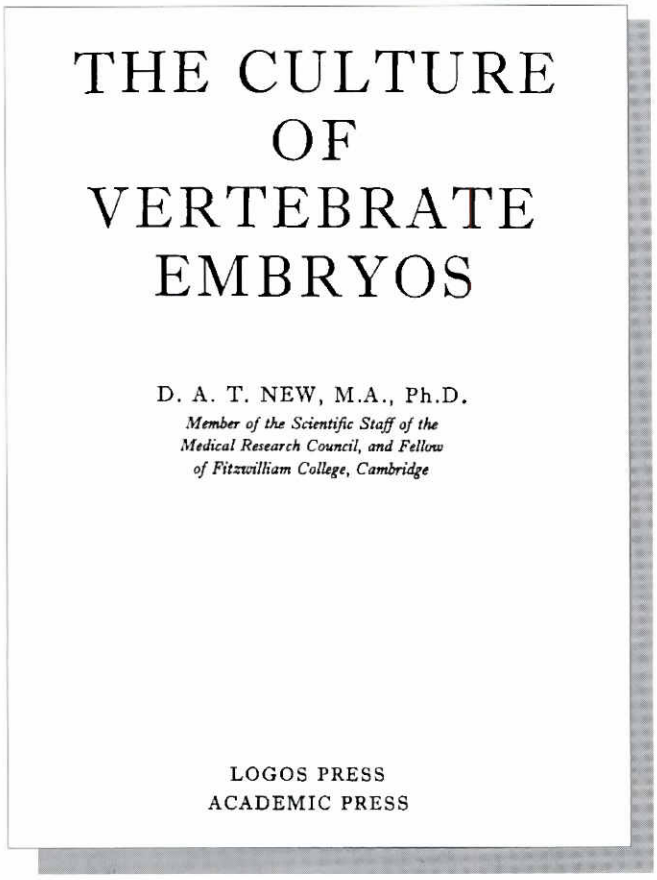

Fig. 6. Cover of Denis New's book *The Culture of Vertebrate Embryos*.

radiation biology, and Michael Webb various aspects of cellular biochemistry. Audrey Glauert was developing the electron microscopy side of the laboratory and, among other applications, was using it to examine the mechanisms of surfactant formation in the fetal lung.

A discovery that had been made a few years previously at the Strangeways Laboratory by Fell and Mellanby had attracted particular attention. They had found that excess vitamin A could completely switch the path of development of embryonic chick skin, so that it formed a mucus-secreting epithelium instead of a keratinizing epithelium. This had stimulated several lines of investigation on the metabolic and developmental effects of vitamin A, which were being pursued by a number of scientists in the Laboratory, particularly John Dingle and Jack Lucy. It had also provided the starting point for an important series of studies by Jack Lucy on theoretical and experimental models of membrane ultrastructure.

During this period, your book "The Culture of Vertebrate Embryos" was published, a classical source book for experimental biologists in the whole world. In your opinion, in what way does embryology depend on *in vitro* techniques?

From the time when I had first started reading about embryological mechanisms, I had been struck by the extent to which experimental embryology, at least in vertebrates, was dominated by studies on amphibia. Everyone knew of the classic experiments of Roux, Spemann, Mangold, Holtfreter and others on frogs and newts, but 'explanations' of development in other vertebrates were usually just speculations based on the amphibian work. A little, but not much, was known from direct observation of chick embryos, and almost nothing from mammals. Although relative costs and availability played a part in this uneven distribution, ease of access to the embryo was obviously also a major factor in determining what kind of investigations could be carried out. I became more than ever convinced of this after the publication of my chick technique. I was astonished at how rapidly, and how widely, it was taken up by other laboratories. It was clear that previously a great deal of experimental embryology on the early chick embryo had been held up simply because of the limited accessibility of the embryo in the egg and the lack of adequate culture methods. And this applied even more strongly to the mammalian embryo in the uterus. Here there were not only problems of accessibility but also experimental complications arising from interactions of the embryo with the maternal metabolism.

