British Society for Developmental Biology

LONDON MEETING **2nd-3rd JANUARY 1975**

The 28th Meeting of the Society will take place in the Anatomy Department, **University College London** on 3rd January 1975, and will be continued at the **Clinical Research Centre, Harrow** on 4th January.

Accommodation and Transport

Accommodation will be in Ramsay Hall, 20 Maple Street, London W1, which is about 5 minutes walk from University College. It is also close to Great Portland Street tube station from which frequent trains run to Northwick Park. The Clinical Research Centre is close to Northwick Park tube station. Members are reminded that parking is very difficult in the vicinity of University College.

Meals

Apart from breakfast, no meals are available at Ramsay Hall. On **Thursday,** 3rd January, lunch will be provided in the University College Upper Refectory and a bar will be available. The Conference Dinner will be held in the Old Refectory. Coffee (before the start of the meeting at 10.30) and tea (in the afternoon) will be served in the Anatomy Department. On **Friday,** 4th January, coffee, lunch and tea will be available at the Clinical Research Centre.

Completed booking forms should be returned before 13th December to:

Dr Ruth Bellairs,
Department of Anatomy and Embryology,
University College,
Gower Street,
London WC1.

PROGRAMME

Thursday, 2nd January

Symposium

10.30 COFFEE.

11.00 R. Bellairs and M. Bou-Resli (University College).
The relationship between oocyte and follicle cells in some large yolked species.

11.30 S. Fonzo and P. Esponda (Madrid).
Nuclear extrusion during spermatogenesis of Acrididae.

12.00 R. Tresman (University College).
An E.M. study of the developing chick optic tectum.

12.30 M. Bancroft and R. Bellairs (University College).
The onset of differentiation in the epiblast of the chick blastoderm.

13.00 LUNCH.

14.00 H. Fox (University College).
Tail muscle ultrastructure and its degeneration at climax in *Rana*.

14.30 M. Perry (Edinburgh).
Microfilaments in the developing amphibian embryo.

15.00 P. J. Hyatt (Bristol).
Characterisation of dense granular deposits found in the extra-cellular spaces of the dorsa
tissues of *Xenopus laevis* gastrulae.

15.30 TEA.

16.00 E. G. Jordan (Queen Elizabeth College).
The nucleolus, ultrastructural changes associated with differentiation in plants.

16.30 N. J. Severs and E. G. Jordan (Queen Elizabeth College).
Nuclear pores in growing and differentiating cells.

17.00 P. Barlow and J. Sargent (Cambridge).
Experimental manipulation of cell organelles in root tips.

17.30 P. Bell (University College).
The ultrastructure of gametogenesis in land plants.

18.00 Continued A.G.M.

Friday, 3rd January

Symposium

09.30 R. G. P. Pugh-Humphreys (Aberdeen).
An ultra-structural study of embryonic chick fibroblasts *in situ* using transmission and scanning electron microscopy and freeze-etching.

10.00 W. A. Hemmings (Bangor).
The direct deposition method of E.M. autoradiography applied to problems of protein transport.

11.30 R. F. Searle, J. Elson, M. H. Sellens and E. J. Jenkinson (Bristol).
Electron microscopical visualisation of cell surface antigen on the mouse embryo by peroxidase-labelled antisera.

11.00 COFFEE.

Open Papers

11.30 I. R. McFadyen (Northwick Park Hospital).
Intra-uterine growth retardation in the human.

12.00 A. B. G. Lansdown (C.R.C., Harrow).
Reduced maternal health and foetal growth impairment in rats and mice.

12.30 D. Taylor-Robinson (C.R.C., Harrow).
The effect of *Mycoplasma pulmonis* on the materno-foetal relationship in mice.

13.00 LUNCH.

14.00 D. O'Dell (University College).
Activation of ascidian eggs.

14.30 A. H. M. Douglas (Aberdeen).
Growth and development of myoblast/fibroblast mixed cultures.

15.00 M. C. Prestige (Edinburgh).
On the role of competition in neurogenesis.

15.30 D. P. Humber (C.R.C., Harrow).
The influence of pregnancy on maternal immunological competence.

16.00 TEA.

ABSTRACTS

R.Bellairs & M.Bou-Rached, University College.

