Exceptional Matters: clinical research from bedside to bench

Keith Peters

The Harveian Oration is given annually at the Royal College of Physicians of London under an indenture of William Harvey in 1656. This article is based on the 2004 Oration given on 19 October 2004 by **Sir Keith Peters** FRCP FRS PMedSci, Regius Professor of Physic, Head of the School of Clinical Medicine, University of Cambridge

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Introduction

In his Harveian Oration of exactly 80 years ago, Archibald Garrod, one of the greatest clinical researchers of the last century, quoted from a letter written by Harvey (only six weeks before his death) to a Dutch physician who had sent him an unusual specimen:[1]

> *It is even so – Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature, by careful investigation of cases of rarer forms of disease. For it has been found, in almost all things, that what they contain of useful or applicable is hardly perceived unless we are deprived of them, or they become deranged in some way.*

Garrod then went on :

> *These words, as true to-day as when they were written, are full of encouragement for those of us for whom the study of Nature's experiments and mistakes has a special attraction. The structural malformation, or the hereditary and inborn departure from the normal of metabolism, although unimportant from the practical standpoint, may throw a ray of light into some dark place of embryology or biochemistry; and not a few of the rare maladies, such as chloroma, polycythaemia vera, sulphaemoglobinaemia, and the disease of which Bence-Jones albuminuria is a sign, offer fascinating and still unsolved problems of physiology and pathology.*

The force of these much-quoted observations remains undiminished today.

Garrod, stimulated by the black urine of a patient with alcaptonuria, introduced the concept of the inborn error of metabolism. Influenced by William Bateson, he suggested that inborn errors are caused by (genetic) defects in enzymes: his 1908 inference that genes encode enzymes antedated by 35 years the Nobel Prize winning rediscovery by Beadle and Tatum.[2]

I believe I can be confident that my title would have found approval with Garrod, and Harvey and Bateson. Bateson declared:[3]

> *...a word of counsel to beginners, it is: 'Treasure your exceptions! When there are none, work gets so dull that no one cares to carry it further. Keep them always uncovered and in sight. Exceptions are like the rough brick-work of a growing building which tells that there is more to come and shews where the next construction is to be'.*

Garrod's Oration in 1924 was titled *The debt of science to medicine*. Today's catch-phrase is 'from bench to bedside' and university medical centres are struggling with the promotion of translational research. But in the relatively recent past, the debt of science to medicine has in fact grown more than even Garrod might have envisaged – often in the most surprising ways.

I shall describe examples which reflect a largely personal experience, where astute observations of single cases or small groups of patients have been the starting point of voyages of scientific discovery – from, as it were, the bedside to the bench – but in doing so I shall illustrate the synergy between clinical and basic biomedical research that now provides medical scientists with investigative tools of hitherto unimagined power. Indeed, I hope to persuade you that the golden age of clinical science does not belong to a long-gone era but that it is about to begin.

My professional life has been divided into two overlapping phases: the first as a practising physician/clinical scientist, and the second with strategic responsibilities for teaching and research at the Royal Postgraduate Medical School (RPMS) and at the Clinical School in Cambridge. I hope to show something of the power of blending the values and cultures of the two institutions: the RPMS, the crucible of clinical science, pioneering medical technology and providing the breeding ground for leaders in clinical academic medicine, and Cambridge with its tradition of basic medical science, especially molecular and cell biology.

I was an undergraduate in Cardiff where I was taught by Harold Scarborough, the Professor of Medicine. He was then well known to medical students as the co-author of 'BDS': Bell, Davidson and Scarborough, *Textbook of physiology and biochemistry*. He was an exceptional teacher who sought to explain disease in physiological and biochemical

terms. I also saw a great deal of Archie Cochrane, for he invited our small band of medical students to his elegant home and tried passionately to convert us to epidemiology by proselytising the virtues of the randomised clinical trial. He had few total converts but his influence was enduring.

In 1965, I moved to Birmingham to join John Squire in the Department of Experimental Pathology; this was my first exposure to experimental models of disease in animals. Squire was in every way exceptional and was planning, as Director Designate, the MRC Clinical Research Centre at Northwick Park, but sadly died five months later. Memorably, I met Emil Unanue who had gone to work with John Humphrey at Mill Hill but had earlier, with Frank Dixon, conducted comprehensive and definitive studies of experimental models of nephritis.[4,5] Unanue, Frank Austen and Hugh McDevitt, who also worked with John Humphrey, have become leading immunologists in the USA: they acknowledge the scientific inspiration that John unselfishly provided. John Humphrey was later to become Professor of Immunology at the RPMS in succession to Peter Lachmann.

Frank Dixon had established the paradigm for pathogenetic mechanisms in glomerulonephritis – namely that there were two underlying immunological mechanisms, the first mediated by autoantibodies (especially to glomerular basement membrane (GBM)) and the second due to accumulation in the glomerulus of circulating antigen–antibody complexes – immune complex disease; and that these two processes were readily distinguishable by immunofluorescent examination of renal biopsies.

From experimental models to the clinic: autoimmunity and plasma exchange

I was appointed to a lectureship in renal medicine at the RPMS in 1969 with the intention of developing research into mechanisms of disease in nephritis, then the principal cause of end-stage renal failure, and application of Dixon's ideas was a natural starting point. Antibodies to GBM were usually associated with a devastating disease (Goodpasture's syndrome) in which the lung basement membranes were also damaged causing life-threatening pulmonary haemorrhage, and where kidney function was rapidly lost due to a severe proliferative nephritis (rapidly progressive glomerulonephritis (RPGN)). We reasoned that if circulating autoantibodies were indeed the cause of disease, then interventions aimed at reducing their generation – immunosuppression, combined with plasma exchange to remove antibody – should alleviate the disease.[6]

The surprising finding was not that disease was alleviated but that antibody production was permanently arrested in some patients in less than a few weeks (Fig 1). It has taken many years of research into immunoregulatory mechanisms to begin to throw light on how this might have been brought about. Only in the last decade has there been a convincing characterisation of regulatory T cells whose engagement (shifting the balance between activatory and regulatory T cells) could explain the termination of an autoimmune response. The subject of regulatory T cells is now a major area of immunological research – and devising immunological interventions specifically to promote the generation of immunological control systems to suppress unwanted immune responses, including immune responses to transplanted organs, is a major goal of modern immunology.[7] (It should be added that despite thousands of publications on the subject of regulatory T cells in mice there is as yet no test which identifies their specific functional presence in regulating autoimmune disorders in man.)

