

An obsession with the chick

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ABSTRACT This paper provides a brief account of some aspects of the career of Ruth Bellairs using selected examples from her research publications, with the emphasis being placed on the early stages of chick embryo development, and in particular, on cell migration. Topics include the role of Hensen's node, the vitelline membrane, the structure and segmentation of somites, the tail bud and the Wolffian duct. Her research approach has involved embryo culture, experimental surgery, transmission and scanning electron microscopy, time-lapse filming and immunostaining techniques.

KEY WORDS: *fibronectin, somites, cell migration, primitive streak, precardiac mesoderm.*

I have always liked domestic fowls, with their beaks and their combs and their gizzards, so it is perhaps not surprising that I chose to become a zoologist. I had been much impressed as a schoolgirl with a book by Lancelot Hogben called "Science for the Citizen", which had enjoyed a wide popularity in the pre-war years, and when I discovered that Hogben was the head of the Zoology Department in the University of Birmingham I decided that that was the place for me. But what a disappointment it was when I arrived in Birmingham in 1944 to find that Hogben was permanently away on war work. He returned to the university two years later when the war ended, but soon it became apparent to the students that Professor Hogben was not an easy man. Within a few months the entire research and teaching staff resigned. Among those who left us was Michael Abercrombie who was then soon appointed as a lecturer in Embryology at UCL (University College London).


As a student during the war I had given little thought to my future career as I knew that once I graduated I would be drafted into one of the women's services. I rather fancied the WAAF (the women's branch of the Royal Air Force). But with the end of the war the compulsory call-up was ended for women and I became free to choose my next step. At the critical moment Michael Abercrombie wrote and invited me to join him as a PhD student to work on the chick embryo. I had looked at parasitology and investigated entomology but Michael's idea appealed much more, and so I went to the Sub-department of Embryology run by Professor G R De Beer (later Sir Gavin) in the Department of Anatomy and Embryology at UCL.

Before the war Michael had been a postgraduate student of CH Waddington at the Strangeways Research Laboratory in Cambridge. It was a period when the search was in full swing to identify the supposed chemical organiser of amphibian embryos, and Waddington had been the first to demonstrate experimentally

that Hensen's node in the chick was in many ways comparable to the dorsal lip of the blastopore of amphibian embryos. His approach was to insert grafts of Hensen's node under the ectoderm in different regions of the area pellucida, and he found this often resulted in the induction of a secondary axis. It is almost impossible to carry out experiments of this type *in ovo* on the early embryo but Waddington (1932) had bi-passed this problem by devising the first successful technique for growing an entire chick blastoderm *in vitro*. Essentially, it consisted of freeing the blastoderm from the yolk and the vitelline membrane and then explanting it onto a bed of clotted blood plasma. It was still in use when I joined Michael's lab in 1947. Suitable plasma was not yet available commercially so we were obliged to anaesthetise fowls in the lab and draw off blood from the carotids with home-made glass canulae that were awkward and slippery. Strict sterility was needed because the plasma was a suitable medium for bacterial infection. Waddington's was a tricky technique but later was successfully simplified and greatly improved by New (1959) who abandoned the plasma clot and replaced it with the vitelline membrane, the embryo's own natural substratum. Embryos grown by Waddington's method could be expected to survive for only about 24 hours and were smaller than control specimens developing simultaneously *in ovo* in their natural environment. Embryos explanted by New's technique could survive for twice as long or longer and were within the normal size range; with minor modifications this technique is still widely used today.

My own research included the replacement of the whole thickness of Hensen's node (at stages no later than stage 4- of Hamburger and Hamilton), with grafts taken from other regions of the area pellucida and demonstrated that the result depended

Abbreviations used in this paper: UCL, University College London.

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Fig. 1. Ruth Bellairs in 1973.

on the site of origin of the graft, indicating that the properties of Hensen's node owed much to the influence of the surrounding area pellucida. It didn't take long for me to realise how fortunate I was in having Michael Abercrombie as my PhD supervisor, always calm, encouraging and stimulating with an immense knowledge of the literature and a keen analytical mind. Over the years I have observed how sometimes PhD students have been left by their supervisor to flounder with impossible tasks, or have suffered from their findings being used without true acknowledgement. The "Embryologists' Club" was initiated at this period, largely at the instigation of Michael Abercrombie and David Newth, (then a lecturer in Zoology at UCL and subsequently Regius Professor in the University of Glasgow). There were about 12 of us at the first meeting, all drawn from London Medical Schools and Zoology Departments. Later, the Embryologists' Club was transformed into the Society for Developmental Biology, then subsequently renamed The British Society for Developmental Biology.

