**General abstracts**

**Number and quality of cell cycles in an *in vitro* regeneration and transdifferentiation system**

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Mononucleated striated muscle of an anthomedusa can be isolated and cultivated in artificial sea water. When treated with bacterial collagenase the isolated muscle can transdifferentiate autonomously into several cell types and regenerate the entire sexual and feeding organ of the medusa, the manubrium. Whereas transdifferentiation from striated to smooth muscle is direct, formation of a glandular cell type needs one cell cycle. Aphidicolin experiments show that this one cell cycle has to be initiated at the right time otherwise transdifferentiation of striated muscle to the glandular cell type does not occur.

**Effects of sodium fluoride on vascular system of early chick embryo**

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The effects of sodium fluoride NaF on development of blood islands, blood vessels, and heart of early chick embryos were investigated. The *in vitro* culturing technique was employed. Embryos at stages 8, 9+, and 11— and NaF at concentrations of 100, 200, and 300 µg/ml medium were used. In embryos at stage 8, and at 100 µg/ml NaF, the extraembryonic circulation was extremely sensitive. Blood islands and blood vessels were absent. The wall thickness of dorsal aortae was reduced, and these vessels were enlarged. The hearts of such embryos (30 %) were enlarged, and their beats per min were reduced relative to those of control embryos. Epimyocardial walls were slightly thinner, and the endocardia were smaller and thicker than those in controls. At 200 µg/ml NaF, embryos of the previous stage (8) exhibited more pronounced effects. Completely inhibited hearts were noticed in about 55 % of treated embryos. A concentration of 300 µg/ml NaF resulted in complete absence of dorsal aortae, and in complete inhibition of heart development in 71 % of treated embryos. The above-mentioned effects were less pronounced in embryos at stages 9+ and 11—, except for the phenomenon of enlarged hearts which increased with rise in concentration of NaF and with increase in age of treated embryos. These effects of fluoride are suggested to be a result of the inhibition of mitotic activity in embryonic cells by this ion.
Developmental abnormalities of the eye and snout region of the Pupoid foetus mouse mutant

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Pupoid foetus (pf) is a recessive lethal mutation in the mouse, previously described by Meredith (1965) and by Watson (1978).

Homozygous pf/pf embryos are first distinguishable at two days gestation, due to the development of a tail twist. Subsequently, hyperproliferation of the epidermis results in deformation of the limbs and tail, abnormalities in the development of pelage and vibrissa follicles, disruption of eye development, and overall reduction in surface detail.

This epidermal hypertrophy is associated with mesodermal disturbances and increased growth and penetration of peripheral nerves. Light and scanning electron microscopy have been performed to investigate the temporal and spatial relationships of the affected tissues. Tissue culture experiments suggest that an epidermal cell surface defect is responsible for its hypertrophy and, secondarily, for defects in the other tissues.


Basal and insulin-stimulated glucose consumption of the early chick embryo

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Until now, no quantitative data have been available about the glucose metabolism of the young chick embryo. We have adapted a method, using deoxyglucose, an analogue of glucose, in order to estimate the glucose consumption of chick embryos during the first 24 h of incubation: from the stage X (Eyal-Giladi and Kochav 1976) to the stage 7 (Hamburger and Hamilton 1951). The glucose consumption increases from 35±8 nmol.h-1 (0.44 pmol.cell-1) in the unincubated stages X-XII, to 51±10 nmol.h-1 (0.14 pmol.cell-1) in the gastrulating stages 3-4, and 91±13 nmol.h-1 (0.16 pmol.cell-1) in the neurulating stages 6-7. Respectively 46, 20 and 20 % of glucose are taken up by the area pellucida at these 3 stages; the rest is taken up by the extraembryonic area opaca. These basal values found in the embryo are as high as those found in the most active differentiated tissues. Likewise, they can be modified by different metabolic conditions. In particular, insulin can modulate the glucose consumptions according to its concentration and to the stage of development. Half-maximal stimulation was obtained for concentrations around 2.5 ng/ml (about 60 μi.u./ml) corresponding nearly to mammalian physiological concentrations. Chick embryonic cells become sensitive to insulin during the pregastrulation period: no response was found at the stages X-XII, whereas 30 % and 50 % increase of glucose uptake occurred at the stages 3-4 and 6-7 respectively. Both area pellucida and opaca were stimulated to the same extent. The presented method provides a valuable tool for studies of the glucose metabolism and its control in embryonic tissues.
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General abstracts

Clonal analysis of senescence and differentiation in diploid human melanocytes

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Diploid human epidermal melanocytes can now be isolated and cultured for many population doublings, after which they senesce (proliferation slows and stops) (Marko et al., 1982). We have modified the culture conditions and developed a strain largely of premelanocytes (with little or no pigment). These too eventually senesce; concomitantly the proportion of pigmented cells rises markedly.

Senescence is of interest as a property of normal but not malignant cells; it is often suggested that it is connected to differentiation. Melanocytes are ideal for studying this possibility, because their differentiation entails a pigmentation visible in living cells. We are therefore analysing clonogenicity and pigmentation in individual cells, using single-cell cloning and timelapse filming, as done previously with melanoma cells (Bennett, 1983). As with diploid fibroblasts, there is a proportion of non-dividing cells in the premelanocyte strain, which rises during senescence. The first results indicate that, although pigmentation and failure to proliferate are clearly correlated, a cell can show either property without the other. Both properties may thus be elements of pigment cell differentiation, but they seem not to be causally connected.


Olfactory bulb vulnerability to in utero tritiated exposure in Swiss albino mice

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Due to a larger uptake and longer retention of tritium, the brain seems to be one of the critical organs for tritiated water (HTO). Tritium, a radionuclide of hydrogen being routinely and accidentally released from nuclear energy operations, recently emerges as a major radiopollutant of neurobiological concern. All the inventories of tritium in the environment are primarily tritated water (HTO). Since the body water is essentially in equilibrium with ambient HTO levels, as a result the embryo and fetus are subjected to continuous, low level, total body irradiation from this source during development. While determining the differential vulnerability of various subdivisions of prenatally developing brain to a continuous tritiated water exposure (5-5 μCi(203-5 kBq)/ml of drinking water; 3-5 μCi(129 KBq)/ml; 0.102 Gy/day, from 11-25, 12-25, 14-25, 15-25 and 16-25 days to 19-25 days, post coitum), the olfactory bulb of Swiss albino mice exhibited the gestational-day-dependent incidence and severity of damage on day 19-25 post coitum which varied directly as the pregnancy advanced. Though olfactory bulb has been reported to be the least radiosensitive to external radiations during prenatal development, the present study indicates the possibility of significant impact of the internal tritiated environment as manifested in the form of various radiopathological lesions viz., the loss of columnar arrangement of mitral layer, disarray of the tissue with an obliteration of olfactory ventricles, enlarged ventricles, necrosis, pycnosis etc. Taking the incidence of rosette formation in the olfactory area as an intense and important end point, 16-25-day-old HTO-exposed fetuses showed the maximum damage with hemorrhage and rosette formation, spongy degeneration of the subependymal layer with hyperaemia and sclerosis in the vasculature. Blood clots present in some fetuses indicated brain hemorrhage. Some showed foot and tail abnormalities. There were noticed incidences of microcephaly with enlarged lateral ventricles in 11-25-day-old exposed fetuses.
Central embryological collection of the Hubrecht Laboratory

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The Hubrecht Laboratory houses an extensive and unique embryological collection, which consists of histological slides and specimens in alcohol pertaining mainly to the normal development of vertebrates. Numerous series of embryonic material of rare and endangered mammalian species are represented, e.g. Fishes, Marsupials, Insectivores, Primates. This Collection is expressly available for morphological, descriptive and comparative embryological and placentalogical studies by research workers in all countries.

The core of the Collection is formed by material collected from about 1890 to 1950 from all over the world by Prof. A. A. W. Hubrecht (Utrecht, Netherlands), and Prof. J. P. Hill (London, UK). The Collection was extended by various personal donations and contributions, e.g. from A. Dohrn, E. Selenka, L. Glaesner, C. G. Hartmann, D. de Lange, F. H. Edgeworth, P. D. Nieuwkoop, J. Pasteels.

In addition some slide collections relating to experimental morphological investigations have been incorporated, donated by e.g. H. Grüneberg, O. Mangold, H. Spemann. The Collection may be consulted at the Hubrecht Laboratory on application. Extensive card indexes, catalogues and original notes are available and material of the collection can be borrowed. Financial support in the form of short-term maintenance grants is available in special cases.

Observations concerning the heterotopic development of distal parts of avian limb buds after interspecific grafting

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The development of distal parts of quail leg buds grafted either to wing bud stumps or to the coelomic cavity of chick embryos was studied. Special attention was given to the origin of the tissues making up the heterotopically grown parts of the limbs. By means of the differences in the nuclear structures, quail and chick cells can be distinguished (Le Douarin & Barq, 1969). The differentiation potential of the somatopleure was found to be restricted to certain types of tissues. Thus, the invasion of the limb bud with cells from different origins is necessary to the development of a limb with all its constituents.
Calcium channel blockers influence muscle cell differentiation in vitro
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Calcium ions are known to influence some aspects of muscle cell differentiation (Shainberg, et al., 1969). We have tested three Ca\(^{2+}\) channel blockers (Mn\(^{2+}\), D600 and nifedipine) on primary cultures of embryonic amphibian muscle cells. Neural tube, notocord and somitic mesoderm from Xenopus laevis embryos (stage 18–20) were dissociated and cultured (Messenger & Warner, 1979). Eighteen h later nerves, mononucleate muscle cells, fibroblasts and epithelial cells had differentiated.

When 2 mM MnCl\(_2\) was included in the culture medium the proportion of muscle cells (recognised morphogically) increased 2–3 fold. No other cell type was effected. A 12 % drop in the total number of cells did not account for this increase. Concentrations greater than 2 mM were toxic. Raising the [Ca\(^{2+}\)] protected and lowering the [Ca\(^{2+}\)] potentiated the effect of the Mn\(^{2+}\). EGTA (a Ca\(^{2+}\) chelator) did not mimic the Mn\(^{2+}\) effect. The organic Ca\(^{2+}\) channel blockers D600 (0.01 mg/ml) and nifedipine (10\(^{-5}\) M) also produced an increase in the proportion of muscle, although slightly smaller than with Mn\(^{2+}\), with no effect on the total number of cells.