Success in the anti-GBM syndromes led to a consideration of other diseases where patients were not critically ill and were therefore easier to evaluate. The first of these was myasthenia gravis (MG), then suspected though not proven to be due to

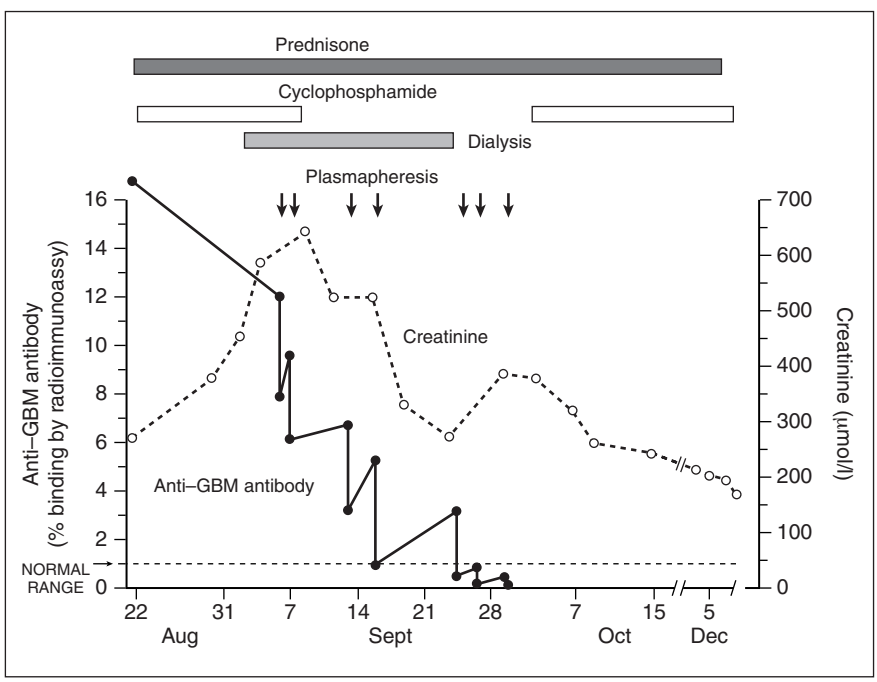

Fig 1. Effect of plasma exchange and immunosuppression on anti-GBM antibody and renal function. Autoantibody production ceased and never recurred (from Ref 6).

autoantibodies to the acetylcholine receptor (AChR). Tony Pinching, who had worked with John Newsom-Davis as a junior doctor at Queen Square, suggested that we invite John to collaborate. To our delight, there was a dramatic response to plasma exchange (Fig 2), later shown to correlate with removal of antibody and fall in circulating anti-AChR antibody concentrations.[8] This clinical experiment established that circulating antibodies to the acetylcholine receptor were indeed the principal cause of the neuromuscular dysfunction characteristic of MG in man; and further that the therapeutic goal was the safe suppression of the generation of these pathogenic autoantibodies.

Key to evaluating plasma exchange was the development of radio-immunoassays (RIA) for the putative pathogenic autoantibodies, so that the effect of therapy on its target – the pathogenic autoimmune response – could be quantitatively assessed. RIA had been devised by Berson and Yalow for the measurement of plasma hormones, research for which Yalow (after Berson's death) won the Nobel Prize.

Returning now to renal disease, anti-GBM nephritis characteristically presents as a rapidly progressive glomerulonephritis (RPGN). But only a minority of patients with RPGN have anti-GBM antibodies. Immunofluorescence of renal biopsies usually shows no evidence of accumulation of immune reactants in the kidney; and despite the fact that the majority have some form of systemic vasculitis, circulating antigen–antibody complexes are rarely detected – findings which led some to use the term 'pauci-immune'. Such patients therefore did not match the Dixon experimental model and the rationale for treating this group of patients with immunosuppressive drugs or plasma exchange was flimsy; nevertheless, cytotoxic drugs such as cyclophosphamide seemed beneficial. Since it could be argued that certain plasma protein constituents, such as complement and fibrinogen, might be important as mediators of inflammation, plasma exchange, which was safe and relatively easy, should be tried. Surprisingly, the response to therapy was better than that found in patients with anti-GBM disease.[9]

What might be the explanation for this? It was not until 1982 that a circulating autoantibody (whose significance was not immediately appreciated)[10] was detected in plasma of this category of RPGN patients; the autoantibody surprisingly was directed to intracellular antigens in neutrophils – antineutrophil cytoplasmic antibody (ANCA) – and later associated with Wegener's granulomatosis.[11,12] In due course, the principal targets of ANCA were identified as myeloperoxidase (MPO) and anti-proteinase-3; ANCA tests proved to be of great diagnostic and practical value in monitoring therapy.[13] But how could an autoantibody to intracellular neutrophil constituents cause disease? The next step was the demonstration *in vitro* that ANCA could activate polymorphs and make them potentially capable of enhancing tissue damage.[14] However, it was still not clear whether ANCA was the cause or consequence of the nephritis or vasculitis with which it was associated. Resolution required the technology of the late twentieth century. This was done by an ingenious experiment in which the gene for MPO was knocked out in a mouse; the knock-out mice were then immunised with mouse MPO to which they made antibodies; these antibodies were then transferred to normal mice (which had MPO in their white cells) and necrotising vasculitis and nephritis was produced.[15]