The fevered search for the supposed chemical organiser was already fading with the onset of the war and had virtually disappeared as I embarked on my PhD and by the time I had finished it there were few interested in the organiser at all. A new fashion and excitement had arisen among experimental biologists - to locate the origin and distribution of alkaline phosphatase in every tissue imaginable - but as this seemed to have little significance for an understanding of the early chick embryo I decided to branch out on my own.

I was appointed as a junior lecturer at UCL in 1949 and began to investigate the origin of the chick foregut endoderm, which until then had been thought to be from cells ingressing at the posterior end of the primitive streak, rather like the gut cells of the amphibian blastopore, but my labelling experiments now indicated that it was derived from the anterior end of the primitive streak, its presumptive area lying around Hensen's node (Bellairs, 1953a). A further paper traced the morphogenetic movements involved (Bellairs, 1953b).

It is worth mentioning here two factors that affected embryologists at this time. *First*, the methods for labelling and tracing

cell movements which are now in use were not available then. Interestingly, as early as 1898 Florence Peebles had pushed a tiny hair into Hensen's node and later found that it had moved toward the posterior end of the area pellucida, probably the first experimental demonstration of node regression using a labelling method. But by the middle of the 20th century the two most widely used techniques to mark areas of vertebrate embryos were either jabbing tiny particles (usually carbon) onto the surface of the cells, or dabbing patches of vital stain (eg Nile blue sulphate) on to them. There were disadvantages to each procedure (see Bellairs and Osmond, 2014). More sophisticated and reliable techniques were yet to be designed. The *second* handicap for chick embryologists at this time was the inadequacy of good Normal tables for recording the stage of development of an embryo. For example, with Waddington's (1932) terminology, the primitive streak was described as either short, medium or long. But the now universally used HH Normal table (Hamburger and Hamilton (1951) which replaced it, was less satisfactory for the all-important primitive streak stages; for example, Waddington's stage *Short* corresponds to HH stage 2, and Waddington's stage *Medium* is comparable with HH stages 3 and 3+, Waddington's stage *Long* is not represented in the HH table, despite the fact that this is the critical stage in early development when the primitive streak is fully formed and node regression and head process formation are about to begin. But good sense has prevailed and Waddington's *Long* stage is now known by most chick workers as stage 4- (see Plate 9 of Bellairs and Osmond, 2014).

During the nineteen fifties the Anatomy department at UCL acquired its first transmission electron microscope (TEM), thanks to the foresight of Professor JZ Young, the head of the department. These were early days for the study of biological tissues by electron microscopy, and despite extensive searches through the literature at that time I was able to find only one paper on the chick embryo, a report by Duncan (1957) on neural tube. These were pioneering days when every scrap of tissue studied yielded new information. When I saw my first electron microscopy sections I was amazed to discover quantities of small intracellular yolk droplets, too small to be seen by light microscopy, in the supposedly yolk-free cells of the area pellucida. Many of my early investigations using transmission electron microscopy and, later scanning electron microscopy,



Fig. 2. Esmond Sanders (foreground), Jay Lash and Ruth Bellairs (right) at a NATO Research Workshop held in Glasgow (1986).

whether alone or with co-workers, were initially designed less to answer specific questions than to describe what we saw. It was still an unknown world and these were exciting times, though soon electron microscopy settled down, as is common with new techniques, and became just another tool in our armoury.

Much of my later work was concerned with aspects of cell migration, especially in the embryo proper, combining electron microscopy, tissue culture, labelling, experimental surgery and aspects of molecular biology. I had become much impressed by the findings of Abercrombie and his colleagues on cell migration in tissue culture (Abercrombie 1980) but felt it was important to establish if migrating cells in an embryo behaved in the same way as monolayers of fibroblasts migrating on glass in tissue culture. I was of course not the only one to raise this point; eg Trinkaus and Erickson (1981) exhibited a beautiful film of cells migrating within living and transparent teleost (*Fundulus*) embryos. An example of the investigations I and certain of my colleagues made during this period was into the structure and composition of the vitelline membrane, the transparent bag that surrounds the yolk of the laid egg. It had long been thought to be composed of collagen, but we found none, either by TEM or by biochemical analysis (Bellairs *et al.*, 1963). Instead we discovered that it consisted of two morphologically distinct layers of non-collagenous fibrous connective tissue, the inner layer secreted by the follicle cells in the ovary, the outer added in the infundibulum of the oviduct. These findings were of especial interest in view of the significant role played by the inner layer of vitelline membrane in the centrifugal expansion of the chick blastoderm. New (1959) had shown that this migration depended on the edge cells of the area opaca clinging to the inner surface of the vitelline membrane and using it as a substrate for migration. These edge cells are bound to their neighbours in an epithelial sheet and we were able to demonstrate by time-lapse studies (Bellairs *et al.*, 1969) that their mode of locomotion on the inner surface of the vitelline membrane was similar to that of epithelial sheets studied by time lapse cinematography in tissue culture (Abercrombie 1982). Our associated studies with scanning electron microscopy showed that the edge cells possessed the usual appearance of migrating cells with long cytoplasmic processes extending out from the leading edge.