So I remained very interested in whole embryo culture, and at about this time I was invited by David Newth, then biology editor of Logos (Academic) Press, to write a book on the subject. I found the proposal intriguing and decided to include in the book culture methods for all classes of vertebrates, and for several species in each class, in the hope that this might encourage a wider use of different types of embryo. Eventually the book appeared under the title *The Culture of Vertebrate Embryos*. It was necessary to write to embryologists all over the world for the required information and, thanks to their generosity, I learnt much that was new to me and greatly enjoyed this aspect of the work. But in other ways the project was less pleasurable. It took me away from the laboratory bench and absorbed far more time than I had expected. The completion date that had been agreed with the publishers was overrun, renegotiated, and overrun again. Common enough experiences perhaps, but they weighed on me. I decided that, much as I admired scientists who manage to write several books, it would be a long time before I contemplated another.

During your stay at the Strangeways Laboratory, you shifted your interest towards the study of mammalian experimental embryology, where you were faced with the great difficulties of *in vitro* culture, especially of postimplantation embryos. What were your first attempts in this respect?

I had been thinking for a long time about trying to devise methods for culturing postimplantation mammalian embryos. In fact several years previously I had made some unsuccessful attempts with Michael Abercrombie in London to maintain rat embryos *in vitro* by explanting the pregnant uterus and perfusing it with nutrient medium. Around the time of my arrival at the Strangeways Laboratory, two events occurred which further concentrated my thoughts on mammalian development. One was a small personal tragedy: June and I had just lost our first baby, which had died shortly after birth with severe brain damage resulting from anoxia caused by a placental defect. The other was the international tragedy of the thalidomide disaster which, as well as horrendously demonstrating the dangers of new drugs, had revealed how little was known of developmental and teratogenic mechanisms in mammals. Laboratory study of mammalian embryos had always been severely impeded by the inaccessibility of the embryos in the

uterus and it seemed to me that, if any real advances were to be made, an essential requirement was the development of reliable culture techniques. Good techniques already existed for maintaining the eggs of mice and rabbits at preimplantation stages, but no reliable method was available for culturing any mammalian embryo during organogenesis, the period of maximum sensitivity to teratogens.

Encouraged by Honor Fell, I began work on this with Kathryn Stein, a visitor from Mount Holyoke College, Massachusetts, who was studying the loop-tail anomaly in mice and wanted a method for observing its development *in vitro*. We explanted mouse and rat embryos of early somite stages onto chick plasma clots in watchglass cultures (the clots also contained 'embryo extract' – a formula used at the Strangeways Laboratory for many years for organ culture) and were delighted to find that many of the embryos developed a blood circulation and continued growing up to 25-30 somites. Soon after, I found that rat serum could be substituted for the plasma clot in the watchglass and gave similar results. I then devised a simple apparatus that I called a 'circulator', in which the embryos were grown in circulating medium. No pumps were required; the circulation was maintained by a stream of bubbles entering from a gas cylinder, which both oxygenated and propelled the medium. Prolonged embryonic development, up to 40 somites or more, could be obtained with this device. Some of this work had been foreshadowed by other studies many years earlier. In the 1930s, Jolly & Lieure in France had grown postimplantation rat and guineapig embryos in watchglass cultures, and Nicholas & Rudnick in the USA had experimented with both watchglass cultures and with circulating medium. But I was able to apply to embryo culture useful information that had accumulated in the intervening years from organ culture, particularly regarding optimum pH and oxygen levels, and there was no doubt that this yielded results which were much in advance of anything obtained previously. Before I left the Strangeways Laboratory I was joined for a short period by Myron Turbow, a young researcher from San Francisco, and he used the cultured embryos to examine the teratogenic effect of trypan blue. This was the first application of my mammalian culture techniques to a specific research problem.