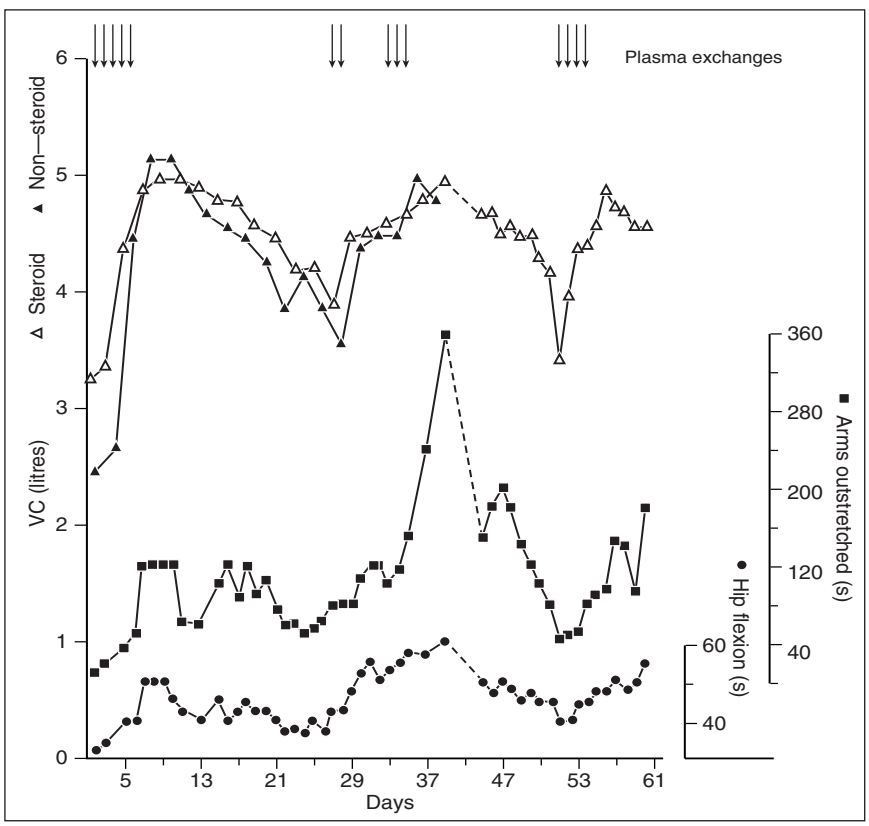

Fig 2. Effect of plasma exchange in a patient with myasthenia gravis. Improvement in neuromuscular function is dramatic. VC = vital capacity. Reprinted with permission from Elsevier (*The Lancet* 1977;**309**:428–9).[8]

It has thus required three decades of research, moving from bedside to bench and from man to mouse, to elucidate the scientific basis of an empirical therapeutic discovery; and in doing so a new paradigm of reno-vascular immunopathology was established, namely that autoantibody activation of neutrophils is the central pathogenetic mechanism in this important group of disorders, an outcome which in turn opens new avenues for therapeutic research.

Complement

At the RPMS, Chris Booth had succeeded John McMichael as Professor of Medicine in 1966 (Fig 3). McMichael had presided over the great physiological era in clinical medicine which had found expression in his own work in cardiology, that of Moran Campbell and his colleagues in respiratory medicine, Cuthbert Cope, Russell Fraser and Iain MacIntyre in endocrinology, Sheila Sherlock in hepatology, and Malcolm Milne in renal disease. Chris Booth was determined to promote research into disease mechanisms and his recruitment of Peter Lachmann as Foundation Professor of Immunology in 1971 was a signal event, and for me the turning point in my career. Peter Lachmann is a complementologist. In the 1960s and 1970s, the complement system in particular and immunology in general were being revolutionised by advances in protein chemistry. Complement functions discovered at the turn of the nineteenth/twentieth century, including the lysis of bacteria and erythrocytes and opsonisation of antigen–antibody complexes, were now being put on a firm molecular basis.

But, as I shall show, a single case changed thinking about the physiology of the system.

The background is that Peter Lachmann had conducted research on a substance known as conglutinogen activating factor (KAF) because its action was needed for conglutinin, the first described mammalian plasma lectin and peculiar to bovidae, to react with C3; and the starting point was a patient in Boston called TJ with recurrent bacterial infections. A blood sample was sent to the laboratory of Chester Alper for elec-

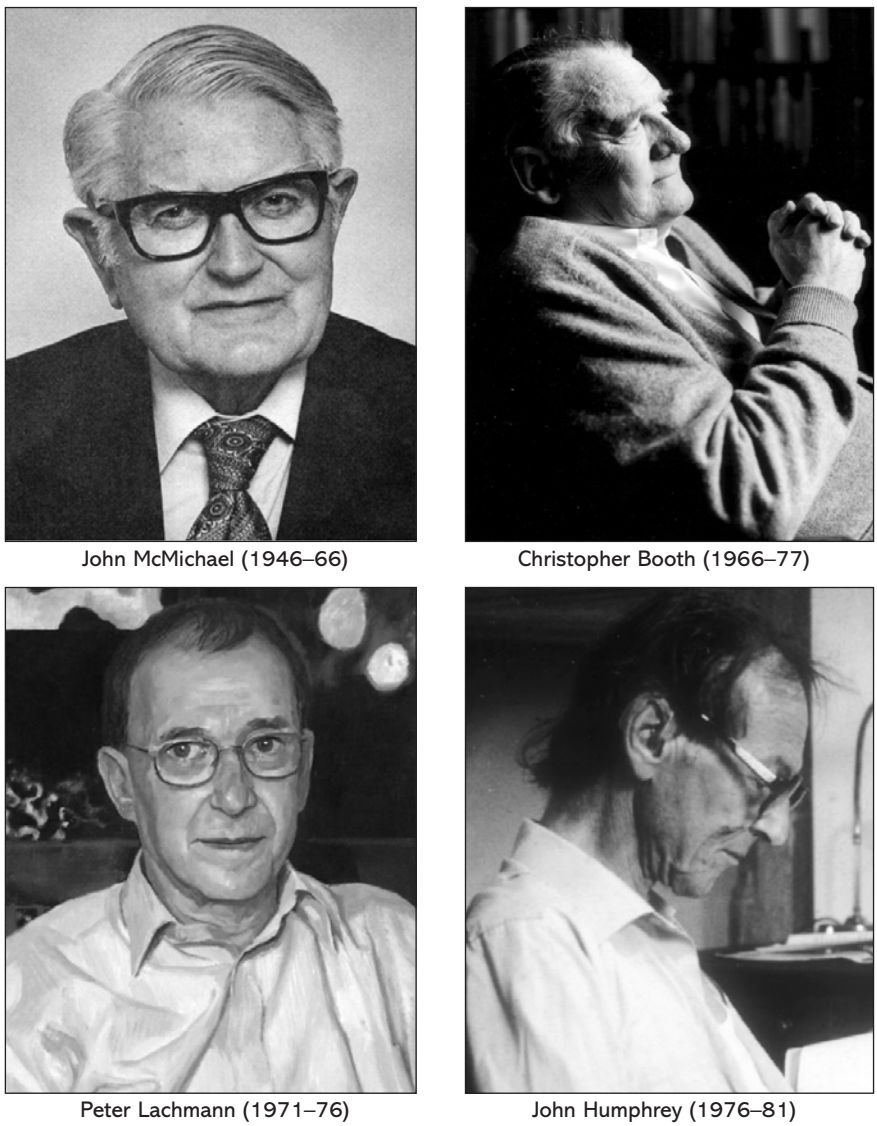

Fig 3. Hammersmith Professors. *Top:* Professors of Medicine; *bottom:* Professors of Immunology. *(Portrait of Peter Lachmann by Jeff Stultiens; from the Academy of Medical Sciences. Photograph of Christopher Booth by Nick Sinclair; from the National Portrait Gallery, London.)*

