*Histochemical Journal* **28**, 531–575 (1996)

REVIEW

# Bromodeoxyuridine: a diagnostic tool in biology and medicine, Part III. Proliferation in normal, injured and diseased tissue, growth factors, differentiation, DNA replication sites and *in situ* hybridization

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Received 30 September 1995 and in revised form 6 February 1996

**Summary**

This paper is a continuation of parts I (history, methods and cell kinetics) and II (clinical applications and carcinogenesis) published previously (Dolbeare, 1995 *Histochem. J.* **27**, 339, 923). Incorporation of bromodeoxyuridine (BrdUrd) into DNA is used to measure proliferation in normal, diseased and injured tissue and to follow the effect of growth factors. Immunochemical detection of BrdUrd can be used to determine proliferative characteristics of differentiating tissues and to obtain birth dates for actual differentiation events. Studies are also described in which BrdUrd is used to follow the order of DNA replication in specific chromosomes, DNA replication sites in the nucleus and to monitor DNA repair. BrdUrd incorporation has been used as a tool for *in situ* hybridization experiments.

## Proliferation in normal tissues

In a number of the references cited in previous parts of this review and particularly in part 2 of *Tumour proliferation*, and *Carcinogenesis*, normal tissues served as controls (Dolbeare 1995a, b). In this section I have intended to deal separately with normal tissues, e.g. central nervous system (CNS), skin, and gastrointestinal system, where studies were aimed primarily at obtaining information on proliferation in normal and not diseased organs and tissues.

Several studies have shown the efficacy of BrdUrd labelling in the intact animal. The important issue was the stability of the incorporated pyrimidine under physiological conditions. Likhachev *et al.* (1983) demonstrated that bromodeoxyuridine (BrdUrd) injected into foetuses or newborn rats labelled cells throughout the animal, and that the label was stable for months beyond the initial pulse. Ward *et al.* (1991) showed also that the BrdUrd label persisted long after the initial injection, especially in tissues with slow turnover, such as brain and uterine stroma, which showed persistent label at 49 weeks.

### *CNS*

Fujita *et al.* (1988) studied postnatal proliferation in the rat cerebrum following intravenous administration of BrdUrd at various times up to 24 weeks after birth of the rats. They showed that only few labelled cells existed in the cerebral cortex and caudate-putamen at birth and that these disappeared by adulthood. In the white matter (corpus callosum), the labelling index (LI) was 5.6–6.3% at birth and became zero later, whereas in the subependymal layer of the lateral ventricle it was high at birth and decreased to 7.5% in the adult. Grenier *et al.* (1989) cultured glial fibrillary acidic protein (GFAP)-positive astrocytes obtained from surgically resected temporal lobes of young adults operated upon to remove epileptogenic foci. They observed a mitotic index of 6% in these cells. In older individuals, astrocytes were non-proliferative. Estes *et al.* (1993) also estab-

lished GFAP+ cell lines from surgically resected tissues from cerebral white matter. They found that the GFAP-positive cells also incorporated BrdUrd in culture. Knapp (1991) showed that immature glial cells may serve as progenitors for oligodendrocytes even before they express galactocerebrosides. Those cells did not express GFAP. In a separate study, Knapp (1992) established cultures of astrocytes and determined that their cell cycle time was 20.5 h, approximately that of glial cells *in vivo*. Scarpini *et al.* (1988, 1992) developed techniques for dissociating adult peripheral nerve cells from myelin and connective tissues to establish cultures of Schwann cells, which were identified by indirect immunofluorescence with antibodies against S-100, galactocerebroside, and laminin. These cells were maintained in culture for 7 days to obtain sufficient numbers for proliferation studies and morphological characterization. Seki and Arai (1993) found in the adult rat the persistent expression, by newly generated granule cells of the dentate gyrus, of a highly polysialylated neural cell adhesion molecule generally specific for developing tissues. They showed that the newly generated granule cells involved in neuronal circuit formation in the hippocampus also transiently expressed the sialylated neural cell adhesion molecule at day 12 but not day 80 after BrdUrd injection.

*Olfactory tissues*

Kimura *et al.* (1990) employed both morphological and immunohistochemical analysis of BrdUrd in olfactory mucosa in mice. They found that BrdUrd label was concentrated in the basal layer of the epithelium after 5 h but became a part of the mid-layer of the epithelium at 15 days and disappeared completely from the epithelium by 30 days. They were also able to follow regeneration of damaged neurons in the olfactory apparatus by this technique. In a similar study, Nakamura (1991) obtained tissue specimens from guinea pigs 7 and 21 days after olfactory nerve axotomy. BrdUrd label was found in the eplithelial layer directly above the basal layer at day 7 and these cells were thought to be precursors to regenerating olfactor receptor cells. Corotto *et al.* (1993) used a combination of BrdUrd and tritiated thymidine labelling and demonstrated both neurogenesis and cell migration in the olfactory bulbs of adult animals where growth had ceased.

*Ear*

Roberson *et al.* (1992) showed that there is ongoing postnatal production of supporting cells and Type II hair cells in all chick vestibular epithelium organs. Similar findings were not obtained in other vertebrates. Takeno *et al.* (1993) used fibroblast-reorganized collagen gels as a matrix for growing long-term cultures of middle ear epithelium of guinea pigs. At the marginal portion of the explants, the cells changed from cuboidal to squamous and exhibited high proliferative activity.

*Oral tissues*

Casasco *et al.* (1988, 1989a, b) applied immunochemical detection of BrdUrd on tissue sections to measure proliferation in developing teeth and other oral tissues in the rat. They found that the immunohistochemical measurement of the labelling index did not agree with earlier autoradiographic data on similar oral tissues. Casasco *et al.* (1992) used double staining of BrdUrd and anti-enamel protein to detect the temporal relationship in developing teeth. Their study suggested that cells withdrew from the cell cycle in the inner enamel epithelium before they started to secrete enamel protein. Casasco *et al.* (1993) confirmed with the use of the P10 monoclonal antibody that PCNA represented G1, S, and G2/M phases of cycling cells in the developing enamel epithelium. Sfrondini *et al.* (1989) found BrdUrd incorporation in periodontal tissue following experimental tooth movement in guinea pigs by a coil spring. Young *et al* (1992) showed that the odontogenic mesenchyme in growth hormone deficient dwarf rats showed only approximately 50% the proliferation of control rats and that treatment of the dwarf rats with bovine growth hormone increased DNA synthesis in the internal enamel epithelium stratum intermedium and Hertwig root sheath to a level equal to that in control rats.

McCulloch and Knowles (1991) labelled hamster gingiva fibroblasts *in vivo* with tritiated thymidine ([$^3$H]dThd) and *in vitro* with BrdUrd. Using anti-vimentin and anti-keratin antibodies as additional labels, they found two fibroblast (vimentin+, keratin–) progenitor populations in early explant cultures of hamster gingiva. Hume (1989) and Hume and Thompson (1990) demonstrated in the mouse tongue a circadian rhythm in the labelling index using double labelling with tritiated thymidine and BrdUrd. There was a period with a short S phase duration (Ts) and a high efflux of cells from S phase, followed by a period of high labelling index and a slow efflux of cells from the S phase. Ultrastructural studies by Savelli *et al.* (1991) also revealed a circadian effect in labelling of epithelium and basal elements of the vocal fold epithelium in rats, with a higher proliferation rate at night.

Baeza-Squiban *et al.* (1991) obtained primary cultures of rabbit tracheal cells from explants, in which epithelial cells, uncontaminated by fibroblasts, grew in a pattern similar to normal tracheal epithelium. The cultures contained basal cells and epithelial polarized cells as well as ciliated cells which functioned in culture.

*Bone/growth plate*

Postnatal bone growth occurs by the process of endochondral ossification in cartilagenous growth plates at

the ends of long bones (Farnum & Wilsman, 1993). These authors examined progression of BrdUrd labelling in time and the spatial pattern of chondrocyte proliferation. They found that cells entered the cell cycle in the proximal part of the growth plate, spent four days in the proliferative cell zone, and then, after leaving the cell cycle, required 48 h to become terminally differentiated chondrocytes. They suggested that this was the time required to complete hypertrophy. Marshall & Davie (1991) injected four-day old mice with BrdUrd and observed labelled osteoclast nuclei in the parietal bone within 24 h, whereas the half-life of the label was 1.3 days. Peltomaki and Hakinen (1992) found BrdUrd labelling in rib costochondral junction, both in the proliferative zone and also in the germinative zone. They proposed that this was evidence that the growth potential of costochondral grafts was related to the length of the cartilagenous portion.

*Skin/hair*

Hair follicles are epidermal derivatives that produce hairs characteristic for thin skin. They develop by ingrowth of the epidermis through the dermis and then into the subcutaneous tissue below. Soft keratin is found in the inner root sheath, and is the soft centre of most hair. Hard keratin is the major component of the hair body. Hair is produced by the proliferation of the matrix at the base of the follicles (Cormack, 1984).

Egawa *et al.* (1988), Tezuka (1990) and Tezuka *et al.* (1990, 1991) studied cell kinetics in anagen (proliferation phase) of generating hair follicles in newborn and 7-day old mice. In anagen hair tissues, a large number of cells in the hair bulb were stained for BrdUrd. They proposed that the suprapapillary cell group might be responsible for the production of the hair cortex through the innermost cell layer of the outer root sheath, and that the outer group of cells may be responsible for hair elongation. In a further comparative study of human and mouse hair follicle proliferation, Tezuka *et al.* (1990) defined the growth of various parts of the inner and outer root sheaths and hair cuticle. In late anagen, the lowest outer portion became BrdUrd-negative, followed by the upper and inner portion and finally, the main germinative portion, where the outer root sheath abruptly increased in thickness.

Aarnaes *et al.* (1990, 1993) developed mathematical models to describe the BrdUrd/DNA bivariate analysis of the basal cells from mouse epidermis. Using the models, they were able to describe the heterogeneity in the proliferation rates that normally occurs in the epidermis. Kirkhus and Clausen (1990, 1992) localized basal cells in the mouse epidermis during the cell cycle at various times during the day and found that progression of cells through S and G2 phase was delayed considerably at night, producing a higher labelling index. Casas & Jefferies (1992a, b) estimated S phase duration and cell turnover time in goat epidermis by an intradermal double labelling technique using BrdUrd and tritiated thymidine. They found that both labels gave similar results with an S phase duration of 7.7 h and a cell turnover time of 22–26 days.

Czernielewski and Demarchez (1990) studied the proliferation of Langerhans cells in human skin and combined localization of BrdUrd with that of the surface antigen, OKT-6, for Langerhans cells. De Fraissinette *et al.* (1988) and Staquet *et al.* (1989) employed the same combination (OKT6 and BrdUrd) for Langerhans cell proliferation with an additional marker for basal keratinocytes.

*Muscle*

Smith *et al.* (1992) developed cultures of adult human atrial myocardium which stained positively for actin, myoglobin, atrial natriuretic peptide, incorporated BrdUrd, and which showed bundles of myofibrils with dispersed dense Z-bodies. Yamamoto *et al.* (1992) used flow cytometry of primary cultures of rabbit arterial smooth muscle to monitor modulation of phenotype and cell cycle analysis. During the first day, the cells exhibited the contractile phenotype but then began to enter the cell cycle and proliferate. Kuyatt *et al.* (1993) utilized multicolour image analysis to assess proliferation in rabbit aorta smooth muscle segments in primary cultures. They were able to follow changes in normal and perturbed muscle segments.

*Gastrointestinal tract*

Replication patterns of normal colon and rectal crypts provide useful information for the evaluation of kinetic changes in dysplastic or neoplastic tissues. Several of the following studies have dealt with the crypt labelling patterns and kinetics. Potten *et al.* (1992a) studied the crypt dimensions and labelling patterns in normal colon and rectal crypts. They found that the average crypt of the large bowel and rectum was 82 cells in height and 41 cells in circumference with a total of about 2000 cells. The highest labelling index in the colonic crypt (30%) was found at the 15th position, and the labelling was much lower at the bottom of the crypt, the probable stem cell zone. In an additional study, Potten *et al.* (1992b) compared the labelling indices of epithelium of ileum, large bowel, and rectum. They found the average labelling index to be 17.8%, 10.3%, and 8.5% respectively, suggesting an inverse relationship between cancer development and cell proliferation. Kellett *et al.* (1992) used combination labelling of tritiated thymidine and BrdUrd to determine the spatial distribution of label within the ileum and colon of mouse and human. They determined that the crypts of the mouse and the human consisted of approximately 250 and 450 cells,

respectively, and the colon of 590 and 2000 cells respectively, the labelling index being approximately two-fold greater in the mouse. In another study by this group, Patel *et al.* (1993) determined proliferative properties of the human gastric crypt and gland cells. They observed that the median length of the crypts was 137 cells for the gastric body (GB) and 188 cells for the gastric antrum with labelling index and duration of S phase of GB 2.8% and 7.7 h for the gastric body and 4.8% and 10.8 h for the gastric autrum. Abe *et al.* (1992) used multiple doses of BrdUrd to determine distribution patterns and kinetics of proliferation in mouse ileum. Other studies of BrdUrd label distribution and cell kinetics in murine intestine include those of Shutte *et al.* (1987) showing intestinal mucosa kinetics and antigen distribution patterns, of Lacy *et al.* (1991) on rat intestinal epithelium, and of Abe *et al.* (1992) on mouse jejunal crypts.

Sato and Ahnen (1992) studied the localization of stem cells and the direction of colonocyte migration in the normal rat colonic crypt. They showed that, in the distal colon, cells migrated up toward the luminal surface. In the proximal colon, the stem cells were located in the midcrypt, and the colonocytes migrated in two directions, up towards the luminal surface and downward towards the base of the crypt. Other kinetic studies of colonic mucosa were performed by Verstijnen *et al.* (1988) using xenografts of normal human colon mucosa, by Darmon *et al* (1990) using crypt labelling for risk assessment, by Wanders *et al.* (1992) using colon and vaginal mucosa, and Welberg *et al.* (1990) using the colonic crypt zone.

Han *et al* (1993) observed that macrophages in the lamina propria at the tips of the villi of small intestine of guinea pigs extended their pseudopodia into the epithelial lining, and internalized fragments of effete enterocytes in their phagosomes. They suggested that effete enterocytes were not simply exfoliated into the lumen, but were damaged by intraepithelial lymphocytes possessing natural killer cytotoxicity, and were subsequently phagocytosed by subepithelial macrophages.

*Liver/pancreas*

Jezequel *et al.* (1991) used BrdUrd labelling to follow cell renewal in normal rat liver. They found that the hepatocyte labelling index was 0.45%, with over 80% of the labelled cells in periportal zones. Kupffer cells showed 0.5% positive labelling, and these labelled cells were distributed randomly through the hepatic lobule.

Yuasa *et al.* (1993) showed that normal rat hepatocytes grew as spheroids in a serum-free supplemented medium, and expressed albumin and glucokinase activity but no DNA synthesis. Hepatocytes grown as monolayers on a collagen substrate became proliferative but did not express albumin or glucokinase activity. Koike *et al.* (1993) used both pulse and cumulative labelling of bile duct epithelium of the rat and demonstrated that these cells underwent a slower renewal rate than other parts of the gastrointestinal tract. They also showed that the renewal time for epithelial cells at the ampulla was shorter than at the intra- or extra-hepatic bile ducts.

A study of proliferation in normal adult pancreatic tissues was performed by Davidson *et al.* (1989), who demonstrated by BrdUrd labelling that there was proliferation in pancreatic beta cells in the rabbit. De Vroede *et al.* (1990) compared three methods of labelling in adult rats and investigated BrdUrd incorporation in endocrine and non-endocrine cells of the pancreas. They showed that there was no evidence of proliferation in the endocrine B cells of the islet.

*Urinogenital tissues*

Hanazono *et al.* (1990a, b) surveyed BrdUrd labelling in luminal and glandular epithelia, in periluminal, in periglandular and deep stromal regions, and in the myometrium in normal adult mice, during the oestrous cycle and during early pregnancy, in prepubertal mice, in ovariectomized mice and in young animals treated with progesterone. They observed a sharp increase in the labelling index of luminal epithelium at metoestrus and on the morning of pro-oestrous, indicating extensive proliferation in the absence of oestrogen stimulation. The change in the labelling index in adult mice was more evident in the luminal epithelium than in the glandular epithelium. Jablonka-Shariff *et al.* (1993) determined growth and cellular proliferation of ovine corpora lutea throughout the oestrous cycle. Their data suggested that growth of the ovine corpora lutea was extremely rapid from days 2–12 due to hyperplasia of, primarily, nonsteroidogenic cells, mostly endothelial.

*Mammary tissue*

Sapino *et al.* (1990) measured amounts of alpha smooth muscle actin in epithelial cells, keratin in luminal epithial cells, alpha lactalbumin and b-casein in secretory cells and followed organogenesis in mouse mammary glands from virgin, primed, and lactating animals, and in glands cultured under specific hormonal stimulation. Proliferation was found mainly in focal areas (end buds) as undifferentiated cells. Oestrogen and progestin stimulation induced increases in proliferation of epithelial and myoepithelial cells. Christov *et al.* (1991) studied the proliferative activity of normal acinar and ductal breast epithelial cells with *in vivo* labelling using BrdUrd in 26 cases with concurrent breast carcinoma. The fraction of labelled epithelial cells declined with age of the individuals and was significantly higher in premenopausal women. In a later study, Christov *et al.* (1993) used xenografts of mammary tissue in nude mice to analyse cell proliferation.

**Proliferation as a result of injury or trauma**

Injury to tissue, in the form of surgery, ablation, or chemical trauma, is generally followed by a proliferative response in order to repair and replenish the damaged tissue zones.

*CNS*

Cockroach neuroglia can be repaired rapidly and orderly, with normal structures appearing within 14 days after selective glial damage (Smith *et al.* 1984, 1987). Smith *et al.* (1990) used incorporation of BrdUrd to determine the sequence and pattern of glial proliferation in the cockroach following selective killing of glial cells in penultimate abdominal connective tissue. They found that cell proliferation occurred in both major glial domains, the perineurium and subperineurium, with all cells in the damaged area participating in proliferation. Bernardini *et al.* (1992) performed unilateral ablation of the brachial or lumbar segment of the spinal cord of larvae of *Xenopus laevis* and also removed related ganglia, or as a control, only removed the related ganglia. With spinal cord ablation and removal of ganglia, restitution of the spinal cord and regeneration of the spinal ganglia occurred following migration of neural elements from the regenerating spinal cord. Clowry and Vrbova (1992) transplanted embryonic spinal cord grafts from rat embryo into the lumbar spinal cord of adult rats, which were depleted of sciatic motoneurons by previous nerve injury, and also implanted a soleus muscle from the leg with the proximal end of the nerve grafted to the graft site. They found that 21–90 days after transplantation, muscle fibres had regenerated with several phenotypes, including immature acetylcholinesterase-positive neurons possibly caused by the new motor innervation. Sieradzan and Vrbova (1993) used their previously reported technique (Sieradzan & Vrbova, 1989) and transplanted small solid grafts of ED-12 embryonic spinal cords, prelabelled with BrdUrd, into the host's hemicord on the side of sciatic nerve injury. Each graft was connected to a neuromuscular implant, either in a newborn rat or in a compatible adult rat. The BrdUrd labelled positive cells of embryonic origin were only observed in adult but not in developing animals.

Tsurushima *et al.* (1993) proposed that cell adhesion molecules play important roles in the host–graft interaction during reconstruction of the injured nervous system by neural transplantation. They reported the expression of adhesion molecules in migration and differentiation of donor Purkinje cells (cerebellar primordium from 14 day gestation) in the adult rat cerebellum. Purkinje cells had been labelled with BrdUrd, and tenascin was detected by a specific antibody. Tenascin was detected adjacent to the migrated Purkinje cells but not remote from that site.

Orita *et al.* (1988) followed the regeneration of cerebral vascular system and Orita *et al.* (1989) studied cerebral endothelial regeneration following cold injury of the brain. Regenerating endothelial cells were evident by the 3rd day post-injury and these cells participated in rebuilding of the blood–brain barrier. Repair of the microvascular architecture had begun by the 3rd day after injury, with haematogenous cells and reactive astrocytes participating in the regenerating process. Swales and Smith (1990) and Swales *et al.* (1992) developed histochemical methods for electron microscopical examination of insect neuroglial tissues, to study repair of the blood–brain barrier following glial damage. High resistance barrier cells did not incorporate BrdUrd, but sheath cells became labelled. They suggested that the cells may be a form of invasive, transitional blood cell producing matrix molecules for neural lamella repair.

Astrocytes are star-shaped cells of the CNS, which attach both to capillaries and to neurons, forming a supporting structural matrix. Takamiya *et al.* (1988) investigated proliferation and expression of cytoskeletal proteins in astrocytes following brain injury in rats. They proposed that astrocytes in the molecular layer of the cortex and white matter adjacent to the lesion proliferated in response to injury and expressed vimentin transiently, then acquired glial fibrillary acid protein (GFAP). Takemoto *et al.* (1989) found that GFAP+ glial cells proliferated between days 1 and 5 following cerebral infarct in the Mongolian gerbil. Nishino *et al.* (1993) infused thrombin into the caudate nucleus in rats and observed an infiltration of inflammatory cells, proliferation of mesenchymal cells, induction of angiogenesis, an increase in vimentin-positive reactive astrocytes, and gliosis. They suggested that antithrombin therapy following brain injury could reduce gliosis and scarring. Yu *et al.* (1993) developed a tissue culture model of astrogliosis in which mechanical injury to astrocytes induced hyperplastic and hypertrophic effects with increased GFAP synthesis. They were able to block the effect with antisense oligonucleotides to the coding region of mouse GFAP.

Schiffer *et al.* (1991, 1993) administered ethylnitrosourea to pregnant rats and then investigated the effect of injury trauma, during the 1st and 2nd month of extrauterine life, on cell proliferation in the cerebral cortex. They found that there was an increase in BrdUrd-positive cells in foetuses injured at 2 months and analysed 15 days later. Miller (1992) found a diurnal variation of cell proliferation in the neocortical ventricular zone. Prenatal exposure of foetuses to ethanol eliminates the foetal circadian rhythm of cell proliferation.

*Head and neck*

Minabe (1989) induced regeneration of acinar cells in submandibular glands of the rat by ligating excretory

ducts. Ligation of the ducts led to disorganization of the gland, with infiltration of inflammatory cells resulting in fibrosis. Major regeneration of the gland was observed at 21 days. Tsuji *et al.* (1989) induced oesophageal ulcers in the rat with acetic acid, which led to regeneration of the mucosa from day 3 to day 14. Marked proliferative activity was observed, with thickening of the oesophageal mucosa by day 14. Hashiguchi *et al.* (1993) studied the role of the autonomic nervous system in proliferation of the gastric mucosa during stress. They showed that the parasympathetic rather than the sympathetic nervous system played a role in stress-induced reduction of BrdUrd labelling in the corpus ventriculi mucosa of Wistar-Kyoto rat.

Hashino and Salvi (1993) determined the spatio-temporal pattern of DNA synthesis following noise damage to chick cochlea. They found that DNA synthesis began approximately 12 h after hair loss and that synthesis accelerated near the end of the 48 h of noise exposure. Warchol *et al.* (1993) showed that supporting cells in the vestibular epithelia from the ears of mature guinea pigs and humans proliferated following aminoglycoside antibiotic treatment that caused sensory hair cells to die. Four weeks after treatment, new cells showing characteristics of immature sensory hair cells began to reappear in culture. Takeno *et al.* (1993) found that in long term culture of guinea pig middle ear epithelium on fibroblast-reorganized hydrated collagen gels, the growth pattern resembled that which occurs in normal respiratory epithelial after mechanical injury or insult.

Hitchcock *et al.* (1992) excised small patches of retina from goldfish, using a trans-scleral surgical approach, and then followed morphology and proliferation for several weeks. They observed that the gap in the existing retina was replaced by new retina with regeneration of new neurons and neuroepithelial cells clustering at the wound margin.

### *Muscle repair*

Muscle regeneration can occur following injury, by the replacement of damaged mature muscle cells with satellite cells. These myeloblasts, which are formed rapidly during the postnatal period, fuse with muscle cells to lengthen the muscle fibres during periods of growth (Hurme & Kalimo, 1992). They examined proliferation of satellite cells in a contusive myofibre rupture in the rat. They found that proliferation occurred within one day and was extensive during the first 4 days in the necrotic zone but not in normal tissue. They showed that a majority of the myoblasts were derived from local precursors and not from surviving parts. Robertson *et al.* (1993) found that several growth factors (platelet derived growth factor, basic fibroblast growth factor, transforming growth factor-β, and leukaemia inhibitory factor), generated by macrophages which were attracted to damaged myofibres, were chemoattractants for myogenic cells and also induced proliferation in these cells.

Hanke and co-workers reported two studies of proliferation of muscle cells following induction of cardiac ischaemia. In one of these, Hanke *et al.* (1990) used balloon angioplasty to induce the ischaemia. They used α-actin labelling to identify smooth muscle cells, and found maximal proliferation in the intimal cells within 7 days while medial proliferation was delayed with a small increase at 21 days after balloon dilation. In a further study, Hanke *et al.* (1991) found that after laser excimer angioplasty, smooth muscle cells proliferated during 14 days after ablation, resulting in intimal thickening within 4 weeks. Zeymer *et al.* (1992) used balloon denudation of rat aorta to compare labelling indices obtained by PCNA, [$^3$H]dThd, and BrdUrd labelling. They found that after 7 days the intimal labelling was 46%, 42% and 40%, by the 3 methods, respectively. Tanaka, N. *et al.* (1993) also examined the effect of balloon injury on rabbit aortas. They showed that local inflammatory activation of endothelium persisted up to 30 days after injury and resulted in epithelial regeneration and migration. Hassenstein *et al.* (1992) performed holmium laser angioplasty to remove calcified intimal plaques in rabbits. The ablation resulted in an initial decrease in BrdUrd incorporation followed by a large increase in proliferation at days 7 and 14 following the angioplasty. Schiffers *et al.* (1992) reported that the arterial endometrium in young rats acted as a modulator to reduce BrdUrd incorporation in the tunical medium in denuded arteries where DNA synthesis was high.

### *Surgical injury*

Surgical injury also can induce proliferation in the zone of repair at the incision site. McDowell *et al.* (1990) used BrdUrd labelling of cells of the wound site, following wounding of the epithelium and submucosa of the hamster trachea. They were able to distinguish the boundary of the wound and found that cells distant from the wound site remained unlabelled. Miyata *et al.* (1989, 1990) showed that wound injury to cultured bovine corneal epithelium induced proliferation after 24 h which became maximal between 48 to 60 h and then decreased rapidly. Van Dierendonck *et al.* (1991) surgically removed primary tumours in a rat mammary tumour model, to determine the proliferative response in local tumour remnants, secondary implants and metastases. They found that in both slow growing and fast growing tumours, the removal of the primary tumour caused a significant rise in labelling index, and that surgical trauma served to modulate the kinetics at distant sites. Svensson & Aldskogius (1993) induced peripheral nerve injury in rats by hypoglossal nerve transection and were able to block microglial cell pro-

liferation with 1-β-D-arabinofuranosylcytosine (ara-C) infusion into the cerebrospinal fluid. Kennerknecht *et al.* (1991) found that stress due to surgical biopsy reduced BrdUrd labelling in chorionic villi, but that a brief culture period allowed recovery of labelling in these cells.

*Chemical injury*

Chemical injury has also been shown to induce proliferation in target tissues. McDowell *et al.* (1989) found that streptozoticin, which destroys islet cells of the pancreas producing mild to severe diabetes, induced proliferation of the beta cells which subsequently reduced hyperglycaemia. Hexachlorobutadiene (HCB) induced nephrotoxicity in goldfish, *Carassius auratus*, is followed by clustering of basophilic cells that incorporated BrdUrd, which elongated and fused with collecting ducts, and subsequently developed into immature nephrons (Reimschuessel *et al.*, 1990). Pan *et al.* (1990) induced duodenal ulcers in rats with cysteamine and then administered BrdUrd one hour prior to laparotomy, to follow cell kinetics during and after healing. They found that neither the ulcer scar nor regenerating mucosa proliferated as rapidly as normal mucosa. Manson *et al.* (1992) found that ethoxyquin-induced liver toxicity in the rat was both age- and sex-dependent. Savage *et al.* (1992) showed that administration of emetine dihydrochloride caused severe depletion of the number of plasma cells after day 3 in the Harderian gland of 5–12 week old chicks with a complete repopulation by days 5–7. Rahman and Tsuyama (1993) studied the reversible epidermal hyperplasia induced by cholera toxin, and found that the basal layer became 2 to 3 times thicker than normal epidermis and that labelling reached a peak during the first 24 h and returned to normal after 7 days.

*Liver regeneration*

Regeneration is the process by which part of a tissue is replaced by controlled growth following loss by injury or disease. Liver regeneration occurs when parts of the liver are removed surgically, after severe hepatic disease such as cirrhosis, or after chemical poisoning.

During postnatal development, the hepatocyte population is mostly mononuclear diploid and shifts towards tetraploidy and binucleation when the animal reaches maturity. Octaploidy and even higher ploidies are found frequently in the hepatocytes of older rats and mice. It is only during regeneration or hepatocarcinogenesis that the hepatocytes revert to lower ploidy classes, with mostly mononuclear hepatocytes being found (Saeter *et al.*, 1988). Several recent reports describing BrdUrd labelling have been used to study the complex problem of ploidy during regeneration. Frederiks *et al.* (1990) studied the relationship between ploidy class and DNA synthesis following partial hepatectomy in the rat, and found that not only mononuclear diploid cells, but also binuclear diploid, mononuclear tetraploid and binuclear tetraploid hepatocytes were in S-phase. Labelled nuclei were found mainly in the periportal zones at 24 h after partial hepatectomy. Vitale *et al.* (1991) observed that hepatocytes were present in early S-phase as early as 18 h after partial hepatectomy, and that the labelling was mainly localized in interchromatin domains of hepatocytes. Thirty-four h after partial hepatectomy, cells were in late S-phase, with specific labelling of the heterochromatic regions. Gerlyng *et al.* (1992) compared image and flow cytometry analysis of incorporated BrdUrd to calculate labelling indices for hepatocytes following partial hepatectomy. The image analysis technique using fluorescence or Feulgen staining appeared to be inferior to flow cytometry for DNA and ploidy analysis, but superior for obtaining information about binucleation and cell identification. The non-immunological method (Latt, 1973), based on BrdUrd quenching of the fluorescence of DNA-bound Hoechst dye, could only be applied to assay isolated nuclei and did not give information about nucleation.

Chemical agents such as ethanol, carbon tetrachloride and chloroform lead to liver injury and subsequent proliferation. Tanaka, Y. *et al.* (1990) used anti-desmin to identify lipocytes, and anti-BrdUrd to quantify BrdUrd incorporation, and found that the labelling index of lipocytes increased from 3.7% in controls to 25.7% at 48 h after chemical induction of proliferation. Tanaka, Y. *et al.* (1991a) demonstrated that lipocytes may differentiate to myofibroblast-like cells, since desmin and smooth muscle actin labelling was found in cells around collagen bundles near to the scar tissue formed after chemical treatment. In another study, Tanaka, Y. *et al.* (1991b) found that ethanol ingestion by rats suppressed the proliferative response of lipocytes following partial hepatectomy, but that the ethanol effect was independent of changes of vitamin A levels in the liver.

Johnson *et al.* (1992) showed expansion of desmin-positive perisinusoidal cells predominantly within the damaged pericentral zones, which reached a peak at 3–4 days after $CCl_4$ administration. The perisinusoidal response was preceded by an increased proliferation of Kupffer cells and an influx of blood monocytes. In another study by this group, Hines *et al.* (1993) investigated the response of macrophages and perisinusoidal cells during biliary cirrhosis after ligation and division of the common bile duct. Proliferation of Kupffer cells, macrophages and monocytes was shown by BrdUrd and ED1 and ED2 labels. Nakamura and Hotchi (1992) showed that acute $CCl_4$ toxicity induced proliferation first in hepatocytes and then in non-parenchymal cells after pericentral necrosis. DNA strand-breaks were also observed in non-parenchymal cells. Burr *et al.* (1993)

used BrdUrd labelling to follow the temporal and spatial distribution pattern of proliferation, and polyclonal antibodies to TGF-α, and observed that this cytokine may play a role in regeneration following $CCl_4$ toxicity. Larson *et al.* (1993) found that chloroform intoxication produced increased necrosis in liver and kidney. Mancini *et al.* (1992) studied proliferation of lipocytes induced by dimethylnitrosamine cirrhosis in rats. They showed that lipocyte proliferation was high (22.6% at day 7 and reduced to 8.5% by day 21 after chemical treatment).

Regulatory effects have also been demonstrated in animals following partial hepatectomy. Katoh *et al.* (1989) found that, after activation of the reticuloendothelial system with K-432, phagocytic activity, the level of cyclic AMP, and DNA synthesis were increased significantly following partial hepatectomy. Chamuleau *et al.* (1991) studied the role of conjugated bilirubin in liver regeneration in Gunn rats, in transport-mutant (TR-) rats, and in rats with extrahepatic biliary obstruction. Conjugated bilirubin levels were positively correlated with proliferation in the Gunn rats, but did not play a role in the other two models. Wagenaar *et al.* (1993) observed loss of the normal lobular distribution pattern of glutamine synthase and carbamoylphosphate synthase after repeated hepatectomies in the same rat. Van Noorden (1995) showed that rats on a fish oil diet had increased beta-oxidation, increased lipid peroxidation and reduced cell proliferation in periportal zones following hepatectomy. The results suggest a regulatory effect of lipid peroxidation products on cell proliferation.

