Science and Medicine

The 11th Science and Medicine Conference, the third held jointly with the Medical Research Society, took place at the Royal College of Physicians of London on 3–4 November, 1994. The plenary sessions of the Medical Research Society and Association of Young Medical Scientists were also incorporated.

Cellular basis of programming—the fetal impact on adult life

Nutritional programming

It was the first speaker, **Dr A Lucas** (Dunn Nutritional Laboratory, University of Cambridge), who proposed the term 'programming' for the concept that a stimulus or insult, when applied at a critical or sensitive period of development, could result in a lifelong effect on the structure or function of an organism. Triggers that act at these sensitive periods could be endogenous—for example, sex hormone exposure *in utero* influencing sexual differentiation in rats; or exogenous, including sensory inputs. Animal studies have shown that nutrition can act as such a trigger. Early nutrition affects such characteristics as growth and development, lipid levels, and possibly even longevity.

The investigation of nutritional programming includes animal studies, but for mankind human epidemiological studies, such as those from Professor Barker's group at the MRC Environmental Epidemiology Unit, Southampton, have pointed to some interesting lines of further research. However, the definitive investigation remains the randomised controlled interventional study. Interventions could be targeted at the critical periods during fetal and early life. Dr Lucas described his studies of preterm babies born in the 1980s who were randomised to receive different milks. These children have been followed up, with an impressive 98% of the original cohort being seen between the ages of 7.5 and eight years. Important differences in bone density at age five and in IQ at age seven to eight were found. The key questions to be answered are: the duration of the critical periods of development, the precise nutritional triggers, the sensing systems in the organism, and, in view of the rapid turnover of most cells, how the memory of these early events is stored. This presentation raised many important issues and provided an excellent introduction to the talks that followed.

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Hepatic metabolism may be programmed by a low protein diet during pregnancy

Professor C N Hales (Department of Clinical Biochemistry, University of Cambridge) described studies on two insulin-sensitive liver enzymes in rats, glucokinase and phosphoenolpyruvate carboxykinase (PEPCK). Glucokinase was reduced and PEPCK raised in pups whose dams had been fed a low protein diet in pregnancy. The ratio of the enzymes changed by 400%, and these changes were still present at 11 months of age. Hepatic zonation of enzymes is known to exist and changes in the relative sizes of these zones as a result of early nutrition may account for the persistence of these enzyme changes. Changes in the relative size of zones could also help to explain the observed association in humans of decreased glucose tolerance with an increased tendency to thrombosis, because clotting factors are also synthesised in particular zones of the liver.

Impaired fetal growth and insulin resistance in adult life

Dr D I W Phillips (MRC Environmental Epidemiology Unit, University of Southampton) discussed the metabolic basis for this association, with reference to metabolic studies carried out in adult human volunteers for whom detailed birth records were available. The metabolic studies were designed to discover whether the observed increased incidence of non-insulin-dependent diabetes mellitus (NIDDM) in adults who were thin at birth was due to a decrease in insulin secretion or an increase in insulin resistance. A strong association was found between thinness at birth and insulin resistance in adult life, a relationship independent of current body mass index. In skeletal muscle, glycolytic metabolism, an important site of insulin action, was decreased in subjects who were thin at birth. These subjects can use fatty acids as muscle fuel for aerobic exercise, but are less able to break down glycogen when this fuel is needed for anaerobic bursts of exercise. An interesting question raised during the discussion was whether all world-class athletes were large babies.

Metabolic capacity and macronutrient intake

Professor A A Jackson (Institute of Human Nutrition, University of Southampton) noted that whereas severe shortages of glucose or energy are needed before they can be shown to influence fetal growth and metabolism, small changes in protein intake have profound effects on the developing fetus. Professor Barker and colleagues had observed that both fetal size at birth and size of the placenta had an independent, but interrelated, effect on blood pressure at the age of 50 (people of low birthweight with relatively large placentae were most likely to suffer from hypertension). An animal model in which to investigate the

possible basis of these effects became available when it was found that rats on a low protein diet before and during pregnancy had pups that were small at birth but with larger placentae. In a further experiment, dams were fed on diets of differing protein content before and during pregnancy, and changed to a normal diet once they had delivered. Blood pressure was measured in the offspring, and a graded but non-linear response was found, with those on the lowest protein diets *in utero* having the highest blood pressures. These changes of maternal diet in pregnancy had long-lasting effects on the blood pressure of the offspring without demonstrable effects on their body size at nine weeks of age. Other changes were noted, including variations in appetite, changes in body composition, in antioxidant capacity, and even in immune function.