trophoresis to see if hypogammaglobulinaemia was the explanation. He noted that there was an absence of a band at the electrophoretic position, β1C (by then identified as C3). Further studies showed that TJ had very low levels of C3 and another recently identified protein of unknown function called glycine-rich beta glycoprotein (GBG). What was striking was that the early components of the complement system, C1, C4 and C2, were normal.[16] At the time it was believed that complement activation by antibody required the participation of these complement components in sequence to produce the enzyme that breaks down C3. But it was clear that C3 itself was indeed being broken down *in vivo*: the technique of crossed immunoelectrophoresis,[17] which Chester had learnt from Carl-Bertil Laurell in Sweden, demonstrated the presence of C3 breakdown products in fresh plasma.

How then was complement being activated? That there might be alternative pathways of complement activation had indeed been suspected in the 1950s,[18] but the idea had fallen into disrepute until the discovery of rare subjects (and animals) with deficiencies of C2 (or C4) whose serum still supported complement activation by gram-negative bacteria. Peter Lachmann met Chester Alper at a complement meeting, and suggested that TJ might be deficient in KAF. Chester sent TJ's serum to Peter Lachmann who confirmed complete absence of KAF, suggesting that KAF was the primary deficiency. Peter Lachmann and Pru Nicol conducted ingenious experiments in which they showed, by depleting normal sera of KAF, that a pattern of activation identical to that of TJ could be produced.[19] Peter Lachmann then proposed that what was by now termed 'the alternative pathway of complement' was normally ticking over at a slow rate, kept in control by KAF; and that deficiency or depletion of KAF led to uncontrolled activation of the pathway. KAF was then renamed C3b inactivator or factor I. It was later shown by Doug Fearon that physiological activators of the alternative pathway acted by preventing complement regulators, such as factor I, and another inhibitor, factor H, from exerting their effects.[20]

Thus we can trace a path beginning with the investigation of patient TJ, with recurrent bacterial infections, to the elucidation of a major physiological system of immunity.

Lipodystrophy and adipocytes: from immunity to metabolism and back

Had TJ not existed, the intricacies of the alternative pathway would almost certainly have emerged from the investigation of another extraordinary clinical syndrome, this time not due to a single gene defect.

Study of sera from a rare form of glomerulonephritis, which histologists had termed membranoproliferative glomerulonephritis (MPGN), revealed a form of complement C3 activation not involving the classical pathway C1, C4 and C2, associated with a circulating factor which caused C3 breakdown when added to normal human serum (the so-called nephritic factor (Nef)).[21,22] It was much later demonstrated that Nef was an autoantibody which blocked the action of the alternative pathway inhibitors, factors I and H, so that the normally labile enzyme which breaks down C3 is stabilised, thereby leading to excessive C3 consumption.[23,24] An identical system of complement activation was also found in patients with the rare disorder, partial lipodystrophy (PLD), itself known to be associated with MPGN.[25,26] These clinical associations indicated that this peculiar form of autoimmunity seemed to predispose to MPGN and/or PLD but the underlying mechanisms, particularly of adipocyte destruction, were obscure. Only lately, by genetic technology, has it been shown in mice that knock-outs of the complement regulatory factor, factor H, which physiologically inhibits the alternative pathway, develop MPGN, establishing beyond doubt the primary and causal role of unrestrained alternative pathway activation in this disorder.[27] Human H deficiency is associated with the haemolytic uraemic syndrome, as first shown by Thompson and Winterborn in 1981.[28]

But what of the adipocyte? The clue came in the late 1980s with the discovery that an enzyme in adipocytes named adipsin was identical with an alternative pathway complement factor, factor D.[29] Elevated concentrations of factor D potentiate alternative pathway activation, and this raised the possibility that under appropriate circumstances activation of the alternative pathway, enhanced by Nef, might cause lysis of fat cells. Peter Mathieson was able to demonstrate that this could indeed take place *in vitro*, [30] and showed that fat cells at those sites in the upper body where PLD is usually found have higher levels of adipsin/factor D than elsewhere. But the finding of complement factor D in adipocytes was of more general significance, for it was one of the earliest unequivocal indications that adipocytes were not just storage cells for excess fat and might have other hitherto unsuspected biological functions. This leads me to the leptin story.

Leptin is the first hormone whose role has been unequivocally demonstrated in the control of appetite. The beginnings were the discovery of the genetically obese mouse, the ob/ob mouse, and the pioneering parabiotic experiments linking ob/ob mice to normal mice – establishing that normal mice provided a factor which reversed the obesity of the ob/ob mice.[31]

Clearly, the ob/ob mouse lacked a factor present in the blood of normal mice, which controlled food intake and fat mass. Then, in 1995, Friedman and his colleagues at the Rockefeller University identified that the ob/ob mouse was deficient in leptin, a hormone secreted by fat cells that acted on the hypothalamus.[32] Early hopes that leptin might be of value in treating obesity were dashed by the finding that under normal circumstances obese humans have high plasma leptin concentrations and that malnourished subjects have low plasma leptin levels, reflecting in both circumstances adipocyte mass. Stephen O'Rahilly's group were the first to identify the human equivalents of the ob/ob mouse in a series of consanguineous families in whom leptin is totally absent.[33] The children had morbid obesity characterised by uncontrollable hyperphagia which was dramatically reversed by administration of recombinant leptin (see Fig 4) – a remarkable demonstration (albeit extreme) of the centrality of elucidating the control mechanisms of satiety to the understanding of obesity.

But an unexpected twist to the story is that leptin turns out to be an immunoregulatory hormone: ob/ob mice[34a] and the leptin-deficient children have impaired T cell function[34b] (Fig 5), posing the question of whether the immunity deficiency associated with starvation has its origins in whole or in part in adipocyte loss and consequent hypoleptinaemia. This has been postulated as an adaptive process of energy homeostasis.[35] Further, since it is now clear that adipocytes secrete a variety of cytokines, the converse question is the extent to which the untoward consequences of a gross excess of adipocyte tissue might be explained by subtle adipocyte-dependent pertubations of the immuno-inflammatory system. This is an area of research which is now attracting great interest.[36,37]

The leptin story is a striking example of the synergy of research in mouse and man.