Loizidou *et al.* (1991) studied the effect of trauma and regeneration on the growth of intraportally injected cancer cells. Tumours grew at the incision scar when partial hepatectomy was performed 2 days before tumour injection, and within the regenerating lobes if hepatectomy was done 4–7 days before cancer cell injection. There were two peaks of BrdUrd labelling, one confined to regenerating hepatocytes, and a second peak coinciding with the beginning of tumour growth in the regenerating lobe. Tanaka, N. *et al.* (1993a, b) showed that interleukin-2 or lymphokine-activated killer cells can destroy not only cancer cells but also syngeneic hepatocytes during regeneration. Yuasa *et al.* (1990) induced liver necrosis by administration of heat-killed *Propionibacterium acnes* to mice, which caused an infiltration of neutrophils and macrophages in the liver. An increase in proliferating neutrophils and macrophages was observed followed by a subsequent proliferation of these types of cells in the spleen.

Vroeman *et al.* (1988) examined the effect of liver regeneration on the cytokinetic behaviour of hepatocytes transplanted into the spleens of rats. They found that there was a short transient rise in the labelling index of the ectopically transplanted hepatocytes to about 10%, which returned rapidly to a control level of 3%.

**Growth factors and cell proliferation**

Growth factors are generally endogenous, have general proliferative actions (e.g., endocrine secretions such as growth hormone and insulin), or can be growth factors with a specific target cell type such as mast cell growth factor. Autocrine secretions by some cell types provide self stimulation of growth. An example is interleukin-2 secretion by T cells, which helps to enhance proliferation of these cells after either antigenic or mitogenic stimulation. Non-endogenous factors include retinoic acid, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and plant lectins. See Table 1 for a summary of studies of growth factors and target tissues in which BrdUrd incorporation has been used to analyse the proliferative action of these factors.

*ACTH and steroids*

Arola *et al.* (1993) demonstrated that ACTH caused strong inhibition of proliferation of foetal rat adrenocortical cells in the first 24 h, but provided a potent stimulation of proliferation during the following 48 h. Hanazono *et al.* (1990a, b) studied the effect of hormonal stimulation on proliferation of uterine cells in adult and prepubertal mice. A marked rise in the labelling index was found in luminal epithelium at metoestrus as well as on the pro-oestrous morning, indicating extensive cell proliferation in the absence of oestrogen stimulation. The change in labelling in adult mice was more evident in luminal epithelium than in glandular epithelium under the same conditions. In immature mice a large increase in labelling was observed in muscle layers and perimetrium.

*Epidermal growth factors (EGF) and transforming growth factor (TGF)*

Di Lorenzo and Steinberg (1990) studied the response of normal laryngeal cells and viral-induced papillomas cells to TGF-β. They observed that both cell types exhibited growth inhibition at 10 ng $ml^{-1}$ in low calcium medium, with less effect in high calcium medium. Liver fat storing cells are stimulated by TGF-α and EGF (Bachem *et al.*, 1989). In contrast, hepatocytes that were treated with diethylnitrosamine and which became gluthathione S-transferase (GST)-positive were resistant to TGF-β, but hepatocytes which were GST-negative were inhibited by TGF-β.

*Interferons*

Lagra *et al.* (1989) reported that 50 IU interferon α-2b caused an increase in cell proliferation of PHA-stimulated T lymphocytes in comparison with control individuals and patients with chronic brucellosis. Increasing the dose to 100 IU caused stimulation of T lymphocytes from anergic patients and controls.

**Table 1**. BrdUrd assay of proliferation induced by growth factors

| *Growth factors* | *Target tissue* | *References* |
|---|---|---|
| Acetylcholine | Mouse eggs | Moore *et al.* (1993) |
| ACHT | Foetal rat adrenocortex | Arola *et al.* (1993) |
| Activin A | Vascular smooth muscle | Kojima *et al.* (1993) |
| Alanine | Hamster thymus | Hagglund & Sandberg (1993) |
| Autocrine growth factors | Lung cancer | Yamaguchi *et al.* (1989) |
| b-FGF | H/N squamous cell carcinoma | Schultz-Hector & Haghayegh (1993) |
| | Pituitary adenomas, prolactin secretion | Atkin *et al.* (1993) |
| b-FGF/TGF-β | Chick embryonic bone cells | Lundy *et al.* (1991) |
| Bile acids | Gastric epithelium | Murata *et al.* (1991) |
| c-*myc* | Myelo-monocytic cells | Yen *et al.* (1992) |
| | | Karn *et al.* (1989) |
| Calcitonin related peptide | Guinea pig tracheal epithelium | White *et al.* (1993) |
| Calcium | Sigmoid colon | Kleibeuker *et al.* (1993) |
| Calmodulin | Rat kidney | Lopez-Girona *et al.* (1992) |
| Cardiac growth factor | Chick embryo cardiac myoblasts | Nagano *et al.* (1993) |
| Cell-to-cell contact by preadipocytes | Human epidermal keratinocytes | Miyashita *et al.* (1992) |
| Cyclin A | HeLa cells | Sobczak-Thepot *et al.* (1993) |
| Dietary fibres | Rat colon | Edwards *et al.* (1992) |
| Dihydroxycholecalciferol | Avian chondrocytes | Farquharson *et al.* (1993) |
| EGF | Head/neck cancer | Ozawa *et al.* (1988) |
| EGF, PDGF | Human diploid fibroblasts | Chen & Rabinovitch (1989) |
| EGF, PHA | Kidney epithelium (LLC-PK1) | Soler *et al.* (1993) |
| Electrical stimulation | Osteoblasts, undifferentiated mesenchymal cells | Matsunaga *et al.* (1993) |
| Fasting and refeeding | Rat colon epithelium | Butler *et al.* (1988) |
| Fats, fibre | Rat colon | Lee *et al.* (1993) |
| | Rat colon | Chapkin *et al.* (1993) |
| Fatty acids short chain | Regenerating liver | Hamada (1993) |
| General growth factors | Human skin mast cells | Rein & Karasek (1992) |
| | Rabbit limbal and corneal epithelium | Kruse & Tseng (1993a,b) |
| General growth factors, serum components | Schwann cells | Stewart *et al.* (1991) |
| Glial cell growth factor | Human glial cells | Yong *et al.* (1988) |
| Glioma derived growth factor | Human astrocytes | Couldwell *et al.* (1992) |
| Glucocorticoids | Chromaffin cells | Yang *et al.* (1990) |
| Glucose | Human endothelial cells | Lorenzi *et al.* (1987) |
| GM-CSF | Human CD34+ bone marrow cells | Lardon *et al.* (1993) |
| GM-CSF, G-CSF | Bone marrow cells | Danova *et al.* (1993) |
| Growth hormone | Pancreas β-cell | Nielsen *et al.* (1989) |
| | Islet cells | Scharfmann *et al.* (1990) |
| | Rat testis | Closset *et al.* (1991) |
| | Osteoblasts | Lundy *et al.* (1991) |
| | Human osteoblast-like cells | Kassem *et al.* (1993) |
| | Lewis dwarf rat odontogenic epithelium mesenchyme | Young *et al.* (1992, 1993) |
| Hepatocyte derived growth factor | Lipocytes | Gressner *et al.* (1992) |
| Hydrocortisone | Chick chorioallantoic membrane | Desbiens *et al.* (1992) |
| IL-1, IL-2, IL-4, IL-7 | T cells | Duschl *et al.* (1992) |
| Insulin | Human alveoli | Mason *et al.* (1990) |
| | Human diploid fibroblast | Chen & Rabinovitch (1989) |
| Insulin-like growth factor | Mouse ectoplacental cone cells | Kanai-Azuma *et al.* (1993) |
| Interferon α | Lung endothelium | Lagra *et al.* (1989); |
| | | Hammar *et al.* (1992) |
| Interferon γ | Human endometrium | Tabibzadeh *et al.* (1988) |
| | Astrocytes | Yong *et al.* (1992) |
| | Rat skeletal muscle | Kelic *et al.* (1993) |
| | Regenerating liver | Sato *et al.* (1993) |
| Interleukin-2 | EBV cell line | Lantz *et al.* (1991) |
| Interleukin-4 | Human T cells | Lernbecher *et al.* (1991) |
| L-glutamate, D-aspartate | NIHG-3T3 | Bussolati *et al.* (1993) |
| Lactation | Rat bone marrow cells | Betancourt *et al.* (1992) |
| Laryngeal growth factor | Laryngeal cells | Vambutas *et al.* (1993) |

**Table 1** *continued*

| *Growth factors* | *Target tissue* | *References* |
|---|---|---|
| Lithium | Human perathyroid cells | Saxe & Gibson (1993) |
| Melatonin | Liver cells | Karasek *et al.* (1992) |
| Myogenin, MyoD | Myoblasts | Wolf *et al.* (1992) |
| Nerve cell growth factor | Anaplastic glioma | Marushige *et al.* (1992) |
| Nutritional factors | Squamous carcinoma | Frank *et al.* (1992) |
| | Colorectal cancer | Levin *et al.* (1992) |
| Oestrogen/progesterone | Mouse uterine | Hanazono *et al.* (1990a, b) |
| | Breast cancer | Scheres *et al.* (1990) |
| | Human osteoblast | Scheven *et al.* (1992) |
| Oestrogen/testosterone | Rat mammary cancer | Ormerod et al. (1993) |
| | Rat ventral prostrate | Murakoshi *et al.* (1992) |
| P70s6k | Rat embryo fibroblasts | Lane *et al.* (1993) |
| Paracrine | Rat embryo atria, ventricles | Love & Tucker (1993) |
| PDGF, TGF-β, b-FDG, LIF | Muscle precursor cells | Robertson *et al.* (1993) |
| Phenobarbital | Rat liver | Jones *et al.* (1993) |
| Pituitary factor | Uterine adenomyosis | Sakamoto *et al.* (1992) |
| Platalet derived growth factor | Human diploid fibroblast | Chen & Rabinovitch (1989) |
| Polyvinyl alcohol sponges | Transplanted rat hepatocytes | Uyama *et al.* (1993) |
| Prolactin (glycosylated, non-glycosylated) | Islet cells | Sinha & Sorenson (1993) |
| Prostaglandin E2, iloprost, TGF-β | Rat arterial endothelium | Schiffers *et al.* (1992) |
| Protein bound polysaccharide (PSK) | Mouse T cells | Hirai *et al.* (1993) |
| Reserpine | Chromaffin cells | Tischler *et al.* (1991, 1992) |
| Retinoblastoma gene product | RB cells | Goodrich *et al.* (1991) |
| Retinoic acid, Ch55 | Embryonic mouse molar | Mark *et al.* (1992) |
| Retinoic acid/vitamin A | Pituitary cancer | Roy *et al.* (1990) |
| | Regenerating hepatocytes | Tanaka *et al.* (1991a) |
| | Murine epidermis | Lutzow-Holm *et al.* (1992) |
| Seric factors | Murine erythroleukaemia cells | Mencherini *et al.* (1992) |
| Somatostatin | ECL cells | Modlin *et al.* (1992) |
| (octreotide) | Rat colonic mucosa | Pawlikowski *et al.* (1993) |
| Stem cell factor | Human fetal liver | Irani *et al.* (1992) |
| Thymic growth peptide | Guinea pig thymus | Sandberg & Ernstrom (1991) |
| Thyroid hormone | Mouse submandibular gland | Fujieda *et al.* (1993) |
| | Rat testis Sertoli cells | Van Haaster *et al.* (1993) |
| Thyroliberin | Thymus | Pawlikowski *et al.* (1992) |
| Tissue necrosis factor | Acute myeloblastic leukaemia | Kleine *et al.* (1992) |
| | Daudi lymphoma | Santavenere *et al.* (1991) |
| | Chick embryo chorioallantoic membrane | Olivo *et al.* (1992) |
| | Human astrocytes | Moretto *et al.* (1993) |
| TPA (phorbol esters) | B cell leukaemia | Stephenson *et al.* (1991) |
| | Oligodendrite | Avossa & Pfeiffer (1993) |
| | Peripheral blood lymphocytes | Vinogradov *et al.* (1991) |
| Transcription factor E2F1 | Quiescent cells | Johnson *et al.* (1993) |
| Transforming growth factor | Human laryngeal epithelial cells | Di Lorenzo & Steinberg (1990) |
| | Human neoplasms | |
| | Hepatocytes | Miller *et al.* (1992) |
| | Rat intestinal epithelium | Burr *et al.* (1993) |
| | Glomerular mesangial cells, glomerular epithelium | Ciacci *et al.* (1993) |
| | | Nitta *et al.* (1993) |
| | Avian embryo | |
| | Transgenic mouse epidermis | Sanders *et al.* (1993a,b) |
| | Hepatocytes | Sellheyer *et al.* (1993) |
| | | Stenius (1993) |

ACTH, adrenocorticotropic hormone; EGF, epidermal growth factor; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; LIF, leukaemia inhibitory factor; PDGF, platelet derived growth factor; PHA, phytohaemagglutinin; PSK, protein-bound polysaccharide; TGF, transforming growth factor; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate.

Tabibzadeh *et al.* (1988) used recombinant interferon-γ (IFN-γ) to induce HLA-DR (human lymphocyte antigen) molecules of the major histocompatibility complex to inhibit proliferation of human endometrial cells and a human endometrial carcinoma cell line (EnCa101AE) in a dose-dependent manner. Labelling with BrdUrd, Ki67, and MPM-2 (a marker for mitotic cells) was markedly lower in the cell line than in control cultures. Hammar *et al.* (1992) showed induction of tubuloreticular structures in cultured human endothelial cells by application of recombinant IFN α and β.

*Endocrine and autocrine factors*

Endocrine or autocrine factors may either stimulate or inhibit cell proliferation. Mashiba *et al.* (1981) found that peripheral blood monocytes from patients with head and neck, gastro-intestinal, uterine, or cervical cancer, or suffering from several inflammatory diseases, showed elevated cytostatic activity, whereas supernatants from cultured monocytes did not differ from those of controls. Stevenson *et al.* (1985) incubated various cancer cell lines in the presence of a tumouricidal macrophages after BrdUrd labelling. They observed that in the presence of the macrophages, the BrdUrd-labelled cohort did not progress through the cell cycle but remained in S-phase during the 12 h culture period. G1 cells did not progress into S-phase. Ackermann *et al.* (1989) showed that YAC-1 lymphoma cells were killed by polymorphonuclear leukocytes (PMNs) within the first 24 h of coculture, or were able to escape killing and eventually reach growth rates faster than control cells. The liver sinusoids also contain Kupffer cells which are cytotoxic against several cancer cell lines. Lukomska *et al.* (1991) investigated the origin of these cytotoxic cells. They transplanted a female liver into a syngeneic male Lewis rat. About 9% of the liver sinusoidal mononuclear cells from both control and transplanted liver showed mitotic activity, with no proliferating cells in the peripheral blood. The proliferating sinusoidal cells were of the male (XY) karyotype indicating that they were recruited from the blood.

Often the growth of a cell type is dependent on activity or secretion of a second cell type., i.e. endocrine or exocrine stimulation. *In vitro* culturing of the target cells in media preconditioned by previous growth of other cell types is one method of determining the origin of specific cell growth factors. Gard and Pfeiffer (1990) tested culture media, preconditioned by either type-1 astrocytes, meningeal cells, or cerebellar interneurons, on the growth of cells from intermediate stages of oligodendrite development. They found that in normal medium, no proliferation was observed, but proliferation of A2B5+O4– cells was stimulated by the above mentioned precursor cells, whereas intermediate O4+GalC– cells were only stimulated by neuronal products.

*Lymphokines and haematopoietic growth factors*

A number of factors affect the growth of specific bone marrow elements and drive proliferation of specific cell types after severe blood loss, radiation, or chemotherapy. These include factors such as granulocyte/macrophage colony-stimulating factor (GM-CSF) that drives the myeloid differentiation and growth, and the interleukins that enhance growth and differentiation of the lymphoid derivatives. Erickson-Miller *et al.* (1990) observed that M-CSF (GM-CSF) stimulated proliferation and maturation of human blood monocytes, whereas platelet-derived growth factor (PDGF), interleukin 1 α (IL1α), lipopolysaccharide (LPS) and interferon γ (IFN-γ) did not show these effects. Lardon *et al.* (1993) followed the kinetics of purified bone marrow progenitor CD34+ cells after stimulation with G-CSF or GM-CSF. They found that these populations were strongly stimulated by either or both of these factors.

*Specific cell cycle action of growth factors*

Chen and Rabinovitch (1989) used the Ho33258/BrdUrd flow cytometric assay and studied effects of platelet-derived growth factor (PDGF) , epidermal growth factor (EGF), somatomedin C (SMC), transferrin (Trf) and dexamethasone on human diploid fibroblasts in culture. They showed that PDGF and EGF regulated the proportion of cells entering the cell cycle from the quiescent state, and SMC regulated the exit of cells from G1, whereas Trf and dexamethasone had only small effects on the cell cycle. Seyschab *et al.* (1989), also using the Ho33258/BrdUrd technique, showed that recombinant interferon, bacterial lipopolysaccharide and anti-IgM mu chain antibodies stimulated a majority of human resting B cells to enter the cell cycle with one subset of B cells with a short Tc (<10 h). Goodrich *et al.* (1991) showed that the retinoblastoma gene product, a nuclear phospholipid, blocked cells at the G1/S border but did not block BrdUrd incorporation in cells already in S-phase.

*Growth stimulation by a combination of factors*

Mason *et al.* (1990) reported that EGF, endothelial cell growth supplement, and acid and basic fibroblast growth factors (a-FGF and b-FGF), but not cholera toxin, were able to induce proliferation in human alveolar type II cells, which are important in recovery of alveolar epithelium following acute lung injury. Kanai-Azuma *et al.* (1993) showed that insulin-like growth factor I and p10 day placental extracts stimulated growth and migration of ectoplacental cone (EPC) cells *in vitro*. TGF-α appeared to have no effect on the EPC cells but b-FGF and EGF stimulated migratory activi-

ties. IGF-II reduced transformation of EPC cells into trophoblast giant cells. Olivo *et al.* (1992) tested a number of angiogenic-like factors on the chick embryo chorioallantoic membrane and found that tissue necrosis factor-α (TNF-α), human angiogenic factor, and b-FGF produced angiogenic reactions in the membrane that were due to chemotaxis rather than cellular proliferation at the site. Two studies by Kruse and Tseng (1993a, b) concerned the growth modulation and differentiation of cultured rabbit corneal and limbal epithelium. In the first report, they showed that several factors (insulin, EGF, FGFa and b, and cholera toxin) were mitogenic, especially in AE-5 negative cells, and that TGF-β1 was strongly inhibitory in AE-5 positive cells. In the second study, they suggested that the cell cycle length of corneal and limbal epithelial cells was correlated with the mitogenic pathway via calcium- and phospholipid-dependent protein kinase C. The kinase was inhibited by prolonged treatment of phorbol ester tumour promoters.

## Proliferation in diseased tissue

Proliferation is increased in a number of diseases such as inflammation, autoimmune diseases and lymphoproliferative disorders. Studies in which BrdUrd was used to measure the proliferation are summarized in Table 2, which lists the disease type, tissue affected and physical or biochemical parameters analysed.

## Differentiation and development

Differentiation is the acquisition of completely individual characteristics, as occurs during the development of various functional tissues from the embryonic state. Immunohistochemical detection of incorporated BrdUrd can be used to determine proliferative characteristics of differentiating tissues, and to obtain birth dates for actual differentiation events. BrdUrd labelling can be used in tracing the course of development in the embryo or foetus, and to follow the migration of prelabelled cells to discrete zones within the developing tissue. Generally a second antigen (surface or cytoplasmic) or visual examination are the techniques employed in the identification of differentiated tissues.

### *Early development: invertebrates*

Prokop and Technau (1991) traced the development of triply labelled single precursor cells of the thoracic ventral nerve cord from the early gastrula stage to late larval stages in *Drosophila melanogaster*. Their results indicated that postembryonic cells always appeared together with embryonic cells in one clone, and that the embryonic neuroblast itself, rather than one of its progenitors, resumed proliferation as a postembryonic neuroblast. Ito and Hotta (1992) studied the spatial and temporal proliferation of postembyronic neuroblast in the central brain region of the supraoesophageal ganglion of *Drosophila melanogaster*. They observed 5 proliferating neuroblasts per hemisphere in larvae just after hatching with 4 of these in the postero-dorso region of the brain continuing to proliferate during the mid larval-early pupal stages until an estimated 800–1200 progenitor cells existed per hemisphere.

Selleck and Steller (1991) concluded that the arrival of photoreceptor axons in the larval brain of *D. melanogaster* initiates cell division, directly or indirectly, to produce lamina neurons. They demonstrated that, without axon ingrowth, lamina precursors did not enter their final S-phase but appeared to arrest in the previous G1 phase. Selleck *et al.* (1992) showed that progress through the cell cycle was regulated at the G2/M transition in the newly cellularized *Drosophila*, whereas regulation later in postembryonic neuronal precursors was at the G1/S transition. Neurogenesis in the ventral CNS of *Drosophila* was studied by Truman and Bate (1988) using Toluidine Blue staining and BrdUrd labelling. The thoracic neuromere consisting of 47 stem cells proliferated for 12 h with a cell cycle time of less than two h and produced 100 progenitor cells, whereas the abdominal neuroblasts with a Tc of less than 2 h, proliferated for 50–90 h of larval life. Neurons found during the larval stage accounted for more than 90% of the cells found in the adult ventral CNS. Ito and Hotta (1992) examined the spatio-temporal proliferation of the oesophageal ganglion of *Drosophila melanogaster* and found that the initial five proliferating neuroblasts per hemisphere in new larvae proliferated to an estimated 800–1200 per hemisphere in the post embryonic stage. Green *et al.* (1993) investigated the development of the *Drosophila* larval photoreceptor (or Bolwig's) organ and the optic lobe, and the role of *Notch* in the development of both. They found that *Notch* limits the number of cells incorporated into Bolwig's organ and that it is required for the maintenance of the epithelial characterer of the optic lobe cells during and after its invagination.

Phillips and Whittle (1993) used the *wingless* genotypes of *Drosophila* to dissect the role of this gene in late stages of wing disc development. *Wingless* activity is also required at the presumptive wing margin and is a necessary precondition for the change in proliferation patterns in this region.

Skaer (1992) examined the patterns of cell activity resulting in the generation of epithelium of Malpighian tubules of *Rhodnius prolixus* during embryogenesis. A unique cell in each tubule was shown not to enter the cell cycle, but was required for proliferation of all the other cells of that tubule, and it was found that these cells imposed on each tubule a clear proximo-distal axis.

**Table 2.** Proliferation in diseased tissue

| *Reference* | *Disease model* | *Tissue* | *Parameters* |
|---|---|---|---|
| Akiya *et al.* (1992) | Rat diabetes | Lingual papillae | LI, labelling pattern |
| Arimura (1991) | Gastric ulcer | Regenerative epithelium | LI |
| Biasco *et al.* (1992) | Gastrointestinal hyperproliferative disorders | Stomach, colon | LI, cell kinetics |
| Chono (1993) | Myelochisis | Rat neural plate | LI, labelling pattern |
| Farmer & Phillips (1993) | Familial adenomatous polyposis | Rectal mucosa | LI |
| Hadrava *et al.* (1992) | Rat hypertension | Arterial smooth muscle | LI, Tc, rate of entry into S phase |
| Hogen *et al.* (1993) | Chronic proliferative dermatitis in mice | Dermal epithelium | LI, Mac-1, CD3 |
| Hong *et al.* (1993) | *Chlonorchis sinensis* infected rats | Biliary mucosa | LI |
| Ito (1993) | Gall bladder disorders | Gall bladder, pancreatico-choledochoductal junction | LI |
| Katelaris *et al.* (1992) | GVH disease in mice | Dermal cells, fibroblasts | LI, labelling pattern |
| Katsuda *et al.* (1993) | Human atherosclerosis | Smooth muscle cells | LI, PCNA |
| Kosaka *et al.* (1990) | Familial polyposis coli | Colonic epithelium | LI |
| Kuni *et al.* (1988) | Liver cirrhosis, chronic hepatitis | Hepatocytes | LI, labelling patterns |
| Marucci *et al.* (1993) | Rat biliary obstruction | Biliary epithelium, hepatocytes | LI, labelling patterns, cytokeratins |
| Matsumoto *et al.* (1988, 1993) | Rat autoimmune, encephalomyelitis | Astrocytes, T cells | LI |
| Mazure & Grierson (1992) | Proliferative vitreoretinopathy | Bovine scleral fibroblasts, retinal pigment epithelium, retinal glia | LI, matrix formation |
| Miura *et al.* (1992) | Xeroderma pigmentosum | Fibroblasts | PCNA, DNA repair |
| Morimoto *et al.* (1991) | Skin diseases | Keratinocytes | LI |
| Nagai *et al.* (1991) | Lung disease | Fibroblasts, macrophages | LI, labelling pattern, DNA repair |
| Nishizaki *et al.* (1990) | Tuberous sclerosis | Subependymal giant cell astocytoma | LI |
| Ohara *et al.* (1992) | Anterior capsule opacification | Human lens epithelium | LI, outgrowth length |
| Ohshio 1992 | Oligodactyly | Hand plate development | Mesenchyme labelling |
| Orton *et al.* (1992) | Hypoxia in calves | Pulmonary arterial cells | |
| Perros & Kendall-Taylor (1992) | Thyroid-associated ophthalmopathy | Porcine extraocular myoblast | IgG effect, LI |
| Poot *et al.* (1992) | Werner syndrome | Lymphoblastoid cell line | S-phase transit |
| Ren *et al.* (1991) | Immune complex nephritis | Glomerular macrophages | LI, labelling patterns, ED1 antibody |
| Risio *et al.* (1991) | Nonfamilial neoplastia of large intestine | Rectal mucosa cells | LI, morphology |
| Sakamoto *et al.* (1992) | Murine uterine adenomyosis | Endometrial epithelium | LI |
| Scarpini *et al.* (1992) | Human diabetes | Schwann cells | LI, laminin, fibronectin, NGF receptor, HLA antigens |
| Schimizu *et al.* (1988) | Liver cirrhosis, chronic active hepatitis | Hepatocytes | LI, serum chemistry |
| Tachibana *et al.* (1993) | Spontaneously hypertensive rats | Cardiac myocytes | LI |
| Tarao *et al.* (1989) | Liver cirrhosis/HCC | Hepatocytes | LI |
| Tarao *et al.* (1991) | Primary biliary cirrhosis | Hepatocytes | HCC development |
| Tazzari *et al.* (1989) | Bovine leukaemia virus infection | Lymphocytes | LI |
| Weissman *et al.* (1992) | Limbic keratoconjunctivitis | Human conjunctival epithelium | Labelling pattern, LI |
| Wheeler *et al.* (1993) | Glomerulonephritis in mouse | Capsular, tubular, and tuft cells | LI |
| Wilson *et al.* (1990) | Colonic polyps | Colonic mucosa | LI, labelling pattern, Tpot |
| Witzig *et al.* (1988) | Monoclonal gammopathies | Peripheral B cells | LI |
| Wolf & Michalopoulos (1992) | Hepatitis | Hepatocytes | LI, regeneration |
| Yokoi *et al.* (1988) | Diabetic corneal epithelialopathy | Basal cells | LI, cell movement |

HCC, hepatocellular carcinoma; LI, labelling index.

Baptista *et al.* (1990) demonstrated that ablation of male genitalia of the leech, *Hirudo medicinalis*, early in embryogenesis, or disruption of the nerves that connect them to ganglia, prevented birth of these neurons. They concluded that generation of the late-appearing neurons depended on a highly localized signal produced by male genitalia, to which only the ganglia that normally innervate these organs have the capacity to respond.

Plickert and Kroiher (1988) were able to follow cellular differentiation pathways with BrdUrd labelling, using macerates of several *Hydra* species. They were also able to trace cellular lineages and migration pathways by this technique. Teragawa and Bode (1990) labelled interstitial cells of *H. vulgaris* to follow their migration in unlabelled host polyps. Migration of these cells was dependent on their position in the body column and not on their origin. Dubel and Schaller (1990) showed that differentiation to either head- or foot-specific ectodermal epithelial cells can start and be completed within the same G2 phase in *Hydra*. Neuron differentiation in *Hydra* has been assumed to follow a pathway where a multipotent stem cell among the large interstitial cells becomes committed to neuron differentiation and divides. The two daughter cells, which are post-mitotic small interstitial cells, differentiate further into neurons (Bode *et al.*, 1990). These investigators examined the neuron pathway in the lower peduncle region of *H. oligactis* and found that the neurons involved were derived from proliferating small interstitial cells, and that the pathway involved proliferating intermediates. Koizumi *et al.* (1992) showed that the nerve ring in this species is a distinct neuronal complex running circumferentially at the border between the hypostome and tentacle zone, and that it was a stable and static structure compared to the dynamic nerve net of *Hydra*.

Ettensohn and Ruffins (1993) labelled small micromeres in the embryo of the sea urchin to show that descendants of those cells did not participate in skeletogenesis in primary mesoderm-depleted larvae. They proposed that skeletogenic secondary mesenchyme cells were derived exclusively from the veg2 blastomeres and that a primary mesenchyme cell-derived signal regulates a switch in the development of the pigment cell phenotype rather than a skeletogenic one.

*Early development: amphibia*

Gaze *et al.* (1993) studied the position of the earliest optic synapses in *Xenopus* and determined the stage at which they start to develop. They found that the earliest mature optic synapses were found in the mid-diencephalic region, where the major diencephalic optic neuropils were beginning to develop. Oinuma *et al.* (1992) compared cellular differentiation in the fundic glands of adult and larval *Xenopus laevis*. They found, using accumulative labelling with BrdUrd, that the proliferative cell zone in the adult was between the surface mucous cells and mucous neck cells, whereas in the immature gland of the larvae, labelling was distributed randomly throughout the developing mucosa.

Stofer and Horn (1993) studied the relation between dates of birth of sympathetic neurons and their subsequent differentiation into cutaneous B cells and vasomotor C cells in the paravertebral ganglia 9 and 10 of the bullfrog tadpole. They established that neurogenesis and gliogenesis occurred during the tadpole stages, and that the last wave of neurogenesis in sympathetic ganglia did not give rise to a specific subclass of sympathetic neurons.

*Early development: chick embryo*

Sanders *et al.* (1993a) used computer-aided reconstruction maps of BrdUrd and PCNA labelling to follow the labelling pattern in the primitive streak and the various cell layers in gastrulating chick embryo. Generation times ranged from 2 h in the caudal region of both epiblast and mesoderm to almost 10 h in rostral regions of the gastrulating embryos. Sanders *et al.* (1993b) showed that TGF-β1 induced cell proliferation among the mesodermal populations during early development.

Seifert *et al.* (1992) suggested, on the basis of cytokinetic studies that not only angiogenesis but also vasculogenesis take place from mesenchymal precursors of differentiated endothelial cells during limb vascularization *in situ* in avian embryos, and appeared to be involved in limb bud vessel formation as well. White *et al.* (1992) provided kinetic data on the progression of chick wing bud mesoderm cells in the cell cycle by continuous BrdUrd labelling of stage 20 embryos. The authors calculated equations for estimating the fraction of unlabelled cells in G2/M which could be used to estimate durations of G2/M ($T_{G2M}$), of G1 ($T_{G1}$), and of S-phase ($T_S$). Desbiens *et al.* (1992) monitored S phase cells at day 6.5 in chick embryos and showed the importance of the spatial pattern of ectodermal and mesodermal cell proliferation during normal feather morphogenesis, and corroborated the role of epidermis both in the establishment of feather rudiments and in the cephalocaudal orientation of the feathers.

Early in development, the chick embryo hindbrain manifests an axial series of bulges, termed rhombomeres. The possible cellular development of this region of the brain may be related to early patterns of cell division (Guthrie *et al.*, 1991). They used BrdUrd labelling of proliferating cells, and Basic Fuchsin, to visualize mitotic cells, to show that S-phase nuclei within the rhombomeres were located towards the pial surface of the neuroepithelium, whereas at the rhombomere boundary, S-phase cells were located close to the ven-

tricular surface. Layer and Alber (1990) used a combination of histochemical localization of cholinesterase, peanut lectin histochemistry, BrdUrd labelling and immunolocalization of neurite-specific G4 protein antibodies to follow development of the rhombomeres of chick hindbrain. They proposed that an early rostrocaudal wave establishes major brain vesicles, and a superimposed pairwise segmentation emanates rostrally and caudally from the otic vesicle, and that segregation of the cerebellar rhombomere is a late step in development.