The relationship between oocyte and follicle cells in some large yolked species"

In vertebrates, each oocyte is enclosed in a capsule of follicle cells, and all raw materials for the growth of the oocyte must pass through this capsule. It is not surprising therefore that in all species examined by electron microscopy, the surface of the oocyte is extended into villi and characterised by the presence of pinocytotic vesicles. It is generally considered that the germ cells arise separately from the somatic cells. No intermingling of the cytoplasm would therefore be expected between them. In the chick, however, large pieces of follicular cytoplasm appear to be engulfed by the oocyte. In the lizard Acanthodactylus scutellatus intercellular bridges are present between follicle and oocyte. The significance of these findings will be discussed.

Sonia Fonzo & Pedro Escaronda, Madrid

Nuclear Extrusions during Spermatogenesis of Acrididae

During meiotic prophase in Chorthippus apicalis and Paracinema tricolor, the nucleolus is constituted by fibrillogranular masses. Eventually spherical fibrillar masses appear associated with the nucleolus; this formation can be observed as free in the nucleoplasm or in relation with a chromatin mass. During these stages it is also possible to find similar structures in the cytoplasm, which on some occasions are in close relation with the nuclear envelope. At this region the nuclear envelope presents a great amount of pores. In later stages of meiotic prophase these bodies are observed only in the cytoplasm in relation with mitochondria and ribosomes. The structures studied are positively stained after Bernhard's method (preferential for RN) and are negative after Feulgen's stain. In late prophase spermatocytes a diffuse fibrillar structure is observed in relation with the outer surface of nuclear membrane. It is suggested that this formation could constitute the chromatoid body.

A fibrillar body surrounded by nuclear envelope, which it is supposed to be a remnant of the nucleolus, is extruded into the cytoplasm of young spermatids. The role of this structure in connection with the evolution of the spermatid nucleolus is discussed.

R.L. Tresman, University College

An Electron Microscopical Study of the Developing Chick Optic Tecum

The avian retinotectal system has been the subject of a number of recent histological autoradiobiographical and electrophysiological studies. These have indicated that this is an appropriate system in which to investigate the phenomena of trophic inter-neuronal relations, specificity of synaptic connections, and axoplasmic transport. In the present study the chick optic tectum has been investigated with the electron microscope before and during the arrival of retinal afferents, to the relationships they establish with tectal neurons, and to the development of tectal synapses. Cationic dyes have been used to stain the surface coats of cells and processes in the tectum.

Small neuronal processes are seen in the outer layers of the tectum at three days in ovo and the first synapses are observed at seven days. The possibility that these processes and synapses are of retinal origin is discussed. There is a consistent increase with embryonic age in the intensity and extent of surface coat material which can be stained with cationic dyes. The possible role played by this material in the establishment of adhesive contacts between cells and processes is discussed.

M.Bancroft & R. Bellairs, University College.

The onset of differentiation in the epiblast of the chick blastoderm.

Chick embryos have been examined by scanning and transmission in electron microscopy from the unincubated to the late head process stages (Hamburger and Hamilton, stages 1-5). Regional variations in cell structure have been found to develop very shortly after incubation begins and these become more clear-cut with further development. In particular, those cells which are destined to invaginate possess fewer microvilli but more globular projections and more vesicles than the cells which are destined to form the ectodermal covering of the embryo.

Harold Fox, University College

Tail muscle ultrastructure and its degeneration at climax in Rana.

Tail muscles of anuran larvae degenerate at metamorphic climax. The tail commences to involute first at the tip and gradually the area of degeneration becomes more widespread; near the end of climax most of the tissue is affected before the tail stub finally disappears.

Tail muscle degenerates by autolysis. It is probable that lysosomal enzymes are concerned in the degradation of the sarcoplasm, though their role cannot be established by the myofibrils. It is considered likely that in most cases tail muscle is fully degraded and unrecognizable as muscle, before it is phagocytosed by mesenchymal macrophages.

Margaret M. Perry, Edinburgh.

Microfilaments in the Developing Amphibian Embryo

Electron microscope observations show that circumferential rings of microfilaments are present at the apical junctions of the superficial ectoderm cells prior to neurulation. In Triturus the microfilaments are 8 nm in diameter, bind heavy meromyosin, contract in dissociated cells, and are disrupted in the presence of cytochalasin B. In Xenopus an additional class of 10 nm microfilaments is associated with the developing desmosomal plaques which first appear at the blastula stage.