Rare cancers

My next example of a clinical investigator catalysing a paradigm shift in a major disease is the work of Alfred (Al) Knudson. Knudson is an American paediatrician who as a student had been influenced by the pioneer geneticist, Thomas Hunt Morgan. In the 1960s, Knudson became fascinated by the rare childhood tumour, retinoblastoma. He noted that whereas the sporadic form was almost invariably unilateral and presented at various times in childhood, the familial form was frequently bilateral and presented soon after birth (Fig 6).

Knudson deduced that in the familial form there was an inherited loss of a gene responsible for preventing tumour formation and that during the hundred million or so cell divisions that take place in retinoblasts during differentiation, the chances of losing a second copy of this gene were sufficiently high to make the development of a tumour likely (but not inevitable); whereas in the non-familial cases the chances of two mutations occurring would be small.[38] The presentation of what has now become known as the 'Knudson two-hit hypothesis' ignited the field of cancer genetics and heralded the era of tumour suppressor biology.

The starting point for confirmation of the hypothesis was another clinical observation: cytogenetic analysis of rare patients with retinoblastoma and mental retardation and other developmental abnormalities revealed constitutional (in the non-tumour cells) deletions on the long arm of chromosome 13.[39] This clue to the location of the retinoblastoma gene was confirmed by the demonstration of linkage of retinoblastoma within families to the locus for esterase D,[40] which lay within the deleted region. This in turn led to the confirmation by Cavanee and White that the 'second hit' was indeed loss of the second allele Rb locus,[41] and ultimately to the cloning of the gene in the laboratory of Robert Weinberg.[42] Somatic mutation of the same Rb gene was found in the tumours of non-hereditary cases, the forerunner of the observation, now well established through the work of Vogelstein and others,[43] that patients with common cancers often have mutations in the same genes that are responsible for the familial forms. (If I may digress, it is easy to imagine a state of affairs where 'customer-driven' policies for medical research would accord low priority to rare patients with inherited malignancies.)

Cancer is now recognised as a genetic disease – in the majority

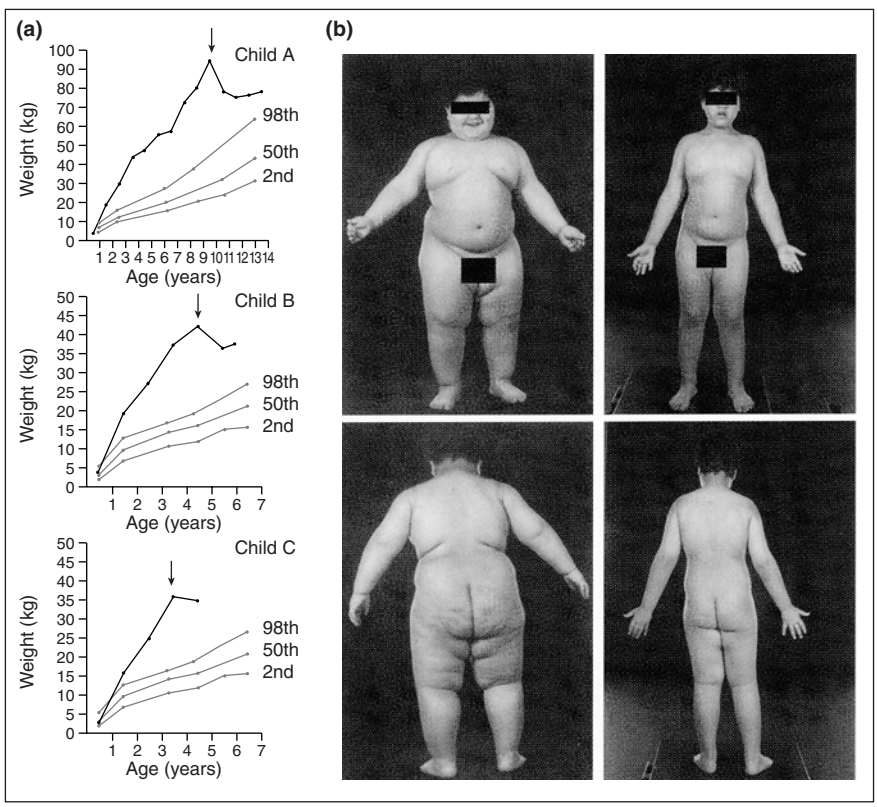

Fig 4. Effects of recombinant leptin in children with total deficiency of leptin (from Ref 34b).

acquired as a result of a series of somatic mutations, vital clues to which have been provided by the study of familial cases. Fast-forward about 30 years from Knudson's pioneering observations: the cancer world is radically altered. The development of genomic technology has enabled the analysis and characterisation of thousands of genes in biopsy material including, importantly, archival material. Much effort is being devoted to finding a gene signature that allows patients to be better characterised, especially in relation to the likely prognosis and response to therapy.[44–46] A feature of this work is the large datasets generated and the need for critical statistical analysis: this in turn requires strong mathematical and bioinformatics teams. The early results of these approaches are promising, suggesting that molecular signatures do indeed predict responses to therapy. Although these early claims may have been exaggerated,[47,48] it seems likely that molecular profiling is here to stay. A striking recent example comes from clinical trials of gefatinib, a small molecule drug targeted to the epidermal growth factor (EGF) receptor which is expressed in many cancers. In trials in non small-cell lung cancer, response rates were very poor; but it was noticed that a few percent of patients had good responses, and most of these proved to have mutations in the tumour which altered the ATP binding domain of the receptor kinase, and thus brought about responsiveness to the drug.[49]

Such studies suggest that patient selection based on detailed knowledge of individual variation in molecular targets will be central to the future development of cancer treatment.

Structural medicine

Max Perutz had witnessed the birth of the modern era of molecular haematology with the publication of Linus Pauling's classical paper in Science in 1949,[50] describing how sickle cell disease was due to a small change in the electric charge of haemoglobulin. Vernon Ingram, working in the Perutz laboratory in Cambridge, showed that the alteration in charge was due to replacement of a single one of the 146 amino acids in the β chain by another.[51]

Max Perutz had been influential in securing Robin Carrell's return to Cambridge to the Chair of Haematology.