Mn\(^{2+}\) has been shown to increase the adhesion and spreading in vitro of some cell types (e.g. BHK cells; Grinnell, 1984). Although Mn\(^{2+}\) did not increase the initial rate of attachment and spreading of the cultures, the length of the muscle cells increased by up to 50 %. Also, labelling the muscle cells with a monoclonal antibody (gift of Dr. J. Brookes) revealed a population of round muscle cells in control cultures (not recognised morphologically) that were converted to bipolar spread cells in the presence of Mn\(^{2+}\), partly accounting for the increase in the proportion of muscle. D600 and nifedipine alone had no effect on muscle cell length. D600 prevented the length increase, but not the increase in muscle cell numbers, caused by Mn\(^{2+}\). The Ca\(^{2+}\) ionophore A23187 did not prevent the Mn\(^{2+}\) effect.

This study reveals that Ca\(^{2+}\) channel blockers specifically increase the number of muscle cells which differentiate in vitro and that Mn\(^{2+}\), in addition, dramatically increases the length and spreading of the muscle cells.


Experiments to the role of Ca\(^{2+}\) during differentiation of Dictyostelium discoideum
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We measured the extracellular Ca\(^{2+}\)-concentration in cell suspensions of Dictyostelium discoideum, strain Ax-2 and mutant strain agip 53, with a Ca\(^{2+}\) sensitive electrode. During differentiation to aggregation competence oscillations in the extracellular Ca\(^{2+}\) concentration were observed with a frequency of 6–9 min and an amplitude of about 5×10\(^6\) calcium ions per cell. Simultaneous measurements of total cyclic AMP by radioimmunoassay showed that peaks of cyclic AMP slightly preceded peaks of Ca\(^{2+}\) uptake during spike formation. Addition of chemoattractants caused a rapid, transient uptake of Ca\(^{2+}\). Adenosine, 5’ AMP and ATP were ineffective. Ca\(^{2+}\) channel blockers such as verapamil and gallopamil inhibited the cyclic AMP-induced Ca\(^{2+}\) uptake. The experiments suggest a regulatory role for Ca\(^{2+}\) during the early stages of chemotaxis.

A constant cyclic AMP stimulus induced a constant reduction of the extracellular Ca\(^{2+}\) concentration of agip 53 cells as long as the stimulus was maintained. No apparent adaptation was observed. We propose that an elevated cellular free Ca\(^{2+}\) concentration leads to an inhibition of cyclic AMP synthesis. Adaptation of cyclic AMP synthesis to constant cyclic AMP stimuli may be a consequence of the non-adapting uptake of Ca\(^{2+}\).
**General abstracts**

**Onset of a large oocyte pool and escape by vitellogenesis in *Xenopus laevis* females**

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A complete analysis of the organization of the adult ovary in *Xenopus* females shows that it consists of about 140,000 oocytes larger than 50 μm; 80% of these cells are previtellogenic and constitute a large oocyte stock in the ovary. This pool is built up by the beginning of the 2nd year of the animal’s life and allows 60 to 80 layings (2000 eggs); consequently some previtellogenic oocytes may be blocked during several years. The ovary of laboratory maintained adult animals contains about 8000 white banded large oocytes (1200–1250 μm) which can be artificially ovulated within a short period (hCG stimulated females); since the growth rate of the large oocytes is very slow – about one year from 1050 to 1250 μm, as observed in growing females (Callen et al., 1980), it is suggested that this population represents the normal stock annually laid by the female: 4 layings separated by periods of about 1-5 months, during the breeding season in natural conditions. Consequently, in absence of follicular atresia, the stock of oocytes we have observed in the ovaries of subadult females seems sufficient to ensure the whole sexual life of the animal (over 20 years) and the continuous gonial multiplication supposed to occur in all amphibians appears unnecessary for the normal functioning of the *Xenopus* ovary.

The middle-sized oocytes (400 to 1100 μm) of large unstimulated females have necessarily a very reduced growth rate; on the contrary, vitellogenic cells of identical size have been shown to grow rapidly in young females. Since we observe an important accumulation of 1150 μm oocytes within a few months after a single laying, we conclude that these middle-sized oocytes resume a rapid growth, similar to that carried in young animals; their metabolic activity is also greatly enhanced compared to the controls. These results suggest that the largest follicles exert a negative control towards the growth of early vitellogenic cells.

As a conclusion, the whole ovary of an unstimulated adult female has a very reduced physiological activity and any cellular or biochemical study concerning oogenesis has to take into account not only the accurate size of the oocyte, but also the physiological status of the female.


**Regional and temporal differences in the parietal endoderm of the mid-gestation mouse embryo**

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The parietal endoderm (PE) of mouse embryos aged between 8.5 and 13.5 days of gestation was studied by light microscopy, scanning-electron microscopy, time-lapse video recording, and autoradiography. At all ages there was a preponderance of 'blebby' cells near the placenta, while the cells situated more distally were relatively smooth and round. This distribution of the 'blebby' phenotype was paralleled by greater motile activity of the parietal endoderm in the vicinity of the placenta, and with greater incorporation of radio-labelled amino acids into the underlying basement (Reichert's) membrane in the same region, suggesting that the blebbing may be associated with basement membrane synthesis.

In addition to the spatial distribution of smooth and blebby cells which was observed at all ages, there were also age-related differences in the morphology of the PE cells. The younger embryos had, in the extreme proximals region of the parietal endoderm, rounded cells with numerous short filopodia, some of which were acquiring blebs. Such cells were not in evidence in the older embryos and appeared to be precursors of the other PE cell types. In the parietal endoderm of the older embryos and appeared to be precursors of the other PE cell types. In the parietal endoderm of the older embryos the cells in the middle and distal (to the placenta) regions were often very large and flattened, and showed variable numbers of short filopodia.

These observations are consistent with a scheme whereby 'primitive' parietal endoderm cells near the placenta give rise to 'blebbby' cells which are engaged in extensive biosynthesis of basement membrane and, as the conceptus expands, move distally with their associated membrane. As the cells move distally their synthetic activity declines (perhaps much of that which remains is associated with remodelling of existing membrane to allow it to accommodate to the changing shape and size of the conceptus) and the cells lose their blebs and become rounded – ultimately becoming large and flattened.

In all but the oldest embryos studied, rounded and dividing cells could be found in both proximal and distal regions of the parietal endoderm, but the possibility that many of the cells were being recruited from elsewhere could not be excluded, and the above scheme implies a greater proliferation in, or recruitment to, the placental region, though this has yet to be demonstrated.
General abstracts

Specific protein markers of neoblasts and differentiated cells in the planarian Dugesia (g) tigrina

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The understanding of pattern formation and cell differentiation mechanisms during planarian regeneration would be greatly enhanced if specific cell markers were known. This would allow one to follow specific cell lineages, to trace the origin of blastema cells, to assess the role of undifferentiated stem-cells (neoblasts), and to characterize specific gene products of interest.

As a first step aimed to this goal, we have isolated and purified undifferentiated cells (neoblasts) and differentiated cells by serial filtration through nylon meshes and discontinuous Ficoll density gradients respectively (purity $\geq 95\%$). Soluble and insoluble protein fractions from both kind of cells were studied by IEF (isoelectric-focusing) and two-dimensional electrophoresis according to O'Farrell (1975).

The pattern obtained shows several polypeptides limited to one or another kind of cells. Also, differences between soluble and insoluble fractions within each population were found. All together, this opens the way to characterize specific markers for each cell population in planarians, and eventually to isolate them.

Demonstration of a parallelism between carbonic anhydrase activity and calcification during enchondral osteogenesis in chick embryo

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The first recognizable expression of osteoblasts – alkaline phosphatase activity – can be observed from stage 28 in chick femur periosteum. Carbonic anhydrase activity and the first Ca deposit can be observed only at stage 33 (8 days), 48 h later in femur diaphyseal periosteum. At stage 36 (10 days) carbonic anhydrase activity extends to the whole diaphysis and at the same time hypertrophic cartilage of this region is entirely calcified. Three days later (stage 39) carbonic anhydrase activity is observed in epiphysis where Ca begins to be deposited.

Anhydrase activity has been visualized by histoenzymologic Hansson's method modified by Carpentier and Coll. Calcium identification has been performed by Kashiwa’s method.

We agree with Gay and Kumpulainen who recently suggest two different roles for carbonic anhydrase during cartilage ossification:

i) carbonic anhydrase appears to maintain pH and HCO$_3$ concentration at a high level, which presumably facilitates Ca precipitation and apatite crystal formation.

ii) the occurrence of high HCO$_3$ – concentration may serve to facilitate the removal of CO$_2$ in this poorly vascularized tissue.

So calcification initiation occurs only in the areas where alkaline phosphatase and carbonic anhydrase activities have just appeared.
Structure and composition of fish egg chorion

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The fully grown fish egg is surrounded by an acellular coat, the 'chorion'. The aim of our work is to analyze the structural composition of this protective envelope as well as its biosynthetic pattern during oocyte growth. The mature chorion of Carassius auratus (Cyprinidae) egg consists of three main layers – Ch 1, Ch 2, Ch 3 – that are progressively synthesized, secreted and assembled by the developing oocyte in three discrete steps; both temporally and spatially detectable at ultrastructural level. We have prepared egg chorions either from homogenates of whole ovaries or from ovulated mature eggs following chorionic gonadotrophic hormone (HCG) stimulation. These chorions can be purified to such an extent that contamination from other cellular structures (i.e. egg microvilli, usually embedded into the chorion mass, or yolk platelets) are no longer detectable under light or electron microscopy observations. SDS-PAGE of these purified preparations show a reproducible pattern of discrete bands with molecular weight ranging from 20 to 250 KD, where no cytoplasmic contaminants are detectable. To follow the time-course appearance of the various chorion components, we also analyzed the electrophoretic pattern of chorion preparations from small sized oocyte where the envelope structure is not complete. The major differences, as compared to the pattern of fully developed chorions, can be observed in the class of high M.W. components, some of which seem to be less represented or even absent in the small sized oocyte preparations. We are presently analyzing chorion components in more detail by two-dimensional electrophoresis to follow their assemblage during oocyte development. At the same time we are now isolating and characterizing the major chorion proteins.