*CNS development*

CNS development, neurogenesis and cellular migration in mammals has been conveniently monitored by administration of BrdUrd to pregnant animals at various times during gestation. In this way it has been possible to determine birthdates of cellular events and paths of migration and differentiation during CNS development. Brown and Stanfield (1989) were able to label foetal brain tissue prior to transplantation because of the stability of BrdUrd incorporation, and could follow migration and location of the transplanted cells. Miller and Nowakowski (1988) administered BrdUrd to pregnant mice and rats and showed that after 1 h BrdUrd-positive cells were distributed throughout the CNS. One to 3 days after BrdUrd administration, intensely positive cells were found in the superficial cortical plate and less intensely labelled cells were scattered throughout the deep cortical plate, the intermediate zone, and the germinal zone. In a recent study by this group, Al-Ghoul and Miller (1993) examined the spatiotemporal sequence of migration of neurons to the principal sensory nucleus of the trigeminal nerve (PSN) of the rat by injecting BrdUrd on the 12th and 14th days of gestation (E12 and E14). Early and late neurons were distributed medially and laterally, respectively following radial glial fibres in migration. Wood *et al.* (1992) labelled the earliest generated cells of murine cerebral cortex when the cells were dividing, and then followed distribution patterns of the cells during development of the foetus. They found many of the labelled cells above the cortical plate, in the marginal zone, or below it before birth. The labelled cells had disappeared by postnatal day 21. During cortical development, neurons generated at the same time in the ventricular zone migrate out into the cortical plate and form a cortical layer (Berry & Eayrs, 1963). Götz and Bolz (1992) studied the migration of rat cortical neurons in slice cultures of embryos and postnatal rats. They found that cortical cells reached their final laminar position only in postnatal animals, whereas cells in embryos became scattered throughout the cortex. Development of the mammalian forebrain involves massive proliferation of ventricular zone cells lining lateral ventricles. Part of this region continues to proliferate in adult life as the subependymal layer (Morshead & van der Kooy, 1992). In a study to ascertain the fate of these proliferating cells, they examined proliferation and fate of mitotic cells of the subependyma of adult mice. They found that the growth fraction was 33% with a $T_S$ of 12.7 h. Following injection of replication-deficient recombinant retrovirus, they found that one of the progeny of each cell division dies. Takahashi *et al.* (1992, 1993) analysed the cytokinetic behaviour and cell number in proliferative populations involved in neocortical histogenesis in the mouse embryo on E14. They observed BrdUrd labelling in two periventricular proliferative zones in the cerebral wall. In a similar study, Del Rio and Soriano (1989) injected pregnant mice with BrdUrd to label postmitotic neuroblasts. The distribution patterns of labelled nuclei after injection at different embryonic stages followed spatiotemporal gradients of cortical and hippocampal neurogenesis previously described in [$^3$H]dThd-labelling studies. McDermott and Lantos (1990) combined thymidine autoradiography and BrdUrd immunohistochemistry to study proliferative activity of the subependymal layer in the marmoset. They found BrdUrd-incorporating cells at all postnatal ages. The labelled cells were most abundant around the anterior lateral ventricle except in neonates, where the proliferating cells were located dorsally and ventrally at the junctions of the corpus callosum and caudate nucleus.

Placzek *et al.* (1993) used antibodies to monitor expression of floor-plate differentiation in neural plate explants of early embryonic stages of rats, and found that contact-mediated signals from both the notochord and the floor plate affected directly the differentiation of neural plate cells. The response of neural plate cells to inductive signals declined with embryonic age.

In a study of the development of the rat meninx (one of the membrane coverings of the brain and spinal cord), Kamiryo *et al.* (1990) observed proliferation in the compact cell layer around the neural tube, the meninx primitiva, in 12- and 13-day foetuses. They found BrdUrd-positive cells from day 12 of gestation to day 15 post partum with the largest percentage of positive cells, 50%, in foetuses 12–13 days old, but decreasing gradually towards the neonatal period. They also found that pronounced cell proliferation in the meninx started after migration of neural crest cells.

Bourrat and Sotelo (1991) used periodic administration of BrdUrd to rats to correlate the birthdate of neurons with their ultimate location within the inferior olivary nucleus (ION). They found that ION neurons that were born at the same time became arranged as clusters in the adult, indicating that the cytoarchitecture of the neuronal nucleus was the consequence of the temporal sequence of the generation of its neurons.

Kitao *et al.* (1993) determined the sequence of origin of the different vestibulospinal projection neurons in

rats, by labelling the foetuses between days E12 and E15. When the rats reached adulthood, they were injected with fluoro-gold as a retrograde tracer into the cervical cord to identify the spinal projection neurons. They suggested that genesis of the vestibulospinal neurons was in the order of LVe, IVe, and finally MVe. In a study to determine whether neuroepithelial cells in the mammmalian CNS express neurotransmitter-synthesizing enzymes, Ma *et al.* (1992) measured glutamate decarboxylase (GAD) immunoreactivity and BrdUrd uptake in cells dissociated from E11–E18 spinal cords of the rat. The ventral-to-dorsal gradient of GAD expression in precursor cells and postmitotic neuroblasts correlated anatomically and temporally with the sequential generation of motoneurons, commissural neurons, and interneurons in the dorsal horn. They suggested that spinal neuron precursors synthesize GAD-related proteins prior to or during the terminal cell cycle. Noll and Miller (1993) applied antibodies to distinguish astrocytes, oligodendrocytes and their precursors following injection of BrdUrd at day E16.5. They found that the majority of proliferating cells differentiated into astrocytes in cultures of dorsal spinal cord whereas cells in ventrally-derived cultures from the same animals differentiated into oligodendrites.

Sejvar *et al.* (1993) suggested that expression of SA-1 antigen is correlated with the ability of rat sympathoadrenal cells to proliferate in the superior cervical ganglions early in embryogenesis, to proliferate in chromaffin cells both during embryogenesis and in the adult, and to proliferate in small intensely fluorescent cells during the transient period of division in the superior cervical ganglion.

Krushel & Van der Kooy (1993) studied *in vitro* the role of cell adhesion in the organization of neurons within the rat telencephalon. Their results suggested that early (E13) but not late (E18) postmitotic striatal and cortical neurons are selectively associated in their respective zones.

### *Eye*

Rod precursor cells in the outer nuclear layer of the mature retina continuously generate photoreceptors. Knight and Raymond (1990) using double antibody labelling for BrdUrd and opsin, followed the time course of the commitment of rod precursor cells to differentiated rod receptor cells. They found that the first double-labelled, thus committed, cells appeared 4 days after BrdUrd injection. The number of double-labelled cells peaked at 10 days and then decreased. The results suggest that the rod precursor cells were probably a multipotent stem cell not committed to rod cells. In a more recent study, Braisted and Raymond (1992) investigated regeneration of dopaminergic receptors in goldfish retina following administration of 6-hydroxydopamine. After 3 weeks of the 6-hydroxydopamine administration, regenerated dopaminergic receptors as well as cones and ganglion cells were found. These results demonstrate that specific ablation of dopaminergic neurons resulted in the generation of a variety of cell types. Larison and Bremiller (1990) developed a monoclonal antibody, FRet 43, that binds specifically to the double cone photoreceptor in the embryonic zebra fish. They observed positivity with this antibody at 48 h postfertilization and found that the onset of this antigenicity occurs within 3 h after the cell division that generates the double cone photoreceptors. Their results indicate that the double cone phenotype is expressed at the cellular birthday and that the cone mosaic pattern occurs within 6 h of this expression. Thus, accurate information about the terminal mitosis of a precursor cell is useful in determining the relationship between proliferation and differentation. Repka and Adler (1992a, b) used a 'window labelling' technique to determine the timing of terminal mitosis in chick retinal precursor cells. They were able to determine the birthdates of differentiated cells within minutes to hours. Willbold and Layer (1992) demonstrated that the inner neuroblastic layer of the retinal primitive neuroepithelium of the embryonic chick is composed exclusively of acetylcholine esterase positive cells representing a primary differentiation zone of the retina.

Watanabe and Raff (1990) described a system of reaggregated embryonic rat cells in culture, in which retinal neuroepithelial cells give rise to rod photoreceptors at a similar schedule *in vitro* and *in vivo*. Ye *et al.* (1993) double-labelled allotransplanted retinal pigment epithelial cells to follow their migration in Bruch's membrane in albino rabbits. The cells possessed distinct basal and apical morphology and were in close contact with outer segments of the photoreceptor of the host without rejection. Fedtsova (1991) showed that lens developed from explants of both retinal and pigment epithelium of chick embryos.

Suzuki and Takeda (1991, 1993) found, using BrdUrd labelling, that the basal region in developing olfactory epithelium of mice was differentiated into either basal proper cells or globose basal cells between embryonic day 18 and postnatal day 1, and that during this period olfactory cells originate from globose basal cells and not from basal cells proper.

### *Pituitary*

Immunochemical detection of BrdUrd in proliferating anterior pituitary was applied *in vivo* by Carbajo-Perez *et al.* (1989) and *in vitro* by McNicol and Duffy (1987). Carbajo-Perez and Watanabe (1990) used the same techniques to study cellular proliferation in the anterior pituitary of the rat during the postnatal period. They observed maximum proliferation in 2-day-old animals,

with a sharp decline in BrdUrd labelling afterwards. Approximately 30% of all BrdUrd labelled cells were also positive for various pituitary hormones. Carbajo *et al.* (1992a, b) followed BrdUrd incorporation in the intermediate and marginal layers of the pituitary gland of developing rats from birth until day 14 postnatally, and found most of the proliferating cells in the marginal layer, suggesting that the marginal layer has a role as germination zone of the intermediate layer during postnatal growth.

*Haematopoietic system*

The haematopoietic system offers a unique opportunity to study not only differentiation of a specialized tissue but also migration, compartmentalization, and activation of cells of that system under a variety of autocrine, antigenic and mitogenic influences. The cells of the bone marrow, lymph nodes, thymus and spleen can be labelled with BrdUrd and then followed from one compartment to another over the course of time after either a pulse or continuous label. Continuous infusion or feeding of BrdUrd in drinking water to animals offers a tool for determining population turnover and migration. Gray (1988) examined the recirculating B cell pool after a 5-day treatment of rats with BrdUrd via drinking water. He found that 3–4% of the peripheral pool is replaced by new B cells each day (approximately 10% of the putative output of the bone marrow of the rat) while 0.3–0.6% became part of activated clones each day. Forster *et al.* (1989) combined BrdUrd measurement with that of specific B cell subset surface markers, to determine the distribution and proliferation of B cells in adult mice. They found two B cell populations in bone marrow, which proliferated and remained in the bone marrow for long periods of time. Only 20% of splenic B cells could be found to proliferate over a period of 8 days, whereas no peritoneal B cell proliferated during that period. In a second study, Forster and Rajewsky (1990) compared the turnover of splenic B cells in young and adult mice. They found that two-thirds of splenic B cells in the young adult have lifetimes from several weeks to several months, but that in 4 week old mice, the same population had a much shorter life time. Crippen and Jones (1989) used continuous perfusion of BrdCyt into mice to label bone marrow, thymus, and spleen cells. They found that after 6 days of infusion, 95% of bone marrow and thymus cells were labelled, but only 50% of the spleen population (Fig. 1). Westermann *et al.* (1989a, b) studied the organ distribution of various lympocyte subsets in lymphoid organs of the adult rat. Rocha *et al.* (1990) looked at the the precursor compartments and kinetic behaviour of both B and T cells in the adult mouse. They found that at least 10% of mature B and T lymphocytes were generated every 24 h. Furthermore, they found that while 90% of S-phase cells in thymus and bone marrow were labelled after a single BrdUrd pulse, continuous labelling with BrdUrd was necessary to label peripheral B and T lymphocytes.

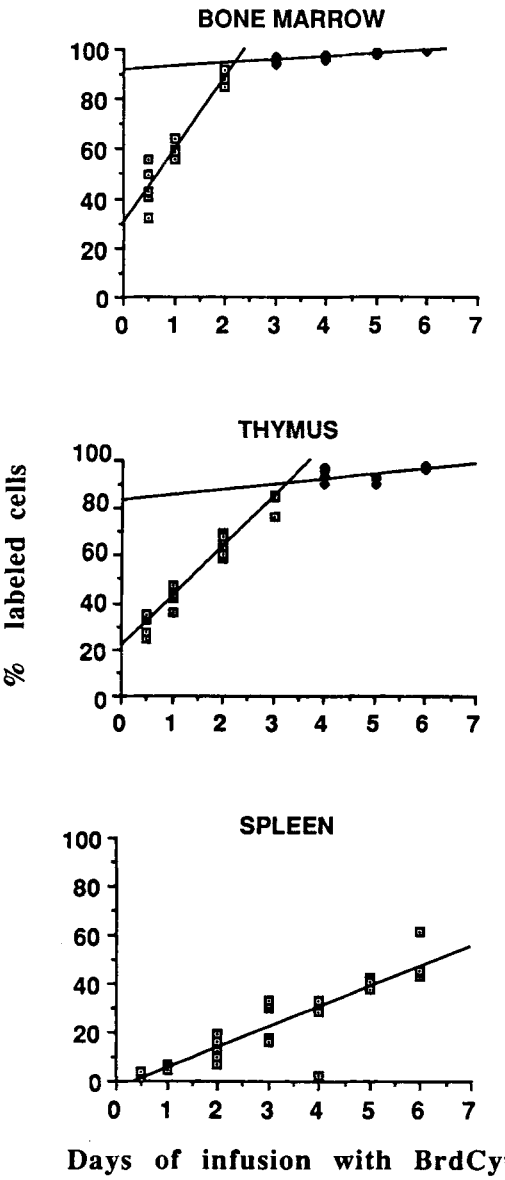


**Fig. 1.** Accumulation of BrdUrd labelled cells in bone marrow, thymus and spleen as monitored by flowe cytometry following *in vivo* exposure to BrdCyt. Each datum is presented as a percentage of the total number of events in all phases of the cell cycle and is the result of analysis of cells from one mouse. Mice were infused with BrdCyt (50 mg $h^{-1}$) for the number of days indicated. The percentage of BrdUrd-labelled cells increased as a function of exposure time. The kinetics of the accumulation of labelled cells is represented by two regression lines for bone marrow and thymus. The first regression line (■) represents those cells which become labelled within 2 days in bone marrow and within 3 days in thymus. The second regression line (●) represents those cells labelled within 6 days. All regression lines had significant slopes ($p<0.05$). From Crippen & Jones (1989). Permission granted by Blackwell Scientific Publications.

Similar results were obtained by Schittek *et al.* (1991), who labelled 87% of S-phase cells in mouse bone marrow and 85% in spleen after only 1.5 h of BrdUrd administration.

Medina *et al.* (1993) demonstrated a reduction of B lymphocyte precursors and IL-7 responsive cells by day 6.5 in normal pregnant mice. They found production and transport of B lymphocytes from the bone marrow. Deenen and Kroese (1993) used continuous labelling of Thy-1+ B cells which represented newly formed bone marrow derived B cells. They found that most of the Thy-1+ B cells were labelled by BrdUrd within a period of 8–16 days, indicating that all of these cells are relatively short lived. Chan and MacLennan (1993) found that a cohort of newly produced virgin B cells, originating in the bone marrow, arrived in the spleen in non-immunized rats and survived less than a week in the red pulp and T zones of the spleen, but that these represented only a small proportion of the splenic B cells in the adult.

The thymus represents both origin and the site of selection for T-cells. Thymus differentiation and T-cell maturation have been described by Penit and co-workers in numerous reports in which they used BrdUrd incorporation to follow the proliferation of various cell populations of the thymus. Penit (1986, 1988) and Penit & Vasseur (1988) followed murine thymus regeneration for 3 days during treatment with hydroxyurea or demicolcine to deplete the thymus of all cycling cells. New cells rapidly entered the cell cycle that were negative for both L3T4+ and BrdUrd then became positive for L3T 4+ and finally positive for BrdUrd. Cycling cells in the regenerating thymus were located first at the corticomedullary junction and then in the subcapsular region, suggesting a reverse migration process to that observed after cessation of proliferation. In a subsequent study, Penit and Ezine (1989) used 6 Gy whole body irradiation to deplete murine thymus of cycling cells. These investigators found that the thymus regenerated in 2 waves at 3–10 days and again at 25–32 days. The depletion affected all subsets, but the CD4+8+ population decreased later than immature cells. When irradiation was followed immediately by intrathymic injection of $10^5$ C57B1/Ka (Thy1.2) bone marrow cells, the relapse in thymus reconstitution was not observed. In a more recent paper, Baron & Penit (1990) studied normal murine thymocytes using bivariate BrdUrd/ DNA analysis 24 h after a single injection of BrdUrd. They found that $T_c$, $T_s$, and $T_{G1}$ and $T_{G2M}$ were 10 h, 6.5 h and 1.5 h respectively. Seventy percent of all labelled cells returned to and remained in a resting state. Cell loss was not observed for cycling cells, indicating that cell death is not directly linked with the proliferative stage in thymus differentiation. Ezine *et al.* (1993) and Penit *et al.* (1995) described a novel CD45RA+CD4+ transient thymic subpopulation in various thymic and peripheral T cell subsets of MRL-lpr/lpr mice, and they suggested that this subpopulation may generate the CD4-CD8- tumour cells. Lucas *et al.* (1993) examined *in vivo* kinetics of T cell differentiation and T cell receptors with BrdUrd and two surface markers in normal murine thymus. Figure 2 demonstrates the method for simultaneous BrdUrd/surface antigen labelling. They found that progression of CD4+8+ cells to CD4+8– and CD4–8+ was restricted to cells containing high levels of T-cell receptor (TCR).

Huesmann *et al.* (1991) demonstrated in T cell receptor (TCR) transgenic mice that CD4+8+ thymocytes generally have a lifespan of 3.5 days, but that binding of TCR to thymic major histocompability complex (MHC) rescued the CD4+8+ cells from programmed death. Only 20% of those cells reach maturity. Chan *et al.* (1993) challenged the instruction model of thymocyte commitment of the CD4 helper versus CD8 cytotoxic lineage using MHC class I, and class II, double-deficient mice to provide data that suggest a double TCR-MHC molecule: the first provokes random downregulation of either CD4 or CD8 and a degree of differentiation; the second requires participation of an appropriate co-receptor, permitting end-stage differentiation. Zhou *et al.* (1993) studied the abnormal development of T cells in the thymus using TCR transgenic MRL-lpr/lpr mice. They found that in the TCR transgenic mouse, lymphoproliferation was inhibited by reduced production of CD4+CD8+TCR null thymocytes that normally

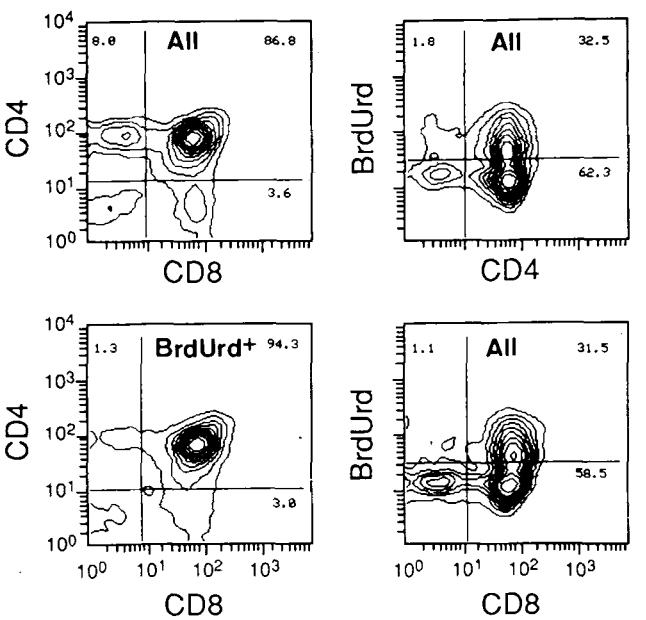


**Fig. 2.** Three-colour labelling of thymocytes. Cycling thymocytes were labelled with BrdUrd, labelled on their surface with PE-conjugated anti-CD4, and biotinylated anti-CD8 revealed using Cy-chrome-conjugated streptavidin. BrdUrd was then detected with the DNAse I technique. From Penit and Vasseur (1993). Permission granted by John Wiley & Sons, Inc.

undergo Fas-antigen mediated apoptosis. In non-transgenic mice this subpopulation was able to escape to the periphery where it could proliferate. Boehme & Lenardo (1993) showed that CD4+ and CD8+ T lymphocytes could be made susceptible to T cell receptor-mediated apoptosis if treated with IL-2 or IL-4 when the cells were in S phase but not when blocked in G1. They suggested that these T-cell growth lymphokines may participate in the down-regulation of T cell responses by an apoptosis pathway.

Mackay *et al.* (1990) addressed the way in which naive and memory T cells recirculate through the body, and the intrinsic rate of division within the naive and memory populations. They found an enrichment of 2 populations in different circulatory compartments, and that memory T cells divided whereas the naive cells did not. A similar study was performed by Schittek and Rajewsky (1990), to explain long lived memory B cells. They were able to determine whether adhering antigen maintained a proliferative memory B cell or whether the life span of the memory B cell was very long. With *in vivo* BrdUrd labelling, they showed that after an initial period of extensive proliferation, the memory B cell persisted for long periods without further division. In a subsequent study, Schittek *et al.* (1991) used BrdUrd labelling *in vivo* to demonstrate that lymphocytes at an early stage of maturation also labelled as efficiently as lymphocytes at a later stage of maturation.

T and B cell proliferation occurs following either allogenic stimulation, antigenic stimulation, endogenous cytokine stimulation, or exogenous mitogen stimulation (phytohaemagglutinin, concanavalin A). McClure & Hein (1989) assessed activation/differentiation of T cells in sheep following *in vivo* challenge by a number of alloantigens or *in vitro* with alloantigens or various mitogens. They found that BrdUrd-labelled CD4+ or CD8+ cells in the blood and lymph rose to a peak at 5–8 days, whereas the percentage of 197+ cells continued to increase even at day 14 following allogenic challenge. Purified 197+ cells were activated *in vitro* by phytohaemagglutinin and concanavalin A but not by B-cell mitogens. Matsuno *et al.* (1989) used triple labelling to resolve the role of the antigen-laden marginal metallophils (MM) and other macrophages in the intrasplenic response of specific B-cells to a thymus independent type-2 antigen, and found that the antigen specific plasma cell accumulated near the antigen-laden MM cells and ED2-positive macrophages, and were stimulated to proliferate in the periarterial lymphoid sheath. Fuller *et al.* (1993) studied antibody reponse to T cell-dependent antigens, which requires the physical interaction between antigen-specific T and B cells. They demonstrated that germinal centres must recruit and retain antigen-specific T cells for the interaction between antigen-specific T and B cells.

Resting peripheral human lymphocytes show considerable heterogeneity in response to IL-2 stimulation. Kubbies *et al.* (1990) applied the BrdUrd/Hoechst flow cytometric method to study the heterogeneous response to reducing agents. They found that α-thioglycol enhanced recruitment of $G_0$ lymphocytes with partial increase in production of IL-2, which alone did not increase lymphocyte stimulation. In another BrdUrd/Hoechst study, Lehrnbecher *et al.* (1991) showed that both IL-2 and IL-4 could drive PHA-stimulated human peripheral lymphocytes through at least one cell cycle, and that the IL-4-stimulated cells appeared to be a subset of the IL-2 stimulated population. Maggio-Price *et al.* (1993), showed that CD4+ and CD8+ T cells in the mutant anaemic mice (sphha/sphha) have increased levels of IL-2 receptor, and consequently increased proliferation, which could not be explained by differences in activation state, transmembrane signalling, accessory cells, or cytokines. Spontaneous stimulation of human T cells *in vitro* after reduction of the proportion of monocytes has been suggested by Treves *et al.* (1980). The proliferating cells were Fc receptor-negative and some were E-rosette-forming.

A number of plant lectins stimulate either B or T cells or both to proliferate within 24 h of treatment. Adriaansen *et al.* (1990) tested the proliferative effects of phytohaemagglutinin, concanavalin (Con A) pokeweed mitogen (PWM) and staphylococcal protein A on the CD4+ and CD8+ subsets of T cells *in vitro*. Both phytohaemagglutinin (PHA) and Con A gave rise to more CD8+ than CD4+ proliferating cells. PHA, but not Con A, induced B cell proliferation. SpA is also a B cell mitogen and stimulated primarily the CD4+ subset of T cells. Larramendy and Knuutila (1990) combined morphology, subset monoclonal antibody labelling and chromosome staining procedures to show that (1) the relative proportions of mitotic B and T cells are similar irrespective of the mitogen used, (2) T and B lymphocytes proliferate faster in cultures stimulated by PWM than by PHA and (3) that T cells enter mitosis sooner than B cells when PHA or PWM were used as mitogens. Schindler *et al.* (1988) used the BrdUrd/Hoechst flow cytometric method and found no correlation between age and PHA stimulatory response in humans. Conversely, PHA response was shown to be reduced in the elderly without a difference in the CD4+ or CD8+ response (De Greef *et al.*, 1992).

In the delayed hypersensitivity reaction induced in the skin of young pigs, Fritz *et al.* (1990) showed that lymphocytes, and mostly CD8+ but not CD4+ T cells, appeared to be clustered around venules, and that the labelling index of these cells was much higher than in surrounding normal skin.

Several methods have been introduced to replace the tritiated thymidine autoradiographic method for evaluation of lymphocyte activation. Huong *et al.* (1991) em-

ployed an ELISA type assay to quantitfy proliferation of human peripheral lymphocytes following antigen stimulation. Duschl *et al.* (1992) compared $^{3}$H-thymidine incorporation with a colorimetric method based on reduction of tetrazolium dyes, and immunochemical detection of incorporated BrdUrd. They found that the three methods gave similar results when lymphocytes from different individuals were stimulated with IL-2, IL-4, and IL-7 but with greater variability in the IL-4 response. Wemme *et al.* (1992) evaluated autoradiography, ELISA, and BrdUrd incorporation as methods for assaying human lymphocyte activation.

Murate *et al.* (1993) demonstrated a close relationship between replication and selection of differentiation lineages of human erythroleukaemia cell lines K562, HEL, and cytokine TF1. TPA induced commitment towards megakaryocytic differentiation, whereas the inhibitor of DNA synthesis, aphidicolin, induced cells towards the erythroid lineage.

*Myogenesis*

During differentiation of skeletal muscle, mononucleate myoblasts proliferate, stop replicating, fuse spontaneously and express a number of genes which determine the muscle phenotype. Kaufman and Robert-Nicoud (1985), using BrdUrd labelling, found that cessation of replication was a prerequisite for final myoblast differentiation as had also been found in previous studies with [$^{3}$H]dThd labelling. Kaufman and Foster (1988) studied the final replication cycles in myoblasts during the last stage of mammalian skeletal myogenesis. They found that the markers desmin and an integral membrane protein, H36, were present in the replicating myoblast prior to BrdUrd incorporation, and that these phenotypes represented a distinct stage in mammalian myogenesis. Harris *et al.* (1989) used BrdUrd to determine myonuclear birthdates, in order to distinguish primary and secondary myotubes in embryonic mammalian skeletal muscles. They concluded that primary myotubes were formed by a nearly synchronous fusion of myoblasts with a similar birthdate, but that the secondary myotubes were formed in a progressive manner. Labrecque *et al.* (1991) labelled rat myoblasts with various concentrations of BrdUrd for varying durations and found that normal myoblast fusion occurred even with myoblasts that had incorporated BrdUrd.

*Spermatogenesis*

Spermatogonia differentiate via 8–10 stages, leading to mature haploid spermatocytes. Studies have been performed with [$^{3}$H]dThd to determine cell cycle duration of the various stages, and whether cell cycle delays occur due to radiation (Clermont, 1972). Clausen *et al.* (1992) isolated testicular tissue from the blue fox (*Alopex lagopus*) following pulse labelling with BrdUrd for 35 days. Using flow cytometric methods, they were able to determine kinetic parameters of various cohorts of cells as they progressed from the stem cell stage to the haploid spermatocyte stage. They determined that the spermatogenic cycle went through the 8 phases in 12 days. BrdUrd appeared to be non-toxic, and incorporated label was not degraded during the course of the study. Miething (1993) labelled hamsters and found that M-prespermatogonial cells proliferated during post-conception days 10.5–15.5, T1-spermatogonial cells were quiescent from days 16.5–23.5, and T2-spermatogonial cells proliferated during days 24.5–27.5. Moreover, the prespermatogonial M-phase of germ cells started at least 24 h before testicular differentiation.

*Fertilization*

Brandriff and Gordon (1989) analysed the first cell cycle after fertilization of hamster eggs by human sperm. They found that S-phase began synchronously at 3–3.5 h after co-incubation of sperm and eggs and 2-cell stages appeared after 16 h. DNA was still synthesized in the pronuclei. In a timed study with BrdUrd pulses lasting from 0.5 to 5 min, the earliest incorporation sites were detectable at 2 min and were distributed throughout the pronuclei. These sites became brighter but not more numerous or differently distributed in the pronuclei during subsequent 5 or 30 minute pulses of BrdUrd (Fig 3). Nomura *et al.* (1991, 1993), examined DNA labelling patterns in the first S-phase of starfish oocytes after fertilization, during or after maturation, using confocal microscopy. They found that label was distributed uniformly at first, and condensed during maturation. Furthermore, a mosaic of fibrillar DNA patterns from the putative female region was confined to the periphery of the nucleus, and condensed sites of DNA replication were found in the male region.

Moore *et al.* (1993) tested the hypothesis that sperm-induced oocyte activation has several features in common with signal transduction pathways mediated by ligand-receptor-effector systems. It was found that treatment of mouse oocytes expressing the human m1 muscarinic receptor, a G protein-coupled receptor, resulted in the events that lead to oocyte activation in the absence of sperm.

Munaut *et al.* (1990) studied the fate of chloroplast DNA in zygote formation in *Chlamydomonas reinhardtii*, and found that mitochondrial– (mt–) nucleoids were lost within a few h of copulation, but that the mt+ DNA was retained in the large majority of zygotes. Mutations of the mat-3 types prevented selective destruction of paternal chloroplast DNA.

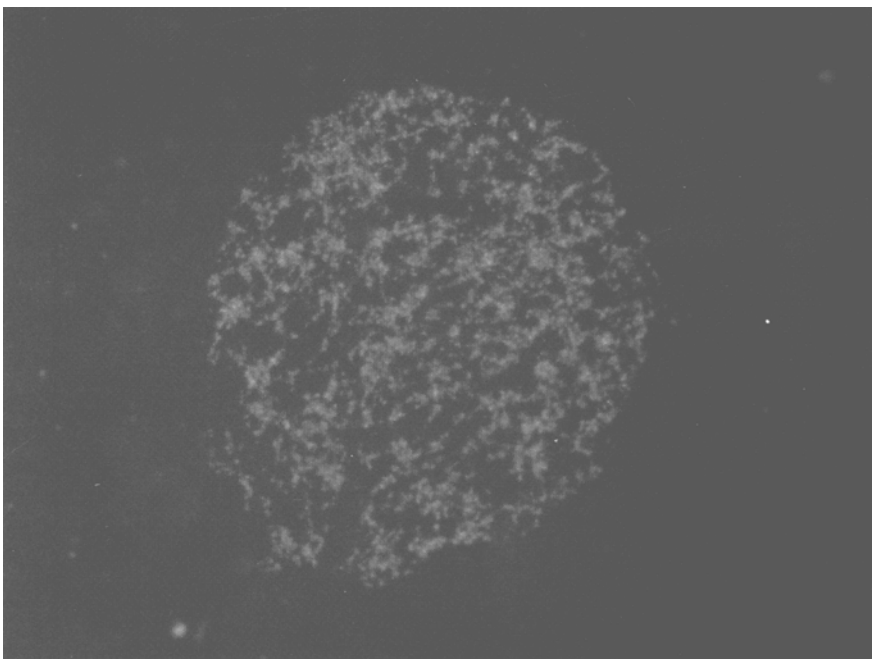

**Fig. 3.** Zygote pronucleus showing BrdUrd incorporating sites after a 5 min pulse. BrdUrd was detected using a monoclonal antibody, followed by a fluorescein-conjugated secondary antibody. From Brandriff & Gordon (1990). Permission granted by Cold Spring Harbor Laboratory Press.

*Oncogenes in development*

Oncogenes and their products play various roles in cell activation, cell cycle regulation, and differentiation (see review by Studzinski, 1989). One such example of the involvement of an oncogene in differentiation is c-*myc*. There are indications that terminal differentiation is often accompanied by a sharp reduction in the levels of the c-*myc* protein (Reitsma *et al.*, 1983; Campisi *et al.*, 1984). Farquharson *et al.* (1992a, b) found that c-*myc* levels increased 74% and 146% in the rat and chick, respectively, when proliferating chondrocytes became differentiated. Almazan & McKay (1992) suggested that conditional oncogenes can establish neural precursor cell lines which are capable of differentiation *in vitro*. They established permanent cell lines from the optic nerve of the rat by incorporation of a temperature sensitive immortalizing oncogene (Simian Virus 40 large T-antigen). At the permissive temperature (33°C) a clonal line expressed some of the properties of oligodendrocyte precursors and at the nonpermissive temperature (39°C), the cells appeared to differentiate further, expressing specific oligodendrocyte components.

Hashiro *et al.* (1991) used antisense oligomers of *c-myc* mRNA to study the inhibitory effect of *c-myc* production on human keratinocytes. They observed a 50% decrease in c-*myc* protein production and almost 80% inhibition of growth, but the oligomers did not induce differentiation in these cells, suggesting that growth and differentiation were not necessarily coupled in these cells. Hirning *et al.* (1991) used *in situ* hybridization to detect c-*myc* and N-*myc* expression in different developing tissues during mouse organogenesis, and found c-*myc* expression only during formation of cartilage, brown adipose tissue, submandibular gland, thymus, and liver, and expression of N-*myc* in brain, retina and eye lens. In gut epithelium, expression of the two oncogenes became diverse during villus formation.

## Chromosome replication patterns and DNA replication sites

BrdUrd incorporation has been used since 1973 for the assay of sister chromatid differentiation (Latt, 1973). Incorporated BrdUrd causes quenching of DNA fluorescent dyes as well as colour differentiation with Giemsa staining. The technique has been used extensively since the mid 1970s to assay sister chromatid exhange (SCE) and related chromosomal abberrations induced by various environmental agents. A survey of chromosomal aberrations and SCEs is beyond the realm of this review. A recent comprehensive survey of the applications of SCEs is the review by Tucker *et al.* (1993). A few references relevant to chromosomal banding in relationship to cell kinetics or DNA replication sites will be covered in the following section.