These results are relevant to human populations since many people in developing countries have a low protein intake comparable to that in the rat experiments. Vegetarians are likely to have a low protein diet, and up to 30% of teenage girls in the UK consider themselves vegetarian, an area that may warrant further study. In answer to a question, Professor Jackson agreed that, as yet, it is not clear whether the effects of low protein diets on the fetus are mediated through changes induced in maternal metabolism, or whether they are a result of deficient nutrients reaching the fetus.

Temperature-dependent sex determination in alligators

Professor M W J Ferguson (School of Biological Sciences, University of Manchester) gave a sparkling talk on the discovery of how the temperature at which alligators' eggs are incubated, programmes not only the reptiles' sex but also their embryonic and adult growth, pigmentation, and thermoregulation. Much of the fieldwork took place along the marshy Florida coastline. The effect of temperature on sex differentiation appears to be some kind of dose mechanism, female being the default sex. Under controlled conditions, temperatures lower than 31.5°C, or temperatures of exactly 35°C, produce females, while other temperatures produce males, and incubation at 32°C produces both sexes. This temperature effect was also found in alligator nests in the wild when egg position was mapped and temperatures measured, the eggs being collected shortly before hatching. Dr Ferguson personally sexed over 8,000 alligators—no mean feat! The observed sex ratio of about 6:1 to 8:1 in favour of females has puzzled geneticists. Professor Ferguson explained that egg-laying alligators tend to pick environments and nest sites close to the temperature at which they themselves were incubated, and this in turn will tend to favour eggs incubating within the female temperature ranges. Prospective PhD students who enjoy living dangerously may well find there is a lot more work planned.

The role of glucocorticoid exposure 'in utero' in programming adult cardiovascular disease

Professor C R W Edwards (Faculty of Medicine, University of Edinburgh) explained that, in the search for mechanisms linking low birthweight to raised blood pressure, his group concentrated on glucocorticoids because, among their many other actions, they can retard fetal growth, modify development, and affect placental growth. The enzyme 11β-hydroxysteroid dehydrogenase (11β-OHSD) reversibly converts inactive cortisone to active cortisol in the human. A newly identified and purified form of this enzyme, type II, found particularly in the kidney and the placenta, irreversibly converts cortisol to cortisone. A rare adult case of apparent mineralocorticoid deficiency due to the absence of type II 11β-OHSD helped to elucidate its action in the kidney. Renal mineralocorticoid receptors are not specific and can be bound by cortisol, in which case hypertension and hypokalaemia result. Type II 11β-OHSD removes cortisol, allowing the much lower levels of mineralocorticoids to bind to these receptors.

In the placenta the enzyme acts as a barrier to protect the fetus from high maternal levels of glucocorticoids. A low enzyme activity in the rat placenta was associated with low birthweight and larger placentae. In studies of pregnant rats given inhibitors of this enzyme, the offspring developed insulin resistance in later life, and, in some studies, raised blood pressure. In pregnant rats on a low protein diet, the levels of placental 11β-OHSD are reduced, thus exposing the fetuses to increased cortisol; and pregnant rats given dexamethasone, a steroid that is not inactivated by placental 11β-OHSD, had offspring with raised blood pressure. Perhaps this indicates a common pathway between these observations and the changes seen in the offspring of protein-restricted rats?

Genes, proteins, and cell proliferation

Mechanisms of signal transduction

Dr J Downward (Imperial Cancer Research Fund, London) focused his talk on the *ras* proteins and their role in the regulation of cell proliferation. These low molecular weight proteins are part of a large family of related proteins showing about 30% homology, including *rho* (involved in regulating/controlling the cytoskeleton), and *rab* (involved in intracellular trafficking). The *ras* proteins are the most commonly activated dominant oncogenes, occuring in 25–30% of all human tumours, and in some, such as pancreatic carcinoma, found in virtually all cells.