Immunogenetic analysis of human muscle surface antigens

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Human-mouse muscle somatic cell hybrids are an attractive antigen source for the generation of human specific monoclonal antibody (McAb) reagents. Skeletal muscle myoblasts were therefore fused using polyethylene glycol 1000 with a HGPRT negative clone of the mouse G8-1 cell line and hybrids selected in culture media supplemented with HAT and ouabain. A fusion frequency of $0.2 \times 10^{-4}$ was found. All hybrid cell clones tested were myogenic and readily fused to form multinucleate myotubes. Clone RC4.J.1 grew rapidly and greater than 70 % of nuclei could be found in myotubes, some of which contracted spontaneously. This clone also expressed and regulated human muscle antigens at the cell surface. Thus muscle specific McAb 5-1H11 was found on myoblasts and myotubes while McAb reactive with Thy-1 antigen was present on myoblasts only; these patterns of gene expression are similar to those found in human muscle cultures (Walsh and Ritter (1981), Nature 289, 60). RC4.J.1 cells contained 96±37 chromosomes at 64 days in vitro (DIV) indicating that it is the product of a 1:1 fusion since G8-1 cells contain 68 chromosomes. Subculturing and cloning has been used to induce muscle hybrids to segregate human chromosomes. Subclone RC4.J.1.2.8 has undergone the most extensive non-disjunction of human chromosomes and at 259 DIV contained only 4 (X, 15, 14, 12) out of 18 chromosomes tested. These hybrid cells have now been used as an immunogen to attempt to identify human gene products encoded by these chromosomes. McAb's that were reactive with human but not mouse cells were identified by differential ELISA assay using human skeletal muscle and G8-1 cells as targets. A number of human specific McAb's have been produced and their profiles of reactivity are being assessed on a hybrid cell panel to locate the chromosome controlling antigen synthesis. McAb A24 has a pattern of immunoreactivity similar to Thy-1 antigen in cell culture but differs from it on cryostat sections. Five McAb's were found to react with antigens of the extracellular matrix and a number of McAb's show reactivity with cytoplasmic antigens. McAb D2AF1 and D1B.C11 are cytoplasmic differentiation antigens whose expression is restricted to certain muscle fibre types.

These data show that hybrid muscle cells are useful for generating McAb's that react with human muscle differentiation antigens.
General abstracts

1,25-dihydroxyvitamin D₃ accelerates differentiation of epithelial cells in cultured embryonic chick intestine

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The diverse effects of 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃), the hormonally acting metabolite of vitamin D₃, are not only of special relevance for calcium and inorganic phosphate homeostasis but may also be of some importance for the differentiation of various cell types including monocytes, lymphoid and epidermal cells. Our recent observations that 1,25-(OH)₂D₃ induces uptake of phosphate, D-glucose and calcium in cultured embryonic small intestine at different stages of embryonic development point to a possible role of the sterol in promoting differentiation also of its classical target organ. To test this hypothesis we monitored the appearance of goblet cells in cultured embryonic chick jejunum under the influence of 1,25-(OH)₂D₃. This system is ideally suited for this type of studies since the embryonic intestine is essentially devoid of 1,25-(OH)₂D₃. Furthermore, intestinal epithelial cells including goblet cells are derived from a single crypt-based undifferentiated cell type. Hence, any effect of 1,25-(OH)₂D₃ on differentiation should be expressed by an increase in the number of any specialized, viz. goblet, cells.

Jejunal segments were excised from chick embryos on day 15, 18 and 20 of embryonic development and cultured for 48 h in serum-free McCoy's 5A mod. medium in the absence or presence of 120 nM 1,25-(OH)₂D₃. Goblet cells were visualized by Alcian-PAS staining of cultured tissue routinely processed for light microscopy. 50-55 villi were randomly selected in each group (for comparison non-cultured jejunum was also examined) and the number of goblet cells in relation to the total number of epithelial cells per villus determined.

Culture conditions had no apparent influence on villous architecture since the number of cells per villus, which expectedly rose from day 15 to 20, was not changed during 48 h culture. Thus, any proliferative effect of vitamin D inferred from observations in intact animals is not apparent in organ culture. However, culture of day 15 and 18 intestinal segments more than doubled the number of goblet cells, suggesting that a control mechanism of differentiation effective in vivo is absent in the culture system. Consistently, this phenomenon is not seen on day 20 prior to hatching. When the hormone-treated groups were compared to controls, a significant increase (35-40 %) of the proportion of goblet cells within the intestinal epithelium became apparent at all stages of maturation investigated. These findings provide evidence for a definite role of 1,25(OH)₂D₃ in enterocyte differentiation. (Supported by Grant No. 4422 of the Fonds zur Förderung der Wissenschaften)

changes in lectin binding patterns during epididymal maturation of mouse sperm

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Mouse sperm are known to undergo important changes in both motility and fertilizing ability during their passage through the epididymis. Sperm from the caput, corpus and cauda regions of the epididymis and from the vas deferens have been examined using a panel of fluorescein labelled lectins. Con. A, LTA, PNA, UEA and WGA all showed characteristic changes in their binding to sperm throughout epididymal maturation. These results suggest that sperm surface carbohydrates undergo distinctive changes as sperm pass through the epididymis and these surface changes may be related to metabolic changes in the maturing sperm.
General abstracts

The phorbol ester TPA stimulates tentacle regeneration in Hydra
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Phorbol esters are known tumor promoters primarily acting on the cell membrane, where they induce a number of changes in membrane structure and function. They are also known to exert both stimulatory or inhibitory effect on proliferation of different cell types and modulate cell differentiation. To understand the phorbol ester’s effect on the regulating processes involved in growth and differentiation, we have studied the effect of TPA (12-o-tetradecanoyl phorbol-13-acetate) on tentacle regeneration in Hydra attenuata. Normal Hydrae, treated with different concentrations of TPA for 48 hrs, were cut in the middle of gastric region, and then were further cultured individually in presence of same concentration of TPA. Regeneration of the animals was monitored daily by determining the number and size of the tentacles formed compared to untreated, decapitated Hydrae grown similarly. Dose curve studies indicated that 2-5 ng/ml of TPA induces the maximum regeneration. Lower concentrations did not show significant effect, and higher concentrations were toxic to the animals. We observed the following at 2-5 ng/ml of TPA treatment as above: the TPA treatment caused more rapid growth of tentacles, and the total number of tentacles per animal was found to be 21% higher than control animals. The length and thickness of the tentacles were also found to be increased compared to those of control. TPA at this concentration did not affect the budding rate in these Hydrae, whereas the length of the tentacles of the buds ensuing from TPA treated Hydrae were found on an average ~40% higher than those of controls. Some treated animals showed the regeneration of two heads, which are formed laterally on the body column. However, this phenomenon was statistically insignificant. On the basis of the results that the general budding rate is not affected by TPA, whereas the regeneration of tentacles in the adults as well as formation of new tentacles in the buds are stimulated, we infer the specificity of tumor promoters on tentacle forming cells in Hydra.

An ultrastructural study on the form and distribution of electron-dense bodies in primordial germ cells (PGCs) of early chick embryo
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A characteristic and specific structure of germ cells is germ plasm or nuage material. It has not been found in all groups and, as Smith, Michael & Williams (1983) wrote, no germ plasm has hitherto been demonstrated in birds, and so the role of germ cell determinant cannot at present be attributed to any particular ultrastructural characteristic of the PGCs. Detailed ultrastructural studies are still rudimentary in early chick embryo and that may explain its non identification in this specie. PGCs of the chick embryo in different phases of its development (stages 3, 9 and 17) have been studied by transmission electron microscopy. At these stages, PGCs show areas in the cytoplasm with specific, distinctive electrondense masses. These structures are ovoid in shape and measure about 0.25 by 0.5 nm. It appears to be composed of many closely packed, small, granular subunits, about 20 nm in diameter, which seems to be embedded in a fibrillar matrix. However, a difference in electron opacity has been found in adjacent granules, even in same stage of development. These electron-dense bodies are not enclosed within a limiting membrane. It lies adjacent to the outer nuclear membrane, as well as near cell periphery. It is often closely associated with mitochondria, rER, ribosomes, as well as lipid droplets. These electron-dense structures resemble the germ cells inclusions present in many groups ranging from coelenterates to mammals. Its detection at an early embryonic stage (stage 3), when the germ cells are first detected, supports the view that they could be cytoplasmic markers playing a role in germ line differentiation in avian embryos. To summarise, observations presented here favour the universality of the germ plasm.

Phagocytosis-defective mutants in *Dictyostelium discoideum*

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Two recessive mutations of the cellular slime mould *Dictyostelium discoideum*, phgA1356 and phgB1351, which express altered phagocytic properties, have been characterized using parasexual genetic analysis. The *phgA* locus was assigned to linkage group VII, and the *phgB* locus to linkage group IV. Complementation analysis of twelve more independently derived phagocytosis mutants showed that each fell into one of these two complementation groups (Duffy & Vogel, 1984).

Closer analysis revealed independent binding sites, and a model was proposed for recognition in phagocytosis: hydrophobic particle binding is via a 'non-specific' receptor, and there is also a specific receptor for glucose. In our mutants, only the non-specific receptor appears to be inactivated (Vogel et al., 1980).

A procedure for the selection of mutants defective in the glucose receptor has been devised, based on the method of Clarke (1978). The mutants described above, which already lack the non-specific receptor, were mutagenized and allowed to ingest *E. coli* B'r bacteria that had been incubated with BUdR. B'r bacteria contain glucose residues, and so should not be taken up by the desired mutants. Those cells which are able to take up bacteria, and thus BUdR, are killed by irradiation at 365 nm following incubation of the cells with the vital dye Hoechst 33258 to potentiate the killing effect of the BUdR (Graetzer & Deering, 1983).


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**Timekeeping by frog embryos**

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The availability of large batches of synchronously developing frog embryos makes them particularly suitable material for the study of precise timekeeping during development. Members of the same ovulation commence development almost synchronously as, in nature, the mother lays her eggs over a short time into cold water. Thereafter, siblings pass through the same stages of development at the same time.

Following heat shock, development is arrested. Embryos that recover develop at the normal rate. The retardation, compared with controls, is measured in hours or days and is proportional to the intensity of shock. Heat shock is injurious; shocks a little less intense than those that cause immediate death, result in retardation equivalent to the time taken to develop from the egg to the gastrula. These embryos die over the next few days with severe and variable abnormalities. Our observations show that within batches of embryos given short, or long, duration shocks viable embryos continue to develop synchronously, notwithstanding retarded and abnormal development. It appears, therefore, that the arrest of development is controlled to the same high precision as normal development.

**Take-home message.**

1. The tempo of development can be readily perturbed by heat shock.
2. Although the morphological effects may vary, the temporal effect is precisely the same for all embryos of the same treatment batch.
3. Therefore, the precision of the temporal response cannot be susceptible to any variable aspect of the phenotype.