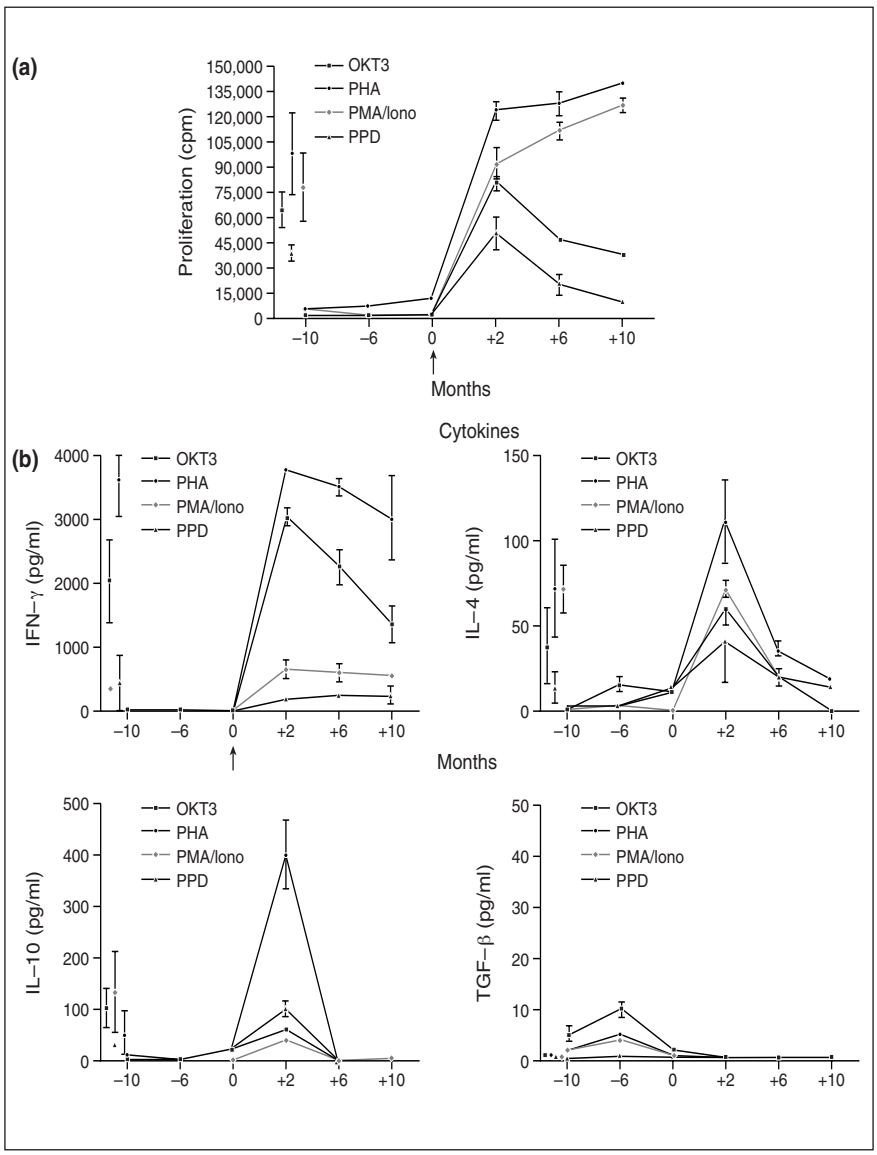

Fig 5. Effects of leptin on assays of cell mediated immunity: (a) proliferation assays; **(b)** cytokines IFN-γ, IL-4, IL-10 and TFG-β. Leptin therapy reverses the T cell hyporesponsiveness (from Ref 34b).

Two case reports.

Carrell (then in his native New Zealand), enthused by Carl-Bertil Laurell's discovery of alpha-1 antitrypsin deficiency as a cause of emphysema,[52] decided to elucidate the structural biology of this class of inhibitors: sequencing alpha-1 antitrypsin identified the mutation responsible for the failure of the proteinase inhibitor to protect the lungs, and thereby revealed the position and nature of the active site that confers specificity as an inhibitor of neutrophil elastase. A similar sequence was also present on another plasma protease inhibitor, antithrombin. Importantly, comparison of the two sites demonstrated that the specificity of inhibition depended on a single amino acid – with clear implications for the mechanism of action of antithrombin and its mode of activation by the anticoagulant drug heparin.[53]

This conclusion was not immediately accepted but the publication of a case report of a 14-year-old boy in Pittsburgh with a fatal haemorrhagic disorder provided final proof. The Pittsburgh investigators had noted that the patient's plasma contained an electrophoretic variant of alpha-1 antitrypsin that acted as an inhibitor of coagulation. Carrell immediately deduced that a methionine in the active site had mutated to arginine, changing the specificity of the inhibitor from an anti-neutrophil protease to an antithrombin. This was indeed the case – a natural experiment in protein engineering showing the way in which a single mutation can completely change the function of a protein.[54] This single case report also set the stage for the elucidation of the molecular basis of the antithrombin-activating drug, heparin – one of the oldest drugs in the pharmacopoeia. A further point is that alpha-1antitrypsin is an acute phase protein: the mutated anticoagulant molecule would therefore increase in concentration during the course of injury or infection to provide an anticoagulant effect equivalent to an excessive dose of heparin.