Replication sites and replication patterns in polytene chromosomes of *Drosophila* larvae during replication have been studied by biologists interested in the sites of initiation of DNA synthesis, as well as in the change of

patterns of DNA synthesis during S-phase (Cave, 1968; Darrow & Clever, 1970; Hägele, 1970; Arcos-Terán, 1972; Kroeger *et al.*, 1973; Achary *et al.* 1981). Generally the patterns revealed a continuous synthesis during early S-phase with a discontinuous synthesis during late S-phase. However, all of these studies were performed with [$^3$H]dThd and lacked the sensitivity of fluorescent immunochemical or gold-immunohistochemical techniques. Lau and Arrighi (1981) reported that the elements necessary for differential chromatid staining were present throughout the cell cycle and that the prematurely condensed chromosome technique allowed banding patterns for G1/S, early, middle and late S-phase replication to be established. Camargo and Cervenka (1982) suggested that DNA synthesis in chromosomes was segmented but co-ordinated on the basis of high resolution chromosome analysis. They showed also that R-bands coincided with initiation sites even in late-replicating late X and Y chromosomes. Vogel *et al.* (1985, 1986, 1989, 1990) and Vogel and Speit (1986) used first the BrdUrd-Ho33258 labelling technique and then the immunohistochemical method for localizing BrdUrd in metaphase chromosomes in the mouse and in various murine cell lines. They used immunogold labelling for analysis at the electron microscopic level. Latos-Bielenska *et al.* (1987) found the two staining techniques comparable but the immunohistochemical technique stained a few GTG bands and constitutive chromatin less intensely. Latos-Bielenska and Vogel (1990, 1992) observed that heterochromatin was mostly synthesized in early S-phase, with most of the synthesis before the initiation of G-banding. Furthermore, they found that the immunohistochemical method was as sensitive as the [$^3$H]dThd incorporation, and more sensitive than the traditional Giemsa differential staining of chromosome bands. Iannuzzi *et al.* (1989) used electron microscopy to find late-replicating segments (G + C bands) in mammalian chromosomes.

In a number of vertebrates, DNA synthesis during S-phase has been shown to be discontinuous (for a review see Holmquist, 1987). DNA synthesis appeared to cease for a period of time up to one hour in mid S-phase. Conversely, flow cytometric bivariate BrdUrd/DNA studies of Dolbeare *et al.* (1983), Dean *et al.* (1984), and Sasaki *et al.* (1986) and the chromosome banding studies of Cawood & Savage (1985) showed clearly that DNA synthesis during S-phase is continuous without interruption. Aghamohammadi & Savage (1990) used 10 min BrdUrd pulses and reversed Giemsa staining procedure (TT chromatin pale, TB chromatin dark) to establish that a clear-cut demarcation does not exist between early R-banding regions and late banding G-banding regions, and that a mid-S-phase pause does not exist. However, Van Dierendonck *et al.* (1989) proposed subdivisions of the S-phase based on distinct changes in S-phase labelling patterns with BrdUrd. Allison *et al.* (1985) observed several patterns of DNA synthesis in polytene chromosomes with the fluorescent BrdUrd technique. In early S-phase, DNA synthesis was first detected in puffs and interbands, and later in bands. Most bands appeared to initiate DNA synthesis synchronously except in the centromeric and heterochromatic regions.

Nakamura *et al.* (1986) demonstrated ring-like structures of replicating replicons in interphase nuclei of mammalian cells with immunofluorescence detection of BrdUrd. They reported that the number of replication sites (126±13.8) remained nearly constant during S-phase, and that the duration of replication for one site was approximately 1 h. Nakayasu & Berezney (1989) compared biotinylated dUTP incorporation patterns with immunocytochemically detected BrdUrd incorporation in 3T3 mouse fibroblasts and kangaroo kidney cells. They found three similar patterns of replication sites in relationship to chromatin domains within the nucleus. Fox *et al.* (1991) followed the temporal course of DNA synthesis in synchronous 3T3 cells with the aid of a charge-coupled device camera and a confocal laser scanning microscope. They showed that replication begins in a small number of interior sites in very early S-phase and then proceeds to form a large number of discrete sites throughout the nucleus. Baumann *et al.* (1992) applied algorithms to generate stereo pairs from three dimensional data, acquired with confocal laser scanning microscopy as described by Fox *et al.* (1991), to provide a map of DNA replication sites within the nuclei of mouse fibroblasts. Humbert and coworkers used BrdUrd alone or in combination with PCNA and newly developed analytical methods to characterize the distribution of newly synthesized DNA within the nucleus (Humbert & Usson, 1992; Humbert *et al.*, 1992; Usson & Humbert, 1992). They used spectral analysis for visual ordering of BrdUrd patterns, followed by k-nearest neighbour cluster technique and application of nine topographical features, to characterize DNA fluorescence patterns. Neri *et al.* (1992) used confocal microscopy and pulse labelling with BrdUrd in Swiss 3T3 cells, to detect five different staining patterns for DNA replication during S phase, with significant differences in the time, suggesting that a different number of replicons is activated at different stages of the S phase.

Manders *et al.* (1992) used two halopyrimidines, CldUrd and IdUrd and confocal microscopy to study temporal and spatial progression of DNA replication in single nuclei of Chinese hamster V79 cells. By following progression of label development in single nuclei, they were able to avoid the problem of orientation of the chromatin in different nuclei. Figure 4 shows the multicolour images of nuclei they labelled first with IdUrd and subsequently with CldUrd. They concluded that during late S-phase, replicon clusters situated in the same region are replicated synchronously and that after

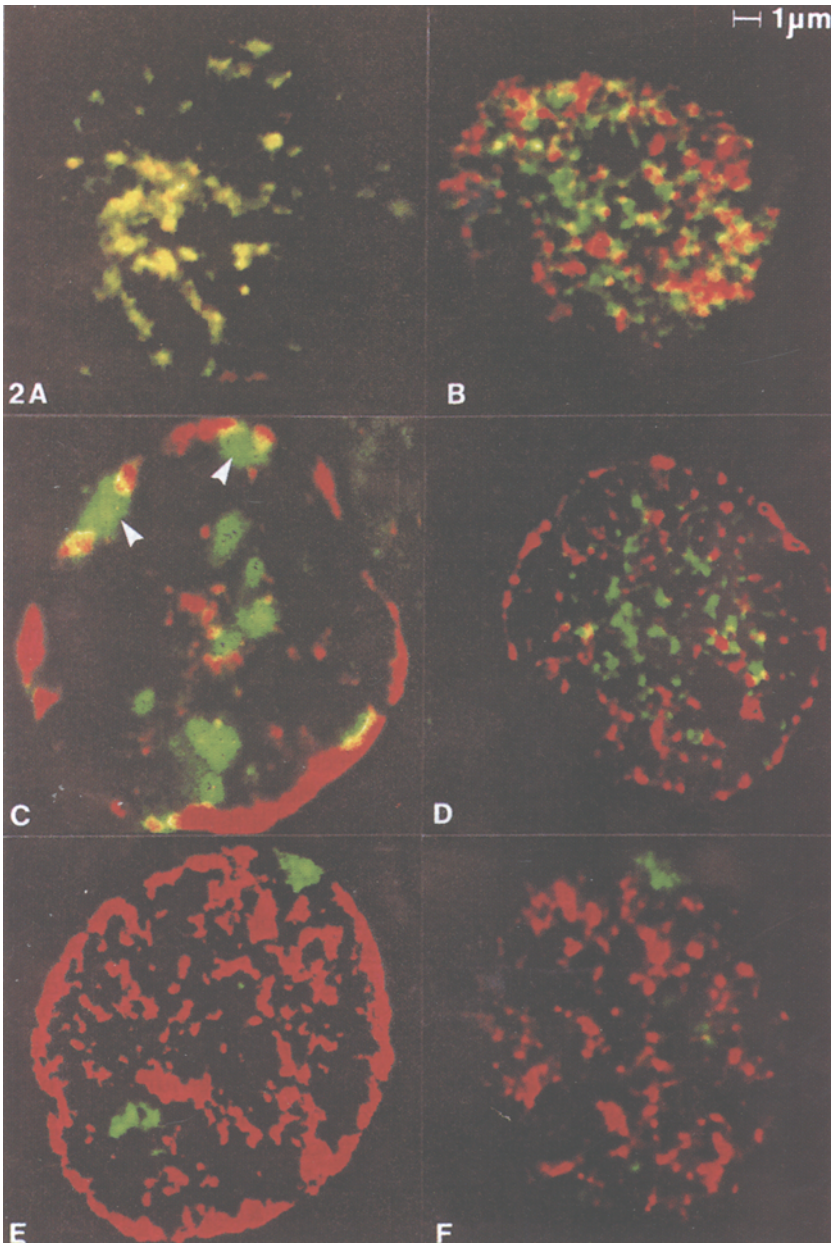


**Fig 4.** Optical sections (CSLM) through the middle of nuclei doubly labelled with two different halogenated nucleotides. The nucleus in (A) was labelled with IdUrd and CldUrd simultaneously. The time interval between the incorporation of the labels in the other nuclei was 1 h (B and C), 3 h (D and E) and 5 h (F). IdUrd-labelled DNA (first label) is depicted colour and CldUrd-labelled DNA is green. (F) shows early and late replicated DNA in the same nucleus. From Manders *et al.* (1992). Permission granted by The Company of Biologists Limited.

a period of replication there are no unreplicated clusters left in the region.

Vassilev and Russev (1988) and Vassilev and Johnson (1989) used a combination of BrdUrd labelling and polymerase chain reaction (PCR) to amplify nascent DNA strands during replication. They isolated BrdUrd-labelled initiation sites by immunoprecipitation with monoclonal antiBrdUrd. Shinomiya and Ina (1993) used 2-dimensional gel electrophoresis to map replicon and BrdUrd labelled nascent strands, to determine that DNA replication is initiated at multiple sites located within the histone gene repeat unit in early embryos of *Drosophila melanogaster*, and to show that there were several replication pause sites located at 5' upstream regions of the histone genes.

Indirect immunogold techniques were applied to analyse incorporated BrdUrd ultrastructurally (Thiry, 1988; Thiry & Drombowicz, 1988). Ultrathin sections were analysed following 22 h BrdUrd incorporation in the presence of FdUrd to examine DNA synthesis sites

in the nucleolus. They found gold particles over the perinuclear chromatin and over its intranucleolar invaginations connected with the fibrillar centres where only light labelling was found. No gold particles were seen in the dense fibrillar regions. Recently, Thiry (1992a, b) described a sensitive method using exogenous terminal deoxynucleotidyl transferase, and either biotin or digoxigenin analogues of dUTP, for the detection of replication sites within the nucleus. Immunogold staining was applied also by Mazzoti *et al.* (1990) to murine erythroleukaemia cells without acid denaturation of DNA. Using electron microscopy, they observed similar labelling patterns to those observed by fluorescent antibody staining. After 5 min labelling, 55% of the cells appeared to be positive. Rizzoli *et al.* (1992) performed immunogold labelling of 3T3 fibroblasts, and observed labelling in the interchromatin region during early S-phase, at the boundary between heterochromatin and euchromatin during middle S, and in the heterochromatin domains during late S-phase. These sites were intrepreted as single replication units being activated during different parts of S phase. About 50% of the positive cells showed labelling at the border between heterochromatin and interchromatin and 30% in the interchromatin domain. Just *et al.* (1993) applied immunoperoxidase and immunogold techniques after partial removal of histones to demonstrate replication patterns in mammalian chromosomes, but without any additional advantages over conventional cytogenetic staining methods.

## Timing of replication and gene expression

DNA sequences in chromosomes of eukaryotic cells appear to be replicated in a defined temporal order during S-phase (Taylor, 1960), and this order is maintained during subsequent cell cycles (for a review see Edenberg & Huberman, 1975). Dutrillaux *et al.* (1976) and Dutrillaux and Viegas-Pequignot (1981) applied techniques based on quenching of Hoechst or quinacrine, and Giemsa staining with BrdUrd incorporation, to determine sequences of replication of R-, Q- and G-bands in human lymphocytes. Not only are the banding patterns of lymphocyte chromosomes of man and *Cebus* analogous (Dutrillaux, 1979) but also the chronology of replication is very similar when lymphocytes from the two species were labelled for 7 h with BrdUrd (Couturier et al., 1979; Couturier & Dutrillaux, 1981). Karube *et al.* (1987), and Karube and Watanabe (1988) compared immunolabelling for BrdUrd in human lymphocyte chromosomes with locations of R- and G-banding using image analysis. They showed that early phase DNA synthesis roughly coincided with R-bands and late phase DNA synthesis with G-bands.

Reddy *et al.* (1988) examined the replication patterns of early and late replicating X chromosomes in fibroblasts and lymphocytes and found similar banding sequences and intervals. They also found that the late X had a delayed start in synthesis but proceeded at the same rate as the early X. Ved Brat et al. (1981) used continuous labelling of human skin fibroblasts beyond two replication cycles, which caused bifilar staining of some chromosome regions, which they intrepreted as late replicating regions.

Furst *et al.* (1981) used bromouracil-substituted DNA that was replicated during selected S-phase intervals and cleaved this DNA with EcoRI, subjected the fragments to agarose gel electrophoresis and then hybridized the DNA to diazobenzyloxymethyl paper with probes to the α-globin gene. They were able to demonstrate that the α-globin gene sequences were replicated early in S-phase. In a later study of this group, Calza *et al.* (1984) applied the same techniques for isolating BrdUrd labelled fragments and concluded that a gene's position in the chromosome rather than its sequence determines the time of replication. Furthermore, they showed that immunoglobulin gene rearrangements can affect the timing not only of the expressed gene but also of genes with which it is linked. Spack *et al.* (1992) performed BrdUrd density labelling of DNA in T-lymphomas and myelomonocyte cell lines, separated cells by centrifugal elutriation, and then isolated the DNA. The temporal order of replication of groups of DNA segments suggested 5 or 6 replicons with similar rates of replication. Schmidt and Migeon (1990) used BrdUrd-sensitive restriction sites and UV irradiation to identify the order of replication of HPRT and factor IX loci in mouse-human hybrids. The hypoxanthine-guanine-phosphoribosyl-transferase (HPRT) gene was replicated early in S-phase when located on the active X chromosome, but late in S-phase when located on the inactive X chromosome. Gonzalez-Fernandez *et al.* (1992) also used UV irradiation to inactivate BrUrd-substituted DNA, to show that portions of the genome replicated in late S phase had a positive effect on initiation of S phase synthesis, while DNA replicated in early S had a negative effect, and replication in mid S phase had no regulatory effect. Haase and Calos (1991) showed that centromeric alpha sequences, which normally replicate late in S phase when they are present in their chromosomal context, replicate earlier when they mediated replication of an extrachromosomal vector.

Sorscher *et al.* (1993) examined the transformed hamster fibroblast cell line BP6T using caesium chloride gradients and hybridization to investigate the correlation between expression and timing of replication of suB+ and suB– cells. Their results suggested that down-regulation of transcription was not always accompanied by a concomitant change in time of gene replication from early to late S phase. Sinnett *et al.* (1993) studied

the chronological order of replication using BrdUrd incorporation, UV photolysis of BrdUrd strands and PCR amplification of remaining strands to permit identification of non-replicated genes. Ma *et al.* (1990) were able to localize three early firing initiation loci of the dihydrofolate reductase amplicon in the Chinese hamster cell line DC3F-A3/4K, and were able to localize the termini between each of the sites and the next origin in the 5′ direction. Mah *et al.* (1993) identified two replication sequences, ors8 and ors12, of nascent CV-1 cells. Ors12 replicated almost exclusively on the matrix in early and mid S phase. Replication sequences of ors8 were also found to be enriched on the matrix in early S-phase.

Hartmann and Rode (1982) and Rode *et al.* (1983) showed, with the use of caesium chloride density chloride gradients, that the higher density DNA due to BrdUrd oversubstitution during early S-phase may influence the metabolism of BrdUrd and thus affect the timing of substitution throughout the S-phase. These findings were similar to that of Brown and Schildkraut (1979), who showed that BrdUrd substitution caused perturbation of both growth and differentiation in murine erythroleukaemia cells.

Maniotis and Schliwa (1991) removed the centrosomes by microsurgery from BSC-1 cells and followed the fate of the experimental karyoplast. They observed that the karyoplasts re-established a juxtanuclear microtubule-organizing centre, an astral array of microtubules, and a compact Golgi apparatus. The karyoplasts completed S-phase but did not regenerate centrioles or undergo cell division.

## Parental DNA strand segregation

DNA strands in mammalian cells synthesized during the same cell cycle but on different chromosomes later segregate at random (Geard, 1973; Mayron & Wise, 1976; Sinkus *et al.*, 1980). In experiments using 3H-thymidine to label proliferating cells in the tongue and intestinal epithelium, Potten *et al.* (1978) found that most of the proliferating cells segregated their DNA randomly at mitosis. Ito and McGhee (1987) found, using BrdUrd labelling to study the fate of gamete DNA in the next generation embryos of the nematode *C. elegans*, that both the number and position of fluorescent spots in the embryo indicated that gamete DNA strands segregate randomly during development. In another study, Ito *et al.* (1988) treated male mice with BrdUrd to label the germ line, and then mated these animals with unlabelled females. Sperm DNA strands in the early embryos derived from those matings could be detected by the fluorescent antibody technique. Again position and number of fluorescent spots indicated that the paternal DNA strands segregated randomly. Neff and Burke (1991) investigated the pattern of chromatid segregation in *Saccharomyces cerevisiae* by labelling the DNA of a strain auxotrophic for thymidine with BrdUrd. Chromatids of the same replicative age were distributed randomly to daughter cells at mitosis. They concluded that BrdUrd was distributed randomly due to mitotic segregation of the chromatids and not via sister chromatid exchanges.

## DNA repair

Mammalian cells possess a variety of mechanisms to restore the integrity of DNA after damage by environmental or endogenous agents. Some DNA damage can be repaired with minimal disturbance to the DNA molecule (e.g. by photoreactivation of cyclobutane pyrimidine dimers by a dimer-specific endonuclease), whereas other repair processes remove not only the lesion, but also a number of adjacent nucleotides. The standard assay for DNA repair during the last 30 years has been the incorporation of [$^3$H]dThd into DNA of non-replicating cells following damage (Rasmussen & Painter, 1964). Other methods for following DNA repair include the analysis of loss of adducts from damaged DNA (Crathorn & Roberts, 1966), the use of caesium chloride density gradients (Painter & Cleaver, 1969), alkaline sucrose gradients (Elkind & Kamper, 1970; Lett *et al.*, 1970), BrdUrd photolysis (Regan & Setlow, 1971), alkaline elution to follow single strand breaks (Kohn & Grimek-Ewig, 1973), and benzoylated naphthoylated diethylaminoethyl cellulose column chromatography (Scudiero *et al.*, 1975). Ahmed & Setlow (1978), Ahmed (1980) and Ley *et al.* (1989) used the BrdUrd-photolysis technique to determine repair patch size in normal human fibroblasts and in xeroderma pigmentosum cell lines following ultraviolet irradiation. Ley *et al.* (1989) found that patch size ranged from 13 nucleotides in an RSV-10denV cell line to 95 nucleotides in normal fibroblasts. Repair appeared to be more efficient in the xeroderma pigmentosum cell lines, with 50% dimer removal in 3–6 h compared with 18 h in normal fibroblasts. Zelle *et al.* (1980) were able to show, using the BrdUrd photolysis technique, that excision repair in a xeroderma pigmentosum fibroblast strain, XP2RO, was not due to shorter lengths of repair regions but a decrease in the number of sites removed. Rosenstein *et al.* (1980b) used a combination of Hoechst 33258 and the UV photolysis technique, and Rosenstein and Rosenstein (1992) combined the BrdUrd photolysis and alkaline elution techniques for analysis of DNA repair.

Cohn & Lieberman (1984) used monoclonal antibodies to isolate DNA patches which had incorporated BrdUrd during the repair process. Russev and Boulikas (1992) combined thymidine synchronization, hydroxyurea inhibition at different intervals, *in situ* hybridization and

BrdUrd immunohistochemistry to determine that most actively transcribed genes are repaired at constant rates throughout S- and G2-phases of the cell cycle, but that non-transcribed genes appeared to be repaired at a slower rate in early S-phase but at faster rates in late S- and G2-phase. Kalle *et al.* (1993), using streptavidin-coated magnetic beads to isolate BrdUrd-labelled repair patches from the adenosine deaminase gene in UV-treated human fibroblasts, showed that after longer repair times, the active gene incorporated more label than the inactive gene. They suggested that this was consistent with the more rapid repair of cyclobutane dimers from housekeeping genes. Mullenders *et al.* (1993) also used immunoextraction to isolate BrdUrd-labelled patches during repair, to investigate repair in transcribed and non-transcribed HPRT genes in V79 Chinese hamster cells. They found no preferential repair of the 6-4 pyrimidine photoproducts at the higher UV dose (10 J $m^{-2}$). In an earlier study, Rosenstein and Setlow (1980) showed that during DNA repair in UV irradiated ICR 2A frog cells, pyrimidine dimers were long acting inhibitors of nascent DNA synthesis. Kusewitt *et al.* (1992) compared UV dimer repair in a repair-efficient murine fibroblast and in fibroblasts transfected with the bacteriophage T4 denV gene. The authors found that the transfected cells were inhibited longer in DNA synthesis, which might be attributed to the differences in excision repair of dimers by endonuclease V instead of cellular repair enzymes.

Herpes Simplex Virus (HSV) infection of mammalian cells induces unscheduled DNA synthesis. Lehtinen *et al.* (1989) were able to demonstrate DNA synthesis in human cells infected with HSV by flow cytometry. Kulomaa *et al.* (1992) compared HSV2 induced unscheduled synthesis in a virus-susceptible HeLa cell line with that in non-susceptible CaSki and C-33A cells. They found that DNA synthesis in the HeLa cells was 100-fold more efficient than in the non-susceptible cell lines. Carty *et al.* (1993) used an *in vitro* replication system of human HeLa and monkey CV-1 cells to replicate a simian virus 40-base shuttle vector plasmid. They showed that UV treatment resulted in inhibition of replication, and that replication of the UV-damaged template occurred in a normal semiconservative manner and that this replication resulted in fixation of mutations within the vector.

Berges *et al.* (1993) used androgen ablation to induce programmed cell death in androgen-dependent prostatic glandular cells. During the process of apoptosis, a number of cells in $G_0$ underwent partial DNA repair.

Navone and Raza (1987) used a combined autoradiographic [$^3$H]dThd and BrdUrd fluorescence technique to double-label proliferating P388 murine leukaemia cells. They were able to distinguish unscheduled DNA synthesis (UDS) in individual cells from S-phase DNA synthesis, and could quantify the level of repair in these cells. Rudolph and Latt (1989) used flow cytometric BrdUrd/DNA analysis to assess the kinetics of ataxia telangiectasia (AT) fibroblasts following X-ray damage. They used [$^3$H]dThd autoradiography to measure the level of repair, since the fluorescent BrdUrd assay was not sensitive enough to measure repair following 200 rads of irradiation. Normal fibroblasts, but not *at/at* cells, were prevented from entering S-phase. Rudolph *et al.* (1989) used the same technique to identify AT heterozygotes by flow cytometric measurement of X-ray damage.

Beisker and Hittelman (1988) developed a flow cytometric BrdUrd/DNA assay to study the level and rate of repair following UV irradiation of human diploid fibroblasts. Their method was based on determination of the increase in fluorescence of either G1 or G2/M cells above controls to quantitate incorporated BrdUrd as a measure of DNA repair. The assay permitted simultaneous detection of both repair and proliferation synthesis. They were able to detect repair following UV irradiation at 0.1 J $m^{-2}$ in a repair time of 15 min. They found that above 5 J $m^{-2}$, the repair process was saturated but that repair continued until all damage was repaired. Selden *et al.* (1993) modified this method to permit greater sensitivity as well as stoichiometry of the measurements of repair in both UV- and chemically-damaged cells. Figure 5 shows flow cytometric analysis of the increased incorporation of BrdUrd into the G0/G1 region of the BrdUrd/DNA bivariate distribution after human diploid fibroblasts (IMR-90) were exposed to 4-nitroquinoline oxide, ICR-170 (acridine orange), ethyl methane sulphonate, or methyl methane sulphonate. In an additional study, Selden *et al.* (1994) developed an *in vitro* rat hepatocyte assay for evaluating DNA repair following the application of a number of chemical mutagens and carcinogens. Srivastava *et al.* (1993) blocked the cells in the plateau stage by serum depletion, and followed repair of damage by UV irradiation or methyl methane sulphonate (MMS) by fluorescent image analysis of incorporated BrdUrd. They suggested that the BrdUrd method was more sensitive for the short patch repair than the [$^3$H]dThd method in the MMS studies.

### *In situ* hybridization

*In situ* hybridization has been used extensively in recent years to identify chromosomal locations of specific genes or DNA sequences. BrdUrd technology has been applied to these studies in three ways: (1) labelling of DNA with BrdUrd, followed by isolation of labelled DNA fragments by density sedimentation and identification on filters; (2) production of specific DNA or RNA probes with incorporated BrdUrd; (3) determination of cell proliferation by BrdUrd incorporation in combina-

tion with *in situ* hybridization of specific genes with specific probes.

Using method 1, Doggett *et al.* (1988) pulse-labelled C3H 10T1/2 cells with BrdUrd for various periods of time throughout S-phase. They then used $^{32}P$ probes to identify isolated fragments from early, middle or late S-phase. They found that Ha-*ras* was among the first genes replicated, at the onset of S-phase. The *myc* proto-oncogene replicated later but within the first hour of S-phase. Ki-*ras*, *raf*, and *mos* were detected between 1 and 2 h after the beginning of S-phase. Selig *et al* (1992), labelled cells for 2 h with BrdUrd, separated the cells by elutriation and then digested the DNA with nucleases, and used DNA probes of the cystic fibrosis (CF) gene. They were able to map replication timing topography of DNA within the flanking DNA of the CF gene locus on chromosome 7.

Using method 2, Sakamoto *et al.* (1987) labelled M13 DNA *in vivo* with BrdUrd. Frommer *et al.* (1988) incorporated BrdUrd into highly repeated satellite sequences cloned into M13 followed by indirect immunochemical detection of a 100 bp repeat from human satellite DNA on chromosome 3. They found that the probe hybridized to the subcentromeric heterochromatin of chromosome 9 and to the centromeric chromatin of chromosomes 1, 16, 17, and 20. Jirikowski *et al.* (1989, 1990) used a combination of BrdUrd and $^{32}P$ labelled probes complimentary to oxytocin mRNA in sections of mouse hypothalamus. They showed that while both kinds of probes provided similar localization of the reaction product, only the immunocytochemical technique showed a clear cellular localization of the reaction product. BrdUrd has also been incorporated into probes using PCR (Tabibzadeh *et al.* 1991) and enzymatic labelling (Kitazawa *et al.* (1992, 1993). Kitazawa *et al.* (1989) used plasmid DNA with human inserts and incorporated BrdUrd for DNA-RNA hybridization, where 15% of the total DNA of the plasmid DNA was substituted with BrdUrd. The probes with incorporated BrdUrd were detectable on nitrocellulose filters at picogram levels. They could detect mRNA on filters and in cells using immunohistochemistry and peroxidase-labelled antibodies. Niedobitek *et al.* (1988) employed both BrdUrd and biotin-labelled probes for the detection of viral DNA in infected tissue. The method was sensitive enough to detect co-infection by two viruses when the combination labelling was used. Niedobitek *et al.* (1988, 1989) attempted to detect cytomegalovirus (CMV) and hepatitis B virus (HBV) in human liver. BrdUrd-labelled probes showed CMV but not HBV. BrdUrd was a useful alternative for biotin probes for detecting viral DNA in liver, because of the high background produced by endogenous liver biotin. Scippo *et al.* (1989) were able to avoid the use of radiolabelled precursors for very sensitive detection of both DNA and RNA in human testis and placenta using probes with free ends contain-

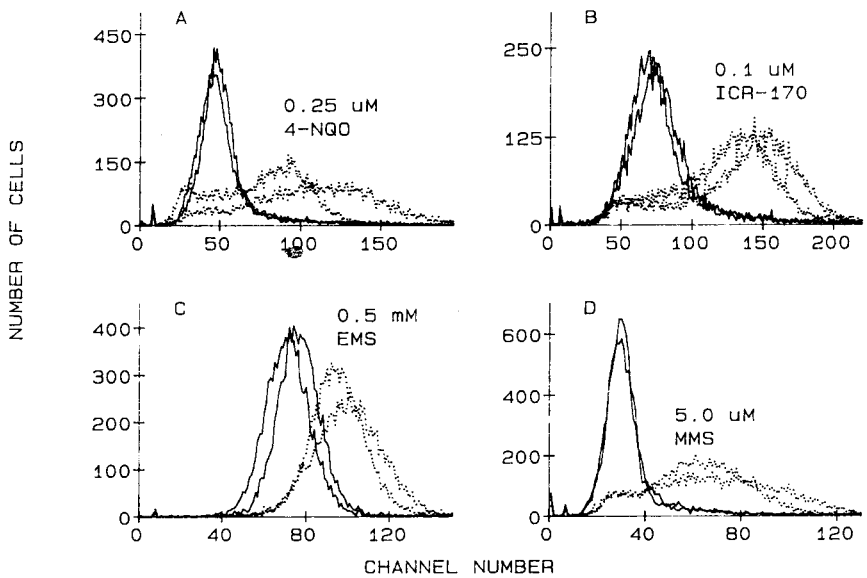


**Fig. 5.** Four composite graphs displaying univariate green fluorescence distribution of $10^4$ cells from pairs of control and chemically-treated cultures receiving doses near the limits of detection for each compound. Graphs plot number of cells (Y-axis) per fluorescence channel (X-axis, 256-channel resolution). **A** presents cultures dosed with 0.25 μM 4-nitroquinoline oxide (4-NQO); **B** presents cultures dosed with 0.1 μM acridine orange (ICR-170); **C** presents cultures dosed with 500 μM ethyl methane sulphonate (EMS); **D** presents cultures dosed with 5.0 μM methyl methane sulphonate (MMS). All samples were assayed as described by Selden *et al.* (1993). Doses of 4-NQO were incubated with cells for the entire 24 h. Distributions of treated samples were significantly different from those of controls (p, .05 for presented doses of all four compounds, t test). Distributions of control samples are represented by solid lines; distributions of treated samples are represented by dotted lines. From Selden *et al.* (1993). Permission granted by John Wiley & Sons, Inc.

ing incorporated BrdUrd, combined with immunogold and silver staining. Yao *et al.* (1993) employed BrdUrd-labelled DNA probes for mRNA detection by both fluorescence and electron microscopy in HL-60 cells. The probes were localized in the nucleus and in the endoplasmic reticulum.

Jirikowski *et al.* (1990) used BrdUrd-labelled probes to detect vasopressin mRNA in ultrathin sections in which mRNA was well-preserved. When they localized both vasopressin mRNA and vasopressin protein with antibodies, they found neurons in the magnocellular nuclei that were positive for either RNA or protein or both, indicating different stages of synthetic and secretory activity. Kitazawa *et al.* (1992) used BrdUrd-labelled cDNA probes for parathyroid hormone-related protein to identify its expression in oxyphil and transitional oxyphil cells of the normal parathyroid. Senger *et al.* (1993) used eight well-characterized probes derived from the human major histocompatibility complex class II region and were able to order the probes with antibody staining of BrdUrd in a linear fashion with distances from 200–1000 kb.

With the use of method 3, i.e. immunocytochemical labelling of incorporated BrdUrd in combination with fluorescent *in situ* hybridization, Van Dekken *et al.* (1991) labelled human bladder cancer cells with BrdUrd-labelled DNA probes to identify the chromosome number. They found that a majority of the BrdUrd-positive cells also exhibited monosomy for chromosome 9. Balazs *et al.* (1991) used a combination of DNA repetitive probes to determine chromosome number and state of cell proliferation in breast tumours. Bessereau *et al.* (1990) coupled BrdUrd labelling, to measure cell proliferation, with *in situ* hybridization for c-*fos* and c-*jun* oncogenes in denervated lower leg muscle cells in mice. They found that the oncogenes were differentially expressed following denervation, cells expressing c-*jun* being more numerous than BrdUrd-labelled cells. Biffo *et al.* (1992) combined *in situ* hybridization to localize calmodulin mRNA simultaneously with immunochemical detection of BrdUrd in embryos at day 20. Decalcified nasal mucosa was shown to be both calmodulin and BrdUrd positive. Dolbeare *et al.* (1993) used biotinylated probes for c-*myc* exon 3 to localize and analyse the level of this oncogene in two cell lines, HC-8 and LC-5, subclones of the recombinant CHO-5AHSMyc cell line that was developed by Wurm *et al.* (1986). LC-5 cells contained 10–50 copies of c-*myc* and the HC-8 cell line contained 450–1300 copies of the oncogene per cell. Flow cytometric measurements of BrdUrd/DNA/c-*myc* could distinguish high-copy cells from low-copy cells, whereas image cytometry was required to distinguish low-copy cells from background. Rosette *et al.* (1990) compared c-*myc* protein simultaneously with levels of BrdUrd incorporation and cellular DNA, and found that BrdUrd incorporation was 4.4 times lower in the high-copy C-*myc* cell line. Dirks *et al.* (1993) combined fluorescent *in situ* hybridization with BrdUrd-labelling of rat 9G cells for localization of intron and exon mRNA. They were able to detect spliced out intron sequences scattered around the nucleus.

## Acknowledgements

Part of this work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under contract no. W-7405-Eng-48.

## Disclaimer

This review has been written to provide as comprehensive a coverage of the literature as possible through a computer search of the international literature. Some omissions may unavoidably occur because abstracts were not published, or the key words proposed did not put the references within the search frame. This review may not cover works on chromosome banding or sister chromatid exchanges since these topics are reviewed elswhere. Also omitted from this review were subjects related to bromodeoxyuridine pharmacology, toxicology, or its use in radiosensitization.