The *ras* proteins are GTPases which are active when GTP-bound and inactive when bound to GDP. Control of *ras* is a balance between inactivating GTPase activating proteins and activating nucleotide exchange factors. *Ras* seems to respond to many stimuli, including

most growth factors, and is one of very few oncogenes which are nearly identical in yeasts and mammals, suggesting an ancient and strongly conserved activating mechanism. *Ras* stimulates cell growth, and interacts with *raf*, another oncogene product; both *ras* and *raf* can activate the MAPK (mitogen-activated protein kinase) cascade which influences transcription in the nucleus. During the discussion, it was emphasised how useful it would be to block the action of *ras*, and that there is ongoing research into candidate drugs.

Homeobox genes and craniofacial development

Professor P J Sharpe (UMDS, Guy's Hospital, London) posed the question of how cells in the developing embryo know their position in relation to other cells, and therefore how they should develop, ie their pattern. This year is the 10th anniversary of the discovery of the homeobox motif. Homeogenes code for homeoproteins and function by controlling the transcription of other genes; they provide spatial information but do not form structures themselves.

The homeobox motif was first found in the homeotic genes of the Drosophila fruit fly. It is now known that mammals have 38 *hox* genes, equivalent to the fly's homeotic genes. They occur in four clusters on the genome and have arisen by duplication of one ancestral cluster. Each *hox* gene is expressed along the axis of the embryo, each having different boundaries which appear to specify the pattern of the body.

Professor Sharpe went on to consider the role of some other homeobox genes in patterning, for example those used to determine two features of mammalian dentition: the *position* in the jaw, and the *pattern*, eg molar or canine. The genes *msx1*, *msx2* and *dlx2* are expressed spatially during development of the face and interact to specify both position and pattern via signalling and adhesion molecules.

Msx genes are expressed in many structures derived from the neural crest and illustrate a general feature of homeobox genes—with increasing complexity of evolutionary development, more genes are required. This has been achieved by duplication of existing genes. There is some evidence for expression of homeobox genes in adults but they may be expressed in a different way in embryos.

The 'erb' family of proteins

Continuing the theme of cell signalling molecules, **Professor W J Gullick** (ICRF Oncology Unit, Royal Postgraduate Medical School, London) gave an overview of one of the many families of cell surface receptors, that of the epidermal growth factors (EGF). The four members (EGFR, *c-erb*B2, 3, and 4) each have a single transmembrane spanning sequence, an extracellular portion which binds ligands, and intracellular tyrosine kinase. Each receptor may be able to bind more than one ligand, which could allow variation in regulation by the receptor in different tissues. Binding of a ligand causes dimerisation of receptors, intracellular phosphorylation and triggering of a second messenger system. There may be homodimerisation, or heterodimerisation with another member of the family; the latter binding seems to have a higher affinity for the ligand, and the capacity for heterodimerisation means that the function of one receptor in a tissue is dependent on the other receptors.

What is the role of these molecules in human cancer? For example, the expression of *c-erb*B2 is low in normal breast tissue, but high in 20% of breast cancers, and in other tumours. Experimental techniques which increase expression of growth factor receptor and cause cell transformation, or conversely, suppress expression and reduce malignancy, support the importance of these changes in cancer. Recent work suggests that overexpression of *c-erb*B2 may indicate a poorer response to chemotherapy.

c-ret

Dr V Pachnis (National Institute for Medical Research, Mill Hill) began a fascinating talk by suggesting that an understanding of the developmental mechanisms that operate during mammalian embryogenesis could help in explaining the pathogenesis of some human diseases. The *c-ret* proto-oncogene is a tyrosine kinase receptor which is somatically rearranged in 25% of thyroid papillary carcinomas. In the past year, a number of germ line mutations have been described which cause multiple endocrine neoplasia (MEN) type 2A and 2B, and Hirschsprung's disease. (MEN 2A/2B are dominantly inherited cancer syndromes.) The mutations leading to each syndrome are localised in different areas of the gene and lead to very different phenotypes.

From early stages of their development, enteric neurons and neuroblasts express a high level of *c-ret* oncogene. Transgenic mice, homozygous for the loss of the *c-ret* gene, are born without enteric neurons and glia throughout their gut; thus there is an analogy with Hirschsprung's disease. These mice also had absent or rudimentary kidneys. The kidney develops from a close interaction between the ureteric bud and metanephric mesenchyme, and the *c-ret* gene is expressed in high levels at this interface; in the absence of the *c-ret* receptor, the ureteric bud fails to grow and branch normally.