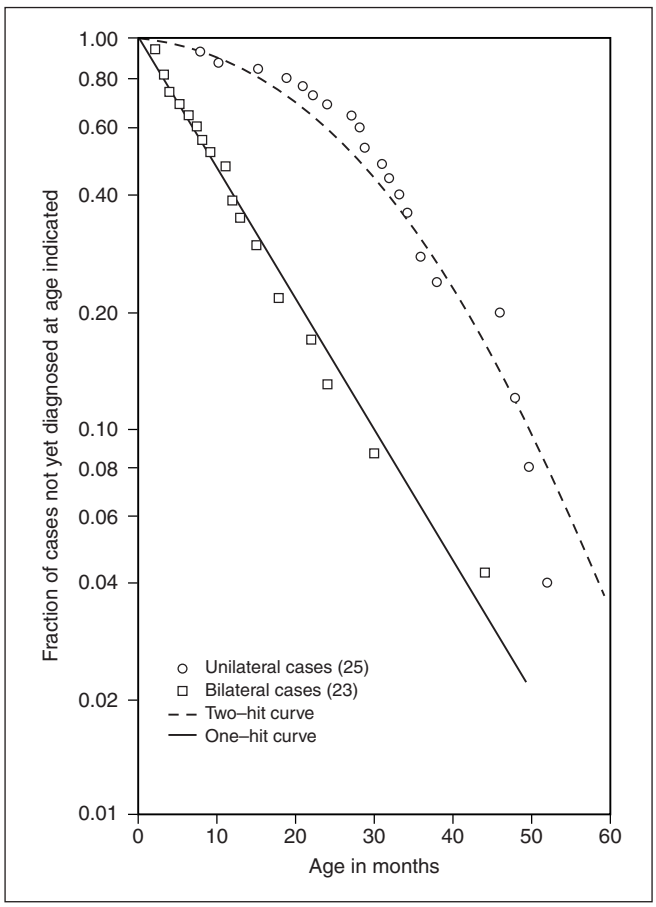

Fig 6. Semilogarithmic plot of fraction of cases of retinoblastoma not yet diagnosed against age in months – unilateral and bilateral separately plotted. Kinetics of bilateral cases indicate 'one hit', whereas kinetics of the unilateral cases indicate 'two hits' (from Ref 38).

The second example from Robin Carrell's group also relates to alpha-1 antitrypsin. David Lomas, a respiratory physician who had decided to train with Carrell, had proposed that intracellular aggregation of the mutated molecule in hepatocytes not only reduced secretion of alpha-1 antitrypsin but also suggested that the aggregated molecules (which could seen by electron microscopy (Fig 7)) were the cause of hepatocyte injury and cirrhosis.[55] Aggregation is concentration- (and temperature-) dependent and the acute phase response would therefore increase the formation of tissue aggregates; this has prophylactic implications.

That this process might be a more general pathogenic mechanism in diseases characterised by intracellular inclusion bodies is graphically demonstrated by a remarkable clinical story. A GP in Upstate New York insisted on an autopsy on a patient aged 56 who died of 'atypical Alzheimer's', and the GP also requested that specialist neuropathological examination be carried out in nearby Syracuse.

Dr Collins, the senior pathologist, noted the presence of unusual neuronal inclusions, and his younger colleague, Dr Richard Davis, with others, isolated the inclusions and found partial sequences identical to those of a newly identified serpin – neuroserpin – known to be specific to neurons. In the course of conversation at a local medical meeting, another practitioner, on hearing the surname of the patient, remarked that another person of that name was under treatment for a personality disorder. Richard Davis established the relationship of the two individuals and their wider cohort, demonstrating the inheritance, with Mendelian dominance, of a trait resulting in the consistent onset of an encephalopathy and dementia between the ages of 45 and 60 years. Because of the similarities of the inclusions with those in alpha-1 antitrypsin deficiency, Robin Carrell was contacted to ask whether it was possible that the neuronal degeneration was due to the polymerisation of neuroserpin. DNA testing at Syracuse soon confirmed Carrell's prediction of the nature of the underlying mutation. David Lomas went on to show that each step in the pathway that had been observed with the abnormal variant of alpha-1 antitrypsin was faithfully reproduced with the mutated neuroserpin. The results were unequivocal. It is difficult to think of any other experiment that could so definitively demonstrate how intracellular polymerisation results in cellular attrition, which with the hepatocyte leads to cirrhosis, and with the neuron leads to encephalopathy and dementia.[56]

An aside: so much for specialist medicine – Lomas, a respiratory physician, underwent his formative research training in a haema-

tology department, and has now made a substantial contribution to dementia research, which he is currently pursuing with the department of genetics by introducing mutated neuroserpin genes into flies!

In 1991, Grant Sutherland, a clinical geneticist working in Adelaide on the fragile X syndrome (so named because of its association with the fragile X chromosome) and the commonest cause of familial mental retardation, made a remarkable discovery: namely that the disorder was associated with hereditable expansion of trinucleotide repeat sequences – CCG – giving rise to the fragile site on the X chromosome.[57] It soon transpired that there are a number of diseases characterised by triplet repeats: AGC repeats are associated with neurological disorders such as myotonic dystrophy and neurodegenerative disorders where the triplet repeat CAG codes for glutamine. These diseases have a characteristic which had no explanation in Mendelian genetics, namely the phenomenon of anticipation, where a genetic disease becomes increasingly severe and presents earlier in successive generations. Genomic analysis provided the explanation, for in successive generations the number of repeats expands. No counterpart to these human disorders has been found in animals.[58]

Max Perutz had earlier noticed alternating positive and negative charged amino acids in the haemoglobin of a parasitic worm and had concluded that the amino-acid chains had the potential to form a molecular 'polar' zipper.[59] He then learned of the triplet repeat expansion characteristic of Huntington's disease[60] and quickly offered an elegant explanation of how such expansion of triplet repeats, creating a long glutamine chain, could by the action of the molecular zipper account for the formation of intracellular aggregates which characterise the disease (Fig 8).[61,62]

Of course, it had long been recognised in different contexts that both physiological and pathological aggregations of protein could be responsible for tissue injury, the former illustrated by immune-complex disease and the latter by amyloid. Here I pay tribute to Mark Pepys for his persistence in investigating the pathophysiology of the acute phase response. His early work purifying C-reactive protein (CRP) led to the recognition of a calcium-dependent binding protein which in due course turned out to be the serum amyloid P component (SAP), so called because of its binding to amyloid fibrils.[63] This binding property led directly to the development of a diagnostic radionuclide test for amyloid (SAP scintigraphy),[64] and allowed for the first time quantification of amyloid load in man – an essential requirement for evaluating therapeutic intervention.

Importantly, SAP scintigraphy provided Mark Pepys with a basis for referral and collection of patients, and in due course the UK NHS National Amyloidosis Centre was established at the Royal Free Hospital in 1999. Rare familial cases of amyloid have again been informative. An excellent example is the case of a family with autosomal dominant disease where sequencing showed that the amyloid fibril was derived from a mutation of the enzyme lysozyme.[65] Lysozyme had been used as a small molecular weight (15 kd) protein as a model for various experimental studies, most notably by the late David Phillips. It was the second protein and the first enzyme to have its complete 3-D structure solved and has been studied extensively with respect to enzyme-substrate reactions and protein-folding, especially by Chris Dobson, who is now in the Department of Chemistry in Cambridge.