## References

AARNAES, E., KIRKHUS, B. & CLAUSEN, O. P. (1990) Mathematical model analysis of mouse epidermal cell kinetics measured by bivariate DNA/anti-bromodeoxyuridine flow cytometry and continuous [3H]-thymidine labelling. *Cell Tissue Kinet.* **23**, 409–24.

AARNAES, E., CLAUSEN, O. P., KIRKHUS, B., DE ANGELIS, P. (1993) Heterogeneity in the mouse epidermal cell cycle analysed by computer simulations. *Cell Prolif.* **26**, 205–19.

ABE, Y., TAKAHASHI, J., FUKUDA, H. & YOSHIDA, K. (1992) Cell kinetic study of murine jejunal crypts during multiple doses per day. *Tohoku J. Exp. Med.* **168**, 311–15.

ACHARY, P. M., JAJUMBAR, K., DUTTAGUPTA, A. & MUKERJEE, A. S. (1981) Replication of DNA in larval salivary glands of *Drosophila* after in vivo synchronization. *Chromosoma* **82**, 505–14.

ACKERMANN, M. F., LAMM, K. R., WIEGAND, G. W. & LUSTER, M. I. (1989) Antitumour activity of murine neutrophils demonstrated by cytometric analysis. *Cancer Res.* **49**, 528–32.

ADRIAANSEN, H. J., OSMAN, C., VAN DONGEN J. J. M., WIJDENES-DE BRESSER, J. H. F. M., KAPPETIJN, VAN TILBORG C. M. J. M. & HOOIJKAAS, H. (1990) Immunological marker analysis of mitogen-induced proliferating lymphocytes using BrdU incorporation or screening of metaphases. Staphylococcal protein A is a potent mitogen for CD4+ lymphocytes. *Scand. J. Immunol.* **32**, 687–94.

AGHAMOHAMMADI, S. Z. & SAVAGE, J. R. (1990) BrdU pulse/reverse staining protocols for investigating chromosome replication. *Chromosoma* **99**, 76–82.

AHMED, F. E. (1980) The combined action of chemical carcinogens on DNA repair in human cells. *Radiat. Environ. Biophys.* **18**, 57–64.

AHMED, F. E. & SETLOW, R. B. (1978) Excision repair in ataxia telangiectasia, Fanconi's anemia, Cockayne syndrome, and Bloom's syndrome after treatment with ultraviolet radiation and *N*-acetoxy-2-acetylaminofluorene. *Biochim. Biophys. Acta* **521**, 805–17.

AKIYA, O., SERIZAWA, N., SUGIHARA, M., KATAGIRI, S. & KAWASHIMA, Y. (1992) A histological study of changes in the lingual papillae of streptozotocin-induced diabetic rats. *Bull. Tokyo Dent. Coll.* **33**, 13–24.

AL-GHOUL, W. M. & MILLER, M. W. (1993) Orderly migration of neurons to the principal sensory nucleus of the trigeminal nerve of the rat. *J. Comp. Neurol.* **330**, 464–75.

ALLISON, L., ARNDT-JOVIN, D. J., GRATZNER, H., TERNYNCK, T. & ROBERT-NICOUD, M. (1985) Mapping of the pattern of DNA replication in polytene chromosome from *Chironomus thummi* using monoclonal anti-bromodeoxyuridine antibodies. *Cytometry* **6**, 584–90.

ALMAZAN, G. & MCKAY, R. (1992) An oligodendrocyte precursor cell line on rat optic nerve. *Brain Res.* **579**, 234–45.

ARCOS-TERÁN, L (1972) Replikation und die Natur der spät replizierenden Orte im X-Chromosom von *Drosophila melanogaster*. *Chromosoma* **37**, 233–96.

ARIMURA, A. (1991) [S phase cells of regenerative epithelium in the healing process of gastric ulcer determined by an in vitro BrdU-anti BrdU method]. *Nippon Shokakibyo Gakkai Zasshi* **88**, 664–71.

AROLA, J., HEIKKILA, P. & KAHRI, A. I. (1993) Biphasic effect of ACTH on growth of rat adrenocortical cells in primary culture. *Cell Tissue Res.* **271**, 169–76.

ATKIN, S. L., LANDOLT, A. M., JEFFREYS, R. V., HIPKIN, L., RADCLIFFE, J., SQUIRE, C. R. & WHITE, M. C. (1993) Differential effects of insulin-like growth factor 1 on the hormonal product and proliferation of glycoprotein-secreting human pituitary adenomas. *J. Clin. Endocrinol. Metab.* **77**, 1059–66.

AVOSSA, D. & PFEIFFER, S. E. (1993) Transient reversion of O4+ GalC- oligodendrocyte progenitor development in response to the phorbol ester TPA. *J. Neurosci. Res.* **34**, 113–28.

BACHEM, M. G., RIESS, U. & GRESSNER, A. M. (1989) Liver fat storing cell proliferation is stimulated by epidermal growth factor/transforming growth factor alpha and inhibited by transforming growth factor beta. *Biochem. Biophys. Res. Commun.* **162**, 708–14.

BAEZA-SQUIBAN, A., ROMET, S., MOREAU, A. & MARANO, F. (1991) Progress in outgrowth culture from rabbit tracheal explants: balance between proliferation and maintenance of differentiated state in epithelial cells. *In Vitro Cell Dev. Biol.* **27A**, 453–60.

BALAZS, M., MAYALL, B. H. & WALDMAN, F. M. (1991) Interphase cytogenetics of a male breast cancer. *Cancer Genet. Cytogenet.* **55**, 243–7.

BAPTISTA, C. A., GERSHON, T. R. & MACAGNO, E. R. (1990) Peripheral organs control central neurogenesis in the leech. *Nature* **346**, 855–7

BARON, C. & PENIT, C. (1990) Study of the thymocyte cell cycle by bivariate analysis of incorporated bromodeoxyuridine and DNA content. *Eur. J. Immunol.* **20**, 1231–6.

BAUMANN, P. H., SCHORMANN, T. & JOVIN, T. M. (1992) Three-dimensional component labelling of digital confocal microscope images enumerates replication centers in BrdUrd labeled fibroblasts. *Cytometry* **13**, 220–9.

BEISKER, W. & HITTELMAN, W. N. (1988) Measurement of the kinetics of DNA repair synthesis after UV irradiation using immunochemical staining of incorporated 5-bromo-2'-deoxyuridine and flow cytometry. *Exp. Cell Res.* **174**, 156–67.

BERGES, R. R., FURUYA, Y., REMINGTON, L., ENGLISH, H. F., JACKS, T. & ISAACS, J. T. (1993) Cell proliferation, DNA repair, and p53 function are not required for programmed death of prostatic glandular cells induced by androgen ablation. *Proc. Natl. Acad. Sci. USA* **90**, 8910–14.

BERNARDINI, S., CANNATA, S. M. & FILONI, S. (1992) Spinal cord and ganglia regeneration in larval *Xenopus laevis* following unilateral ablation. *J. Hirnforsch* **33**, 241–8.

BERRY, M. & EAYRS, J. T. (1963) Histogenesis of the cerebral cortex. *Nature* **197**, 984–5.

BESSEREAU, J. L., FONTAINE, B. & CHANGEUX, J. P. (1990) Denervation of mouse skeletal muscle differentially affects the expression of the jun and fos proto-oncogenes. *New Biol.* **2**, 375–83.

BETANCOURT, M., ORTIZ, R. & GONZALEZ, C. (1992) Proliferation index in bone marrow cells from severely malnourished rats during lactation. *Mutat. Res.* **283**, 173–7.

BIASCO, G., PAGANELLI, G. M., MIGLIOLI, M. & BARBARA, L. (1992) Cell proliferation biomarkers in the gastrointestinal tract. *J. Cell. Biochem. (suppl.)* **16G**, 73–8.

BIFFO, S., VERDUN DI CANTOGNO L. & FASOLO, A. (1992) Double labeling with non-isotopic in situ hybridization and BrdU immunohistochemistry: calmodulin (CaM) mRNA expression in post-mitotic neurons of the olfactory system. *J. Histochem. Cytochem* **40**, 535–40.

BODE, H. R., GEE, L. W. & CHOW, M. A. (1990) Neuron differentiation in hydra involves dividing intermediates. *Dev. Biol.* **139**, 231–3.

BOEHME, S. A. & LENARDO, M. J. (1993) Propriocidal apoptosis of mature T lymphocytes occurs at S phase of the cell cycle. *Eur. J. Immunol.* **23**, 1552–60.

BOURRAT, F. & SOTELO, C. (1991) Relationships between neuronal birthdates and cytoarchitecture in the rat inferior olivary complex. *J. Comp. Neurol.* **313**, 509–21.

BRAISTED, J. E. & RAYMOND, P. A. (1992) Regeneration of dopaminergic neurons in goldfish retina. *Development*, **114**, 913–19.

BRANDRIFF, B. F. & GORDON, L. A. (1989) Analysis of the first cell cycle in the cross between hamster eggs and human sperm. *Gamete Res.* **23**, 299–308.

BROWN, D. B. & STANFIELD, B. B. (1989) The use of bromodeoxyuridine-immunohistochemistry to identify transplanted fetal brain tissue. *J. Neural Transplant* **1**, 135–9.

BROWN, E. H. & SCHILDKRAUT, C. L. (1979) Perturbation of growth and differentiation of Friend murine erythroleukemia cells by 5-bromodeoxyuridine incorporation in early S-phase. *J. Cell. Physiol.* **99**, 261–77.

BURR, A. W., CARPENTER, M. R., HINES, J. E., GULLICK, W. J. & BURT, A. D. (1993) Intrahepatic distribution of transforming growth factor-alpha (TGF alpha) during liver regenera-

tion following carbon tetrachloride-induced necrosis. *J. Pathol.* **170**, 95–100.

BUSSOLATI, O., UGGERI, J., ROTOLI, B. M., FRANCHI-GAZZOLA, R. & GAZZOLA, G. C. (1993) The relationship between sodium-dependent transport of anionic amino acids and cell proliferation. *Biochim. Biophys. Acta* **1151**, 153–60.

BUTLER, R. N., LAWSON, M. J., GOLAND, G. J., JARRETT, I. G. & ROBERTS-THOMSON, I. C. (1988) Proliferative activity in the proximal and distal colon of the rat after fasting and refeeding. *Immunol. Cell Biol.* **66**, 193–8.

CALZA, R.E., ECKHARDT, L.A., DELGIUDICE, T. & SCHILDKRAUT, C.L. (1984) Changes in gene position are accompanied by a change in time of replication. *Cell* **36**, 689–96.

CAMARGO, M. & CERVENKA, J. (1982) Patterns of DNA replication of human chromosomes. II. Replication map and replication model. *Am. J. Hum. Genet.* **34**, 757–80.

CAMPISI, J., GRAY, H. E., PARDEE, A.B., DEAN, M. & SONENSHEIN, G.E. (1984) Cell-cycle control of c-*myc* but not c-*ras* expression is lost following chemical transformation. *Cell* **36**, 241–7.

CARBAJO, S., GONZALEZ DEL POZO F. & CARBAJO-PEREZ, E. (1992a) Quantification of the cellular proliferation on freshly dispersed cells from rat anterior pituitaries after *in vivo* and *in vitro* labelling with bromodeoxyuridine. *Histochem. J.* **24**, 137–43.

CARBAJO, S., HERNANDEZ, J. L. & CARBAJO-PEREZ, E. (1992b) Proliferative activity of cells of the intermediate lobe of the rat pituitary during the postnatal period. *Tissue Cell* **24**, 829–34.

CARBAJO-PEREZ, E. & WATANABE, Y. G. (1990) Cellular proliferation in the anterior pituitary of the rat during the postnatal period. *Cell Tissue Res.* **261**, 333–8.

CARBAJO-PEREZ, E., MOTEGI, M. & WATANABE, Y. G. (1989) Cell proliferation in the anterior pituitary of mice during growth. *Biomed. Res* **10**, 275–81.

CARTY, M. P., HAUSER, J., LEVINE, A. S. & DIXON, K. (1993) Replication and mutagenesis of UV-damaged DNA templates in human and monkey cell extracts. *Mol. Cell. Biol.* **13**, 533–542.

CASAS, F. C. & JEFFERIES, A. R. (1992a) Estimation of S phase duration in goat epidermis by an in vivo intradermal double labelling technique using bromodeoxyuridine and tritiated thymidine. *Res. Vet. Sci.* **52**, 5–9.

CASAS, F. C. & JEFFERIES, A. R. (1992b) Comparison of intradermal injections of bromodeoxyuridine and tritiated thymidine in the in vivo measurement of epidermal cell turnover time in goats. *Res. Vet. Sci.* **52**, 10–14.

CASASCO, A., CALLIGARO, A., CASASCO, E., MARCHETTI, C., POGGI, P. & CASASCO, M. (1988) An immunocytochemical method for studying embryo cytokinetics. *Basic Appl. Histochem.* **32**, 293–6.

CASASCO, A., CALLIGARO, A., MARCHETTI, C., POGGI, P., BRUGNATELLI, S., DANOVA, M. & FIOCCA, R. (1989a) Immunocytochemical detection of proliferating cells in the rat tooth germ by monoclonal antibodies against 5-bromo-2'-deoxyuridine. *Arch. Oral Biol.* **34**, 65–9.

CASASCO, A., CALLIGARO, A., MARCHETTI, C., POGGI, P., CASASCO, M. & MENGHINI, P. (1989b) [Immunocytochemical study of the development of the oral cavity in the rat] *Bull. Group Int. Rech. Sci. Stomatol. Odontol.* **32**, 13–17.

CASASCO, A., CALLIGARO, A. & CASASCO, M. (1992) Proliferative and functional stages of rat ameloblast differentiation as revealed by combined immunocytochemistry against enamel matrix proteins and bromodeoxyuridine. *Cell Tissue Res.* **270**, 415–23.

CASASCO, A., GIORDANO, M., DANOVA, M., CASASCO, M., ICARO CORNAGLIA A. & CALLIGARO, A. (1993) PC10 monoclonal antibody to proliferating cell nuclear antigen as probe for cycling cell detection in developing tissues. A combined immunocytochemical and flow cytometric study. *Histochemistry* **99**, 191–9.

CAVE, M.D. (1968) Chromosome replication and synthesis of non-histone proteins in giant polytene chromosomes. *Chromosoma* **25**, 392–401.

CAWOOD, A. H. & SAVAGE, J. R. (1985) Uninterrupted DNA synthesis during S-phase in untransformed diploid hamster fibroblasts. *Chromosoma* **91**, 164–6.

CHAMULEAU, R. A., ARONSON, D. C., FREDERIKS, W. M., BOSMAN, D. K., SMIT, J. J., MAAS, M. A. & JANSEN, P. L. (1991) Liver regeneration after partial hepatectomy in rats with defective bilirubin conjugation or biliary excretion. *Dig. Dis. Sci.* **36**, 510–12.

CHAN, E. Y. & MACLENNAN, I. C. (1993) Only a small proportion of splenic B cells in adults are short-lived virgin cells. *Eur. J. Immunol.* **23**, 357–63.

CHAN, S. H., COSGROVE, D., WALTZINGER, C., BENOIST, C. & MATHIS, D. (1993) Another view of the selective model of thymocyte selection. *Cell* **73**, 225–36.

CHAPKIN, R. S., GAO, J., LEE, D. Y. & LUPTON, J. R. (1993) Dietary fibers and fats alter rat colon protein kinase C activity: correlation to cell proliferation. *J. Nutr.* **123**, 649–55.

CHEN, Y. & RABINOVITCH, P. S. (1989) Platelet-derived growth factor, epidermal growth factor, and insulin-like growth factor I regulate specific cell-cycle parameters of human diploid fibroblasts in serum-free culture. *J. Cell. Physiol.* **140**, 59–67.

CHONO, Y. (1993) [Overgrowth and DNA synthesis of neuroepithelium in embryonic stages of induced Long-Evans rat myeloschisis] *Hokkaido Igaku Zasshi* **68**, 8–17.

CHRISTOV, K., CHEW, K. L., LJUNG, B. M., WALDMAN, F. M., DUARTE, L. A., GOODSON, W. H., SMITH, H. S. & MAYALL, B. H. (1991) Proliferation of normal breast epithelial cells as shown by in vivo labelling with bromodeoxyuridine. *Am. J. Pathol.* **138**, 1371–7.

CHRISTOV, K., SWANSON, S. M., GUZMAN, R. C., THORDARSON, G., JIN, E., TALAMANTES, F. & NANDI, S. (1993) Kinetics of mammary epithelial cell proliferation in pituitary isografted BALB/c mice. *Carcinogenesis* **14**, 2019–25.

CIACCI, C., LIND, S. E. & PODOLSKY, D. K. (1993) Transforming growth factor beta regulation of migration in wounded rat intestinal epithelial monolayers. *Gastroenterology* **105**, 93–101.

CLAUSEN, O. P., BERG, K. A., KIRKHUS, B., DE ANGELIS, P. & HUITFELDT, H. (1992) BrdUrd incorporation studies for evaluation of spermatogenesis in the blue fox. *Cytometry* **13**, 374–80.

CLERMONT, Y. (1972) Kinetics of spermatogenesis in mammals. Seminiferous epithelium cycle and spermatogonial renewal. *Physiol. Rev.* **52**, 198–232.

CLOSSET, J., DOMBROWICZ, D., VANDENBROECK, M. & HENNEN, G. (1991) Effects of bovine, human and rat growth hormones on immature hypophysectomized rat testis. *Growth Regul.* **1**, 29–37.

CLOWRY, G. J. & VRBOVA, G. (1992) Observations on the development of transplanted embryonic ventral horn neurones grafted into adult rat spinal cord and connected to skele-

tal muscle implants via a peripheral nerve. *Exp. Brain Res.* **91**, 249–58.

COHN, S. M. & LIEBERMAN, M. W. (1984) The use of antibodies to 5-bromo-2'-deoxyuridine for the isolation of DNA sequences containing excision-repair sites. *J. Biol. Chem.* **259**, 12456–62.

CORMACK, D. H. (1984). *Introduction to Histology.* Philadelphia: J. B. Lippincott Company.

COROTTO, F. S., HENEGAR, J. A. & MARUNIAK, J. A. (1993) Neurogenesis persists in the subependymal layer of the adult mouse brain. *Neurosci. Lett.* **149**, 111–14.

COULDWELL, W. T., FRASER, G., DE VELLIS, G., ANTEL, J. P., VILLEMURE, J. G. & YONG, V. W. (1992) Malignant glioma-derived soluble factors regulate proliferation of normal adult human astrocytes. *J. Neuropathol Exp. Neurol.* **51**, 506–13.

COUTURIER, J. & DUTRILLAUX, B. (1981) Conservation of replication chronology of homologous chromosome bands between four species of the genus Cebus and man. *Cytogenet. Cell Genet.* **29**, 233–40.

COUTURIER, J., DUTRILLAUX, B., GARBER, P., RAOUL, O., CROQUETTE, M., FOURLINNIE, J. C. & MAILLARD, E. (1979). Evidence for correlation between late replication and autosomal gene inactivation in a familial translocation. *Hum. Genet.* **49**, 319–26.

CRATHORN, A. R. & ROBERTS, J. J. (1966) Mechanisms of the cytotoxic action of alkylating agents in mammalian cells and evidence for the removal of alkylated groups from deoxyribonucleic acid. *Nature* **211**, 150–2.

CRIPPEN, T. L. & JONES, I. M. (1989) Cell proliferation in the bone marrow, thymus and spleen of mice studied by continuous, in vivo bromodeoxyuridine labelling and flow cytometric analysis. *Cell Tissue Kinet.* **22**, 203–12.

CZERNIELEWSKI, J. M. & DEMARCHEZ, M. (1990) Further evidence for the self-reproducing capacity of Langerhans cells in human skin. *J. Invest. Dematol.* **88**, 17–20.

DANOVA, M., LUCOTTI, C., ROSTI, V., BERGAMASCHI, G., RICCARDI, A. & CASSOLA, M. (1993) In vivo growth characteristics of human CD34+ bone marrow cells after chemotherapy plus GM- or G-CSF. *Blood* **82** (suppl.) 497.

DARMON, E., PINCU-HORNSTEIN, A. & ROZEN, P. (1990) A rapid and simple *in vitro* method for evaluating human colorectal epithelial proliferation. *Arch. Pathol. Lab. Med.* **114**, 855–7.

DARROW, J. M. & CLEVER, U. (1970) Chromosome activity and cell function in polytene cells. III. Growth and replication. *Dev. Biol.* **21**, 331–48.

DAVIDSON, P. M., CAMPBELL, I. L., OXBROW, L., HUTSON, J. M. & HARRISON, L. C. (1989) Pancreatic beta cell proliferation in rabbits demonstrated by bromodeoxyuridine labelling. *Pancreas* **4**, 594–600.

DEAN, P. N., DOLBEARE, F., GRATZNER, H., RICE, G. C. & GRAY, J. W. (1984) Cell-cycle analysis using a monoclonal antibody to BrdUrd. *Cell Tissue Kinet* **17**, 427–36.

DEENEN, G. J. & KROESE, F. G. (1993) Kinetics of B cell subpopulations in peripheral lymphoid tissues: evidence for the presence of phenotypically distinct short-lived and long-lived B cell subsets. *Int. Immunol.* **5**, 735–41.

DE FRAISSINETTE A., STAQUET, M. J., DEZUTTER-DAMBUYANT, C., SCHMITT, D. & THIVOLET, J. (1988) Langerhans cells in S-phase in normal skin detected by simultaneous analysis of cell surface antigen and BrdU incorporation. *J. Invest. Dermatol.* **91**, 603–5.

DE GREEF, G. E., VAN STAALDUINEN, G. J., VAN DOORNINCK, H., VAN TOL, M. J. & HIJMANS, W. (1992) Age-related changes of the antigen-specific antibody formation in vitro and PHA-induced T-cell proliferation in individuals who met the health criteria of the Senieur protocol. *Mech. Ageing Dev.* **66**, 1–14.

DEL RIO, J. A. & SORIANO, E. (1989) Immunocytochemical detection of 5'-bromodeoxyuridine incorporation in the central nervous system of the mouse. *Brain Res. Dev. Brain Res.* **49**, 311–17.

DESBIENS, X., TURQUE, N. & VANDENBUNDER, B. (1992) Hydrocortisone perturbs the cell proliferation pattern during feather morphogenesis: evidence for disturbance of cephalocaudal orientation. *Int. J. Dev. Biol.* **36**, 373–80.

DE VROEDE, M. A., IN'T VELD, P. A. & PIPELEERS, D. G. (1990) Deoxyribonucleic acid synthesis in cultured adult rat pancreatic B cells. *Endocrinology* **127**, 1510–16.

DI LORENZO, T. P. & STEINBERG, B. M. (1990) Laryngeal keratinocytes show variable inhibition of replication by TGF-beta. *J. Cell Sci.* **96**, 115–19.

DIRKS, R. W., VAN DE RIJKE, F. M., FUJISHITA, S., VAN DER PLOEG, M. & RAAP, A. K. (1993) Methodologies for specific intron and exon RNA localization in cultured cells by haptenized and fluorochromized probes. *J. Cell Sci.* **104**, 1187–97.

DOGGETT, N. A., CORDEIRO-STONE, M., CHAE, C. B. & KAUFMAN, D. G. (1988) Timing of proto-oncogene replication: a possible determinant of early S phase sensitivity of C3H 10T1/2 cells to transformation by chemical carcinogens. *Mol. Carcinogen.* **1**, 41–9.

DOLBEARE, F. (1995a) Bromodeoxyuridine: a diagnostic tool in biology and medicine. Part I: Historical perspectives, histochemical methods and cell kinetics. *Histochem. J* **27**, 339–69.

DOLBEARE, F. (1995b) Bromodeoxyuridine: a diagnostic tool in biology and medicine. Part II: Oncology, chemotherapy and carcinogenesis. *Histochem. J* **27**, 923–64.

DOLBEARE, F., GRATZNER, H., PALLAVICINI, M. G. & GRAY, J. W. (1983) Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. *Proc. Natl. Acad. Sci. USA* **80**, 5573–7.

DOLBEARE, F., SELDEN, J. R. & LACOMBE, F. (1993) Non kinetic applications of the bromodeoxyuridine immunochemical technique in *DNA Cytometric Analysis* (edited by SAMPEDRO, A. & ORFAO, A.) pp. 195–218. Oviedo: Oviedo University Press.

DÜBEL, S. & SCHALLER, H. C. (1990) Terminal differentiation of ectodermal epithelial stem cells of *Hydra* can occur in G2 without requiring mitosis or S phase. *J. Cell Biol.* **110**, 939–45.

DUSCHL, A., JAHN, U., BERTLING, C. & SEBALD, W. (1992) A comparison of assays for the response of primary human T-cells upon stimulation with interleukin-2, interleukin-4 and interleukin-7. *Eur. Cytokine Network* **3**, 97–102.

DUTRILLAUX, B. (1979) Very large analogy of chromosome banding between *Cebus capucinus* (Platyrrhini) and man. *Cytogenet. Cell Genet.* **24**, 84–94.

DUTRILLAUX, B. & VIEGAS-PEQUIGNOT, E. (1981) High resolution R- and G-banding on the same preparation. *Hum. Genet.* **57**, 93–5.

DUTRILLAUX, B., COUTURIER, J., RICHER, C. L. & VIEGAS-PEQUIGNOT, E. (1976) Sequence of replication in 277 R- and Q-bands

of human chromosomes using a BrdU treatment. *Chromosoma* **58**, 51–61.

EDENBERG, H. & HUBERMAN, J. A. (1975) Eucaryotic chromosome replication. *Ann. Rev. Genet.* **9**, 245–84.

EDWARDS, C. A., WILSON, R. G., HANLON, L. & EASTWOOD, M. A. (1992) Effect of the dietary fibre content of lifelong diet on colonic cellular proliferation in the rat. *Gut* **33**, 1076–9.

EGAWA, K., YOSHIMURA, K., KAGESHITA, T. & ONO, T. (1988) [Cell kinetics in human anagen hair and hair follicles studied with bromodeoxyuridine (BrdU) and anti-BrdU monoclonal antibody] *Nippon Hifuka Gakkai Zasshi* **98**, 61–4.

ELKIND, M. M & KAMPER, C. (1970) Two forms of repair of DNA in mammalian cells following irradiation. *Biophys. J.* **10**, 237–42.

ERICKSON-MILLER, C. L., BRENNAN, J. K. & ABBOUD, C. N. (1990) Examination of survival, proliferation and cell surface antigen expression of human monocytes exposed to macrophage colony-stimulating factor (M-CSF). *Int. J. Cell Cloning* **8**, 346–56.

ETTENSOHN, C. A. & RUFFINS, S. W. (1993) Mesodermal cell interactions in the sea urchin embryo: properties of skeletogenic secondary mesenchyme cells. *Development* **117**, 1275–85.

EZINE, S., LUCAS, B., VICARI, A., DAUTIGNY, N., VASSEUR, F. & PENIT, C. (1993) A novel CD45RA+CD4+ transient thymic subpopulation in MRL-lpr/lpr mice: its relation to non-proliferating CD4-CD8-CD45RA+ tumour cells. *Int. Immunol.* **5**, 89–96.

FARMER, K. C. & PHILLIPS, R. K. (1993) Colectomy with ileorectal anastomosis lowers rectal mucosal cell proliferation in familial adenomatous polyposis. *Dis. Colon Rectum* **36**, 167–71.

FARNUM, C. E. & WILSMAN, N. J. (1993) Determination of proliferative characteristics of growth plate chondrocytes by labelling with bromodeoxyuridine. *Calcif. Tissue Int.* **52**, 110–19.

FARQUHARSON, C., HESKETH, J. E. & LOVERIDGE, N. (1992a) The proto-oncogene c-myc is involved in cell differentiation as well as cell proliferation: studies on growth plate chondrocytes in situ. *J. Cell. Physiol.* **152**, 135–44.

FARQUHARSON, C., WHITEHEAD, C., RENNIE, S., THORP, B. & LOVERIDGE, N. (1992b) Cell proliferation and enzyme activities associated with the development of avian tibial dyschondroplasia: an in situ biochemical study. *Bone* **13**, 59–67.

FARQUHARSON, C., WHITEHEAD, C. C., RENNIE, J. S. & LOVERIDGE, N. (1993) In vivo effect of 1,25-dihydroxycholecalciferol on the proliferation and differentiation of avian chondrocytes. *J. Bone Miner. Res.* **8**, 1081–8.

FEDTSOVA, N. G. (1991) [The possibility of lens formation in explants of the retina and pigment epithelium of the eye in chick embryos] *Ontogenez* **22**, 237–44.

FORSTER, I. & RAJEWSKY, K. (1990) The bulk of the peripheral B-cell pool in mice is stable and not rapidly renewed from the bone marrow. *Proc. Natl. Acad. Sci. USA* **87**, 4781–4.

FORSTER, I., VIEIRA, P. & RAJEWSKY, K. (1989) Flow cytometric analysis of cell proliferation dynamics in the B cell compartment of the mouse. *Int. Immunol.* **1**, 321–31.

FOX, M. H., ARNDT-JOVIN, D. J., JOVIN, T. M., BAUMANN, P. H. & ROBERT-NICOUD, M. (1991) Spatial and temporal distribution of DNA replication sites localized by immunofluorescence and confocal microscopy in mouse fibroblasts. *J. Cell Sci.* **99**, 247–53.

FRANK, J. L., LAWRENCE, W. J. R., BANKS, W. L. JR., MCKINNON, J. G., CHAN, W. M. & COLLINS, J. M. (1992) Modulation of cell cycle kinetics in human cancer with total parenteral nutrition. *Cancer* **69**, 1858–64.

FREDERIKS, W. M., MARX, F., CHAMULEAU, R. A., VAN NOORDEN, C. J. & JAMES, J. (1990) Immunocytochemical determination of ploidy class-dependent bromodeoxyuridine incorporation in rat liver parenchymal cells after partial hepatectomy. *Histochemistry* **93**, 627–30

FRITZ, F. J., PABST, R. & BINNS, R. M. (1990) Lymphocyte subsets and their proliferation in a model for a delayed-type hypersensitivity reaction in the skin. *Immunology* **71**, 508–16.

FROMMER, M., PAUL, C. & VINCENT, P. C. (1988) localization of satellite DNA sequences on human metaphase chromosomes using bromodeoxyuridine-labelled probes. *Chromosoma* **97**, 11–18.

FUJIEDA, M., MURATA, Y., HAYASHI, H., KAMBE, F., MATSUI, N. & SEO, H. (1993) Effect of thyroid hormone on epidermal growth factor gene expression in mouse submandibular gland. *Endocrinology* **132**, 121–5.

FUJITA, T., YOSHIMINE, T., HAYAKAWA, T., USHIO, Y., TAKEMOTO, O., MARUNO, M., KANO, M. & MOGAMI, H. (1988) [Postnatal cell proliferation in the rat cerebrum: immunohistochemical study with bromodeoxyuridine (BrdU)] *No To Shinkei* **40**, 651–5.

FULLER, K. A., KANAGAWA, O. & NAHM, M. H. (1993) T cells within germinal centers are specific for the immunizing antigen. *J. Immunol.* **151**, 4505–12.

FURST, A., BROWN, E. H., BRAUNSTEIN, J. D., & SCHILDKRAUT, C. L. (1981) Globin sequences are located in a region of early-replicating DNA in murine erythroleukemia cells. *Proc. Natl. Acad. Sci.* USA **83**, 2924–8.

GARD, A. L. & PFEIFFER, S. E. (1990) Two proliferative stages of the oligodendrocyte lineage (A2B5+O4- and O4+GalC-) under different mitogenic control. *Neuron* **5**, 615–25.

GAZE, R. M., WILSON, M. A. & TAYLOR, J. S. (1993) Optic synapses are found in diencephalic neuropils before development of the tectum in *Xenopus*. *Anat. Embryol. (Berl.)* **187**, 27–35.

GEARD, C. R. (1973) Chromatid distribution at mitosis in cultured *Wallabia bicolor* cells. *Chromosoma* **44**, 301–8.

GERLYNG, P., STOKKE, T., HUITFELDT, H. S., STENERSEN, T., DANIELSEN, H. E., GROTMOL, T. & SEGLEN, P. O. (1992) Analytical methods for the study of liver cell proliferation. *Cytometry* **13**, 404–15.

GONZALEZ-FERNANDEZ, A., ALLER, P., SANS, J. & DE LA TORRE, C. (1992) Early and late replicating DNA involved in the G1 to S transition in *Allium cepa* L meristematic cells. *Biol. Cell* **74**, 243–7.

GOODRICH, D. W., WANG, N. P., QIAN, Y. W., LEE, E. Y. & LEE, W. H. (1991) The retinoblastoma gene product regulates progression through the G1 phase of the cell cycle. *Cell* **67**, 293–302.

GÖTZ, M. & BOLZ, J. (1992) Formation and preservation of cortical layers in slice cultures. *J. Neurobiol.* **23**, 783–802.

GRAY, D. (1988) Population kinetics of rat peripheral B cells. *J. Exp. Med.* **167**, 805–16.

GREEN, P., HARTENSTEIN, A. Y. & HARTENSTEIN, V. (1993) The embryonic development of the *Drosophila* visual system. *Cell Tissue Res.* **273**, 583–98.

GRENIER, Y., RUIJS, T. C., OLIVIER, A., ROBITAILLE, Y. & ANTEL, J. P. (1989) DNA synthesis by young adult human-derived astrocytes in vitro. *Brain Res.* **480**, 87–91.

GRESSNER, A. M., LOTFI, S., GRESSNER, G. & LAHME, B. (1992) Identification and partial characterization of a hepatocyte-derived factor promoting proliferation of cultured fat-storing cells (parasinusoidal lipocytes). *Hepatology* **16**, 1250–66.