Frontiers in transplantation

Pigs as organ donors

Transplantation has become a victim of its own success, with demand for human organs outstripping supply. **Dr D J G White** (University of Cambridge) described genetic engineering to produce transgenic

pigs to provide organs for human use. He believes that the action of complement is all-important in the hyperacute xenograft rejection. Inhibitors of the complement cascade include decay-accelerating factor (DAF), which is species specific. Having first shown *in vitro* that mouse fibroblasts expressing human DAF were protected from human anti-mouse antibodies, the team have worked to incorporate the gene for this complement regulator into pigs. These pigs showed a wide variation in the expression of DAF. A mechanical system was used to test the ability of the excised pig heart to pump fresh human blood. As yet the 'best' hearts can pump for up to four hours but, after this test, hearts that express human DAF show virtually no staining for complement. The next step is to find a primate recipient where complement is at least partly down-regulated by human DAF.

Haemopoietic stem cell transplantation

Bone-marrow transplants can be autologous (patient's own cells given back), syngeneic (from an identical twin), or allogeneic (from a sibling). **Professor D Linch** (University College Hospital, London) discussed the use of peripheral blood stem cell transfusion (PSCT), which has been shown to work well in an autologous context. The important cells in a bone-marrow transplant are the primitive stem cells because they retain the potential to form all normal blood cells. Within a few weeks one stem cell in the marrow can produce a million mature cells in the circulation. A few stem cells are also found in normal peripheral blood and their numbers increase after chemotherapy prior to the reappearance of neutrophils.

Differentiation of stem cells is governed by growth factors, including granulocyte colony stimulating factor (G-CSF). G-CSF also encourages stem cells to enter the peripheral circulation. In lymphoma, the inclusion of G-CSF in initial chemotherapy markedly increases the number of stem cells that can be harvested by leukophoresis as the marrow recovers. It is possible to purify 250 ml of leukophoresed cells into 2.5 ml of 78% pure stem cells, screening out malignant cells, removing T cells that could cause graft-versus-host reactions, and leaving cells suitable for genetic manipulation. In patients with lymphoma, giving PSCT instead of bone-marrow transplant can halve the length of hospital stay. It is unclear whether this procedure would be ethical in allogeneic transplants, since it would involve giving G-CSF, a new drug, to healthy volunteers.

Transplantation of insulin-secreting tissues

Mr N J M London (University of Leicester) explained that the islets of Langerhans make up 2% of pancreatic weight but use 25% of its blood supply. Pancreatic transplants for people with type 1 diabetes include a large amount of unnecessary tissue. Moreover, long-term immunosuppression is required and is possibly more dangerous than diabetes itself. Pancreatic transplants have therefore only been performed in patients with type 1 diabetes who require kidney transplants, as they would then require immunosuppressive treatment anyway.

Islet cell harvesting uses collagenase to break down the pancreas and a semi-automated method of islet cell separation. To obtain enough cells, islet transplants tend to be from multiple donors and this increases the risks of rejection. The islets need a well-vascularised milieu, and do well if injected into the portal vein. Function can then be measured indirectly by measuring c-peptide which is produced in equal amounts to insulin. Since 1990 there have been 111 islet allografts; 20 no longer need insulin injections, and one graft has been working for over three years. The endothelium appears to be most closely involved in hyperacute rejection, and rapid vascularisation of islets by host endothelium may reduce the chances of serious reactions. Other options, such as encapsulating the islets and xenotransplantation, are being explored. It is encouraging that we may soon be able to offer young people with diabetes the chance of a well-matched single donor islet allograft without the need for long-term immunosuppression.

Professor R I Lechler (Department of Immunology, Royal Postgraduate Medical School, London) gave a broad overview of the principles of transplant tolerance and the prospects for inducing such tolerance.

Plenary sessions

The plenary sessions of the Medical Research Society and the Association of Young Medical Scientists were an important part of the conference. The standard of presentation was consistently high and the subjects wide-ranging, as we hope the following selection will illustrate.

Dr M R Thursz *et al* (St Mary's Hospital Medical School, London and John Radcliffe Hospital, Oxford) studied the relationship between MHC polymorphisms and the outcome of hepatitis B virus infection. In a two-stage case-control study in the Gambia, they found that HLA DRBI* 1302 was associated with clearance of infection. During the discussion it was noted that the same allele protects against severe malaria and has also recently been associated with a reduced risk of papilloma virus-induced cervical carcinoma.