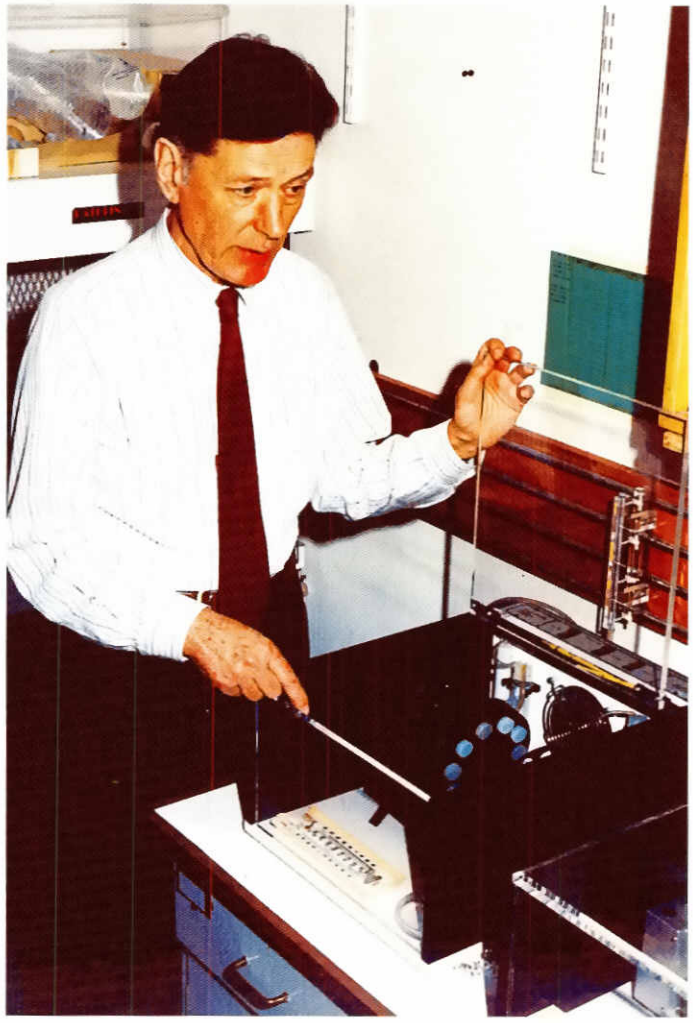

Fig. 7. Denis A.T. New showing his original incubator for rotating culture of postimplanted mammalian embryos.

In 1967, Bunny (C.R.) Austin was appointed Charles Darwin Professor of Animal Embryology in the Physiological Laboratory of the University of Cambridge, and you joined him there and stayed for almost 30 years. Robert Edwards was also there, making *in vitro* fertilisation experiments which were followed by the birth of the first test-tube babies. And other young scientists in the group were also pioneering many aspects of mammalian experimental embryology. I guess those were exciting times, of which you must have many recollections.....

Bunny Austin, Bob Edwards and I all held tenured University teaching posts. Although our department was rather quaintly called the 'Physiological Laboratory', it was in fact the university physiology department and we were expected to teach. But our expertise was relevant only to the courses in reproductive physiology and these, when divided among three, made very modest demands on each of us. Compared with the staff in many universities, we were very lucky. There was enough teaching to be stimulating and enjoyable, without it ever becoming onerous. And it left more time for other activities, which for me meant the opportunity to edit for several years, with Donald Ede, John Paul and Lewis Wolpert, the *Journal of Embryology and Experimental Morphology* (now *Development*).

As regards research, Bunny's laboratory was certainly a very exciting place to be. There was a succession of enthusiastic research students and other talented young research workers, many of whom have since become internationally distinguished leaders in their own fields. Among these were Kamal Ahuja, Barry Bavister, Simon Fishel, Richard Gardner, Robert Gore-Langton, Roger Gosden, Alan Handyside, Peter Hollands, Martin Johnson, Matt Kaufman, Carol Redhead, Azim Surani and David Whittingham. Visiting scientists were also continually arriving to carry out specific projects. And shortly after I arrived, the news of Bob Edwards' success in fertilising human eggs *in vitro* burst on the world, with the expectation that it would soon be followed by the birth of 'test-tube babies'. This led to widespread discussion of the possible ethical and legal issues involved, plus much sensationalism in the tabloid press. The laboratory became a focus of media attention which lasted until, and beyond, the birth of the first test-tube baby, Louise

Fig. 8. (Top) Rat embryos growing in flowing culture medium in the embryo chamber of a "circulator". (Bottom) Rat embryos with culture medium in a roller bottle.

Brown, ten years later. During this period we all became adept at fending off the more importune breeds of reporter. In my case this meant saying repeatedly, "I only work on rats", and, "No, I do not know where Dr. Edwards is". It all added to the variety of academic life.