GUTHRIE, S., BUTCHER, M. & LUMSDEN, A. (1991) Patterns of cell division and interkinetic nuclear migration in the chick embryo hindbrain. *J. Neurobiol.* **22**, 742–54.

HAASE, S. B. & CALOS, M. P. (1991) Replication control of autonomously replicating human sequences. *Nucleic Acids Res.* **19**, 5053–8.

HADRAVA, V., TREMBLAY, J., SEKALY, R. P. & HAMET, P. (1992) Accelerated entry of aortic smooth muscle cells from spontaneously hypertensive rats into the S phase of the cell cycle. *Biochem. Cell Biol.* **70**, 599–604.

HÄGELE, K. (1970) DNS-Replikationsmuster der Speicheldrüsen-Chromosomen von Chironomiden. *Chromosoma* **31**, 91–138.

HAGGLUND, B. & SANDBERG, G. (1993) Effect of L-alanine and some other amino acids on thymocyte proliferation in vivo. *Immunobiology* **188**, 62–9.

HAMADA, H. (1993) [Effects of medium chain triglyceride administration on liver regeneration after partial hepatectomy in rats] *Hokkaido Igaku Zasshi* **68**, 96–109.

HAMMAR, S. P., LUU, J. Y., BOCKUS, D. E., REMINGTON, F. L., LUU, J. W., FRIEDMAN, S. & BEAN, M. A. (1992) Induction of tubuloreticular structures in cultured human endothelial cells by recombinant interferon alfa and beta. *Ultrastruct. Pathol.* **16**, 211–18.

HAN, H., IWANAGA, T., UCHIYAMA, Y. & FUJITA, T. (1993) Aggregation of macrophages in the tips of intestinal villi in guinea pigs: their possible role in the phagocytosis of effete epithelial cells. *Cell Tissue Res.* **271**, 407–16.

HANAZONO, M., YOSHIKI, A., OTA, K., KITOH, J. & KUSAKABE, M. (1990a) DNA replication in uterine cells of adult and prepubertal mice under normal and hormonally stimulated conditions detected by bromodeoxyuridine labelling method. *Endocrinol. Jpn.* **37**, 183–91.

HANAZONO, M., YOSHIKI, A., OTA, K., KITOH, J. & KUSAKABE, M. (1990b) Immunohistochemical detection of DNA replication in mouse uterine cells by bromodeoxyuridine labeling of wax- and resin-embedded tissue sections. *Stain Technol.* **65**, 139–49.

HANKE, H., STROHSCHNEIDER, T., OBERHOFF, M., BETZ, E. & KARSCH, K. R. (1990) Time course of smooth muscle cell proliferation in the intima and media of arteries following experimental angioplasty. *Circ. Res.* **67**, 651–9.

HANKE, H., HAASE, K. K., HANKE, S., OBERHOFF, M., HASSENSTEIN, S., BETZ, E. & KARSCH, K. R. (1991) Morphological changes and smooth muscle cell proliferation after experimental excimer laser treatment. *Circulation* **83**, 1380–9.

HARRIS, A. J., DUXSON, M. J., FITZSIMONS, R. B. & RIEGER, F. (1989) Myonuclear birthdates distinguish the origins of primary and secondary myotubes in embryonic mammalian skeletal muscles. *Development* **107**, 771–84.

HARTMANN, C. & RODE, A. (1982) [Changes, during a synchronous cell cycle, of the level of incorporation of 5-bromodeoxyuridine into DNA of chick embryo fibroblasts] *C. R. Seances Acad. Sci. III* **295**, 69–72.

HASHIGUCHI, J., ITO, M. & SEKINE, I. (1993) The effect of the autonomic nervous system on cell proliferation of the gastric mucosa in stress ulcer formation. *J. Autonom Nerv. Syst.* **43**, 179–87.

HASHINO, E. & SALVI, R. J. (1993) Changing spatial patterns of DNA replication in the noise-damaged chick cochlea. *J. Cell Sci.* **105**, 23–31.

HASHIRO, M., MATSUMOTO, K., OKUMURA, H., HASHIMOTO, K. & YOSHIKAWA, K. (1991) Growth inhibition of human keratinocytes by antisense c-myc oligomer is not coupled to induction of differentiation. *Biochem. Biophys. Res. Commun.* **174**, 287–92.

HASSENSTEIN, S., HANKE, H., KAMENZ, J., OBERHOFF, M., HANKE, S., RIESSEN, R., HAASE, K. K., BETZ, E. & KARSCH, K. R. (1992) Vascular injury and time course of smooth muscle cell proliferation after experimental holmium laser angioplasty. *Circulation* **86**, 1575–83.

HINES, J. E., JOHNSON, S. J. & BURT, A. D. (1993) In vivo responses of macrophages and perisinusoidal cells to cholestatic liver injury. *Am. J. Pathol.* **142**, 511–18.

HIRAI, R., OGUCHI, Y., SUGITA, N., MATSUNAGA, K. & NOMOTO, K. (1993) Enhancement of T-cell proliferation by PSK. *Int. J. Immunopharmacol.* **15**, 745–50.

HIRNING, U., SCHMID, P., SCHULZ, W. A., RETTENBERGER, G. & HAMEISTER, H. (1991) A comparative analysis of N-myc and c-myc expression and cellular proliferation in mouse organogenesis. *Mech. Dev.* **33**, 119–25.

HITCHCOCK, P. F., LINDSEY MYHR K. J., EASTER, S. S. JR, MANGIONE-SMITH, R. & JONES, D. D. (1992) Local regeneration in the retina of the goldfish. *J. Neurobiol.* **23**, 187–203.

HOGEN ESCH, H., GIJBELS, M. J. J., OFFERMAN, E., VAN HOOFT, J., VAN BEKKUM, D. W. & ZURCHER, C. (1993) A spontaneous mutation characterized by chronic proliferative dermatitis in C57BL mice. *Am. J. Pathol.* **143**, 972–82.

HOLMQUIST, G. P. (1987) Role of replication in the control of tissue-specific gene expression. *Am. J. Hum. Genet.* **40**, 151–73.

HONG, S. T., KHO, W. G., KIM, W. H., CHAI, J. Y. & LEE, S. H. (1993) Turnover of biliary epithelial cells in *Clonorchis sinensis* infected rats. *Kisaengchunghak Chapchi* **31**, 83–9.

HUESMANN, M., SCOTT, B., KISIELOW, P. & VON BOEHMER, H. (1991) Kinetics and efficacy of positive selection in the thymus of normal and T cell receptor transgenic mice. *Cell* **66**, 533–40.

HUMBERT, C. & USSON, Y. (1992) Eukaryotic DNA replication is a topographically ordered process. *Cytometry* **13**, 603–14.

HUMBERT, C., SANTISTEBAN, M. S., USSON, Y. & ROBERT-NICOUD, M. (1992) Intranuclear co-location of newly replicated DNA and PCNA by simultaneous immunofluorescent labelling and confocal microscopy in MCF-7 cells. *J. Cell Sci.* **103**, 97–103.

HUME, W. J. (1989) DNA-synthesizing cells in oral epithelium have a range of cell cycle durations: evidence from double-labelling studies using tritiated thymidine and bromodeoxyuridine. *Cell Tissue Kinet.* **22**, 377–82.

HUME, W. J. & THOMPSON, J. (1990) Double labelling of cells with tritiated thymidine and bromodeoxyuridine reveals a circadian rhythm-dependent variation in duration of

DNA synthesis and S phase flux rates in rodent oral epithelium. *Cell Tissue Kinet.* **23**, 313–23.

HUONG, P. L., KOLK, A. H., EGGELTE, T. A., VERSTIJNEN, C. P., GILIS, H. & HENDRIKS, J. T. (1991) Measurement of antigen specific lymphocyte proliferation using 5-bromo-deoxyuridine incorporation. An easy and low cost alternative to radioactive thymidine incorporation. *J. Immunol. Methods* **140**, 243–8.

HURME, T. & KALIMO, H. (1992) Activation of myogenic precursor cells after muscle injury. *Med. Sci. Sports Exercise* **24**, 197–205.

IANNUZZI, L., GUSTAVSSON, I., DI MEO G. P. & FERRARA, L. (1989) High-resolution studies on late-replicating segments (G + C bands) in mammalian chromosomes. *Hereditas* **110**, 43–50.

IRANI, A. M., NILSSON, G., MIETTINEN, U., CRAIG, S. S., ASHMAN, L. K., ISHIZAKA, T., ZSEBO, K. M. & SCHWARTZ, L. B. (1992) Recombinant human stem cell factor stimulates differentiation of mast cells from dispersed human fetal liver cells. *Blood* **80**, 3009–21.

ITO, K. & HOTTA, Y. (1992) Proliferation pattern of postembryonic neuroblasts in the brain of *Drosophila melanogaster*. *Dev. Biol.* **149**, 134–48.

ITO, K. & MCGHEE, J. D. (1987) Parental DNA strands segregate randomly during embryonic development of *Caenorhabditis elegans*. *Cell* **49**, 329–36.

ITO, K., MCGHEE, J. D. & SCHULTZ, G. A. (1988) Paternal DNA strands segregate to both trophectoderm and inner cell mass of the developing mouse embryo. *Genes Devel.* **2**, 929–36.

ITO, Y. (1993) [DNA synthesis of the mucosa of the gallbladder estimated by bromodeoxyuridine (BrdU)-anti BrdU *in vitro* method in patients with various gallbladder disorders] *Nippon Shokakibyo Gakkai Zasshi* **90**, 665–72.

JABLONKA-SHARIFF, A., GRAZUL-BILSKA, A. T., REDMER, D. A. & REYNOLDS, L. P. (1993) Growth and cellular proliferation of ovine corpora lutea throughout the estrous cycle. *Endocrinology* **133**, 1871–9.

JEZEQUEL, A. M., PAOLUCCI, F., BENEDETTI, A., MANCINI, R. & ORLANDI, F. (1991) Enumeration of S-phase cells in normal rat liver by immunohistochemistry using bromodeoxyuridine-antibromodeoxyuridine system. *Dig. Dis. Sci.* **36**, 482–4.

JIRIKOWSKI, G. F., RAMALHO-ORTIGAO, J. F., LINDL, T. & SELIGER, H. (1989) Immunocytochemistry of 5-bromo-2'-deoxyuridine labelled oligonucleotide probes. A novel technique for in situ hybridization. *Histochemistry* **91**, 51–3.

JIRIKOWSKI, G. F., RAMALHO-ORTIGAO, J. F., KESSE, K. W. & BLOOM, F. E. (1990) In situ hybridization of semithin Epon sections with BrdU labelled oligonucleotide probes. *Histochemistry* **94**, 187–90.

JOHNSON, D. G., SCHWARZ, J. K., CRESS, W. D. & NEVINS, J. R. (1993) Expression of transcription factor E2F1 induces quiescent cells to enter S phase. *Nature* **365**, 349–52.

JOHNSON, S. J., HINES, J. E. & BURT, A. D. (1992) Macrophage and perisinusoidal cell kinetics in acute liver injury. *J. Pathol.* **166**, 351–8.

JONES, H. B., CLARKE, N. A. & BARRASS, N. C. (1993) Phenobarbital-induced hepatocellular proliferation: anti-bromodeoxyuridine and anti-proliferating cell nuclear antigen immunocytochemistry. *J. Histochem. Cytochem* **41**, 21–7.

JUST, W., AUTENRIETH, M. & VOGEL, W. (1993) Demonstration of replication patterns on histone-depleted chromosomes. *Cytogenet. Cell Genet.* **62**, 60–3.

KALLE, W. H. J., HAZEKAMP-VAN DOKKUM, A-M., LOHMAN, P. H. M., NATARAJAN, A. T., VAN ZEELAND. A. A. & MULLENDERS, L. H. F. (1993) The use of streptavidin-coated magnetic beads and biotinylated antibodies to investigate induction and repair of DNA damage: analysis of repair patches in specific sequences of uv-irradiated human fibroblasts. *Anal. Biochem.* **208**, 228–36.

KAMIRYO, T., ORITA, T., NISHIZAKI, T. & AOKI, H. (1990) Development of the rat meninx: experimental study using bromodeoxyuridine. *Anat. Rec.* **227**, 207–10.

KANAI-AZUMA, M., KANAI, Y., KUROHMARU, M., SAKAI, S. & HAYASHI, Y. (1993) Insulin-like growth factor (IGF)-I stimulates proliferation and migration of mouse ectoplacental cone cells, while IGF-II transforms them into trophoblastic giant cells in vitro. *Biol. Reprod.* **48**, 252–61.

KARASEK, M., LIBERSKI, P., KUNERT-RADEK, J. & BARTKOWIAK, J. (1992) Influence of melatonin on the proliferation of hepatoma cells in the Syrian hamster: in vivo and in vitro study. *J. Pineal Res.* **13**, 107–10.

KARN, J., WATSON, J. V., LOWE, A. D., GREEN, S. M. & VEDECKIS, W. (1989) Regulation of cell cycle duration by c-myc levels. *Oncogene* **4**, 773–7.

KARUBE, T. & WATANABE, S. (1988) Analysis of the chromosomal DNA replication pattern using the bromodeoxyuridine labelling method. *Cancer Res.* **48**, 219–22.

KARUBE, T., OCHI-TAKEUCHI, H. & WATANABE, S. (1987) A new immunocytochemical method to measure cell kinetics of human lymphocytes by using monoclonal antibody against bromodeoxyuridine. *Nippon Ketsueki Gakkai Zasshi* **50**, 862–7.

KASSEM, M., BLUM, W., RISTELLI, J., MOSEKILDE, L. & ERIKSEN, E. F. (1993) Growth hormone stimulates proliferation and differentiation of normal human osteoblast-like cells in vitro. *Calcif. Tissue Int.* **52**, 222–6.

KATELARIS, A., PENNY, R. & BREIT, S. N. (1992) Mitotic activity in the dermis of mice during graft versus host disease: the role of fibroblast replication in dermal fibrosis. *J. Invest. Dermatol.* **99**, 779–83.

KATOH, K., KASAI, S. & MITO, M. (1989) [Effect of activation of the reticuloendothelial system on hepatocyte regeneration in partially hepatectomized rats] *Nippon Geka Gakkai Zasshi* **90**, 250–7.

KATSUDA, S., COLTRERA, M. D., ROSS, R. & GOWN, A. M. (1993) Human atherosclerosis. IV. Immunocytochemical analysis of cell activation and proliferation in lesions of young adults. *Am. J. Pathol.* **142**, 1787–93.

KAUFMAN, S. J. & FOSTER, R. F. (1988) Replicating myoblasts express a muscle-specific phenotype. *Proc. Natl. Acad. Sci. USA* **85**, 9606–10.

KAUFMAN, S. J. & ROBERT-NICOUD, M. (1985) DNA replication and differentiation in rat myoblasts studied with monoclonal antibodies against 5-bromodeoxyuridine, actin, and alpha 2-macroglobulin. *Cytometry* **6**, 570–7.

KELIC, S., OLSSON, T. & KRISTENSSON, K. (1993) Interferon-gamma promotes proliferation of rat skeletal muscle cells *in vitro* and alters their AChR distribution. *J. Neurol. Sci.* **114**, 62–7.

KELLETT, M., POTTEN, C. S. & REW, D. A. (1992) A comparison of *in vivo* cell proliferation measurements in the intestine of mouse and man. *Epithelial Cell Biol.* **1**, 147–55.

KENNERKNECHT, I., BAUR-AUBELE, S. & VOGEL, W. (1991) Proliferation kinetics in native chorionic villus cells. *Prenat. Diagn.* **11**, 591–5.

KIMURA, Y., KAMIDE, M., FURUKAWA, M., MIWA, T., SAKUMOTO, M. & UMEDA, R. (1990) [Evaluation of turnover of olfactory epithelium in mice by using anti-BrdU monoclonal antibody]. *Nippon Jibiinkoka Gakkai Kaiho* **93**, 165–70.

KIRKHUS, B. & CLAUSEN, O. P. (1990) Cell kinetics in mouse epidermis studied by bivariate DNA/bromodeoxyuridine and DNA/keratin flow cytometry. *Cytometry* **11**, 253–60.

KIRKHUS, B. & CLAUSEN, O. P. (1992) Circadian variations in cell cycle phase progression of mouse epidermal cells measured directly by bivariate BrdUrd/DNA flow cytometry. *Epithelial Cell Biol.* **1**, 32–8.

KITAO, Y., OKOYAMA, S., MORIIZUMI, T. & KUDO, M. (1993) Neurogenetical segregation of the vestibulospinal neurons in the rat. *Brain Res.* **620**, 149–54.

KITAZAWA, R., KITAZAWA, S., FUKASE, M., FUJITA, T., KOBAYASHI, A., CHIHARA, K. & MAEDA, S. (1992) The expression of parathyroid hormone-related protein (PTHrP) in normal parathyroid: histochemistry and in situ hybridization. *Histochemistry* **98**, 211–15.

KITAZAWA, S., TAKENAKA, A., ABE, N., MAEDA, S., HORIO, M. & SUGIYAMA, T. (1989) In situ DNA-RNA hybridization using in vivo bromodeoxyuridine-labeled DNA probe. *Histochemistry* **92**, 195–9.

KITAZAWA, S., KITAZAWA, R., GOTOH, A., FUJIMORI, T. & MAEDA, S. (1993) In situ hybridization using bromodeoxyuridine labelled DNA probe and RNase H. *Biotech Histochem.* **68**, 137–41.

KLEIBEUKER, J. H., WELBERG, J. W., MULDER, N. H., VAN DER MEER, R., CATS, A., LIMBURG, A. J., KREUMER, W. M., HARDONK, M. J. & DE VRIES, E. G. (1993) Epithelial cell proliferation in the sigmoid colon of patients with adenomatous polyps increases during oral calcium supplementation. *Br. J. Cancer* **67**, 500–3.

KLEINE, H. D., WAGNER, U., POLIWODA, H. & FREUND, M. (1992) Effects of tumor necrosis factor alpha on bone marrow aspirates of patients with acute myelogenous leukemia determined by flow-cytometric cell-cycle analysis. *J. Cancer Res. Clin. Oncol.* **118**, 56–60.

KNAPP, P. E. (1991) Studies of glial lineage and proliferation *in vitro* using an early marker for committed oligodendrocytes. *J. Neurosci. Res.* **30**, 336–45.

KNAPP, P. E. (1992) The cell cycle of glial cells grown in vitro: an immunocytochemical method of analysis. *J. Histochem. Cytochem.* **40**, 1405–11.

KNIGHT, J. K. & RAYMOND, P. A. (1990) Time course of opsin expression in developing rod photoreceptors. *Development* **110**, 1115–20.

KOHN, K. W. & GRIMEK-EWIG, R. A. (1973) Alkaline elution analysis, a new approach to the study of DNA single-strand interruptions in cells. *Cancer Res.* **33**, 1849–53.

KOIKE, N., SAITOH, K., TODOROKI, T., KAWAMOTO, T., IWASAKI, Y. & NAKAMURA, K. (1993) Cell proliferation kinetics of the bile duct epithelium of the rat. *Cell Prolif.* **26**, 183–93.

KOIZUMI, O., ITAZAWA, M., MIZUMOTO, H., MINOBE, S., JAVOIS, L. C., GRIMMELIKHUIJZEN, C. J. & BODE, H. R. (1992) Nerve ring of the hypostome in hydra. I. Its structure, development, and maintenance. *J. Comp. Neurol.* **326**, 7–21.

KOJIMA, I., MOGAMI, H., KAWAMURA, N., YASUDA, H. & SHIBATA, H. (1993) Modulation of growth of vascular smooth muscle cells by activin A. *Exp. Cell Res.* **206**, 152–6.

KOSAKA, T., KAMATA, T., OOYAMA, S., KANNO, M., YAMAGUCHI, A., YONEMURA, Y., MIWA, K. & MIYAZAKI, I. (1990) [A flow cytometric cell cycle analysis of familial polyposis coli]. *Gan No Rinsho* **36**, 2141–5.

KROEGER, H., GETTMANN, W. & KALATER, C. H. (1973) Zur Kontrolle der DNA-Replication in Riesenchromosomen explantierter Speicheldrüsen. *Cytobiologie* **7**, 65–88.

KRUSE, F. E. & TSENG, S. C. G. (1993a) Growth factors modulate clonal growth and differentiation of cultured rabbit limbal and corneal epithelium. *Invest. Ophthalmol. Vis. Sci.* **34**, 1963–76.

KRUSE, F. E. & TSENG, S. C. G. (1993b) A tumor promoter-resistant subpopulation of progenitor cells is larger in limbal epithelium than in corneal epithelium. *Invest. Ophthalmol. Vis. Sci.* **34**, 2501–11.

KRUSHEL, L. A., & VAN DER KOOY, D. (1993) Pattern formation in the developing mammalian forebrain: selective adhesion of early but not late postmitotic cortical and striatal neurons within forebrain reaggregate cultures. *Dev. Biol.* **158**, 145–62.

KUBBIES, M., FRIEDL, R., KOEHLER, J., RABINOVITCH, P. S. & HOEHN, H. (1990) Improvement of human lymphocyte proliferation and alteration of IL-2 secretion kinetics by alpha-thioglycerol. *Lymphokine Res.* **9**, 95–106.

KULOMAA, P., PAAVONEN, J. & LEHTINEN, M. (1992) Herpes simplex virus induces unscheduled DNA synthesis in virus-infected cervical cancer cell lines. *Res. Virol.* **143**, 351–9.

KUNI, Y., TARAO, K., SHIMIZU, A., NAKAMURA, N., HARADA, M., ITO, Y., IIMORI, K. & TAMAI, S. (1988) [Evaluation of DNA synthesis using antibromodeoxyuridine antibody in liver cirrhosis and chronic active hepatitis] *Nippon Shokakibyo Gakkai Zasshi* **85**, 2187–92.

KUSEWITT, D. F., BUDGE, C. L., NOLLA, H. A., EDWARDS, B. S. & LEY, R. D. (1992) Cell cycle progression in denV-transfected murine fibroblasts exposed to ultraviolet radiation. *Mutat. Res.* **274**, 163–76.

KUYATT, B. L., REIDY, C. A., HUI, K. Y. & JORDAN, W. H. (1993) Quantitation of smooth muscle proliferation in cultured aorta. A color image analysis method for the Macintosh. *Anal. Quant. Cytol. Histol.* **15**, 83–7.

LABRECQUE, C., HUARD, J., DANSEREAU, G., ALBERT, N. & TREMBLAY, J. P. (1991) In vitro bromodeoxyuridine labelling of nuclei: application to myotube hybridization. *J. Histochem. Cytochem.* **39**, 1421–6.

LACY, E. R., KUWAYAMA, H., COWART, K. S., KING, J. S., DEUTZ, A. H. & SISTRUNK, S. (1991) A rapid, accurate, immunohistochemical method to label proliferating cells in the digestive tract. A comparison with tritiated thymidine. *Gastroenterology* **100**, 259–62.

LAGRA, F., RAPTOPOULOU-GIGI, M., ORPHANOU-KOUMERKERIDOU, H. & GOULIS, G. (1989) In vitro effect of interferon alpha-2b on T lymphocyte transformation and leukocyte migration inhibition in patients with chronic brucellosis. *Immunopharmacol. Immunotoxicol.* **11**, 223–32.

LANE, H. A., FERNANDEZ, A., LAMB, N. J. & THOMAS, G. (1993) p70s6k function is essential for G1 progression. *Nature* **363**, 170–2.

LANTZ, O., ALARD, P., CHAVANEL, G., FRIES, D., SENIK, A. & CHARPENTIER, B. (1991) EBV cell-lines (LCL) and E- cells as stimulator cells for limiting dilution analysis (LDA) of alloreactive IL-2-secreting cells (IL-2-SC) and cytotoxic precursors (CTLp). Comparison of their stimulator capacity and ability to generate specific alloreactivity. *J. Immunol. Methods* **137**, 121–32.

LARDON, F., VAN BOCKSTAELE, D. R., SNOECK, H. W. & PEETERMANS, M. E. (1993) Quantitative cell-cycle progression analysis of the first three successive cell cycles of granulocyte colony-stimulating factor and/or granulocyte-macrophage colony-stimulating factor-stimulated human CD34+ bone marrow cells in relation to their colony formation. *Blood* **81**, 3211–16.

LARISON, K. D. & BREMILLER, R. (1990) Early onset of phenotype and cell patterning in the embryonic zebrafish retina. *Development* **109**, 567–76.

LARRAMENDY, M. L. & KNUUTILA, S. (1990) Immunophenotype and sister chromatid differentiation: a combined methodology for analyzing cell proliferation in unfractionated lymphocyte cultures. *Exp. Cell Res.* **188**, 209–13.

LARSON, J. L., WOLF, D. C. & BUTTERWORTH, B. E. (1993) Acute hepatotoxic and nephrotoxic effects of chloroform in male F-344 rats and female B6C3F1 mice. *Fundam. Appl. Toxicol.* **20**, 302–15.

LATOS-BIELENSKA, A. & VOGEL, W. (1990) Frequency and distribution of chiasmata in Syrian hamster spermatocytes studied by the BrdU antibody technique. *Chromosoma* **99**, 267–72.

LATOS-BIELENSKA, A. & VOGEL, W. (1992) Demonstration of replication patterns in the last premeiotic S-phase of male Chinese hamsters after BrdU pulse labelling. *Chromosoma* **101**, 279–83.

LATOS-BIELENSKA, A., HAMEISTER, H. & VOGEL, W. (1987) Detection of BrdUrd incorporation in mammalian chromosomes by a BrdUrd antibody. III. Demonstration of replication patterns in highly resolved chromosomes. *Hum. Genet.* **76**, 293–5.

LATT, S. A. (1973) Microfluorimetric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc. Natl. Acad. Sci.* USA. **70**, 3395–9.

LAU, Y. F. & ARRIGHI, F. E. (1981) Studies of mammalian chromosome replication. II. Evidence for the existence of defined chromosome replicating units. *Chromosoma* **83**, 721–41.

LAYER, P. G. & ALBER, R. (1990) Patterning of chick brain vesicles as revealed by peanut agglutinin and cholinesterases. *Development* **109**, 613–24.

LEE, D.Y., CHAPKIN, R.S., LUPTON, J.R. (1993) Dietary fat and fiber modulate colonic cell proliferation in an interactive site-specific manner. *Nutr. Cancer* **20**, 107–18.

LEHRNBECHER, T., MERZ, H., SEBALD, W. & POOT, M. (1991) Interleukin 4 drives phytohemagglutinin-activated T cells through several cell cycles: no synergism between interleukin 2 and interleukin 4. *Cytokine* **3**, 593–7.

LEHTINEN, M., KULOMAA, P., KALLIONIEMI, O. P., PAAVONEN, J. & LEINIKKI, P. (1989) Analysis of DNA synthesis in herpes simplex virus infected cells by dual parameter flow cytometry. *Arch. Virol.* **107**, 215–23.

LETT, J. T., KLUCIS, E.A. & SUN, C. (1970) On the size of the DNA in the mammalian chromosome. *Biophys. J.* **10**, 277–82.

LEVIN, B. (1992) Nutrition and colorectal cancer. *Cancer* **70**, 1723–6.

LEY, R. D., APPLEGATE, L. A., DERIEL, J. K. & HENDERSON, E. E. (1989) Excision repair characteristics of DENV-transformed Xeroderma pigmentosum cells. *Mutat. Res.* **217**, 101–7.

LIKHACHEV, A. J., TOMATIS, L. & MARGISON, G. P. (1983) Incorporation and persistence of 5-bromodeoxyuridine in newborn rat tissue DNA. *Chem. Biol. Interact.* **46**, 31–8.

LOIZIDOU, M. C., LAWRANCE, R. J., HOLT, S., CARTY, N. J., COOPER, A. J., ALEXANDER, P. & TAYLOR, I. (1991) Facilitation by partial hepatectomy of tumor growth within the rat liver following intraportal injection of syngeneic tumor cells. *Clin. Exp. Metastasis* **9**, 335–49.

LOPEZ-GIRONA, A., COLOMER, J., PUJOL, M. J., BACHS, O. & AGELL, N. (1992) Calmodulin regulates DNA polymerase alpha activity during proliferative activation of NRK cells. *Biochem. Biophys. Res. Commun.* **184**, 1517–23.

LORENZI, M., NORDBERG, J. A. & TOLEDO, S. (1987) High glucose prolongs cell-cycle traversal of cultured human endothelial cells. *Diabetes* **36**, 1261–7.

LOVE, T. C. & TUCKER, D. C. (1993) Possible paracrine facilitation of ventricular growth in oculo by atrial tissue. *Am. J. Physiol.* **265**, 1082–8.

LUCAS, B., VASSEUR, F. & PENIT, C. (1993) Normal sequence of phenotypic transitions in one cohort of 5-bromo-2'-deoxyuridine-pulse-labeled thymocytes. Correlation with T cell receptor expression. *J. Immunol.* **151**, 4574–82.

LUKOMSKA, B., PIENKOWSKA, B., ANDRZEJEWSKI, W. & OLSZEWSKI, W. L. (1991) Liver sinusoidal cytotoxic cells are recruited from blood and divide locally. *J. Hepatol.* **12**, 332–5.

LUNDY, M. W., HENDRIX, T., WERGEDAL, J. E. & BAYLINK, D. J. (1991) Growth factor-induced proliferation of osteoblasts measured by bromodeoxyuridine immunocytochemistry. *Growth Factors* **4**, 257–64.

LUTZOW-HOLM, C., DE ANGELIS, P. & CLAUSEN, O. P. (1992) Retinoic acid provokes a regeneration-like proliferative response in murine epidermis. A bivariate DNA/bromodeoxyuridine flow cytometric study. *Arch. Dermatol. Res.* **284**, 418–23.

MA, C., LEU, T. H. & HAMLIN, J. L. (1990) Multiple origins of replication in the dihydrofolate reductase amplicons of a methotrexate-resistant chinese hamster cell line. *Mol. Cell. Biol.* **10**, 1338–46.

MA, W., BEHAR, T., MARIC, D., MARIC, I. & BARKER, J. L. (1992) Neuroepithelial cells in the rat spinal cord express glutamate decarboxylase immunoreactivity in vivo and in vitro. *J. Comp. Neurol.* **325**, 257–70.

MACKAY, C. R., MARSTON, W. L. & DUDLER, L. (1990) Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J. Exp. Med.* **171**, 801–17.

MAGGIO-PRICE, L., GROSSMANN, A., SHIOTA, F. & ENGEL, D. (1993) Increased proliferative capacity of CD4+ and CD8+ T lymphocytes from mutant sphha/sphha mice is associated with increased IL-2 receptor expression. *Cell. Immunol.* **148**, 346–56.

MAH, D. C. W., DIJKWEL, P. A., TODD, A., KLEIN, V., PRICE, G. B. & ZANNIS-HADJOPOULOS, M. (1993) *ors12*, a mammalian autonomously replicating DNA sequence, associates

with the nuclear matrix in a cell cycle-dependent manner. *J. Cell Sci.* **105**, 807–18.

MANCINI, R., JEZEQUEL, A. M., BENEDETTI, A., PAOLUCCI, F., TROZZI, L. & ORLANDI, F. (1992) Quantitative analysis of proliferating sinusoidal cells in dimethylnitrosamine-induced cirrhosis. An immunohistochemical study. *J. Hepatol* **15**, 361–6.

MANDERS, E. M. M., STAP, J., BRAKENHOFF, G. J., VAN DRIEL, R. & ATEN, J. . (1992) Dynamics of three-dimensional replication patterns during the S-phase, analysed by double labelling of DNA and confocal microscopy. *J. Cell Sci.* **103**, 857–62.

MANIOTIS, A. & SCHLIWA, M. (1991) Microsurgical removal of centrosomes blocks cell reproduction and centriole generation in BSC-1 cells. *Cell* **67**, 495–504.

MANSON, M. M., GREEN, J. A., WRIGHT, B. J. & CARTHEW, P. (1992) Degree of ethoxyquin-induced nephrotoxicity in rat is dependent on age and sex. *Arch. Toxicol.* **66**, 51–6.

MARK, M. P., BLOCH-ZUPAN, A. & RUCH, J. V. (1992) Effects of retinoids on tooth morphogenesis and cytodifferentiations, in vitro. *Int. J. Dev. Biol.* **36**, 517–26.

MARSHALL, M. J. & DAVIE, M. W. (1991) An immunocytochemical method for studying the kinetics of osteoclast nuclei on intact mouse parietal bone. *Histochem. J.* **23**, 402–8.

MARUCCI, L., BARONI, G. S., MANCINI, R., BENEDETTI, A., JEZEQUEL, A. M. & ORLANDI, F. (1993) Cell proliferation following extrahepatic biliary obstruction. Evaluation by immunohistochemical methods. *J. Hepatol* **17**, 163–9.

MARUSHIGE, Y., MARUSHIGE, K. & KOESTNER, A. (1992) Growth inhibition of anaplastic glioma cells by nerve growth factor. *Anticancer Res.* **12**, 2069–73.

MASHIBA, H., MATSUNAGA, K., YAGAWA, K., NOMURA, Y., YOSHINAGA, H., FURUSAWA, M. & JIMI, S. (1981) Elevated cytostatic activity of monocytes from cancer patients. *Gann* **72**, 848–53.

MASON, R. J., APOSTOLOU, S., POWER, J. & ROBINSON, P. (1990) Human alveolar type II cells: stimulation of DNA synthesis by insulin and endothelial cell growth supplement. *Am. J. Respir. Cell Mol. Biol.* **3**, 571–7.

MATSUMOTO, Y., KAWAI, K. & FUJIWARA, M. (1988) Hemorrhagic autoimmune encephalomyelitis induced by adoptive transfer of activated semiallogeneic spleen cells into irradiated rats. *Am. J. Pathol.* **133**, 306–15.