P.J. Hyatt, Bristol

Characterisation of Dense Granular Deposits found in the Extracellular Spaces of the Dorsal Tissues of Xenopus laevis Gastrulae.

Dense granular deposits have been observed between the dorsal ectoderm and mesoderm cells of X. laevis gastrulae by several workers, but attempts to characterise the granules cytochemically have led to controversy. Different observers have concluded that the granules contain glycogen or RNA or both. In the present study, the use of a specific stain for glycogen, and α-amylase digestion, on araldite sections prepared for examination with the electron microscope indicate that the granules are amyloid in nature. They have the ultrastructural appearance of β-particles of glycogen. The consistently strong affinity of the granules for a high pH lead post-stain after fixation with osmium tetroxide also suggests that the granules are carbohydrate in nature and are not ribonucleoprotein particles, as has been suggested previously.

E.G. Jordan (Queen Elizabeth College)

The nucleolus, ultrastructural changes associated with differentiation in plants

The nucleolus consists of three or four components which can be distinguished in conventional electron microscopy of sections. One zone, which has an electron opacity less than all others except the vacuoles, has been shown to have a distribution which is characteristic of the state of activity of the cell. The other zones show changes which can be related to changes in this first lightly staining zone or 'L' region.

The 'L' region which seems to be the key to the understanding of the various nucleolar morphologies is thought to be the chromatin which contains the genes for ribosomal RNA synthesis.

Evidence in support of such an identification is growing but rests almost exclusively on botanical studies.

Some of the details of the structural rearrangements of the nucleolus and the possible explanations for them will be discussed.

N.J. Severs and E.G. Jordan (Queen Elizabeth College).

Nuclear pores in growing and differentiating cells.

The Bullivant-Ames freeze-fracture replication method has been used to investigate the nuclear pore complex in cell growth. The technique is particularly suited to studies of this kind because it can be used to reveal large expanses of membrane face. Thus, changes in 1) distribution and 2) numbers of nuclear pores, in relation to cell metabolism, can be investigated more easily than by using thin-sectioning techniques.

The systems investigated include activated slices of higher plant storage tissue and synchronised growing yeast cells. In both systems, regardless of metabolic state, four distinct appearances of nuclear pores are observed. These are related to the different ways in which a pore may fracture rather than representing differences associated with cell function. In both systems, the onset of cell activity is marked by an increase in size of the nucleus.

In the higher plant system, nuclear pores are evenly distributed over the surface of nuclei from dormant cells but become slightly grouped with cell activity. The pore frequency remains unchanged, although as a consequence of the increase in nucleus size, the total number of pores does increase.

In the yeast system, the nuclear pores of dormant cells are densely packed on one part of the nuclear surface leaving a single large pore-free area. As metabolism commences, the nuclear pores become evenly dispersed between a series of smaller pore-free areas, and an increase in total pore number occurs.

Possible explanations for these observations will be presented in the context of current theories of the functional role of the nuclear pore complex.

P.Barlow and J. Sargent, Cambridge

Experimental manipulation of cell organelles in root tips.

With the electron microscope it is possible to follow the developmental sequence of cell organelles, such as amyloplasts, mitochondria, dictyosomes and endoplasmic reticulum, in the root cap of Zea mays as the cells within this tissue are displace from the site of their birth in the cap meristem to the exterior of the cap.

We shall describe these changes, and discuss the results of experiments designed to investigate whether the pattern of organelle development is dependent on the time that elapses since the cells in which the organelles are housed are 'born', or is dependent on the position of the cells in the cap tissues.

P.Bell (University College)

The ultrastructure of gametogenesis in land plants

Examples will be given of how electron microscopy has provided new information abo the essential processes of gametogenesis in land plants. Features already known to light microscopists are now revealed in much greater detail, and the problems of gametogenesis can correspondingly be profitably considered in terms of macromolecu

R.G.P. Pugh-Humphres, Aberdeen.

An ultrastructural study of embryonic chick fibroblasts in situ using transmission scanning electron microscopy and freeze etching.