The analogy of genotype and phenotype may be appropriate. The results suggest a possible function of heat shock proteins in the stabilisation of the developmental tempo.
The regulation of myoblast fusion by calcium and prostaglandin E₁

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The differentiation of embryonic myoblasts to myotubes involves cessation of cell replication, specialisation of the plasma membrane, cell-cell fusion and the formation of the contractile apparatus. Cell fusion can be prevented by: lowering external Ca++ , inhibitors of prostanoid synthesis (Zalin, 1977) and the Ca ++ channel antagonist D600 (David and Higginbotham, 1982). The Ca++ ionophore A23187, prostaglandin E₁ and eicosatrienoate all stimulate fusion in myoblasts. These results suggest that both an influx of Ca++ and prostanoid synthesis are involved in the regulation of fusion.

For these experiments, chick myoblasts were isolated by dissection and mechanical dissociation, seeded at 1·2×10⁵ cells/35 mm dish and grown in medium 199 with 10% horse serum and 2% embryo extract. In controls, fusion begins at 43 h and by 53 h 50±5 % of the cells have fused. Either chloroquine (l·8×10⁻⁶ M), a putative inhibitor of phospholipases or indomethacin (3×10⁻⁶ M) prevent myoblast fusion, imposing 70 % and 60 % inhibitions respectively. At these concentrations the drugs achieve a specific inhibition of cell fusion without affecting the pattern of cell proliferation. The fusion inhibited cells exhibit a number of the properties of terminally differentiated muscle. The increases in two parameters of differentiation, creatine phosphokinase activity and acetylcholine receptors is normal. A stable acetylcholine analogue, carbacol (10⁻⁴ M), completely reverses the indomethacin imposed inhibition, implying that the inhibited myoblasts possess active acetylcholine receptors. Finally, the kinetics of reversal by high external K+ (Entwistle, Warner and Zalin, 1983) infers that, like young myotubes, the inhibited cell has a high resting membrane potential and contains voltage dependent calcium channels.

Reversal of these fusion specific blocks are also achieved by PGE₁ (10⁻⁵ M) or its precursor fatty acid eicosatrienoate (10⁻⁶ to 10⁻⁹ M). Both the PGE₁ and K⁺ reversals of the inhibitions are prevented by a brief preincubation with the calcium channel antagonists, D600 (0·01 g/ml) and La³⁺ (1 mM).

We conclude that fusion is regulated by an influx of calcium and that this is triggered by the synthesis of a factor, which is almost certainly a prostanoid of the one series.


Induction of scleral ossicles: the role of epithelial papillae in development of ectomesenchymal membrane bones

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A prerequisite for differentiation of virtually all cartilages and bones of the facial skeleton of the chick is an interaction between ectomesenchymal cells derived from the cranial neural crest and epithelium or ectodermal derivatives (Hall, 1978, Le Lievre, 1978, Noden, 1978). One such interaction was investigated. 14 Epithelial papillae appear on the surface of the eye to encircle the cornea at 6½-12 d of incubation (Hamburger & Hamilton 1951, H. H. stages 29-38). One scleral ossicle forms beneath each degenerating papilla from the 12th day of incubation (H. H. stage 37). One papilla was removed in ovo (papilla 12 ° to reduce anatomical variability) and wounds were either left uncovered (after Coulombre et al., 1962) or covered with Nucleopore filters (1·5 mm², 10 μm thickness, 0·2 or 0·8 μm pore sizes). Post-operative incubation was for 1-8 days before histological analysis of wound healing and osteogenesis. Removal of papilla 12 ° without implanted filters at H. H. stages 29-30 completely inhibited ossicle 12 ° or at H.H. stages 31-35 resulted in small or normal ossicle 12 °. Uncovered wounds sometimes healed with epithelial blastemae or remained partially open. Nucleopore filters were internalized in 24-48 h and covered by layers of ectomesenchyme and epithelia. Direct contact between superficial epithelia and ectomesenchyme with deeper layers of ectomesenchyme was blocked. Embryos operated on at H. H. stages 29-31 never developed ossicles or at H. H. stages 32-35 developed small or normal ossicles beneath filters of both pore sizes. Early stages of papillae with direct epithelial-ectomesenchymal contact were essential for osteogenesis in the sclera.

General abstracts

Catecholamines and development of the mammalian palate: identification of \( \beta \)-adrenergic receptors and demonstration of catecholamine regulated palate mesenchymal cell growth

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Hormonally regulated levels of cyclic AMP have been suggested to play a role in differentiation of the developing secondary palate. Moreover, catecholamines stimulate adenylate cyclase in the developing palate during the period of palatal cellular differentiation (Anat. Rec. (1976) 185, 125). Using HPLC, we have demonstrated the presence of catecholamines in the developing murine palate and characterized a functionally-coupled \( \beta \)-adrenergic receptor system in this tissue. Binding of the \( \beta \)-adrenergic antagonist \( ^3 \)H-Dihydroalprenolol was saturable (B\(_{\text{max}}\) 16 fmol/mg protein), reversible, of high affinity (\( K_D \) 1.5 nM) and was displaced by the competitive \( \beta \)-adrenergic antagonist, propranolol, in a concentration-dependent manner. Functionality of receptors was assessed by demonstrating that fetal palatal mesenchymal cells responded to catecholamine agonists with dose-dependent accumulations of intracellular AMP. The relative order of potency of catecholamines in eliciting an elevation of cAMP was characteristic of a \( \beta_2 \)-adrenergic receptor-mediated response: (-) Isoproterenol > (-) Epinephrine > (-) Norepinephrine.

Development of the palate is accompanied by variations in mesenchymal cell proliferative activity, the proper temporal regulation of which is necessary for normal ontogenesis to ensue. Growth of primary cultures of palate mesenchymal cells was synchronized by 48 h serum deprivation and cells subsequently released from growth arrest by repletion of medium with serum. This protocol resulted in an enriched population of S-phase cells whose growth (labeling index) was assessed in presence of 10 \( \mu \)M (±) isoproterenol administered at time of release. Preliminary data indicate that isoproterenol delayed the rate of cellular entry into S-phase by 2 h, after which time the rate of cell cycle traverse was comparable to control cultures. Alteration in the kinetics of palate mesenchymal cell S-phase DNA synthesis may thus serve as a mechanism whereby catecholamines modulate normal palatal growth and development.

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Glomerular injury in rat neonate kidney following in utero exposure to gentamicin

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Aminoglycosides damage both renal tubule and glomerulus (glomer). Tubule injury is depending on the uptake ability of the epithelium but little is known about mechanism(s) underlying glom. alterations. Numerous investigators have suggested that glom. endothelium changes occurred in response to tubule cell damage. In these conditions before the achievement of the tubule maturation one would not expect glom. lesions. The purpose of this study is to test this hypothesis by treating with gentamicin, developing rat kidneys which until birth present simultaneously mature nephrons (in the juxtamedullary part of the cortex) and immature nephrons (in the superficial part of the cortex).

Wistar female received for the two last weeks of pregnancy Gentamicin (G) (I.P. 75 mg/kg/B.W./day) or the same amount of saline (S). Pregnancy durations were identical for all; but, at birth G neonates were smaller than S-neonates G: 5-416 g±541 n = 58- S: 6-083 g±0.691 n = 50. Kidneys were removed, weighed and fixed for light or electron microscopy. Kidney weights were dimished G: 34-55 mg±6-01 n = 43 - S: 37-68 mg±6-38 n = 43. Numeration of the differentiated glomeruli performed on the more sagittal and median paraffin section showed a significant decrease G: 72-2±8±8 n = 14, S = 100-7±12-8 n = 25.

Both light and electron microscopy demonstrated damage in the juxtamedullary cortical tissues as in the tubular epithelium as in the most mature glomeruli. Tubule lesions can range from cellular swelling to necrosis, glomeruli damage consists of capillary loops elargement, endothelium alterations and severe mesangial ultrastructural changes.

In contrast, under the renal capsule the least mature nephrons were ultrastructurally unchanged. These results show: i) the rat nephron is highly sensitive to the nephrotoxic effect of gentamicin administered to the pregnant female; ii) that the apparition of histological signs of degeneration depends on the functional maturity of the nephron; iii) in addition gentamicin has a general effect in reducing the size of the kidney (number of nephrons). Further study must be performed to determine the mechanism and the reversibility of this underdevelopment.
Impact of the GVHR on the testis development and function in the chick embryo


The testis provides an immunologically tolerant microenvironment. Different allograft systems show very weak or incomplete reaction when performed within this special site.

In the acute model of GVH in the chick embryo it has been shown that the different organ lesions are depending on the local homing of donor immunocompetent cells and the mounting of a local allograft reaction by these cells against the host foreign determinants. So, it was interesting to compare the testis reactions with the usual GVH target organs in chick embryo allografted with adult spleen cells.

Allografts were carried out on 9-day-old embryos with adult spleen pieces from histoincompatible donors. Histocompatible (B14-B14) animals were used as controls. For histomorphometric, enzymatic and ultrastructural studies testis were sampled 2,5,8 days after the graft. Blood samples were taken at the same time for biochemical analysis.

Discreet colonisation of the host testis by donor cells is detectable from the 11th day followed by a massive granulocytic infiltrate which starts from the 14th day.

On the 16th day of incubation, testis appears severely underdeveloped. The semi-niferous cord surface is reduced; the total number of germinal cells is significantly less than in controls; focal necrosis is often seen at the ultrastructural level.

The number of Leydig cells (revealed by the paraphenylenediamine method) is unchanged but histologic observations show modifications in the lipid partition and more frequently myelin whorl images. In addition the Δ5-3β steroidodeshydrogenase activity is clearly depressed.

As soon as 14 days i.e. 5 days after the grafting the fall of the serum testosterone becomes striking. On the 16th day, the mean serum testosterone concentration is 334±136 (pmol/L) in GVH embryos and 731 ±158 (pmol/L) in the controls.

The testis is an important target organ in the acute GVH in the chick embryo; both interstitial and seminiferous tissues are damaged. It is not possible to decide whether the injury proceeds from local reaction mounted by the donor cells homed in the testis or from an indirect testicular mechanism (via the hypophysis for example).