Dr J M Gleadle *et al* (John Radcliffe Hospital, Oxford) investigated the relationship between hypoxia and synthesis of vascular growth factors. Erythropoietin (EPO) is produced in response to hypoxia, but only in the kidney and liver. Other cells can also respond to hypoxia, but do not synthesise EPO, suggesting that other genes may be regulated by an oxygen-sensing system. Several vascular growth factors showed a significant response to hypoxia in tissue

culture and this response, and that of EPO, could be mimicked by cobaltous ions and iron chelators, suggesting that the sensor might be a ferroprotein.

In view of the previous session on cellular programming and discussion of the Barker hypothesis, the work presented by **Dr D Churchill** *et al* (City Hospital NHS Trust, Birmingham), could be regarded as somewhat controversial. Their study involved 24-hour ambulatory blood pressure monitoring of 209 women at three stages in pregnancy. They found that maternal blood pressure was inversely correlated with infant birthweight, and suggested that the work relating low birthweight with adult hypertension could partly be due to an inherited tendency to hypertension.

Dr G J Bellingan (Respiratory Medicine Unit, Edinburgh) described a series of elegant experiments to investigate the fate of inflammatory macrophages during resolution of inflammation, using a mouse model. Few macrophages die at the site of inflammation; most migrate to draining lymph nodes and eventually to the reticulo-endothelial system.

Two papers discussed aspects of HIV infection. **Dr D Lalloo** *et al* (John Radcliffe Hospital, Oxford) investigated mechanisms by which the virus evades the immune response. They showed that a high mutation rate and variation in the region of the viral epitope can cause loss of recognition by cytotoxic T lymphocytes, or produce epitopes which act, instead, as antagonists. A second paper, from **Dr I P Everall** *et al* (Institute of Psychiatry, London), noted that HIV infection is associated with neuronal loss in symptomatic patients and *in vitro* evidence suggests that glutamate is involved in this neurotoxicity.

Conclusions

What were our overall impressions of the conference? Mostly very good, but with a few criticisms. The enthusiasm of the presenters for their work was obvious, and they took the opportunity to give the audience an insight into some exciting areas of biomedical research, and perhaps to encourage students and others to enter this field. In view of this, we were surprised that there were no women among the speakers, although there were many women in the audience who participated in the discussions. An important point for speakers to note at such a wide-ranging conference is that they should avoid using abbreviations, or if that is impossible, they should explain them fully and perhaps more than once during their presentations; otherwise members of the audience not familiar with that field may find it difficult to follow the argument.

Apart from these few reservations, we enjoyed a fascinating two days.

Regional conference in Nottingham

The September 1994 Regional Royal College of Physicians Conference was held in the newly refurbished Postgraduate Medical Education Centre at the Nottingham City Hospital. Over 100 participants enjoyed a broadly based programme where topical issues in many of the specialty areas of general medicine were discussed and posters of local research were displayed. The presentations were given by both local and national experts and were of a uniformly high standard.

Use of radioiodine—efficacy and potential hazards

Dr J A Franklyn (University of Birmingham) identified a number of problems with radioactive iodine treatment of thyrotoxicosis. Assessing the optimum dose for individual patients remains largely empirical. Her policy is to use a single dose of 5 millicuries which cures approximately two-thirds of patients. The prospect of cure is higher and of hypothyroidism lower in those with toxic nodular goitre than in patients with Graves disease. Evidence of severe disease (particularly Graves disease) predicts a poor response to radioactive iodine treatment.

Many non-specialists think that radioactive iodine treatment increases the risk of malignancy and infertility, although several large scale retrospective studies do not support this view and the Administration of Radioactive Substance Advisory Committee has stated that the age restriction for radioactive iodine treatment is unnecessary.

Specialists are concerned over a recent report that Graves ophthalmopathy deteriorated more in patients treated with radioactive iodine than in a matched group treated with methimazole. Although the finding of this study has been disputed by an American retrospective study, many remain concerned about the use of radioactive iodine in patients with Graves ophthalmopathy.

Dr Franklyn recommended radioactive iodine as the treatment of choice in elderly patients and in those with toxic nodular goitre, but that in young subjects with Graves disease a 12 or 18 months course of antithyroid drug, which offers a 30% chance of long-term remission, should be tried first.

Hypoglycaemia in insulin-dependent diabetes

Dr S Heller (Northern General Hospital, Sheffield) gave a fascinating account of hypoglycaemia, the com-

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