I continued working on postimplantation embryos in culture and was very fortunate in having in the laboratory a series of excellent collaborators. With Pat Coppola and David Cockroft, I developed methods for growing the embryos in rotating bottles. These were much simpler than the circulator technique and, for many types of experiment, were preferable. We also began to look into ways of growing the placenta, and successfully set up cultures of rat embryos in which a rudimentary placenta developed complete with a fetal placental blood circulation. Chris Steele made the important discovery that embryonic development, particularly at the younger stages, was much improved if the serum used in the culture medium was (i) obtained from blood centrifuged immediately after extraction, before it had clotted, and (ii) heat-inactivated. The use of such 'IC heat-inactivated serum' has now become standard. David Cockroft devised a method for growing more advanced embryos by opening the yolk sac to increase the surface of exchange with the nutrient medium, and Stephanie Ellington joined me in improving the culture methods for very young (primitive streak) embryos.

Your "rotating" and "circulating" methods have made possible significant advances in mammalian *in vitro* development during the organogenesis period, even supporting growth up to 45-55 somites in rodents. What are the main limitations from this stage?

Some limitations come before this stage. First, our culture methods are only really successful with embryos of rats and mice. No other species that have been tried are anything like as good. Second, growth of the rat or mouse embryos *in vitro* identical to that *in vivo*, as regards both frequency of embryos developing and the extent of their development, can be obtained only over a two-day period between the head-fold stage and about 25-30 somites. This is the period when the embryo in these species is supported mainly by nutritional and respiratory exchanges mediated by the yolk sac. If younger embryos are cultured, *some* of them may develop very well, but the proportion doing so is never as high as *in vivo*. And for older embryos, over 25-30 somites, the lack of an allantoic placenta in culture becomes noticeable. If these embryos are in flowing medium and high oxygen, they continue to differentiate up to 50 somites or more (depending on the stage at the beginning of culture) but have a lower protein content than in vivo; i.e. they are well formed but smaller than normal. Even older embryos (fetuses) can be kept alive for short periods in culture but, lacking sufficient placental support, their growth is negligible.

At one time you were working on marsupials, especially the opossum. What scientific questions did you want to answer with these studies, and what were your most significant results?

So far, rat and mouse embryos have proved to be the best for culturing at postimplantation stages. This is probably related to the importance of the yolk sac during organogenesis in these embryos and to the capacity of the yolk sac to grow *in vitro* and provide a large surface for respiratory and nutritional exchange between embryo and substrate. But from limb-bud stages onwards, the support of the chorio-allantoic placenta is increasingly needed and it has not yet proved possible to grow this placenta in culture for more than a very short period. This sets a limit on the amount of

embryonic/fetal development that can be obtained. An idea that stayed with me for some time was to try to avoid the placental problem by using marsupial embryos, which usually lack a chorio-allantoic placenta and are born at stages of development similar to those of our most advanced rat and mouse in culture. If a marsupial embryo could be grown to 'birth' in culture, it could be transferred to the pouch of a female for continued development, making possible long-term studies of experimental treatments.

There being no indigenous marsupials in Europe, David Cockroft and I began a collaboration with Merle Mizell of Tulane University, New Orleans. Merle periodically sent us batches of mated female American opossums, *Didelphys marsupialis virginiana,* and we endeavoured to grow their embryos in culture. But there were many problems. Most serious was the problem of the culture medium. The opossum embryo in the uterus is nourished by the secretions of uterine glands. Lacking information on the composition of these secretions, we rather arbitrarily concocted substitute mixtures of serum and standard tissue culture media. These did give some development of opossum embryos in culture, but not much. Other problems arose over the animals. Although the opossum is a common rural, and even urban, animal over large areas of the USA and is apparently of little concern there to anyone, it is treated by the UK authorities with extreme suspicion, and the strictest quarantine, caging, housing and transport regulations had to be observed. And when our animals finally arrived, many turned out not to be pregnant (there is no simple way of diagnosing pregnancy in an opossum). Since they had all travelled from New Orleans by air, the cost of the project per embryo rose alarmingly. In the end we regretfully decided that, although the aim of the project was good, Cambridge was not the place to pursue it. We published our limited results in 1977, made a teaching film of opossum reproduction and development, bequeathed our idea to friends in the New World with readier access to opossums, and returned to rats and mice.