Measurements of extracellular pH during neural tube formation in chick embryos

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Neural crest cells separate from the neural tube and migrate into the periphery in a cranio-caudal sequence. Little is known about the mechanisms underlying these processes though a variety of evidence suggests that the environment through which the crest cells migrate can influence the eventual fate of these cells (LeDouarin, 1982). Holtfreter (1944) has proposed that the ionic composition of the extracellular environment and in particular the pH and Ca\(^{2+}\) concentration may be regulated and may influence cell-cell adhesion and promote cell migration in amphibian and echinoderm gastrulae. To examine the possible existence of such mechanisms in neural crest migration ion-sensitive microelectrodes have been used to measure extracellular pH and Ca\(^{2+}\) concentration in chick embryos of 4-22 somites. Embryos were explanted into an organ bath and bathed in a HEPES buffered medium pH 7-4, temperature 22 °C. Voltage recording and ion-sensitive microelectrodes were inserted under the ectoderm at various locations where the neural tube was forming. The potential difference across the ectoderm was uniform and small (-3 mV to +8 mV) in all embryos examined. In the segmented mesoderm measurements of pH were made where the somites near their lateral border and in the unsegmented mesoderm 150 μm from the midline. In embryos of 5-7 somites the pH (mean ± S.D.) in the region of somites 1–3 was 8·12 ± 0·06 (n=5), while in embryos of 14–16 somites the pH was slightly higher, 8·25 ± 0·09 (n = 6). The pH remained constant or fell by approximately 0·1–0·2 pH units at more caudal locations in the segmented mesoderm, but in all cases the pH fell steeply beyond the last somite in the unsegmented mesoderm reaching a value at the end of the primitive streak 0·3–0·5 pH units lower than at the last somite. Furthermore, at any one level a lateral pH gradient was observed. Adjacent to the neural tube, 50 μm from the midline, the pH was 0·1–0·2 units lower than at the lateral border of the same somite while more laterally 300 μm from the midline the pH was 0·1–0·3 units higher. Additional measurements of extracellular Ca\(^{2+}\) further suggest that the concentration of Ca\(^{2+}\) is low at all stages studied (< 1 mM). These results suggest that in the chick the sequential development of an alkaline pH and a low extracellular Ca\(^{2+}\) may influence the onset and possibly the direction of neural crest cell migration since such conditions in vitro are known to influence cell adhesions.


Prostanoid regulation of the in vitro cytodifferentiation of muscle
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The terminal differentiation of embryonic skeletal muscle is characterised by fusion of myoblasts to form myotubes and the appearance of muscle specific proteins for example creatine phosphokinase (CPK). This enzyme is a dimer and exists in three isozymic forms, BB, MB and MM. The myoblast synthesises only the B monomer. Associated with differentiation the M form (muscle specific monomer) appears and the rate of synthesis of B increases. Production of both forms of the enzyme involves de novo synthesis of its mRNA (Perriard, J-C. (1979) J. Biol. Chem. 254, 7036). Indomethacin, an inhibitor of prostanoid synthesis markedly inhibits (90%) the increase in enzyme activity normally associated with the initial period of differentiation (i.e. 44–72 h of culture). This inhibition is reversed by either prostaglandin E1 or isoproterenol.

Here we have examined the ability of two other prostanoid synthesis inhibitors to block the increase in CPK activity, aspirin an inhibitor of cyclooxygenase and chloroquine an inhibitor of phospholipases. As a first step in elucidating their mechanism of action within the differentiating muscle cell, the isozyme composition of CPK in control cells and in cells prevented from differentiating by chloroquine have been compared. Chick myoblasts were isolated from 12 day embryos by mechanical agitation, seeded at 3-5x10^5 cells/60 mm dish and grown in medium 199 plus 10% horse serum and 2% embryo extract. Aspirin (5-6x10^-4 M) or chloroquine (3-2x10^-6 M) were added to the medium at 12 h intervals from 24 h of culture. Both inhibitors of prostanoid synthesis prevent the normal increase in CPK activity. The inhibition by chloroquine is only partial (40% inhibition at 72 h), whereas aspirin, like indomethacin, imposes an almost complete block upon the enzyme increase (90% inhibition at 72 h). Both inhibitions are reversed by either prostaglandin E1 or isoproterenol. This suggests 1) all three drugs have their effects by blocking prostanoid synthesis; 2) the inhibition can be reversed by raising intracellular cAMP. Employing polyacrylamide gel electrophoresis (Morris et al., 1976 Nature 263, 76), to determine the isozymic composition of control and chloroquine treated cultures has revealed that in the inhibited cells there is a normal level of BB, but reduced MB and no measurable MM enzyme. A similar pattern of inhibition is found after separation of the isozymic forms by ion-exchange chromatography (Lough and Bischoff (1977) Dev. Biol. 57, 330).

These findings implicate prostanoid synthesis in the expression of muscle specific proteins. They also suggest that inhibition of prostanoid synthesis specifically inhibits the production of the muscle-specific isozymic form of CPK.

Isolation and expression of sequences specific to early mesenchyme cells in the sea urchin embryo
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At the 4th cleavage of the sea urchin embryo, three cell types arise by unequal division. One of these cell types, the micromeres, is at this stage committed to the production of the larval skeleton. This skeletal formation occurs autonomously in cultured micromeres isolated at the sixteen cell stage (Okazaki, 1975). The descendants of the micromeres in the embryo are the primary mesenchyme cells. Differentiation of these cells is characterized by delamination of these cells from the blastula wall into the blastocoel, migration in the blastocoel, and ultimately the secretion of a magnesium calcite skeleton.

We have isolated primary mesenchyme cells from early gastrula by the method of Harkey and Whiteley (1980). RNA from these cells has been used to carry out a plus/minus screen of a sea urchin poly A+ gastrula cDNA library. We have isolated two clones which we are examining in more detail. One clone is a tissue specific actin. The other clone is a 1.5 kb transcript of unknown identity.

These sequences are developmentally regulated. They do not appear to be present in the egg or 16 cell stage embryo. The prevalence of these sequences is low in the early gastrula RNA used to isolate them, but increases dramatically by late gastrula. These sequences show developmental regulation different from that of late mesenchyme specific sequences (Michael A. Harkey, personal communication) and may be involved with the early migratory phase of mesenchyme development. Supported by USPHS grant HD06902 to R.A.R. D.A.H. is a pre-doctoral trainee supported by USPHS award T32 GMO7227.


**General abstracts**

**Myogenesis and chondrogenesis in explants of brachial somites isolated before the onset of tissue differentiation in the somites**

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Somites in the brachial region of avian embryos (at the level of somite pairs 15–20) not only differentiate to form axial cartilage and bone and the body wall musculature but they are also the source of cells which migrate into the wing primordia to form the skeletal muscles of the wings. This migration occurs before the onset of cellular differentiation in the somites and before, or concomitant with the formation of the sclero-, dermo- and mytomal parts of the somite. The aim of this study was to determine the differentiative capacities of the brachial somitic cells immediately before and during the stages of migration into wing territories to see if the cells are committed to particular developmental pathways at these stages.

The brachial somites were isolated from HH stages 12, 15 and 18 chick embryos, either by microdissection or enzymatic digestion, and grown in organ culture, in explant culture on different substrata, or on the chorioallantoic membrane (CAM) of host chicks, either on their own or in combination with adjacent tissues.

Myogenesis, assessed by the presence of multinucleated myotubes and/or positive immunostaining with antibodies to skeletal muscle myosin and actin, occurred in all HH stage 18 enzymatically separated somites, regardless of the growth environment but was reduced in HH stage 15 explants and could not be confirmed at all in HH stage 12 explanted somites. The presence of the overlying epithelium was found to increase the incidence of myogenesis in HH stage 12 somite explants. Chondrogenesis also occurred in all HH stage 18 explants but, like myogenesis, its incidence decreased in the younger explants. Unlike its effect on myogenic expression, the presence of the overlying epithelium resulted in a dramatic decrease in chondrogenesis in both HH stages 12 and 15 explanted somites.

These results suggest that by the time the migration of somitic cells into wing regions is finishing, brachial somitic cells have become stabilized in their ability to undergo both myogenesis and chondrogenesis for they will do so under a variety of growth conditions and independently of adjacent tissues. However, immediately before (HH stage 12) and shortly after (HH stage 15) the onset of migration, both myogenic and chondrogenic expression by brachial somitic cells are still under the influence of interactions with adjacent tissues.

**Regulation of carbamoylphosphate synthetase and phosphoenolpyruvate carboxykinase levels in cultures of embryonic hepatocytes**

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Dexamethasone, triiodothyronine and dibutyryl cyclic AMP induce the synthesis of carbamoylphosphate synthetase (CPS) and phosphoenolpyruvate carboxykinase (PEPCK) in embryonic hepatocytes of the rat. Using an enzyme immunoassay and hepatocyte cultures fixed in situ, we have demonstrated that the capacity of hepatocytes to synthesize CPS and PEPCK in the presence of hormones is present as soon as the cells become recognizable as hepatocytes (11 days p.c.). However, the capacity of these cells to accumulate CPS and PEPCK protein is 50-fold lower than that of adult hepatocytes. This difference can be explained by a five fold smaller size and a ten fold smaller synthetic capacity of the embryonic compared to the adult (diploid) hepatocytes. No difference in turn-over rate of the enzymes was found.

This smaller synthetic capacity of the embryonic hepatocyte for CPS and PEPCK may in part be explained by the finding that hepatocytes do not acquire or express the capacity to accumulate the enzymes at high rates synchronously as shown by immunochemical staining of the cultures. This heterogeneity in hepatocyte enzyme levels was also observed in liver sections of fetal rats.