MATSUMOTO, Y., HANAWA, H., TSUCHIDA, M. & ABO, T. (1993) In situ inactivation of infiltrating T cells in the central nervous system with autoimmune encephalomyelitis. The role of astrocytes. *Immunology* **79**, 381–90.

MATSUNAGA, S., IJIRI, K., SAKOU, T. & MORIMOTO, N. (1993) Immunohistochemical study of activating osteoblasts from undifferentiated mesenchymal cells by electrical stimulation. *Bone* **14**, 327–32.

MATSUNO, K., EZAKI, T. & KOTANI, M. (1989) Splenic outer periarterial lymphoid sheath (PALS): an immunoproliferative microenvironment constituted by antigen-laden marginal metallophils and ED2-positive macrophages in the rat. *Cell Tissue Res.* **257**, 459–70.

MAYRON, R., & WISE, D. (1976). Random distribution of centromere regions at mitosis in cultured cells of *Muntiacus muntjak*. *Chromosoma* **55**, 69–74.

MAZURE, A. & GRIERSON, I. (1992) In vitro studies of the contractility of cell types involved in proliferative vitreoretinopathy. *Invest. Ophthalmol. Vis Sci.* **33**, 3407–16.

MAZZOTTI, G., RIZZOLI, R., GALANZI, A., PAPA, S., VITALE, M., FALCONI, M., NERI, L. M., ZINI, N. & MARALDI, N. M. (1990) High-resolution detection of newly synthesized DNA by anti-bromodeoxyuridine antibodies identifies specific chromatin domains. *J. Histochem. Cytochem.* **38**, 13–22.

MCCLURE, S. J. & HEIN, W. R. (1989) Functional characteristics of 197+ CD4– CD8– sheep T lymphocytes: expansion and differentiation of peripheral T cells. *Immunol. Cell Biol.* **67**, 223–31.

MCCULLOCH, C. A. & KNOWLES, G. (1991) Discrimination of two fibroblast progenitor populations in early explant cultures of hamster gingiva. *Cell Tissue Res.* **264**, 87–94.

MCDERMOTT, K. W. G. & LANTOS, P. L. (1990) Cell proliferation in the subependymal layer of the postnatal marmoset, *Callithrix jacchus*. *Dev. Brain Res.* **57**, 269–77.

MCDOWELL, E. M., DE SANTI, A. M. & STRUM, J. M. (1989) Correlations between blood glucose levels and bromodeoxyuridine labelling indices of pancreatic islet cells following streptozotocin administration to pregnant Syrian golden hamsters. *Virchows Arch. B Cell Pathol.* **57**, 223–30.

MCDOWELL, E. M., ZHANG, X. M. & DE SANTI, A. M. (1990) Localization of healing tracheal wounds using bromodeoxyuridine immunohistochemistry. *Stain Technol.* **65**, 25–9.

MCNICOL, A. M. & DUFFY, A. E. (1987) A study of cell migration in the adrenal cortex of the rat using bromodeoxyuridine. *Cell Tissue Kinet.* **20**, 519–26.

MEDINA, K. L., SMITHSON, G. & KINCADE, P. W. (1993) Suppression of B lymphopoiesis during normal pregnancy. *J. Exp. Med.* **178**, 1507–15.

MENCHERINI, F., KHOCHBIN, S., CHABANAS, A. & LAWRENCE, J-J. (1992) Seric factors influence cell cycle modifications during commitment of murine erythroleukemia cells to differentiate. *Cytometry* **13**, 518–24.

MIETHING, A. (1993) Proliferative activity of the developing seminiferous epithelium during prespermatogenesis in the golden hamster testis measured by bromodeoxyuridine labelling. *Anat. Embryol. (Berl.)* **187**, 249–58.

MILLER, M. A., BOKHARI, S. A., QADIR, K. & RAZA, A. (1992) Simultaneous assessment of TGFB and cell cycle kinetics using IUdR/BrdU infusions in human neoplasms from plastic-embedded tissue. *J. Histochem. Cytochem.* **40**, 427–30.

MILLER, M. W. (1992) Circadian rhythm of cell proliferation in the telencephalic ventricular zone: effect of in utero exposure to ethanol. *Brain Res.* **595**, 17–24.

MILLER, M. W. & NOWAKOWSKI, R. S. (1988) Use of bromodeoxyuridine-immunohistochemistry to examine the proliferation, migration and time of origin of cells in the central nervous system. *Brain Res.* **457**, 44–52.

MINABE, M. (1989) [Regeneration of the rat submandibular glands after duct ligation] *Kanagawa Shigaku* **24**, 484–500.

MIURA, M., DOMON, M., SASAKI, T. & TAKASAKI, Y. (1992) Induction of proliferating cell nuclear antigen (PCNA) complex formation in quiescent fibroblasts from a xeroderma pigmentosum patient. *J. Cell. Physiol.* **150**, 370–6.

MIYASHITA, M., BABA, S. & SUZUKI, H. (1992) Localized proliferative effect of preadipocytes on cultured human epidermal keratinocytes. *Acta Derm. Venereol. (Stockh)* **72**, 413–17.

MIYATA, K., MURAO, M., SAWA, M. & TANISHIMA, T. (1989) [Kinetic study of cell proliferation in a new wound healing model using tissue cultured corneal endothelial cells] *Nippon Ganka Gakkai Zasshi* **93**, 287–93.

MIYATA, K., MURAO, M., SAWA, M. & TANISHIMA, T. (1990) New wound-healing model using cultured bovine corneal endothelial cells. 2. Role of migration and mitosis studied by immunohistochemistry. *Jpn. J. Ophthalmol.* **34**, 267–74.

MODLIN, I. M., KUMAR, R., NANGIA, A., SOROKA, C. J., PASIKHOV, D. & GOLDENRING, J. R. (1992) Gastrin-dependent inhibitory effects of octreotide on the genesis of gastric ECLomas. *Surgery* **112**, 1048–58.

MOORE, G. D., KOPF, G. S. & SCHULTZ, R. M. (1993) Complete mouse egg activation in the absence of sperm by stimulation of an exogenous G protein-coupled receptor. *Dev. Biol.* **159**, 669–78.

MORETTO, G., YOO, A. & KIM, S. U. (1993) Human astrocytes and cytokines: tumour necrosis factor alpha and interferon gamma do not promote astrocytic proliferation. *Neurosci. Lett.* **151**, 17–20.

MORIMOTO, Y., SAGA, K., BANDO, M. & TAKAHASHI, M. (1991) In-vitro DNA synthesis of keratinocytes in normal human skin, psoriasis, seborrhoeic keratosis, Bowen's disease and basal cell carcinoma. *Br. J. Dermatol.* **125**, 9–13.

MORSHEAD, C. M. & VAN DER KOOY, D. (1992) Postmitotic death is the fate of constitutively proliferating cells in the subependymal layer of the adult mouse brain. *J. Neurosci.* **12**, 249–56.

MULLENDERS, L. H., HAZEKAMP-VAN DOKKUM, A. M., KALLE, W. H., VRIELING, H., ZDZIENICKA, M. Z. & VAN ZEELAND, A. A. (1993) UV-induced photolesions, their repair and mutations. *Mutat. Res.* **299**, 271–6.

MUNAUT, C., DOMBROWICZ, D. & MATAGNE, R. F. (1990) Detection of chloroplast DNA by using fluorescent monoclonal anti-bromodeoxyuridine antibody and analysis of its fate during zygote formation in *Chlamydomonas reinhardtii*. *Curr. Genet.* **18**, 259–63.

MURAKOSHI, M., TAGAWA, M., INADA, R. & SUZUKI, M. (1992) Effects of testosterone, and testosterone plus estrogen, in the castrated rat ventral prostate–histopathological and immunocytochemical studies. *Tokai J. Exp. Clin. Med.* **17**, 133–7.

MURATA, T., WADA, R., ABE, H., KUWABARA, N. & FUKUDA, Y. (1991) [Studies of morphometric analysis and cell cycle of gastric epithelia of rat administered minimal mixed bile acids]. *Nippon Shokakibyo Gakkai Zasshi* **88**, 1532–8.

MURATE, T., SAGA, S., HOTTA, T., ASANO, H., ITO, T., KATO, K., TSUSHITA, K., KINOSHITA, T., ICHIKAWA, A., YOSHIDA, S. & SAITO, H. (1993) The close relationship between DNA replication and the selection of differentiation lineages of human erythroleukemia cell lines K562, HEL, and TF1 into either erythroid or megakaryocytic lineages. *Exp. Cell Res.* **208**, 35–43.

NAGAI, A., MATSUMIYA, H., SAKAMOTO, K., YAMAGUCHI, E., AOSHIBA, K. & TAKIZAWA, T. (1991) [Consequences of DNA damage and the relationship between respiratory diseases in childhood and in adult hood]. *Nippon Kyobu Shikkan Gakkai Zasshi* **29**, 7–10.

NAGANO, M., OHKUBO, T., ARINO, T., TSUCHIYA, M. & NAGAI, M. (1993) Growth factor for cardiac hypertrophy. *Mol. Cell. Biochem.* **119**, 17–22.

NAKAMURA, H. (1991) Immunohistochemical observations of dividing cells in olfactory epithelium using anti-BrdU antibody. *Eur. Arch. Otorhinolaryngol.* **248**, 298–301.

NAKAMURA, H., MORITA, T. & SATO, C. (1986) Structural organizations of replicon domains during DNA synthetic phase in the mammalian nucleus. *Exp. Cell Res.* **165**, 291–7.

NAKAMURA, T. & HOTCHI, M. (1992) Changes in DNA strand breaks in non-parenchymal cells following hepatocyte regeneration in $CCl_4$-induced rat liver injury. *Virchows Arch. B. Cell Pathol.* **63**, 11–16.

NAKAYASU, H. & BEREZNEY, R. (1989) Mapping replicational sites in the eucaryotic cell nucleus. *J. Cell Biol.* **108**, 1–11.

NAVONE, A. & RAZA, A. (1987) The detection of DNA repair in bromodeoxyuridine versus thymidine containing DNA of individual cells. *Anticancer Res.* **7**, 13–16.

NEFF, M. W. & BURKE, D. J. (1991) Random segregation of chromatids at mitosis in *Saccharomyces cerevisiae*. *Genetics* **127**, 463–73.

NERI, L. M., MAZZOTTI, G., CAPITANI, S., MARALDI, N. M., CINTI, C., BALDINI, N., RANA, R. & MARTELLI, A. M. (1992) Nuclear matrix-bound replicational sites detected in situ by 5-bromodeoxyuridine. *Histochemistry* **98**, 19–32.

NIEDOBITEK, G., FINN, T., HERBST, H., BORNHOFT, G., GERDES, J. & STEIN, H. (1988) Detection of viral DNA by in situ hybridization using bromodeoxyuridine-labeled DNA probes. *Am. J. Pathol.* **131**, 1–4.

NIEDOBITEK, G., FINN, T., HERBST, H. & STEIN, H. (1989) Detection of viral genomes in the liver by *in situ* hybridization using 35S-, bromodeoxyuridine-, and biotin-labeled probes. *Am. J. Pathol.* **134**, 633–9.

NIELSEN, J. H., LINDE, S., WELINDER, B. S., BILLESTRUP, N. & MADSEN, O. D. (1989) Growth hormone is a growth factor for the differentiated pancreatic beta-cell. *Mol. Endocrinol.* **3**, 165–73.

NISHINO, A., SUZUKI, M., OHTANI, H., MOTOHASHI, O., UMEZAWA, K., NAGURA, H. & YOSHIMOTO, T. (1993) Thrombin may contribute to the pathophysiology of central nervous system injury. *J. Neurotrauma* **10**, 167–79.

NISHIZAKI, T., ORITA, T., ABIKO, S., AOKI, H. & ITO, H. (1990) Subependymal giant cell astrocytoma associated with tuberous sclerosis: with special reference to cell kinetic studies – case report. *Neurol. Med. Chir. (Tokyo)* **30**, 695–7.

NITTA, K., UCHIDA, K., MURAI, K., HORITA, S., TSUTSUI, T., OZU, H., KAWASHIMA, A., YUMURA, W. & NIHEI, H. (1993) [Role of glomerular mesangial cells in the regulation of glomerular endothelial cell growth] *Nippon Jinzo Gakkai Shi* **35**, 663–9.

NOLL, E. & MILLER, R. H. (1993) Oligodendrocyte precursors originate at the ventral ventricular zone dorsal to the ventral midline region in the embryonic rat spinal cord. *Development* **118**, 563–73.

NOMURA, A., MARUYAMA, Y. K. & YONEDA, M. (1991) Initiation of DNA replication cycle in fertilized eggs of the starfish, *Asterina pectinifera*. *Dev. Biol.* **143**, 289–96.

NOMURA, A., YONEDA, M. & TANAKA, S. (1993) DNA replication in fertilized eggs of the starfish, *Asterina pectinifera*. *Dev. Biol.* **159**, 288–97.

OHARA, K., ITAKURA, K. & IBARAKI, N. (1992) Anterior capsule opacification: a cell culture model. *Acta Ophthalmol.* (suppl.) 29–33.

OHSHIO, I. (1992) [Quantitative analysis of DNA synthesis and cell condensation in the oligodactylic rat hand plate] *Hokkaido Igaku Zasshi* **67**, 322–41.

OINUMA, T., KAWANO, J-I. & SUGANUMA, T. (1992) Bromodeoxyuridine-immunohistochemistry on cellular differentiation and migration in the fundic gland of *Xenopus laevis* during development. *Cell Tissue Res.* **269**, 205–12.

OLIVO, M., BHARDWAJ, R., SCHULZE-OSTHOFF, K., SORG, C., JACOB, H. J. & FLAMME, I. (1992) A comparative study on the effects of tumour necrosis factor-alpha (TNF-alpha), human angiogenic factor (h-AF) and basic fibroblast growth factor (bFGF) on the chorioallantoic membrane of the chick embryo. *Anat. Rec.* **234**, 105–15.

ORITA, T., NISHIZAKI, T., KAMIRYO, T., HARADA, K. & AOKI, H. (1988) Cerebral microvascular architecture following experimental cold injury. *J. Neurosurg.* **68**, 608–12.

ORITA, T., AKIMURA, T., KAMIRYO, T., NISHIZAKI, T., FURUTANI, Y., HARADA, K., IKEYAMA, Y. & AOKI, H. (1989) Cerebral endothelial regeneration following experimental brain injury. Variation in the regeneration process according to the severity of injury. *Acta Neuropathol (Berl.)* **77**, 397–401.

ORMEROD, M. G., TITLEY, J. C., SMITH, T. A., TOMBS, A. L. & ECCLES, S. (1993) Proliferative behaviour of an oestrogen sensitive rat mammary tumour: evidence for a paracrine interaction between tumour and stroma. *Br. J. Cancer* **67**, 107–11.

ORTON, E. C., LA RUE, S. M., ENSLEY, B. & STENMARK, K. (1992) Bromodeoxyuridine labelling and DNA content of pulmonary arterial medial cells from hypoxia-exposed and nonexposed healthy calves. *Am. J. Vet. Res.* **53**, 1925–30.

OZAWA, S., UEDA, M., ANDO, N., ABE, O. & SHIMIZU, N. (1988) Epidermal growth factor receptors in cancer tissues of esophagus, lung, pancreas, colorectum, breast and stomach. *Jpn. J. Cancer Res.* **79**, 1201–7.

PAINTER, R. B. & CLEAVER, J. E. (1969) Repair replication, unscheduled DNA synthesis and the repair of mammalian DNA. *Radiat. Res.* **37**, 451–66.

PAN, S. A., OKAZAKI, Y. & TAKEMOTO, T. (1990) In vivo cell kinetics studies of the duodenum during and after healing of cysteamine-induced duodenal ulcer in rats using bromodeoxyuridine incorporation. *J. Gastroenterol. Hepatol* **5**, 281–7.

PATEL, S., REW, D. A., TAYLOR, I., POTTEN, C. S., OWEN, C. & ROBERTS, S. A. (1993) Study of the proliferation in human gastric mucosa after in vivo bromodeoxyuridine labelling. *Gut* **34**, 893–6.

PAWLIKOWSKI, M., ZEREK-MELEN, G. & WINCZYK, K. (1992) Thyroliberin (TRH) increases thymus cell proliferation in rats. *Neuropeptides* **23**, 199–202.

PAWLIKOWSKI, M., ZEREK-MELEN, G., WINCZYK, K., STEPIEN, H., LACHOWICZ, A. & JANECKA, A. (1993) Comparison of the antiproliferative effects of colon mitosis inhibitor, thyroliberin and the somatostatin analog octreotide on rat colonic mucosal epithelial cells. *Cytobios* **73**, 25–30.

PELTOMAKI, T. & HAKKINEN, L. (1992) Growth of the ribs at the costochondral junction in the rat. *J. Anat.* **181**, 259–64.

PENIT, C. (1986) In vivo thymocyte maturation. BUdR labelling of cycling thymocytes and phenotypic analysis of their progeny support the single lineage model. *J. Immunol.* **137**, 2115–20.

PENIT, C. (1988) Localization and phenotype of cycling and post-cycling murine thymocytes studied by simultaneous detection of bromodeoxyuridine and surface antigens. *J. Histochem. Cytochem.* **36**, 473–8.

PENIT, C. & EZINE, S. (1989) Cell proliferation and thymocyte subset reconstitution in sublethally irradiated mice: compared kinetics of endogenous and intrathymically transferred progenitors. *Proc. Natl. Acad. Sci. USA* **86**, 5547–51.

PENIT, C. & VASSEUR, F. (1988) Sequential events in thymocyte differentiation and thymus regeneration revealed by a combination of bromodeoxyuridine DNA labelling and antimitotic drug treatment. *J. Immunol.* **140**, 3315–23.

PENIT, C., LUCAS, B. & VASSEUR, R. (1995) Cell expansion and growth arrest phases during the transition from precursor (CD4-8-) to immature (CD4+8+) thymocytes in normal and genetically modified mice. *J. Immunol.* **154**, 5103-13.

PERROS, P. & KENDALL-TAYLOR, P. (1992) Biological activity of autoantibodies from patients with thyroid-associated ophthalmopathy: in vitro effects on porcine extraocular myoblasts. *Q. J. Med.* **84**, 691–706.

PHILLIPS, R. G. & WHITTLE, J. R. (1993) *wingless* expression mediates determination of peripheral nervous system elements in late stages of *Drosophila* wing disc development. *Development* **118**, 427–38.

PLACZEK, M., JESSELL, T. M. & DODD, J. (1993) Induction of floor plate differentiation by contact-dependent, homeogenetic signals. *Development* **117**, 205–18.

PLICKERT, G. & KROIHER, M. (1988) Proliferation kinetics and cell lineages can be studied in whole mounts and macerates by means of BrdU/anti-BrdU technique. *Development* **103**, 791–4.

POOT, M., HOEHN, H., RUNGER, T. M. & MARTIN, G. M. (1992) Impaired S-phase transit of Werner syndrome cells expressed in lymphoblastoid cell lines. *Exp. Cell Res.* **202**, 267–73.

POTTEN, C. S., HUME, W. J., REID, P. & CAIRNS, J. (1978) Segregation of DNA in stem cells. *Cell* **15**, 899–906.

POTTEN, C. S., KELLETT, M., REW, D. A. & ROBERTS, S. A. (1992a) Proliferation in human gastrointestinal epithelium using bromodeoxyuridine in vivo: data for different sites, proximity to a tumour, and polyposis coli. *Gut* **33**, 524–9.

POTTEN, C. S., KELLETT, M., ROBERTS, S. A., REW, D. A. & WILSON, G. D. (1992b) Measurement of in vivo proliferation in human colorectal mucosa using bromodeoxyuridine. *Gut* **33**, 71–8.

PROKOP, A. & TECHNAU, G. M. (1991) The origin of postembryonic neuroblasts in the ventral nerve cord of *Drosophila melanogaster*. *Development* **111**, 79–88.

RAHMAN, S. A. & TSUYAMA, S. (1993) Immunohistochemical study of cell proliferation and differentiation in epidermis of mice after administration of cholera toxin. *Arch. Dermatol. Res.* **285**, 27–31.

RASMUSSEN, R.E. & PAINTER, R.B. (1964) Evidence for repair of ultraviolet damaged deoxyribonucleic acid in cultured mammalian cells. *Nature* **203**, 1360–2.

REDDY, K. S., SAVAGE, J. R. & PAPWORTH, D. G. (1988) Replication kinetics of X chromosomes in fibroblasts and lymphocytes. *Hum. Genet.* **79**, 44–8.

REGAN, J. D. & SETLOW, R. B. (1971) Two forms of repair in the DNA of human cells damaged by chemical carcinogens and mutagens. *Cancer Res.* **34**, 3318–25.

REIMSCHUESSEL, R., BENNETT, R. O., MAY, E. B. & LIPSKY, M. M. (1990) Development of newly formed nephrons in the goldfish kidney following hexachlorobutadiene-induced nephrotoxicity. *Toxicol. Pathol.* **18**, 32–8.

REIN, G. & KARASEK, M. A. (1992) Factors affecting the growth and maintenance of human skin mast cells in cell culture. *Int. Arch. Allergy Immunol.* **98**, 211–19.

REITSMA, P. H., ROTHBERG, P. J., ASTRIN, S. M., TRIAL, J., BAR-SHAVIT, Z., HALL, A., TEITELBAUM, S. L. & KAHN, A. J. (1983) Regulation of myc gene expression in HL-60 leukemia cells by vitamin D metabolism. *Nature* **206**, 848–50.

REN, K. Y., BRENTJENS, J., CHEN, Y. X., BRODKIN, M. & NOBLE, B. (1991) Glomerular macrophage proliferation in experimental immune complex nephritis. *Clin. Immunol. Immunopathol.* **60**, 384–98.

REPKA, A. M. & ADLER, R. (1992a) Accurate determination of the time of cell birth using a sequential labelling technique with [3H]-thymidine and bromodeoxyuridine ("window labelling"). *J. Histochem. Cytochem.* **40**, 947–53.

REPKA, A. & ADLER, R. (1992b) Differentiation of retinal precursor cells born in vitro. *Dev. Biol.* **153**, 242–9.

RISIO, M., LIPKIN, M., CANDELARESI, G., BERTONE, A., COVERLIZZA, S. & ROSSINI, F. P. (1991) Correlations between rectal mucosa cell proliferation and the clinical and pathological features of nonfamilial neoplasia of the large intestine. *Cancer Res.* **51**, 1917–21.

RIZZOLI, R., BARATTA, B., MARALDI, N. M., FALCONI, M., GALANZI, A., PAPA, S., VITALE, M., RIZZI, E., MANZOLI, L. & MAZZOTTI, G. (1992) DNA synthesis progression in 3T3 synchronized fibrolasts: a high resolution approach. *Histochemistry* **97**, 181–7.

ROBERSON, D. F., WEISLEDER, P., BOHRER, P. S. & RUBEL, E. W. (1992) Ongoing production of sensory cells in the vestibular epithelium of the chick. *Hearing Res.* **57**, 166–74.

ROBERTSON, T. A., MALEY, M. A., GROUNDS, M. D. & PAPADIMITRIOU, J. M. (1993) The role of macrophages in skeletal muscle regeneration with particular reference to chemotaxis. *Exp. Cell Res.* **207**, 321–31.

ROCHA, B., PENIT, C., BARON, C., VASSEUR, F., DAUTIGNY, N. & FREITAS, A. A. (1990) Accumulation of bromodeoxyuridine-labeled cells in central and peripheral lymphoid organs: minimal estimates of production and turnover rates of mature lymphocytes. *Eur. J. Immunol.* **20**, 1697–709.

RODE, A., DE TAISNE, C. & HARTMANN, C. (1983) Incorporation of 5-bromodeoxyuridine in the total and ribosomal DNA of synchronously dividing chick embryo fibroblasts. *Experientia* **39**, 1134–6.

ROSENSTEIN, B. S. & ROSENSTEIN, R. B. (1992) Survival, excision repair and inhibition of DNA synthesis in normal human skin fibroblasts exposed to simulated sunlight. *J. Photochem. Photobiol. B.* **16**, 247–55.

ROSENSTEIN, B. S. & SETLOW, R. B. (1980) DNA repair after ultraviolet irradiation of ICR 2A frog cells. Pyrimidine dimers are long acting blocks to nascent DNA synthesis. *Biophys. J.* **31**, 195–205.

ROSENSTEIN, B. S., SETLOW, R. B. & AHMED, F. E. (1980) Use of the dye Hoechst 33258 in a modification of the bromodeoxyuridine photolysis technique for the analysis of DNA repair. *Photochem. Photobiol.* **31**, 215–22.

ROSETTE, C. D., DE TERESA, P. S. & PALLAVICINI, M. G. (1990) Simultaneous flow cytometric detection of cellular c-myc protein, incorporated bromodeoxyuridine, and DNA. *Cytometry* **11**, 547–51.

ROY, B., FUJIMOTO, N. & ITO, A. (1990) Growth-promoting effect of retinoic acid in transplantable pituitary tumor of rat. *Jpn. J. Cancer Res.* **81**, 878–83.

RUDOLPH, N. S. & LATT, S. A. (1989) Flow cytometric analysis of X-ray sensitivity in ataxia telangiectasia. *Mutat. Res.* **211**, 31–41.

RUDOLPH, N. S., NAGASAWA, H., LITTLE, J. B. & LATT, S. A. (1989) Identification of ataxia telangiectasia heterozygotes by flow cytometric analysis of X-ray damage. *Mutat. Res.* **211**, 19–29.

RUSSEV, G. & BOULIKAS, T. (1992) Repair of transcriptionally active and inactive genes during S and G2 phases of the cell cycle. *Eur. J. Biochem.* **204**, 267–72.

SAETER, G., SCHWARZE, P.E., NESLAND, J.M., JUUL, N., PETTERSEN, E. & SEGLEN, P.O. (1988) The polyploidizing growth pattern of normal rat liver is replaced by divisional, diploid growth in hepatocellular nodules and carcinomas. *Carcinogenesis* **9**, 939–45.

SAKAMOTO, H., TRAINCARD, F., VO-QUANG, T., TERNYNCK, T., GUESDON, J. L. & AVRAMEAS, S. (1987) 5-Bromodeoxyuridine in vivo labelling of M13 DNA, and its use as a non-radioactive probe for hybridization experiments. *Mol. Cell. Probes* **1**, 109–20.

SAKAMOTO, S., MORI, T., SINGTRIPOP, T., KAWASHIMA, S., SUZUKI, S., KUDO, H., SAWAKI, K. & NAGASAWA, H. (1992) Increase of DNA synthesis in uterine adenomyosis in mice with ectopic pituitary isograft. *Acta Anat. (Basel)* **145**, 162–6.

SANDBERG, G. & ERNSTROM, U. (1991) In vivo stimulation of thymocyte proliferation by thymocyte growth peptide (TGP). *Int. J. Immunopharmacol.* **13**, 649–54.

SANDERS, E. J., PRASAD, S. & HU, N. (1993a) The involvement of TGF beta 1 in early avian development: gastrulation and chondrogenesis. *Anat. Embryol. (Berl.)* **187**, 573–81.

SANDERS, E. J., VAREDI, M. & FRENCH, A. S. (1993b) Cell proliferation in the gastrulating chick embryo: a study using BrdU incorporation and PCNA localization. *Development* **118**, 389–99.

SANTAVENERE, E., CATALDI, A., RANA, R., VITALE, M., LISIO, R., DI DOMENICANTONIO, L., ZAMAL, L., TRUBIANI, O. & MISCIA, S. (1991) Nuclear metabolic changes induced by tumor necrosis factor in Daudi lymphoma cells; a multiparametric analysis. *Cell Biol. Int. Rep.* **15**, 1235–42.

SAPINO, A., MACRI, L., GUGLIOTTA, P. & BUSSOLATI, G. (1990) Evaluation of proliferating cell types in human and mouse mammary gland by a double immunostaining procedure. *Acta Histochem. Suppl.* **40**, 81–4.

SASAKI, K., MURAKAMI, T., OGINO, T., TAKAHASHI, M. & KAWASAKI, S. (1986) Flow cytometric estimation of cell cycle parameters using a monoclonal antibody to bromodeoxyuridine. *Cytometry* **7**, 391–5.

SATO, M. & AHNEN, D. J. (1992) Regional variability of colonocyte growth and differentiation in the rat. *Anat. Rec.* **233**, 409–14.

SATO, Y., TSUKADA, K., MATSUMOTO, Y. & ABO, T. (1993) Interferon-gamma inhibits liver regeneration by stimulating major histocompatibility complex class II antigen expression by regenerating liver. *Hepatology* **18**, 3406–9.

SAVAGE, M. L., OLAH, I. & SCOTT, T. R. (1992) Plasma cell proliferation in the chicken Harderian gland. *Cell Prolif.* **25**, 337–44.

SAVELLI, V., RIZZOLI, R., RIZZI, E., GALANZI, A., BUFFA, A., RANA, R., LATTANZI, G. & BARATTA, B. (1991) Cell kinetics of vocal fold epithelium in rats. *Boll Soc. Ital. Biol. Sper* **67**, 1081–8.

SAXE, A. & GIBSON, G. (1993) Effect of lithium on incorporation of bromodeoxyuridine and tritiated thymidine into human parathyroid cells. *Arch. Surg.* **128**, 865–9.

SCARPINI, E., KREIDER, B. Q., LISAK, R. P. & PLEASURE, D. E. (1988) Establishment of Schwann cell cultures from adult rat peripheral nerves. *Exp. Neurol.* **102**, 167–76.

SCARPINI, E., DORONZO, R., BARON, P., MOGGIO, M., BASELLINI, A. & SCARLATO, G. (1992) Phenotypic and proliferative properties of Schwann cells from nerves of diabetic patients. *Int. J. Clin. Pharmacol. Res.* **12**, 211–15.

SCHARFMANN, R., BASMACIOGULLARI, A. & CZERNICHOW, P. (1990) Effect of growth hormone and glucose on rat islet cells replication using 5-bromo-2-deoxyuridine incorporation. *Diabetes Res.* **15**, 137–41.

SCHERES, H. M., SCHUTTE, B., DE GOIJ, A. F. & BOSMAN, F. T. (1990) Estrogen and progesterone receptor expression and proliferation in breast cancer cells, studied with flow cytometry. *Acta Histochem.* Suppl. **40**, 71–6.

SCHEVEN, B. A., DAMEN, C. A., HAMILTON, N. J., VERHAAR, H. J. & DUURSMA, S. A. (1992) Stimulatory effects of estrogen and progesterone on proliferation and differentiation of normal human osteoblast-like cells in vitro. *Biochem. Biophys. Res. Commun.* **186**, 54–60.

SCHIFFER, D., GIORDANA, M. T., VIGLIANI, M. C. & CAVALLA, P. (1991) Relationship between glial reaction to a stab wound and tumour development after receiving transplacental ethylnitrosourea in the rat. *Acta Neuropathol (Berl.)* **83**, 30–8.

SCHIFFER, D., GIORDANA, M. T., CAVALLA, P., VIGLIANI, M. C. & ATTANASIO, A. (1993) Immunohistochemistry of glial reaction after injury in the rat: double stainings and markers of cell proliferation. *Int. J. Dev. Neurosci.* **11**, 269–80.

SCHIFFERS, P. M. H., JANSSEN, G. M. J., FAZZI, G. E., STRUIJKER-BOUDIER, H. A. J. & DE MEY, J. G. R. (1992) Endothelial modulation of DNA synthesis in isolated arteries of the rat. *J. Cardiovasc. Pharmacol.* **20**, Suppl 12, S124–7.

SCHINDLER, D., KUBBIES, M., PRIEST, R. E., HOEHN, H. & RABINOVITCH, P. S. (1988) The phytohemagglutinin response of human peripheral blood lymphocytes as a function of donor age: a re-examination using BrdU-Hoechst flow cytometry. *Mech. Ageing Dev.* **44**, 253–63.

SCHITTEK, B. & RAJEWSKY, K. (1990) Maintenance of B-cell memory by long-lived cells generated from proliferating precursors. *Nature* **346**, 749–51.

SCHITTEK, B., RAJEWSKY, K. & FORSTER, I. (1991) Dividing cells in bone marrow and spleen incorporate bromodeoxyuridine with high efficiency. *Eur. J. Immunol.* **21**, 235–8.

SCHMIDT, M. & MIGEON, B. R. (1990) Asynchronous replication of homologous loci on human active and inactive X chromosomes. *Proc. Natl. Acad. Sci. USA* **87**, 3685–9.

SCHULTZ-HECTOR, S. & HAGHAYEGH, S. (1993) Beta-fibroblast growth factor expression in human and murine squamous cell carcinomas and its relationship to regional endothelial cell proliferation. *Cancer Res.* **53**, 1444–9.

SCHUTTE, B., REYNDERS, M. M., BOSMAN, F. T. & BLIJHAM, G. H. (1987) Studies with anti-bromodeoxyuridine antibodies: II. Simultaneous immunocytochemical detection of antigen expression and DNA synthesis by in vivo labeling of mouse intestinal mucosa. *J. Histochem. Cytochem* **35**, 371–374.

SCIPPO, M. L., DOMBROWICZ, D., IGOUT, A., CLOSSET, J. & HENNEN, G. (1989) A non-radioactive method to detect RNA or DNA using an oligonucleotide probe with bromodeoxyuridine free ends, a monoclonal antibody against bromodeoxyuridine and immunogold silver staining. *Arch. Int. Physiol. Biochim.* **97**, 279–84.

SCUDIERO, D., HENDERSON, E., NORIN, A. & STRAUSS, B. (1975). The measurement of chemically-induced DNA repair synthesis in human cells by BND cellulose chromatography. Mutat. Res. **29**, 473–9.

SEIFERT, R., ZHAO, B. & CHRIST, B. (1992) Cytokinetic studies on the aortic endothelium and limb bud vascularization in avian embryos. *Anat. Embryol. (Berl.)* **186**, 601–10.