It is well known that human postimplantation development is significantly different from that of rodents. However, apart from ethical, psychological or legal considerations, could your methods be applicable to human embryos?

The only way to obtain a certain answer to this would be to do some actual trials. But it seems to me very unlikely that our present culture methods would be applicable to human embryos, if "applicable" means obtaining as much growth *in vitro* of the human embryo as we do from the rat embryo. Culture methods in general often give very different results even with embryos of closely related species. There is no reason therefore to suppose, *a priori*, that our techniques would be successful with human embryos. And there is the large difference in the placental systems of rodent and human embryos. Rodent embryos develop a large yolk sac which, at the time of embryonic organogenesis (and before the chorio-allantoic placenta has acquired a major function), effects most of the respiratory exchanges between mother and embryo. In culture, these activities of the yolk sac continue, though now mediating exchanges between embryo and culture medium, and this contributes much to the success of the culture system in supporting organogenesis. The human embryo on the other hand has only a very small yolk sac and relies during organogenesis on a precociously developed chorio-allantoic placenta, which differs from the rodent yolk sac both in overall form and in many details of cell structure and function. Unlike the yolk sac, the chorio-allantoic placenta contains maternal as well as fetal tissues, and an intricate system of enmeshed maternal and fetal blood flows. Although we have obtained development of the rodent chorio-allantoic placenta for brief periods in culture, including the formation of the fetal blood circulation, no 'maternal' circulation of culture medium in such growing placentas has yet supported additional growth of the embryo. Until this can be done, it seems unlikely that human embryos would develop *in vitro* through more than a small part of organogenesis.

Your postimplantation embryo culture methods are being used world wide, mainly in teratologic or metabolic studies. In what experimental approaches have these methods been used up to now, and what other applications might they have in the future?

Over the years we have looked at many aspects of postimplantation embryonic development and have been joined in this by many visiting scientists from other laboratories. Space does not permit mention of all these studies here but I will try to give some representative examples.

In 1968, one of our earliest visitors, Joe Daniel from the University of Colorado, joined us in a study of the development in culture of very young (7-8 day) postimplantation rat embryos. We found that such embryos commonly form double hearts, as well as other abnormalities. Later, Chris Steele obtained much better results by the use of 'IC serum', and further improvements have continued to be made from time to time, up to the procedures developed here very recently by Renée van der Most. The '70s and '80s brought more collaborative projects. These included work with Bob Brent, which showed by injection experiments on embryos in culture that yolk-sac antibody, known to be teratogenic in pregnant rats, had little or no direct effect on the embryo and that the teratogenicity almost certainly resulted from a primary effect on the visceral yolk-sac endoderm. Maurice Robkin, from the University of Washington (and inventor of the 'plasmom' culture system) joined David Cockroft to examine the response of cultured embryos to exposure to carbon monoxide and reduced oxygen, and found that the embryos can at least partially adapt to anoxia by increasing their rate of anaerobic glycolysis. Pat Coppola and I had previously examined the overall effects of different oxygen levels (including hyperbaric oxygen) on embryonic growth and, in 1979, Gillian Morriss from Oxford and I studied the detailed effects of oxygen concentration on the morphogenesis of the cranial neural folds and neural crest. Daphne Trasler, from McGill University, joined Chris Steele here in an *in vivo/in vitro* evaluation of the teratogenic action of excess vitamin A and concluded that the effects of the teratogen on cultured embryos were similar to those on embryos growing in the uterus. Chris also collaborated with Alan Ashford and Graham Copping from May & Baker Ltd. in a study of the embryotoxicity of two new hypolipidaemic agents. A few years later, Bill Webster, from the University of Sydney, came to study the effects of isotretinoin on cultured rat embryos and found that the teratogenic concentration of the drug needed to induce malformations *in vitro* was much lower than in the pregnant rat but similar to that in the pregnant human, differences which could be related to the different pharmakokinetics of the drug in the two species. David Cockroft and I looked at the