These observations point to a dichotomy in the differentiative process, i.e. that the acquisition of the capacity to synthesize liver-specific proteins (cytodifferentiation) precedes the capacity to synthesize these proteins at adult rates (maturation). These two processes therefore reflect changes in functional capacities leading to what can be defined as the protodifferentiated and differentiated state respectively. Both enzymes are therefore good parameters in studies that aim to establish the mechanisms that underly the ontogenesis of the hepatic phenotype.
Polyamine depletion causes inhibition of transcription in early chick embryos and blocks development at the primitive streak stage

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In the developing chick embryo there is a precipitous increase in polyamine synthesis at the time of gastrulation. To determine the role of the polyamines in developmental events associated with gastrulation, polyamine synthesis was inhibited by treatment with α-difluoromethylornithine (DFMO), an enzyme-activated irreversible inhibitor of ornithine decarboxylase. Ornithine decarboxylase is the initial and rate-limiting enzyme in polyamine synthesis. In these experiments, DFMO-induced polyamine depletion was found to interfere with nucleolar formation and to arrest development at the end of gastrulation. The present study addresses the question whether the observed effects on nucleolar development in polyamine-depleted embryos are due to interference with rRNA synthesis. Chick embryos, incubated for 7 h at 37 °C, were exposed to DFMO (0-42 μmoles in 100 μl of 0-93 % saline, injected just beneath the embryo). This dose completely prevented the accumulation of polyamines in the developing embryo. After 16 h of DFMO treatment in ovo, the embryos were excised and incubated in Pannett-Compton saline supplemented with 20 % homogenized egg white and 3H-uridine (100 μCi, 40–60 Ci/mmmole) for 30 min at 37 °C in vitro. After a 15-min chase with unlabeled uridine, the embryos were sonicated in 20 mM Tris- HC1 buffer (pH 7-2) containing 2-0 mM EDTA, 0-5 % sodium dodecyl sulphate, and 1 mg/ml Proteinase K. The homogenates were incubated for 20 min at 37 °C. The embryo digests were extracted once with saturated phenol and desalted with Sephadex G-25. The RNA was precipitated overnight at 20 °C in 1-0 M NaCl and 70 % ethanol. The precipitates were collected by centrifugation and dissolved in 0-2 % sodium dodecyl sulphate in Tris-HCl buffer (pH 8-0). The RNA species were separated by electrophoresis in 1 % agarose gels. The gels were stained with toluidine blue for determination of the RNA concentrations, and saturated with Enhance for fluorographic estimation of the rates of RNA synthesis. DFMO treatment caused a 90 % reduction in the incorporation of 3H-uridine into RNA. Furthermore, it appeared to suppress the synthesis and accumulation of all RNA species. Although our morphological observations on polyamine-depleted embryos suggest a specific role for the polyamines in rRNA synthesis, the present biochemical analyses indicate that polyamines are generally required for transcription.

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Observations on the development of ‘naked’ veliger larvae of Crepidula fornicta L. (Gastropoda prosobranchia)

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The application of the scanning electron microscopy to morphological studies of molluscan larvae is limited by the presence of the larval shell. Removal of the shell by either mechanical or chemical methods is difficult and detrimental for the specimens. Crepidula embryos however, were found to lose their shells if egg capsules (from gastrula to late trochophore stages) were kept in a sea water medium with a lowered pH (≈7-7). Gastrulation occurred normally but the subsequent secretion by the shell gland was only a boat-shaped membrane: the periostracum and the organic matrix, lacking in CaCO3 crystals as shown by x-ray diffraction analyses. Radiotracer experiments also showed that there was no 45Ca deposition in the primordium of the shell. The membrane was flexible and only superficially rooted to the mantle which was released at pediveliger stage when the animals were still within the egg capsules.

Self-hatched veligers under these circumstances, successfully completed the free swimming period, settlement and metamorphosis, although 15 days after metamorphosis none were able to produce a normal shell even when transferred to normal pH sea water.

The differences between the morphologies of the naked and shelled animals are discussed as well as the possible reasons for the incapability of the deshelled larave to secrete a second normal shell.
Analysis of control of cell death in the anterior necrotic zone (ANZ) of the chick wing bud

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The anterior necrotic zone (ANZ) appears at the anterior border of the developing chick wing bud at late stage 21. The role is, as yet, unclear. The ANZ is greater in extent and longer in duration than the posterior necrotic zone (PNZ). Carbon mapping techniques have been applied from stage 16. Marking both the flank and adjacent somite and studying the relative movements of each mark, it has been shown that the prospective ANZ (pANZ) shifts posteriorly by one somite to its final position opposite somite 16 where it is detectable by the first appearance of cell death.

Saunders, et al. (1962) demonstrated by grafting prospective PNZ (pPNZ) tissue to the dorsal wing bud, that the grafts of pPNZ become necrotic after stage 22 in about 60 % of cases. They thus deduced that the pPNZ becomes committed to death by stage 22. This is two developmental stages prior to its appearance as in the case of the PNZ. In contrast to the PNZ, there are two differences with the results obtained in the grafts of pANZ. First the proportion of grafts becoming necrotic is less (i.e. 30 %-50 %), and second the appearance of death is as a transitory wave between 6 and 12 hours post operation.

These two differences are considered to be significant and may point to a different system of control in the ANZ compared to the PNZ.


Mode of action of erythropoietin (EP) and glucocorticoids on the erythroid precursor cells: role of the prostaglandins (PG)


Glucocorticoids control the involution of the erythropoietic tissue which occurs in the liver of the fetal rat during the last days of gestation. In vitro, the ability of the precursor cells from this tissue to form erythroid colonies (CFUE) is EP-dependent and dexamethasone (DEX) decreases by roughly 50 per cent the number of colonies obtained at EP concentrations $\geq 25$ mU/ml. PGE$_2$ ($10^{-9}$ M) increases by roughly 100 per cent the number of CFU E observed when no EP is added to the culture medium, whereas its action is negligible in the presence of 50 mU/ml EP. Indomethacin (IND) (10 $\mu$g/ml) decreases by roughly 30 per cent the number of CFU E obtained in the presence of EP. PGE$_2$ ($10^{-9}$ M) relieves the inhibitions due to IND to DEX ($10^{-7}$ M). EP has no effect on PGE$_2$ release in the medium until 5 h incubation. But, already after 3 h incubation, DEX inhibits PGE$_2$ release and, after 5 h incubation, this effect is dose-dependent between $10^{-10}$ M and $5\times10^{-8}$ M, in good agreement with the measured apparent dissociation constant for specific binding at equilibrium, $K_d = 5\times10^{-9}$ M.

These observations do not sustain the hypothesis that PGE$_2$ is a second messenger of EP; they rather suggest that PG might be modulators of EP action.
General abstracts

Presence of nucleolar vesicles and vacuoles in the rat oocyte

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The present investigations were undertaken to study the modifications of the nucleolus under the influence of gonadotropines in the follicular oocytes of immature rats. Nucleolar vacuoles and vesicles in the follicular oocytes of mature and immature rats were namely researched.

First, 28-day-old rats are injected with 40 UI of FSH and the oocytes were observed after 24 h, 48 h, 72 h and 96 h. Secondly, other 28-day-old rats are injected once, twice or three times with 40 UI of FSH with an interval between the injections of 24 h; in this last case, the oocytes are observed 24 h after the last injection. Oocytes of mature rats are also examined at the prooestrus, oestrus, meteoestrus and dioestrus time.

In the three experiments, some nucleoli presented vesicles or vacuoles but their frequency is very different for each condition. In mature rats nearly all nucleoli show closed cavities. In immature rats the presence seems to be dependent upon the stimulation with FSH.

LH does not seem to influence the presence of nucleolar vesicles: one injection of 25 UI of LH, 24 h after an injection of 40 UI of FSH and the observation of nucleolus 1 h after this injection does not show a change in the percentage of nucleoli with vacuoles or vesicles. The nature and the significance of these vacuoles and vesicles are discussed.

Mitochondrial biogenesis in Xenopus laevis oocytes: DNA accumulation and DNA binding proteins

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The mitochondrial biogenesis is not a uniform process along the oogenesis of the toad Xenopus laevis, the replication activity of the mitochondrial DNA (mt DNA) varies according to the phases. The frequency of DNA molecules bearing a displacement loop (D-Loop) can be correlated with the intensity of the mitochondriogenesis (1). The peculiarities of the region containing the D-loop suggest a regulatory role for this structure; protein-DNA interactions are expected; an attempt has been made to identify such mitochondrial proteins. DNA binding proteins have been purified from mitochondrial extracts by ion exchange and affinity chromatography, their in vitro properties have been studied by filter binding assays. A protein fraction has been isolated showing a specific affinity for supercoiled DNA molecules containing the mt DNA D-loop region (2). One protein has been purified to apparent homogeneity, $M^* = 15-5$ Kd (3). It is polymeric in its native form, it binds preferentially and co-operatively to single stranded DNA with a high efficiency. In reconstitution experiments with mt DNA, it covers the single stranded part of the D-loop thereby stabilizing the structure. Apparently similar mt DNA complexes can be isolated from mitochondrial lysate. The amino acid composition of this basic protein has been determined and compared with other DNA binding proteins.

These proteins could be a tool to study heterogeneous distribution and replication activity of mitochondria in Xenopus laevis oocytes (4).

General abstracts

The use of chorioallantoic membrane cultured 14-day-old chick femurs to investigate bone growth, differentiation and repair
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The chorioallantoic membrane (CAM) of the 9-day embryonic chick has been used for over 70 years for the culture of living tissue. These studies have been very successful for the investigation of early limb growth and differentiation, cell interactions and the control of tumour growth. These methods usually involved the culture of tissues below 2 mm in diameter. This was in the mistaken view that larger tissues could not be cultured. We have extended CAM culture methodology by using 14-day-old embryonic chick femurs cultured for up to 10 days on the CAM. These have an initial length of approximately 13 mm and a wet weight of approximately 45 mgs.

Intact femurs cultured for 10 d consistently had a tenfold increase in net weight, dry weight, collagen and calcium content. This growth was matched by near-normal external morphology, including well-developed epiphyses and normal histology, i.e. both growth and morphology were considerably better than can be obtained in organ culture.

From this, using matched experimental and control femurs, we were able to investigate the effect on growth and development of serial dilutions of substances applied in solution to the exposed CAM. These included dexamethasone and calcitonin.

In separate experiments we investigated the role of the fibroblastic and osteogenic layers of the periosteum in growth and fracture repair. This involved either single fractured femurs or damaged pairs of femurs in co-culture on a single host chick.

Using living and dead intact and traumatised bone grafts, we were able to investigate the relative importance of vascularity and osteoblasts in the stimulation of osteoclastic invasion from the host chick.

We have, therefore, a model which, with its vascularity, good morphology and tenfold growth, has considerable potential in investigating bone growth differentiation and repair.

An hypothesis implicating a founder cell population in the regulation of wool follicle formation and distribution in sheep skin
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Wool follicles of Merino sheep develop as a consequence of three waves of initiation during foetal life. The first-formed, primary (P) follicles begin to appear at 60-70 d of gestation, secondary original (SO) follicles at 85-90 days and secondary-derived (SD) follicles after 100 d. SD follicles are not initiated at new sites in the skin but arise by branching from the distal regions of developing SO follicles.