Tissue dissected from hind limbs of Hamilton-Hamburger stage 36 White Leghorn chicken embryos was processed for transmission electron microscopy using standard procedures or freeze etched following the procedure described by Moor (1966). Whole hind limbs which were partially dissected to reveal muscle and connective tissue elements were fixed and freeze dried in preparation for scanning electron microscopy.

The ultrastructure of the tissue fibroblasts, in particular their cell surface structure and its relationship to exudate and "fibre" formation as well as morphological aspects of intercellular contacts within the tissue as revealed by each of the ultrastructural techniques employed will be described.

W.A. Hemmings, University College of N.Wales

The direct deposition method of E.M. Autoradiography applied to problems of protei transport.

The classical method of emulsion autoradiography has not proved of much utility in tracing proteins through cells. The tracks are much too large at the required magnifications. Ferritin and to a lesser extent peroxidase are much more specific localised, but have characteristic disadvantages. With the introduction of the Normandin technique in 1973 it became possible to visualise labelled proteins a other molecules localised at specific sites in cells at very high resolution: loc isation is as good as with ferritin, and more specific than peroxidase. A variety of isotopes may be used. Initial results obtained in the rabbit yolk-sac and youn rat small intestine systems will be used as illustrations.

R.F.Searle, J.Elson, M.H. Sellens & E.J.Jenkinson, Bristol

Electron microscopical visualization of cell surface antigen on the mouse embryo peroxidase-labelled antisera.

Horse-radish peroxidase conjugated antiserum has been used as an electron dense label for the identification of antigenic sites on the cell surface of the mouse embryo. The findings will be discussed in relation to the expression of antigens at various stages of development and on different embryonic cell types.

I.R.McFadyen, Northwick Park Hospital, Harrow.

Intrauterine growth retardation in the human.

The malnourished human foetus may be stillborn. In survivors the incidence of physical and mental maldevelopment is much greater than for normally nourished foetuses. Delivery at the optimal time improved perinatal mortality and the quali of postnatal life.

Intrauterine malnutrition may be detected clinically by laboratory investigation hormonal excretion, bu ultrasonographic measurements and by other means. Investig ions are being conducted to improve detection and to improve intrauterine nutriti

A.B.G. Lansdown, Clinical Research Centre, Harrow.

Reduced maternal health and foetal growth impairment in rats and mice.

Foetal growth impairment may occur as a result of maternal infection with certai viruses in the course of pregnancy. In the case of infection with Influenza or P Influenza viruses, infection of the foetuses does not seem to occur and foetal wastage, prolonged gestation and intrauterine growth retardation have been associated with the ability of the virus to reduce the state of health of the mothers by causing respiratory distress and hyperthermia. Coxsackieviruses of the B group although they have veen slow to infect the foetus and placenta, are believed to induce increased foetal wastage and intrauterine growth retardation by indirect means, that is by inducing a severe maternal pancreatic exocrine insufficiency with the consequence that she is incapable of digesting nutrients important for normal foetal growth.

Alasdair Douglas, Aberdeen

Growth and Development of Myoblast/Fibroblast Mixed Cultures.

Dissociated myoblasts and fibroblasts from chick thigh muscle proliferate during the early *in vitro* period. Myotube formation is prominent reaching a maximum during the third day. The myotube: mononucleated cell ration is increased by supplementing culture medium with extracts of collagen. In time, however, myotubes tend to degenerate, and are replaced by vigorous overgrowth of fibroblasts. The factors involved in the development of the myotubes and in their maintenance *in vitro* will be discussed.

M. Prestige, Edinburgh

On the role of competition in neurogenesis

Various mechanisms for map making will be discussed. The implications of these are tested by computer simulation and the conditions for their success evaluated.

D.S. O'Dell, University College London

Activation of Ascidian Eggs

Eggs of the tunicate *Ascidia meleca* afford convenient material for the study of fertilization and maturation, since they lack some complicating processes such as the rupture of cortical granules. Studies will be presented on the programme of events which follows fertilization, especially on the timing of the onset of DNA replication, which occurs in both pronuclei before they fuse. It has been found that eggs can be activated with the ionophase A23187; from this, successful predictions have been made of other treatments which will activate eggs and evidence will be given for a role of calcium ions in triggering the changes which follow fertilization.