In the early stages of incubation gap junctions and desmosomes are scarce but tight junctions are present in the area opaca and the borders of the area pellucida and play an important role in keeping the entire blastoderm under tension. This tension is brought about by the adhesion of the edge cells as they migrate under the vitelline membrane. When we reduced the tension in various ways the morphogenetic movements, such as regression, still took place, though the embryos remained small (Bellairs and Veini, 1984). Locomotion is possible only by the temporary making and breaking of cell adhesions to the substrate, brought about by changes in the extra-cellular materials and/or in the adhesivity of the cells themselves.

As development proceeds, the adhesivity of the cells changes (Bellairs *et al.*, 1980) and the composition of the basement membrane and the extracellular matrix change too. This may lead to changes in the shape of cells, eg. the neural crest cells as they begin to migrate away from the neural tube settle down flatly on the cells beneath them, as was well illustrated by Bancroft and Bellairs (1976).

Any migrating cell needs to be in harmony with its substrate and

this is often related to the production of adhesion molecules by the migrating cells. For example, we have been able to demonstrate that fibronectin plays an important role in the migration of the edge cells under the inner layer of the vitelline membrane. Using both immunocytochemical staining and experimental treatment with GRGDS, which contains the specific adhesion molecule RGD, the edge cells detach and cannot advance again for some time (Lash *et al.*, 1990). Fibronectin is also important in the migration of the pre-cardiac mesoderm, forming a haptotactic gradient of adhesiveness up which the cells migrate (Easton *et al.*, 1990). By contrast however the nephric duct, which crawls toward the cloaca at about stage 10 of HH (at the astonishing rate of about 400 μ m per hour) requires a different adhesion molecule, PDSA (polysialic acid). Treatment with Endo-N, which destroys sialic acid, prevents further migration (Bellairs *et al.*, 1995).

The formative role that cell locomotion plays in early development is not restricted to gastrulation nor ends when all the undifferentiated mesoderm has ingressed beneath the ectoderm. Cell locomotion, especially the regression of Hensen's node, and changing cell relationships continue to play an important role in later stages. For example, presumptive somite mesoderm cells are unable to become committed until they have passed through the primitive streak (Bellairs, 1980) and become part of the segmental plate. Like others, my colleagues and I investigated a variety of aspects of the control of somite formation, from commitment, segmentation itself, to the control of somite numbers, sizes and shapes. At first the cells are loosely arranged as somitomeres (Bellairs and Sanders, 1986), but with increasing adhesivity (Bellairs *et al.*, 1978) become more closely packed together and clearly segmented. Alongside these events there is a changing pattern of extracellular materials. Eventually the fully segmented somites extend right down the body into the tail, but a small region remains unsegmented at the tip of the tail where it is gradually removed by pyknosis; similar patches of pyknosis are visible in most amniote embryos and may play a



Fig. 3. Ruth Bellairs in the lab (1989).

role in monitoring somite numbers. Our experimental analysis led to a new theory of how the embryo controls the sequences of somite formation (Bellairs, 1985).

Much of my research life has involved the chick embryo though I have strayed occasionally to amphibians, reptiles and even to millipedes (which have the most unusual and interesting segmentation), but have always returned to the chick. During recent years I have enjoyed preparing "The Atlas of Chick Development" (Bellairs and Osmond, 2014).

I have been most fortunate in my colleagues and collaborators, not just the ones mentioned here, but all those who have shared my obsession with the formative stages of the young chick embryo. I owe much to them for their input of ideas, their commitment and, not least, their unending cheerfulness. I consider this an opportunity to thank them for all they have done for the subject and for me.

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