Fibre characteristics and the numbers of P and SO+SD follicles/mm² were obtained in sheep from four lines selected for high and low wool fibre diameter (D±) or staple length (L±) from a single medium-woolled flock. The animals responded to selection and also exhibited marked variations in follicle numbers. However, the four lines all produced approximately the same amount of wool, indicating that selection for a single fleece character resulted in compensatory changes in fibre structure or follicle distribution. Between the four lines, fibre diameter was negatively correlated with follicle number (r = -0.99). In particular, the mean number of follicles/mm² in D- sheep (85-2±2.3) was twice that of the D+ line (41.3±1.8). The differences were due to the numbers of secondary follicles since P density in the four lines remained relatively constant (3-4/mm²). Estimates of the relative contributions of SO and SD follicles to the total population indicated that the last-formed, SD follicles were predominantly responsible for the differences in numbers between the four lines. The fact that SD follicles were formed by branching from SO follicles showed that the skin had a developmental capacity to produce more follicles than available initiation sites. It was hypothesized that the 'developmental capacity' manifested itself as a population of founder cells in the skin which became committed before the first wave of follicle initiation. These cells constituted the total amount of follicle-forming tissue of the skin and all follicles had their origin in them. The inverse relationship between follicle number and fibre diameter in the four lines is thus explained as due to differing contributions of founder cells to follicle formation. For example, relatively more founder cells would participate in the development of large P and SO follicles to make thick fibres in D+ sheep than in D- sheep with small follicles and fine fibres. After occupation of all initiation sites, the residual founder cell population would be smaller in D+ than D- sheep. Fewer SD follicles would therefore result.
General abstracts

Peroxisomes and amphibian development

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Spontaneous metamorphosis of Rana catesbeiana is characterized by a substantial increase in frequency, size and catalase activity of hepatic, renal and intestinal peroxisomes and/or microperoxisomes. These modifications are observed during the post-climax in the liver and mesonephros (Dauca et al., 1982, J. Exper. Zool., 223, 57-65), and they appear later, after a few weeks of terrestrial life, in the intestine (Dauca et al., 1982, J. Exper. Zool., 220, 235-241). Similar increases in the number and in the enzymatic activities of hepatic and renal peroxisomes have been noted in the developing chick at the time of hatching, and in mammals during early postnatal life. For higher vertebrates as well as for amphibians, these modifications occur during critical periods of life when they leave the aquatic habitat for a terrestrial one. On the other hand, no change in catalase activity is detected in the liver and kidney during the entire development of Xenopus laevis, which remains aquatic after metamorphosis.

Do peroxisomes play a role in this adaptation to a terrestrial habitat? In order to answer this question, adult Xenopus have been kept out of water respectively for 7 and 20 d. The experimental animals have shown low metabolic rates. A drastic decrease has also been observed in the hepatic, renal and intestinal catalase activity. These results suggest that a functional correlation exists between peroxisomes and mitochondria.

While it is well known that thyroid hormones (TH) play an important role in regulating the metabolism of amphibians during spontaneous metamorphosis, the in vivo effect of these hormones on the ontogenesis of peroxisomes has also been investigated. 16 days after the beginning of the hormonal treatment, 5-4~ and 2-4~ fold increases have been found for the specific activities of hepatic and renal catalase, respectively. A temporal coordination exists between the structure and the metabolism of peroxisomes and mitochondria during TH-induced metamorphosis (Dauca et al., 1983, J. Exper. Zool., 227, 413-422.

Immunofluorescent localization of the regulatory subunit R II of cAMP-dependent protein kinase in epithelial, fibroblastic and myogenic cells

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Using monospecific antibodies against bovine heart regulatory subunit R II of cAMP-dependent protein kinase, we have carried out an immunofluorescence study to determine the intracellular distribution of R II during cell division and differentiation. Identical immunofluorescent staining patterns were observed using immune-sera from three different rabbits or affinity-purified antibodies. Staining was completely abolished, however, by pre-absorption of sera with purified R II subunit. When bovine epithelial cells (MDBK) or chick embryo fibroblasts were examined during interphase, R II was found to be concentrated in conspicuous perinuclear areas highly reminiscent of the Golgi apparatus. Superimposed over the Golgi area, labeling of the microtubule-organizing center (MTOC) was occasionally apparent. Interestingly, during cell division prominent staining of the poles of mitotic spindles (but not the spindles themselves!) was observed. To study the distribution of R II in a differentiating system, myogenic cells were prepared from breast muscles of 11-day chick embryos and allow to differentiate in vitro. In mononucleated myoblasts, staining of the Golgi area and the MTOC was apparent similar to the results obtained with fibroblastic and epithelial cells. By contrast, in differentiated multinucleated myotubes, prominent labeling of the nuclear envelopes was observed in addition to some cytoplasmic staining. Further studies will be required to determine whether the redistribution of R II in these multinucleated cells reflects a specific translocation of R II or merely a re-arrangement of the entire Golgi apparatus. (Supported by the Swiss NSF and the Deutsche Forschungsgemeinschaft).
General abstracts

Similar membrane antigens on male and female germ cells during early development of the common carp (Cyprinus carpio L.); a study with monoclonal antibodies

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Using four monoclonal antibodies (MoAb) raised against carp spermatozoa, the appearance of membrane antigens specific for spermatogenic as well as premeiotic female germ cells (Parmentier et al., 1984), was studied during development of carp until twenty-five weeks after fertilization (22 °C). Indirect immunofluorescence studies revealed MoAb-WCS-29 stained primordial germ cell membranes in both sexes from three days after fertilization onwards. The MoAb-WCS-3 and 17 reacted with germ cell membranes from seven weeks onwards, simultaneously with the start of germ cell proliferation. At the age of eighteen weeks secondary oogonia and spermatogonia were stained by MoAb-WCS-28. These antigens were not detected on later oogenic stages in the female but they remained present on later spermatogenic stages. It was concluded that in carp germline specific antigens are present on male and female germ cells from early development onwards.


Embryonic skeletal tissue stores ascorbate during culture

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Collagen synthesis requires ascorbate, yet the vitamin deteriorates rapidly under culture conditions. So how can one ensure an adequate supply during long-term organ culture?

In the culture medium of 15-day-old embryonic chick femurs, supplemented daily with 50 or 200 μg/ml ascorbate, less than 1 μg/ml was present after 6 h. Tissue ascorbate levels of pre-culture femurs were 48.0±5.6 μg/g wet weight in the cartilage epiphyses and 71.4±6.0 μg/g in the bone shafts, but, in contrast to the free ascorbate in the medium, tissue ascorbate disappeared less rapidly – even after 6 d in culture without ascorbate around 2 μg/g was still present in cartilage and 5 μg/g in bone. Daily feeding with ascorbate maintained levels of 20–50 % of pre-culture controls.

To observe in more detail how declining media levels influenced tissue ascorbate content, femurs were cultured for 4 days without the vitamin. Then either 50 or 200 μg/ml were added and tissue levels measured at intervals over 24 h. Ascorbate was taken up extremely rapidly (within 1–5 h) in both bone and cartilage. 50 % of the initial uptake was due to diffusion since that was the % with which thrice frozen and thawed bones accumulated the vitamin. Viable bones maintained concentrations above medium levels such that cartilage ascorbate was 30 fold and bone ascorbate 60 fold higher than medium levels, whereas levels in frozen bones fell to medium levels within 6 h.

In spite of high levels before and accumulation during culture, the ascorbate threshold below which collagen synthesis was impaired was very low: only if available ascorbate was less than 5–10 μg/g wet weight could progressive and rapid decrease in collagen synthesis be measured.

This suggests that tissue stores ascorbate far in excess of actual requirements, which may represent an adaptive mechanism to the instability of the vitamin.
General abstracts

Catch-up growth in planarians: quantitation with fluorescent microspheres
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Whereas homeotherms have ample literature concerning 'catch-up growth' phenomena (acclerated growth which occurs after periods of prolonged starvation to compensate the loss of 'potential' tissue), less attention has been given to it in poikilotherms and even less in invertebrates. In homeotherms, 'catch-up growth' is mainly due to higher ingestion rates and higher conversion efficiencies. The scarce data so far available in invertebrates support the existence of some sort of 'catch-up growth' which depend too on higher ingestion uptake and conversion efficiencies. However, how this is affected by body size, food ration, and external temperature is at present uncertain.

We have studied 'catch-up growth' in planarians, organisms which can stand very long periods of starvation followed by rapid growth when feeding resumes. To quantitate it, we have made use of fluorescent microspheres (Fluoresbrite beads, 1.83 μm in diameter; Polysciences) mixed with a sort of artificial food. Organisms of different lengths, reared at different temperatures and feeding regimes were studied, and the ingestion rate measured as number of fluorescent beads absorbed per individual per unit of time. The values obtained were compared to data obtained on number of cells born per cell and per unit of time (cell birth rate, \( k_B \)) which can be taken as an indirect estimate of the conversion efficiency (transformation of energy into soma).

The results show that 'catch-up growth' does exist in planarians for all feeding regimes, body sizes and temperatures, and that this kind of growth regulation is mainly due to higher ingestion uptake than to higher conversion efficiencies.

Isolated, mononucleated striated muscle can undergo pluripotent transdifferentiation and form a complex regenerate

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Isolated mononucleated striated muscle of a medusa can be activated by collagenase treatment to undergo transdifferentiation to various new cell types and to regenerate autonomously the sexual and feeding organ of the animal. All isolated muscle fragments produce under these circumstances smooth muscle cells and a glandular cell type. When culture conditions are appropriate, endoderm is also formed, followed by regeneration of a complex organ of eight further new cell types.
Double embryos: an S.E.M. study of dorsal tissue developing under the influence of two ‘organizing’ regions

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Xenopus embryos with double dorsal structures were produced by performing ‘organizer’ grafts on early gastrulae (stage 10). The resulting embryos were allowed to develop until their synchronously fertilized controls reached stage 20 to stage 28. They were then dissected and prepared for observation by Scanning Electron Microscopy.

The largest part of this study was concerned with the development of the notochord and somites at various stages during double embryo formation. This was done by comparing the apparent maturity of the tissue, and degree of segmentation occurring in the primary and secondary axes of the double, with a single-axis control embryo. An interesting but less detailed study was made on embryos in which doubling was induced by rotation of the precleavage egg. This provided a useful basis upon which to assess the effects of the ‘organizing’ regions on early as well as late embryonic material.

The double embryo presents an opportunity to investigate the way in which the ‘organizer’ exerts its influence. This is also being studied at the light microscope level, to ascertain the origin of the cells which form the second set of dorsal structures. The answers to these questions are fundamental to an understanding of the resoluteness of initial cell specification: can cells ‘specified’ by the primary organizer during early embryonic development, change their migration path (or even their fate) through the exertion of a secondary influence?