Fig. 9. Group photo at the laboratory of Professor Kazuhiro Eto in Tokyo, taken during a visit of Denis New in 1988. *(Prof. Eto is on the right of the front row).*

development of embryos in culture under various levels of hyperthermia and observed deleterious effects with a rise of temperature of as little as 2°C. Stivelia Kachilele, from the University of Malawi, examined the tolerance of the embryos to cooling and found that they could be stored for 5-10 h at room temperature without noticeable effect on their subsequent development; but results were poor after storage at 0° or 5°C. Greg Kesby, from the University of New South Wales, made a re-examination of early claims that embryos could be grown in heparinised plasma and found that heparin, at levels sufficient to inhibit coagulation, has severe effects on cephalic neural tube and eye development. And recently, Leonid Penkov, from the Institute of General Genetics, Moscow, has succeeded in growing parthenogenetic mouse embryos in culture to stages much more advanced than they normally attain *in vivo*, a result that promises to be useful in the study of the mechanisms of parthenogenesis and genomic imprinting.

Besides these collaborative projects, a number of lines of research have been pursued solely by members of our own group. To those already mentioned should be added the detailed analyses by David Cockroft of the nutrient and vitamin requirements of embryos at different stages of development, and of the effects of different glucose concentrations in the culture medium. Stephanie Ellington has taken the glucose work further and has extended it to a number of investigations, some with Penny Rashbass, on diabetes-related problems. She has also made a special examination of the development of the embryonic membranes and is currently investigating, in collaboration with John Brown, the effects of natriuretic peptide on development. And in one of our most recent studies, Rowan Hardy, prompted by some publicised concern over the "morning-after-pill", examined the effects of the abortifacient, RU 38486 (Mifepristone) on cultured rat embryos and concluded that there was no evidence of a teratogenic effect at the levels currently used in clinical practice.

We have also been glad to welcome numerous visiting scientists who have come just to learn the culture techniques or to find out more about them. Many of these visitors then put the techniques to good use in their own laboratories and some of the results are described in the following pages of this issue. In 1978, I wrote an account in *Biological Reviews* of all the embryo culture work up to then, and in the same year we decided that the frequency of enquiries about our methods justified holding a special course in embryo culture. A 4-day course was arranged, with David Cockroft, Stephanie Ellington, Azim Surani and myself as instructors. Limitations on the availability of laboratory space and apparatus restricted the number of places on the course to 24, but we were able to include participants from Brazil, Egypt, Finland, France, Germany, Hong Kong, India, Italy, Japan, Russia, Spain, UK, USA, Yugoslavia. The course was well received and we adapted some parts of it for a course that we have run annually ever since for our own final-year students.

Outside our own laboratory, the embryo culture techniques have been used in many teratological studies. Their advantages for this are obvious. The period of development covered, early organogenesis, is the period of maximum sensitivity to teratogens. The experimental conditions can be tightly controlled and the results

closely monitored. Single factors of the environment (e.g. temperature) can be varied and tested independently of all other factors, often very difficult to achieve with embryos in the uterus. Expensive reagents may be used in much smaller quantities in culture than when injected into pregnant animals. Maternal tissues may be entirely eliminated, or selected maternal cells, microsomes etc, may be cultured with the embryos. And so on. A large number of studies have now been made of the action of different teratogens on embryos in culture, resulting in many important advances in the understanding of teratogenic mechanisms. It has also been shown repeatedly that the response (positive or negative) of embryos *in vitro* to different known teratogens or non-teratogens correlates well with that *in vivo*. This has led to serious consideration of the possibilities of incorporating embryo culture into teratogen screening programmes – indeed, to a limited extent embryo culture is already being so used.