SEJVAR, J. J., LANDIS, S. C. & HALL, A. K. (1993) SA-1 antigen expression in small intensely fluorescent cells is associated with proliferation. *Dev. Biol.* **157**, 547–52.

SEKI, T. & ARAI, Y. (1993) Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat. *J. Neurosci.* **13**, 2351–8.

SELDEN, J. R., DOLBEARE, F., CLAIR, J. H., NICHOLS, W. W., MILLER, J. E., KLEEMEYER, K. M., HYLAND, R. J. & DE LUCA, J. G. (1993) Statistical confirmation that immunofluorescent detection of DNA repair in human fibroblasts by measurement of bromodeoxyuridine incorporation is stoichiometric and sensitive. *Cytometry* **14**, 154–67.

SELDEN, J.R., DOLBEARE, F., CLAIR, J. H., MILLER J. E., MCGETTIGAN, K., DIJOHN, J.A., DYSART, G.R. & DELUCA, J.G. (1994) Validation of a flow cytometric *in vitro* rat hepatocyte DNA repair (UDS) assay in rat hepatocytes. *Mutat. Res.* **315**, 147–67.

SELIG, S., OKUMURA, K., WARD, D. C. & CEDAR, H. (1992) Delineation of DNA replication time zones by fluorescence in situ hybridization. *EMBO J.* **11**, 1217–25.

SELLECK, S. B. & STELLER, H. (1991) The influence of retinal innervation on neurogenesis in the first optic ganglion of *Drosophila*. *Neuron* **6**, 83–99.

SELLECK, S. B., GONZALEZ, C., GLOVER, D. M. & WHITE, K. (1992) Regulation of the G1-S transition in postembryonic neuronal precursors by axon ingrowth. *Nature* **355**, 253–5.

SELLHEYER, K., BICKENBACH, J. R., ROTHNAGEL, J. A., BUNDMAN, D., LONGLEY, M. A., KRIEG, T., ROCHE, N. S., ROBERTS, A. B. & ROOP, D. R. (1993) Inhibition of skin development by overexpression of transforming growth factor beta 1 in the epidermis of transgenic mice. *Proc. Natl. Acad. Sci. USA* **90**, 5237–41.

SENGER, G., RAGOUSSIS, J., TROWSDALE, J. & SHEER, D. (1993) Fine mapping of the human MHC class II region within chromosome band 6p21 and evaluation of probe ordering using interphase fluorescence in situ hybridization. *Cytogenet. Cell Genet.* **64**, 49–53.

SEYSCHAB, H., FRIEDL, R., SCHINDLER, D., HOEHN, H., RABINOVITCH, P. S. & CHEN, U. (1989) The effects of bacterial lipopolysaccharide, anti-receptor antibodies and recombinant interferon on mouse B cell cycle progression using 5-bromo-2'-deoxyuridine/Hoechst 33258 dye flow cytometry. *Eur. J. Immunol.* **19**, 1605–12.

SFONDRINI, G., GANDINI, P., BOVARI, M. & BOTTONE, M. G. (1989) [Histological evaluation of tooth movement using immunocytochemical research] *Mondo Ortod.* **14**, 697–702.

SHIMIZU, A., TARAO, K., TAKEMIYA, S., HARADA, M., INOUE, T. & ONO, T. (1988) S-phase cells in diseased human liver determined by an in vitro BrdU-anti-BrdU method. *Hepatology* **8**, 1535–9.

SHINOMIYA, T. & INA, S. (1993) DNA replication of histone gene repeats in *Drosophila melanogaster* tissue culture cells: multiple initiation sites and replication pause sites. *Mol. Cell. Biol.* **13**, 4098–110.

SIERADZAN, K. & VRBOVA, G. (1989) Replacement of missing motoneurons by embryonic grafts in the rat spinal cord. *Neuroscience* **31**, 115–30.

SIERADZAN, K. & VRBOVA, G. (1993) Observations on the survival of grafted embryonic motoneurons in the spinal cord of developing rats. *Exp. Neurol.* **122**, 223–31.

SINHA, Y. N. & SORENSON, R. L. (1993) Differential effects of glycosylated and nonglycosylated prolactin on islet cell division and insulin secretion. *Proc. Soc. Exp. Biol. Med.* **203**, 123–6.

SINKUS, A., WACHTLER, F. & SCHWARZACHER, H. G. (1980) Random position of human heterochromatin-bearing chromosomes in first and third mitoses of lymphocyte cultures. *Hum. Genet.* **54**, 125–6.

SINNETT, D., FLINT, A. & LALANDE, M (1993) Determination of DNA replication kinetics in synchronized human cells using a PCR-based assay. *Nuclei Acids Res.* **21**, 3227–32.

SKAER, H. (1992) Cell proliferation and rearrangement in the development of the Malpighian tubules of the hemipteran, *Rhodnius prolixus*. *Dev. Biol.* **150**, 372–80.

SMITH, D. A., TOWNSEND, L. E., GLOVER, J. L., SEYMOUR, M. L. & REITZ-VICK, D. M. (1992) Atrial myocytes cultured from explanted human adult myocardium demonstrate DNA replication. *Am. Surg.* **58**, 577–82.

SMITH, P. J., LEECH, C.A. & TREHERNE, J. E. (1984) Glial repair in an insect central nervous system; effects of selective glial disruption. *J. Neurosci.* **4**, 2698–711.

SMITH, P. J., HOWE, E. A. & TREHERNE, J. E. (1987). Mechanisms of glial regeneration in an insect central nervous system. *J. Exp. Biol.* **132**, 59.

SMITH, P. J., HOWES, E. A. & TREHERNE, J. E. (1990) Cell proliferation in the repairing adult insect central nervous system: incorporation of the thymidine analogue 5-bromo-2-deoxyuridine in vivo. *J. Cell Sci.* **95**, 599–604.

SOBCZAK-THEPOT, J., HARPER, F., FLORENTIN, Y., ZINDY, F., BRECHOT, C. & PUVION, E. (1993) Localization of cyclin A at the sites of cellular DNA replication. *Exp. Cell Res.* **206**, 43–8.

SOLER, A. P., LAUGHLIN, K. V. & MULLIN, J. M. (1993) Effects of epidermal growth factor versus phorbol ester on kidney epithelial (LLC-PK1) tight junction permeability and cell division. *Exp. Cell Res.* **207**, 398–406.

SORSCHER, D. H., BARRETT, J. C. & CORDEIRO-STONE, M. (1993) Timing of replication of differentially transcribed genes in Syrian hamster embryo fibroblasts. *Mol. Carcinogen.* **8**, 28–33.

SPACK, E. G., LEWIS, E. D., PARADOWSKI, B., SCHIMKE, R. T. & JONES, P. P. (1992) Temporal order of DNA replication in the H-2 major histocompatibility complex of the mouse. *Mol. Cell. Biol.* **12**, 5174–88.

SRIVASTAVA, V., MILLER, S. & BUSBEE, D. (1993) Immunofluorescent evaluation of DNA repair synthesis using interactive laser cytometry. *Cytometry* **14**, 144–53.

STAQUET, M. J., DE FRAISSINETTE A., DEZUTTER-DAMBUYANT, C., SCHMITT, D. & THIVOLET, J. (1989) A combined method for detection of cell surface marker expression and bromodeoxyuridine (BrdU) uptake by epidermal cells in suspension. *J. Immunol. Methods* **116**, 287–92.

STENIUS, U. (1993) Different inhibition of DNA synthesis by transforming growth factor beta and phenobarbital on GST-P-positive and GST-P-negative hepatocytes. *Carcinogenesis* **14**, 159–61.

STEPHENSON, C. F., DESAI, Z. R. & BRIDGES, J. M. (1991) The proliferative activity of B-chronic lymphocytic leukaemia lymphocytes prior to and after stimulation with TPA and PHA. *Leukemia. Res.* **15**, 1005–12.

STEVENSON, A. P., CRISSMAN, H. A. & STEWART, C. C. (1985) Macrophage-induced cytostasis: kinetic analysis of bromodeoxyuridine-pulsed cells. *Cytometry* **6**, 578–83.

STEWART, H. J., ECCLESTON, P. A., JESSEN, K. R. & MIRSKY, R. (1991) Interaction between cAMP elevation, identified growth factors, and serum components in regulating Schwann cell growth. *J. Neurosci. Res.* **30**, 346–52.

STOFER, W. D. & HORN, J. P. (1993) Neurogenesis and differentiation of sympathetic B and C cells in the bullfrog tadpole. *J. Neurosci.* **13**, 801–7.

STUDZINSKI, G. P. (1989) Oncogenes, growth, and the cell cycle: an overview. *Cell Tissue Kinet.* **22**, 405–24.

SUZUKI, Y. & TAKEDA, M. (1991) Basal cells in the mouse olfactory epithelium after axotomy: immunohistochemical and electron-microscopic studies. *Cell Tissue Res.* **266**, 239–45.

SUZUKI, Y. & TAKEDA, M. (1993) Basal cells in the mouse olfactory epithelium during development: immunohistochemical and electron-microscopic studies. *Dev. Brain Res.* **73**, 107–13.

SVENSSON, M. & ALDSKOGIUS, H. (1993) Infusion of cytosine-arabinoside into the cerebrospinal fluid of the rat brain inhibits the microglial cell proliferation after hypoglossal nerve injury. *Glia* **7**, 286–98.

SWALES, L. S. & SMITH, P. J. (1990) Immunohistochemical localization of the thymidine analogue 5-bromo-2-deoxyuridine in insect tissue: preservation of cellular ultrastructure. *Tissue Cell* **22**, 311–17.

SWALES, L. S., HOWES, E. A. & SMITH, P. J. (1992) Cell specific DNA-labelling in the repairing blood-brain barrier of the insect *Periplaneta americana*. *Cell Tissue Res.* **267**, 535–43.

TABIBZADEH, S. S., SATYASWAROOP, P. G. & RAO, P. N. (1988) Antiproliferative effect of interferon-gamma in human endometrial epithelial cells in vitro: potential local growth modulatory role in endometrium. *J. Clin. Endocrinol. Metab.* **67**, 131–8.

TABIBZADEH, S., BHAT, U. G. & SUN, X. (1991) Generation of non-radioactive bromodeoxyuridine labeled DNA probes by polymerase chain reaction. *Nucleic Acids Res.* **19**, 2783.

TACHIBANA, H., INOUE, S., OKI, H., ANDOU, H., KATAGIRI, T. (1993) Sequence in DNA synthetic phase ratio of spontaneous hypertensive rat (SHR) myocardium – study on development of cardiomegaly. *Jpn. Circ. J.* **57**, 442–8.

TAKAHASHI, T., NOWAKOWSKI, R. S. & CAVINESS, V. S., JR (1992) BUdR as an S-phase marker for quantitative studies of cytokinetic behaviour in the murine cerebral ventricular zone. *J. Neurocytol.* **21**, 185–97.

TAKAHASHI, T., NOWAKOWSKI, R. S. & CAVINESS, V. S. JR (1993) Cell cycle parameters and patterns of nuclear movement in

the neocortical proliferative zone of the fetal mouse. *J. Neurosci.* **13**, 820–33.

TAKAMIYA, Y., KOHSAKA, S., TOYA, S., OTANI, M. & TSUKADA, Y. (1988) Immunohistochemical studies on the proliferation of reactive astrocytes and the expression of cytoskeletal proteins following brain injury in rats. *Brain Res.* **466**, 201–10.

TAKEMOTO, O., YOSHIMINE, T., HAYAKAWA, T., FUJITA, T., NAKAJIMA, S., TANEDA, M. & MOGAMI, H. (1989) [Astrocytic proliferation in the brain adjacent to infarcted lesion: immunohistochemical study of astroprotein (GFAP) and bromodeoxyuridine (BrdU)] *No To Shinkei* **41**, 361–5.

TAKENO, S., HAMAI, Y., TATSUKAWA, T., HAMAMURA, N., HIRAKAWA, K. & HARADA, Y. (1993) [Tissue culture of guinea pig middle ear epithelium – morphological characterization and proliferative activities of cultured cells on fibroblast-reorganized collagen gels] *Nippon Jibiinkoka Gakkai Kaiho* **96**, 41–7.

TANAKA, H., SUKHOVA, G. K., SWANSON, S. J., CLINTON, S. K., GANZ, P., CYBULSKY, M. I. & LIBBY, P. (1993) Sustained activation of vascular cells and leukocytes in the rabbit aorta after balloon injury. *Circulation* **88**, 1788–804.

TANAKA, N., TATEMOTO, A., URABE, T., ONO, M., HIZUTA, A., NAOMOTO, Y., GOTOH, K., MOREIRA, L. F. & ORITA, K. (1993a) Inhibition of liver regeneration in mice following extended hepatectomy by transfusion of lymphokine activated killer cells. *Acta Med. Okayama* **47**, 21–28.

TANAKA, N., YAMAMOTO, H., TATEMOTO, A., URABE, T. & ORITA, K. (1993b) Regulation of liver regeneration by interleukin-2 and its inhibitors: cyclosporine A and FK 506. *Int. J. Immunopharmacol.* **15**, 211–18.

TANAKA, Y., MAK, K. M. & LIEBER, C. S. (1990) Immunohistochemical detection of proliferating lipocytes in regenerating rat liver. *J. Pathol.* **160**, 129–34.

TANAKA, Y., FUNAKI, N., MAK, K. M., KIM, C. & LIEBER, C. S. (1991a) Effects of ethanol and hepatic vitamin A on the proliferation of lipocytes in regenerating rat liver. *J. Hepatol.* **12**, 344–50.

TANAKA, Y., NOUCHI, T., YAMANE, M., IRIE, T., MIYAKAWA, H., SATO, C. & MARUMO, F. (1991b) Phenotypic modulation in lipocytes in experimental liver fibrosis. *J. Pathol.* **164**, 273–8.

TARAO, K., SHIMIZU, A., HARADA, M., KUNI, Y., ITO, Y., TAMAI, S., IIMORI, K., SUGIMASA, Y., TAKEMIYA, S., OKAMOTO, T., MOTOHASHI, H., SAIRENJI, M., INOUE, T. & KANISAWA, M. (1989) Difference in the *in vitro* uptake of bromodeoxyuridine between liver cirrhosis with and without hepatocellular carcinoma. *Cancer* **64**, 104–9.

TARAO, K., SHIMIZU, A., OHKAWA, S., HARADA, M., ITO, Y., TAMAI, S., KUNI, Y., NAGAOKA, T. & HOSHINO, H. (1991) Increased uptake of bromodeoxyuridine by hepatocytes from early stage of primary biliary cirrhosis. *Gastroenterology* **100**, 725–30.

TAYLOR, J. H. (1960) Asynchronous duplication of chromosomes in cultured cells of Chinese hamster. *J. Biophys. Biochem. Cytol.* **7**, 455–64.

TAZZARI, P. L., CIPONE, M., TASSI, C., BONTADINI, A., CREMONINI, A. M., GOBBI, M., GENTILE, G. & TURA, S. (1989) S-phase evaluation with bromodeoxyuridine in lymphocytes from cattle infected with bovine leukemia virus (BLV). *Zentralbl. Veterinarmed. [B]* **36**, 691–8.

TERAGAWA, C. K. & BODE, H. R. (1990) Spatial and temporal patterns of interstitial cell migration in *Hydra vulgaris*. *Dev. Biol.* **138**, 63–81.

TEZUKA, M. (1990) [Cell kinetic study in generating hair tissues in using anti-bromodeoxyuridine monoclonal antibody]. *Nippon Hifuka Gakkai Zasshi* **100**, 153–61.

TEZUKA, M., ITO, M., ITO, K. & SATO, Y. (1990) Cell kinetic study of human and mouse hair tissues using anti-bromodeoxyuridine monoclonal antibody. *J. Dermatol. Sci.* **1**, 335–46.

TEZUKA, M., ITO, M., ITO, K., TAZAWA, T. & SATO, Y. (1991) Investigation of germinative cells in generating and renewed anagen hair apparatus in mice using anti- bromodeoxyuridine monoclonal antibody. *J. Dermatol. Sci.* **2**, 434–43.

THIRY, M. (1988) Immunoelectron microscope localization of bromodeoxyuridine incorporated into DNA of Ehrlich tumour cell nucleoli. *Exp. Cell Res.* **179**, 204–13.

THIRY, M. (1992a) Highly sensitive immunodetection of DNA on sections with exogenous terminal deoxynucleotidyl transferase and non-isotopic nucleotide analogues. *J. Histochem. Cytochem.* **40**, 411–19.

THIRY, M. (1992a) Ultrastructural detection of DNA within the nucleolus by sensitive molecular immunocytochemistry. *Exp. Cell Res.* **200**, 135–44.

THIRY, M. & DOMBROWICZ, D. (1988) Anti-bromodeoxyuridine monoclonal antibody: an alternative tool for the identification of replicated DNA at the electron microscope level. *Biol. Cell* **62**, 99–102.

TISCHLER, A. S., MCCLAIN, R. M., CHILDERS, H. & DOWNING, J. (1991) Neurogenic signals regulate chromaffin cell proliferation and mediate the mitogenic effect of reserpine in the adult rat adrenal medulla. *Lab. Invest.* **65**, 374–6.

TISCHLER, A. S., RUZICKA, L. A. & RISEBERG, J. C. (1992) Immunocytochemical analysis of chromaffin cell proliferation in vitro. *J. Histochem. Cytochem.* **40**, 1043–5.

TREVES, A. J., BARAK, V. & FUKS, Z. (1980) Characterization of human lymphocytes which proliferate "spontaneously" in vitro. *Eur. J. Immunol.* **10**, 883–7.

TRUMAN, J. W. & BATE, M. (1988) Spatial and temporal patterns of neurogenesis in the central nervous system of *Drosophila melanogaster*. *Dev. Biol.* **125**, 145–57.

TSUJI, H., FUSE, Y., KAWAMOTO, K., FUJINO, H. & KODAMA, T. (1989) Healing process of experimental esophageal ulcers induced by acetic acid in rats. *Scand. J. Gastroenterol.* Suppl. **162**, 6–10.

TSURUSHIMA, H., YUASA, S., KAWAMURA, K. & NOSE, T. (1993) [Migration of donor Purkinje cells in the host adult rat cerebellum] *No To Shinkei* **45**, 255–62.

TUCKER, J. D., AULETT, A., CIMINO, M. C., DEARFIELD, K.L., JACOBSON-KRAM, D., TICE, R. R. & CARRANO, A.V. (1993) Sister-chromatid exchange: second report of the Gene-tox Program. *Mutat. Res.* **297**, 101–80.

USSON, Y. & HUMBERT, C. (1992) Methods for topographical analysis of intra-nuclear BrdUrd-tagged fluorescence. *Cytometry* **13**, 595–602.

UYAMA, S., KAUFMANN, P. M., TAKEDA, T. & VACANTI, J. P. (1993) Delivery of whole liver-equivalent hepatocyte mass using polymer devices and hepatotrophic stimulation. *Transplantation* **55**, 932–5.

VAMBUTAS, A., DI LORENZO, T. P. & STEINBERG, B. M. (1993) Laryngeal papilloma cells have high levels of epidermal growth factor receptor and respond to epidermal growth factor

by a decrease in epithelial differentiation. *Cancer Res.* **53**, 910–14.

VAN DEKKEN H., SCHERVISH, E. W., PIZZOLO, J. G., FAIR, W. R. & MELAMED, M. R. (1991) Simultaneous detection of fluorescent in situ hybridization and in vivo incorporated BrdU in a human bladder tumour. *J. Pathol.* **164**, 17–22.

VAN DIERENDONCK, J. H., KEIJZER, R., VAN DE VELD, C. J. H. & CORNELISSE, C. J. (1989) Subdivision of S-phase by analysis of nuclear 5-bromodeoxyuridine staining patterns. *Cytometry* **10**, 143–50.

VAN DIERENDONCK J. H., KEIJZER, R., CORNELISSE, C. J. & VAN DE VELDE, C. J. (1991) Surgically induced cytokinetic responses in experimental rat mammary tumor models. *Cancer* **68**, 759–67.

VAN HAASTER, L. H., DE JONG, F. H., DOCTER, R. & DE ROOIJ, D. G. (1993) High neonatal triiodothyronine levels reduce the period of Sertoli cell proliferation and accelerate tubular lumen formation in the rat testis, and increase serum inhibin levels. *Endocrinology* **133**, 755–60.

VAN NOORDEN, C. J. (1995) Effects of n-3 and n-6 polyunsaturated fatty acid-enriched diets on lipid metabolism in periportal and pericentral compartments of female rat liver lobules and the consequences for cell proliferation after partial hepatectomy. *J. Lipid Res.* **36**, 1708–20.

VASSILEV, L. & JOHNSON, E. M. (1989) Mapping initiation sites of DNA replication in vivo using polymerase chain reaction amplification of nascent strand segments. *Nucleic Acids Res.* **17**, 7693–7712.

VASSILEV, L. & RUSSEV, G. (1988) Purification of nascent DNA chains by immunoprecipitation with anti-BrdU antibodies. *Nucleic Acids Res.* **16**, 10397.

VED BRAT, S., VERMA, R. S. & DOSIK, H. (1981) Sister chromatid differentiation and isolabeling of chromosomes. *Hum. Genet.* **56**, 305–8.

VERSTIJNEN, C. P., KATE, J. T., ARENDS, J. W., SCHUTTE, B. & BOSMAN, F. T. (1988) Xenografting of normal colonic mucosa in athymic mice. *J. Pathol.* **155**, 77–85.

VINOGRADOV, A. E., EZHEVSKY, S. A., ROSANOV, J. M., KAZHDAN, I. A. & ZWEIBACH, A. S. (1991) Loosening of cell cycle controls of human lymphocytes under the action of tumour promoter TPA. *Cell Prolif.* **24**, 493–505.

VITALE, M., RIZZOLI, R., MAZZOTTI, G., ZAMAI, L., GALANZI, A., RIZZI, E., MANZOLI, L., MATTEUCCI, A. & PAPA, S. (1991) Characterization and cell cycle kinetics of hepatocytes during rat liver regeneration: in vivo BrdUrd incorporation analysed by flow cytometry and electron microscopy. *Cell Prolif.* **24**, 331–8.

VOGEL, W. & SPEIT, G. (1986) Cytogenetic replication studies with short thymidine pulses in bromodeoxyuridine-substituted chromosomes of different mouse cell lines. *Hum. Genet.* **72**, 63–7.

VOGEL, W., BOLDIN, S., REISACHER, A. & SPEIT, G. (1985) Characterization of chromosome replication during S-phase with bromodeoxyuridine labelling in Chinese hamster ovary and HeLa cells. *Chromosoma* **92**, 363–8.

VOGEL, W., AUTENRIETH, M. & SPEIT, G. (1986) Detection of bromodeoxyuridine-incorporation in mammalian chromosomes by a bromodeoxyuridine-antibody. I. Demonstration of replication patterns. *Hum. Genet.* **72**, 129–32.

VOGEL, W., AUTENRIETH, M. & MEHNERT, K. (1989) Analysis of chromosome replication by a BrdU antibody technique. *Chromosoma* **98**, 335–41.

VOGEL, W., MEHNERT, K. & PENTZ, S. (1990) Demonstration of chromosome replication by BrdU antibody technique and electron microscopy. *Hum. Genet.* **84**, 237–40.

VROEMEN, J. P., BUURMAN, W. A., SCHUTTE, B., MAESSEN, J. G., VAN DER LINDEN, C. J. & KOOTSTRA, G. (1988) The cytokinetic behavior of donor hepatocytes after syngenic hepatocyte transplantation into the spleen. *Transplantation* **45**, 600–7.

WAGENAAR, G. T. M., CHAMULEAU, R. A. F. M., POOL, C. W., DE HAAN, J.G., MAAS, M. A. W., KORFAGE, H. A. M. & LAMERS, W. H. (1993) Distribution and activity of glutamine synthase and carbamoylphosphate synthase upon enlargement of the liver lobule by repeated partial hepatectomies. *J. Hepatol.* **17**, 397–407.

WANDERS, S. L., TEN KATE J., VAN DER LINDEN, E., DERHAAG, J., DINJENS, W. N. & BOSMAN, F. T. (1992) Does ex vivo labelling of proliferating cells in colonic and vaginal mucosa reflect the S-phase fraction in vivo? *Histochemistry* **98**, 267–70.

WARCHOL, M. E., LAMBERT, P. R., GOLDSTEIN, B. J., FORGE, A. & CORWIN, J. T. (1993) Regenerative proliferation in inner ear sensory epithelia from adult guinea pigs and humans. *Science* **259**, 1619–22.

WARD, J. M., HENNEMAN, J. R., OSIPOVA, G. & ANISIMOV, V. N. (1991) Persistence of 5-bromo-2'-deoxyuridine in tissues of rats after exposure in early life. *Toxicology* **70**, 345–52.

WATANABE, T. & RAFF, M. C. (1990) Rod photoreceptor development in vitro: intrinsic properties of proliferating neuroepithelial cells change as development proceeds in the rat retina. *Neuron* **4**, 461–7.

WEISSMAN, S. S., CHAR, D. H., HERBORT, C. P., OSTLER, H. B. & KALETA-MICHAELS, S. (1992) Alteration of human conjunctival epithelial proliferation. *Arch. Ophthalmol.* **110**, 357–9.

WELBERG, J. W., DE VRIES, E. G., HARDONK, M. J., MULDER, N. H., HARMS, G., GROND, J., ZWART, N., KOUDSTAAL, J., DE LEY, L. & KLEIBEUKER, J. H. (1990) Proliferation rate of colonic mucosa in normal subjects and patients with colonic neoplasms: a refined immunohistochemical method. *J. Clin. Pathol.* **43**, 453–6.

WEMME, H., PFEIFER, S., HECK, R. & MULLER-QUERNHEIM, J. (1992) Measurement of lymphocyte proliferation: critical analysis of radioactive and photometric methods. *Immunobiology* **185**, 78–89.

WESTERMANN, J., RONNEBERG, S., FRITZ, F. J. & PABST, R. (1989a) Proliferation of lymphocyte subsets in the adult rat: a comparison of different lymphoid organs. *Eur. J. Immunol.* **19**, 1087–93.

WESTERMANN, J., RONNEBERG, S., FRITZ, F. J. & PABST, R. (1989b) Proliferation of macrophage subpopulations in the adult rat: comparison of various lymphoid organs. *J. Leukocyte Biol.* **46**, 263–9.

WHEELER, J., ROBERTSON, H., MORLEY, A. R. & APPLETON, D. R. (1993) Anti-glomerular basement membrane glomerulonephritis (anti-GBM GN) in the mouse: BrdU-labelling indices and histological damage. *Int. J. Exp. Pathol.* **74**, 9–19.

WHITE, R. A., FALLON, J. F. & SAVAGE, M. P. (1992) On the measurement of cytokinetics by continuous labelling with bromodeoxyuridine with applications to chick wing buds. *Cytometry* **13**, 553–6.

WHITE, S. R., HERSHENSON, M. B., SIGRIST, K. S., ZIMMERMANN, A. & SOLWAY, J. (1993) Proliferation of guinea pig tracheal epithelial cells induced by calcitonin gene-related peptide. *Am. J. Respir. Cell Mol. Biol.* **8**, 592–6.

WIENS, D. J., MANN, T. K., FEDDERSON, D. E., RATHMELL, W. K. & FRANCK, B. H. (1992) Early heart development in the chick embryo: effects of isotretinoin on cell proliferation, alpha-actin synthesis, and development of contractions. *Differentiation* **51**, 105–12.

WILLBOLD, E. & LAYER, P. G. (1992) Formation of neuroblastic layers in chicken retinospheroids: the fibre layer of Chievitz secludes AChE-positive cells from mitotic cells. *Cell Tissue Res.* **268**, 401–8.

WILSON, R. G., SMITH, A. N. & BIRD, C. C. (1990) Immunohistochemical detection of abnormal cell proliferation in colonic mucosa of subjects with polyps. *J. Clin. Pathol.* **43**, 744–7.

WITZIG, T. E., GONCHOROFF, N. J., KATZMANN, J. A., THERNEAU, T. M., KYLE, R. A. & GREIPP, P. R. (1988) Peripheral blood B cell labelling indices are a measure of disease activity in patients with monoclonal gammopathies. *J. Clin. Oncol.* **6**, 1041–6.

WOLF, H. K. & MICHALOPOULOS, G. K. (1992) Hepatocyte regeneration in acute fulminant and nonfulminant hepatitis: a study of proliferating cell nuclear antigen expression. *Hepatology* **15**, 707–13.

WOLF, J. R., HIRSCHHORN, R. R. & STEINER, S. M. (1992) Growth factor responsiveness: role of MyoD and myogenin. *Exp. Cell Res.* **202**, 105–12.

WOOD, J. G., MARTIN, S. & PRICE, D. J. (1992) Evidence that the earliest generated cells of the murine cerebral cortex form a transient population in the subplate and marginal zone. *Dev. Brain Res.* **66**, 137–40.

WURM, F. M., GWINN, K. A. & KINGSTON, R. E. (1986) Amplification and inducible overproduction of the mouse c-myc protein in mammalian cells. *Proc. Natl. Acad. Sci. USA* **83**, 5414–18.

YAMAGUCHI, K., IMANISHI, K., MARUNO, K., MIYAKE, Y., SHIMOSATO, & ABE, K. (1989) Lung cancer and autocrine growth factors. *Chest* **96**, 9S–31S.

YAMAMOTO, M., FUJITA, K., SHINKAI, T., YAMAMOTO, K. & NOUMURA, T. (1992) Identification of the phenotypic modulation of rabbit arterial smooth muscle cells in primary culture by flow cytometry. *Exp. Cell Res.* **198**, 43–51.

YANG, G., MATOCHA, M. F. & RAPOPORT, S. I. (1990) Increased numbers of extra-adrenal chromaffin cells in the abdominal paraganglia of senescent F344 rats: a possible role for the glucocorticoid receptor. *Cell Tissue Res.* **259**, 233–8.

YAO, C. H., KITAZAWA, S., FUJIMORI, T. & MAEDA, S. (1993) In situ hybridization at the electron microscopic level using a bromodeoxyuridine labeled DNA probe. *Biotech. Histochem.* **68**, 169–74.

YE, J., WANG, H. M., OGDEN, T. E. & RYAN, S. J. (1993) Allotransplantation of rabbit retinal pigment epithelial cells double-labelled with 5-bromodeoxyuridine (BrdU) and natural pigment. *Curr. Eye Res.* **12**, 629–39.

YEN, A., SAMUEL, V. & FORBES, M. (1992) Regulation of cell proliferation: late down-regulation of c-myb preceding myelomonocytic cell differentiation. *J. Cell. Physiol.* **153**, 147–56.

YOKOI, N., OKAMOTO, S., AKAGI, Y., TERUBAYASHI, H., IBARAKI, N., TSUTSUMI, M. & ITOI, M. (1988) [Investigation of the movement and proliferation of basal cells by BrdU immunohistochemistry in control group of diabetic corneal epitheliopathy] *Nippon Ganka Gakkai Zasshi* **92**, 1112–16.

YONG, V. W., KIM, S. U., KIM, M. W. & SHIN, D. H. (1988) Growth factors for human glial cells in culture. *Glia* **1**, 113–23.

YONG, V. W., TEJADA-BERGES, T., GOODYER, C. G., ANTEL, J. P. & YONG, F. P. (1992) Differential proliferative response of human and mouse astrocytes to gamma-interferon. *Glia* **6**, 269–80.

YOUNG, W. G., ZHANG, C. Z., LI, H., OSBORNE, P. & WATERS, M. J. (1992) The influence of growth hormone on cell proliferation in odontogenic epithelia by bromodeoxyuridine immunocytochemistry and morphometry in the Lewis dwarf rat. *J. Dent. Res.* **71**, 1807–11.

YOUNG, W. G., ZHANG, C. Z., LI, H., LOBIE, P. E. & WATERS, M. J. (1993) A bromodeoxyuridine immunocytochemical and morphometric study of the influence of growth hormone on cell proliferation in odontogenic mesenchyme of the Lewis dwarf rat. *Arch. Oral Biol.* **38**, 207–14.

YU, A. C., LEE, Y. L. & ENG, L. F. (1993) Astrogliosis in culture: I. The model and the effect of antisense oligonucleotides on glial fibrillary acidic protein synthesis. *J. Neurosci. Res.* **34**, 295–303.

YUASA, K., ARAI, T. & YAMADA, S. (1990) [Histochemical study of proliferating cells in the spleen in mice with acute hepatic injury]. *Nippon Shokakibyo Gakkai Zasshi* **87**, 989–96.

YUASA, C., TOMITA, Y., SHONO, M., ISHIMURA, K. & ICHIHARA, A. (1993) Importance of cell aggregation for expression of liver functions and regeneration demonstrated with primary cultured hepatocytes. *J. Cell. Physiol.* **156**, 522–30.

ZELLE, B., BERENDS, F. & LOHMAN, P. H. (1980) Repair of damage by ultraviolet radiation in xeroderma pigmentosum cell strains of complementation groups E and F. *Mutat. Res.* **73**, 157–69.

ZEYMER, U., FISHBEIN, M. C., FORRESTER, J. S. & CERCEK, B. (1992) Proliferating cell nuclear antigen immunohistochemistry in rat aorta after balloon denudation. Comparison with thymidine and bromodeoxyuridine labelling. *Am. J. Pathol.* **141**, 685–90.

ZHOU, T., BLUETHMANN, H., ELDRIDGE, J., BERRY, K. & MOUNTZ, J. D. (1993) Origin of CD4-CD8-B220+ T cells in MRL-lpr/lpr mice. Clues from a T cell receptor beta transgenic mouse. *J. Immunol.* **150**, 3651–67.