The effect of hyaluronate on amnion endothelial cell structure and invasion of SV40-3T3 cells through human placental amnion

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The structure and integrity of cells which line blood vessels in vivo or extraembryonic structures (such as chick chorio allantoic membranes or human placental amnion) in vitro invasion assays appear to be critical to the penetration of tumor cells. We investigated the effect of a number of biopolymers on the structure of epithelial cells lining human placental amnion and the consequent effect of these alterations on the penetration of SV40-transformed 3T3 cells through the amnion. Amnions were pretreated with pronase, hyaluronate, heparin or chondroitin sulphate, and then washed prior to exposure to radiolabeled cells. Pronase increased penetration of cells by two-fold while hyaluronate (1 mg/ml) pretreatment caused a four-fold increase in the percentage of invading cells. Neither chondroitin sulphate nor heparin had an effect. The structure of pretreated amnions was investigated using histology and scanning electron microscopy. Pronase treatment removed epithelial cells. Although tumor cells were observed to attach to the remaining basement membrane structure, they were rounded; this suggests that enzyme treatment may have also removed attachment factors. Hyaluronate promoted microvillus formation and rounding of the epithelial cells, resulting in exposure of the underlying basement membrane. Flattened, apparently motile tumor cells could be seen to penetrate at these exposed sites. Sulphated glycosaminoglycan treatment did not promote epithelial cell rounding to the extent that hyaluronate did. These results suggest that integrity of epithelia is critical to invasion, and indicate that biopolymers, as well as enzyme, may effect this integrity. When considered in context with recent evidence these results implicate hyaluronate in invasion.
Regionalisation in the molluscan embryo

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In this poster we will present some new aspects of egg polarity in Patella. The results of experiments in which fertilized and unfertilized eggs are subdivided equatorially in an animal and vegetal half will be dealt with. They will concern cleavage rhythm, cleavage pattern, the appearance of dorsoventral polarity, and the induction of the mesoderm in both halves. Special attention will be paid the formation of an extra cellular matrix by the animal micromeres in the two parts.

The Voltage-dependence of sperm-egg interactions and the timing of the blocks to polyspermy in Xenopus laevis.

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Fertilization of Xenopus eggs is associated with a transient membrane depolarization, the fertilization potential, whereas cleavage is accompanied by a hyperpolarization. By injecting current via a second electrode, the membrane could be held at different potentials during insemination. Fertilization was prevented at around 0 mV. If the membrane potential was now returned to its unclamped value (about -20 mV), in 2 mV increments each lasting about 1 min, a potential was eventually reached at which a fertilization potential or current ensued depending on the clamp conditions. Under current clamp fertilization could be blocked at -15.6 mV ± 3.9 S.D. (12). Both clamp techniques enabled measurement of the time interval between the start of the potential increment that elicits fertilization and the first sign of the fertilization potential. This interval increased, apparently exponentially, from 0.33 ± 0.32 (8) at -40 mV, to 3.58 ± 4.47 (8) at -16 mV, under voltage clamp conditions.

In a series of eight current clamp experiments the membrane potentials of two control eggs were monitored at the same time as the clamped egg. Control eggs fertilized within 1.2 min ± 1.7 of each other. The clamped eggs were fertilized 19.7 min ± 12.1 after the controls. The time interval from fertilization to onset of the hyperpolarization at first cleavage for the controls was 87.3 min ± 10.9 and for the clamped eggs it was 90.4 min ± 13.6. The two control eggs began to hyperpolarize within 3.6 min ± 3.4 of each other, whereas the previously clamped eggs began 22.3 min ± 12.9 later. Monospermy could be ensured using voltage clamp by hyperpolarizing the membrane for a short time to about -20 mV from a holding potential of 0 mV. If a second, much longer pulse was then given immediately after the first, polyspermy resulted. A maximum interval between the two pulses of about 2 min was reached at which polyspermy did not occur and the cortical reaction (slow block) was considered complete.

Experiments of this kind allowed more precise measurement of the timing of sperm-egg interaction and the relative timing of the fast block and the end of the slow block.
General abstracts

The effect of protamine upon the development of the extraembryonic vasculature in the chick

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Angiogenesis, the growth of new capillary blood vessels occurs during the development of the embryo, certain immune reactions, wound healing and neoplasia. Recently it has been reported that protamine (a basic protein of molecular weight 5-10,000) specifically inhibits angiogenesis in all these processes (Taylor & Folkman, 1982). The exact mechanism by which protamine inhibits angiogenesis is unknown, but it has been suggested that it specifically binds to heparin sulphate or to some heparin fragments on the capillary endothelial cell and thus blocks directional migration of the endothelial cells (Folkman, Taylor & Spillberg, 1983). In view of this suggestion, it is important to discover whether the angiogenesis involved in embryonic development is modulated by heparin. Here I report that mast cells (cells which produce heparin) are present on the area vasculosa at early stages of vascular development and on the basis of their distribution and morphology it is suggested that mast cells play a role in the development of the embryonic vasculature.

The effect of protamine upon angiogenesis in the area vasculosa was re-examined using the method described by Taylor & Folkman (1982). The results confirmed the observation that protamine inhibits the outward migration of the capillary bed, but when examined histologically the inhibition did not appear to be the result of blocked chemical modulation, but a result of hyperplasia within the extraembryonic membrane.


Differentiation tendencies of pregut entoderm after intracoelomic grafting in chick embryos

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Entero-endocrine cells (eec) are aminergic peptide containing cells in the epithelium of the gut. In the development of ectodermal aminergic cells the notochord plays an essential role as can be deduced from Nieuwkoop's (1952) theory. Andre (1982) and Inokuchi (1983) have given conclusive evidence that eec stem from entodermal precursor cells. However, the hypothesis is put forward that, presumably in the developmental phase it is containing catecholamines (Kirby & Newgreen 1977), the notochord plays a role in the differentiation of entoderm cells into eec. So it is expected that in grafts, consisting of isolated parts of pregut entoderm (generally + adjacent mesoderm) no eec can be found. In corresponding grafts plus a fragment of notochord, eec do develop. The experiments consist of cultivating presumptive gut entoderm in the coelomic cavity of a host aged ±50 hrs. In a second series of experiments a notochord fragment of a donor is added to the original graft. The embryos are cultivated up to 16 d; specimens are analysed at regular intervals. The following results have been obtained. Pregut entoderm develops into gut epithelium in the host's somatopleura, visceropleura or mesonephros after 1-3 days of cultivation. Parts of the graft protruding in the coelom develop poorly. In exceptional cases grafts develop abundantly. After 5-7 days of culture most grafts tend to degenerate. In embryos cultivated 12-14 days no implants can be found. If a fragment of notochord from a donor is added to the original graft, well differentiated enteric structures occur in the host after 15 d of cultivation. In this epithelium eec can be observed. From our experiments it is observed that grafts of pregut entoderm (± mesoderm) have the competence to develop during at least three days into gut epithelium. After 5-7 d a critical period occurs, in which an additional factor is required to enable further differentiation. In presence of notochord enteric structures and differentiated epithelium including eec develop. Absence of an additional stimulus in the development may account for the fact that several authors describe negative results in culturing entoderm obtained from early stages (4, 5). Experiments to test the influence of the plexus myentericus and submucosus on pregut entoderm differentiation are in progress. The tentative conclusion drawn from these experiments is that the notochord plays an important role in maintenance and further differentiation of the entoderm.
**General abstracts**

**H-Y Antigen in differentiated quail gonads: induction in the testis by estrogens, inhibition in the ovary by androgens**

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The serologically identified H-Y antigen is closely associated with the vertebrate heterogametic sex. Thus in birds, the H-Y antigen is expressed in female cells (H-Y positive) and not in male cells (H-Y negative). Furthermore, the early estrogen treatment of a male embryo induces partial gonadal sex inversion, and, as previously demonstrated in quail (1), the left testis differentiates into an ovotestis that becomes H-Y positive while the right one is not feminized and remains H-Y negative. Thus the presence of gonadal H-Y antigen is associated with the development of ovarian structures, suggesting a role for H-Y in the ovary differentiation. However, a prerequisite for the study of this role, if any, was to establish the physiologic conditions of the H-Y expression in differentiated normal quail gonads since it seemed dependent on sex hormones.

Posthatched and adult male and female quails were given total doses of 1–2.5 mg diethylstilbestrol (DES) or 17 beta-estradiol (E2) or 1–4 mg dihydrotestosterone (DHT). Individuals were sacrificed two days after the last injection and the H-Y typing of gonad cells was performed by immunoabsorption of mouse H-Y antiserum followed by detection of residual anti-H-Y activity using an immunobacterian test on target mouse spermatozoa (2).

- The DES or E2 treatment caused the appearance of H-Y antigen in the left testis without any phenotypic feminization, while the right one remains H-Y negative.
- The DHT treatment inhibited the H-Y antigen expression in the ovary without any masculinization.

This study provides direct evidence of the hormonally controlled H-Y expression in quail gonads, even in adults. Moreover, it gives additional information on the problem of gonad asymmetry in avian: the adult left testis retains the potentiality to express a female feature, the H-Y antigen.


**Growth and development of offspring of rats under hypokinesia**

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It has been established experimentally that prolonged hypokinesia (HK) has an adverse effect on skeletal development and mineral metabolism, as well as on growth of offspring. Against this background the objective of this investigation was to evaluate the effect of 60 d of HK on some parameters of growth and development of the offspring of 36 white male and female rats. They were divided into two groups: the 1st group of rats (10 males and 10 females) was subjected to HK and the 2nd group (8 males and 8 females) placed under ordinary vivarium conditions and served as control. For the reproduction of HK the 1st group of rats was kept in small individual cages made of plexiglas which restricted their movements in all directions without hindering feed and water intake. Both groups of animals received feed and water ad lib. The rats were weighed on the 10th, 20th, 30th, 40th and 60th day of HK. Principal biological parameters, fertility and growth indices were calculated. Following the exposure to HK, males from corresponding groups were put with females. The males were removed from the females a few days prior to giving birth. The offspring from the rats in each group were weighed on the 1st and 10th days. The baby rats were sacrificed under anesthesia at the age of 30 and 60 d, and internal organs were weighed. The results were processed statistically. Young rats born from the hypokinetic rats opened their eyes and became covered with hair at a later date. The lowest percentage of female reproductivity (13 %) was referable to the hyperkinetic rats, and this was apparently related to changes in the neuroendocrine system that regulates reproductive function. In the initial 30–40 days after birth the weight and size of offspring lagged considerably behind the young rats born from the control group. The weight of most viscera of baby rats (both males and females) born from the hypokinetic rats was markedly lower than the rats born from the control group. It was concluded that hypokinetic conditions induced significant alterations in growth and development of the offspring of rats as well as reproductive function of adult animals.