Less use has been made so far of the culture methods for investigating the normal development and physiology of the embryo, but the work that has been done in these areas includes a number of really excellent studies. The early experiments of Tom Shepard and his colleagues in Seattle, involving the analysis of metabolic pathways by the administration of labelled glucose to cultured embryos, have become classic examples of what can be achieved with this type of approach. The work of Maurice Robkin and others in the same laboratory on the effects of cardioactive drugs on the embryonic heart rate showed how the accessibility and clear visibility of the early heart and blood circulation in culture can be exploited experimentally. Embryos in culture are also ideal for studies involving labelling and/or transfer of cells and tissues, as in the remarkable experiments of Rosa Beddington and Gillian Morriss-Kay at Oxford, and of Kirstie Lawson at Utrecht, on cell lineages and morphogenetic movements. Although the cultured embryo is usually surrounded by the yolk sac and amnion, quite large holes can be made in these membranes without affecting embryonic development, allowing access to the embryo for many operative techniques. Such operations were made by Elizabeth Deuchar in the 70's and have more recently been refined by Andrew Copp in Oxford in a study of neurulation mechanisms. The membranes themselves are readily available for analysis of their metabolic and transport mechanisms, as in the pioneer work of Felix Beck's group at Leicester, and John Lloyd and Stewart Freeman at Keele, on uptake and transport of proteins and amino acids by the yolk sac. I am sure that in the future all these different approaches will be explored much further. And at the same time, work will continue on improving the culture methods. Recently Masahiko Fujinaga and Jeffrey Baden in California have obtained improved development of primitive-streak stage embryos by removal of the ectoplacental cone and destruction of the adjacent cavity. This has been an important breakthrough and has already made possible a fascinating study by these authors and their collaborators on the mechanisms determining left/right sidedness in the developing embryo. But we still lack any reliable culture techniques for the peri-implantation stages of development, although Yu-Chih Hsu at Baltimore has been able to grow a small percentage of embryos *in vitro* from preimplantation to organogenesis stages. Growth of more advanced (fetal) stages will probably require the further development of methods for growing the allantoic placenta in culture with the embryo. And equally valuable for long term studies of the effects of experimental treatments would be a method for returning post-implantation embryos to the uterus. We do not yet have a sufficiently reliable method for doing this but Rosa Beddington has obtained continued development of about 7% of mouse embryos returned to the uterus at the head-fold stage.

How much have you lectured and worked abroad on mammalian embryo culture and its applications?

In 1969, Tom Shepard invited me to Seattle to visit his laboratory and to present my embryo culture work, such as it then was, at a meeting of The Teratology Society at Crystal Mountain. It was a wonderful opportunity to meet many of the world's leading teratologists and for me a source of much inspiration. As part of the same trip, I included visits to some other centres of learning in the USA and developed a taste for visiting and working in laboratories abroad that I have subsequently indulged whenever possible. It would be impossible to do justice here to all the kindness and hospitality that I have received on such visits. But – if only because they endured me the longest – I would particularly like to record my gratitude to Arthur and Marjorie Hughes, and Peggy and Joe Egar at Case Western Reserve University, Cleveland; to Tom and Alice Shepard, and Maurice and Nancy Robkin at the University of Washington, Seattle; to Joe Daniel at the University of Colorado; to Merle and Lorraine Mizell at Tulane University, New Orleans; to Bob Brent at the Thomas Jefferson University, Philadelphia; to Jan Langman, Dave Kochhar, Tom and Debbie Sadler at the University of Virginia, Charlottesville; to Norman Klein at the University of Connecticut; to Kazuhiro and Mariko Eto at the Medical and Dental University, Tokyo; to Mineo and Iku Yasuda at the University School of Medicine, Hiroshima; to Yasukazu Akita at Kamakura; to Takashi Kobayashi at the Tanabe Seiyaku Company, Osaka; to Akinori Miki at the University School of Medicine, Kobe; to Ichiro Naruse at the National Institute for Minamata Disease, Minamata City; to Kohei Shiota at the University School of Medicine, Kyoto; to Toshiaki Watanabe at the University School of Medicine, Yamagata; to Samuel Chan at the University of Hong Kong; to Boris and Tanya Konyukhov, and Evgeny and Tanya Platonov at the Vavilov Institute of General Genetics, Moscow; to Boris and Margarita Leonov at the Laboratory for Clinical Embryology, Moscow; to Boris and Dina Protasov at the Research Institute for the Breeding and Genetics of Farm Animals, St. Petersburg; to Marta and Laszlo Bencsath at the Semmelweis University of Medicine, Budapest. To these and many, many more friends and colleagues throughout the years, I remain grateful for generous help and invaluable encouragement. Such friendships have left me with some of the best memories of my life in biological research.

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