Like my contemporaries of the 1960s, I had been familiar with the notion that protein–protein interactions could be pathogenic as in immune-complex disease, but few anticipated how

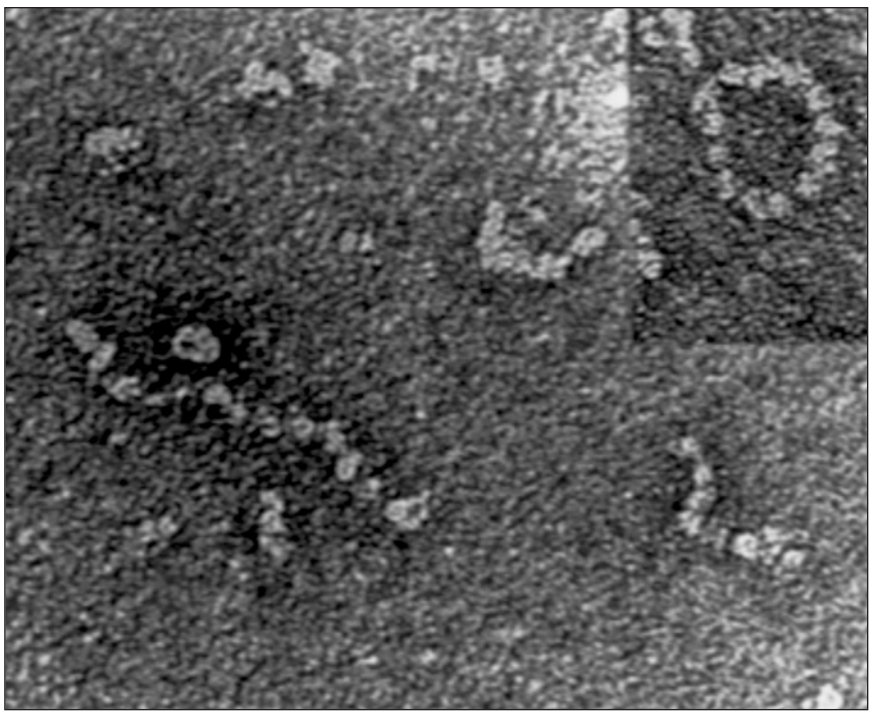

Fig 7. Electron microscopy showing molecular aggregates of mutated α-1 antritrypsin molecules in hepatocytes (from Ref 55).

important a general paradigm abnormal protein-folding would become. Prusiner's discovery of prions, infectious protein agents which produce their effects by inducing conformational changes in the equivalent normal brain proteins and subsequent protein aggregation, is the most dramatic example.[66] It was, however, the astute observation of William Hadlow, an American veterinary neuropathologist, of the similarity between the histopathogy of kuru and scrapie that was the first clue to the aetiology of the transmissible spongiform encephalopathies.[67] Prusiner's work remained controversial until Charles Weissmann's key experiment demonstrated that mice in which the normal prion protein had been 'knocked out' were resistant to inoculation by infectious prion particles[68] (another excellent example of the immense power of transgenic mice as a biomedical research tool).

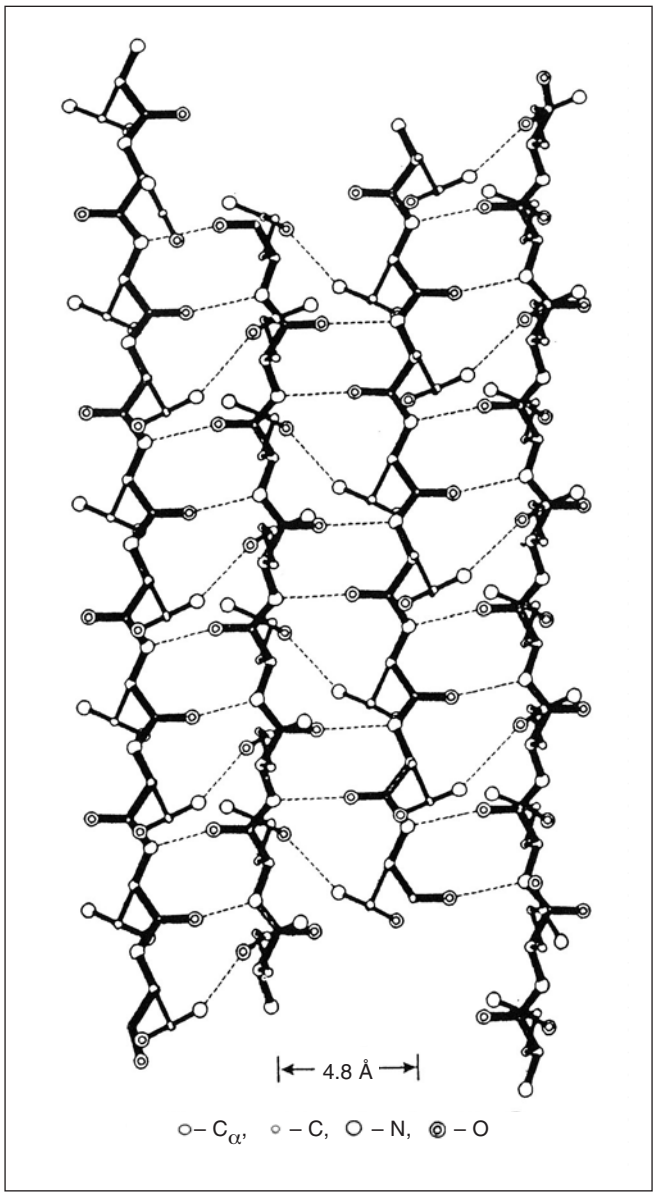

Fig 8. Four chains of poly-L-glutamine linked by hydrogen bonds between their main chain and side chain amides (from Ref 62).

In the UK, we need no reminder of the economic consequences of the prion disease of cattle, bovine spongiform encephalopathy, and the effect its transmission to man has had on relations between scientists, politicians and public.

Monoclonal antibodies

Monoclonal immunoglobulins (Ig) were first recognised in patients with myeloma. Much of our knowledge of Ig structure, function and genetics comes from the studies of myeloma proteins by Kunkel,[69] Putnam[70] and others – an earlier example of knowledge transfer from bedside to laboratory. This work also provided the basis of the technology devised by Milstein and Kohler for the production of monoclonal antibodies in mice by fusion of lymphocytes with myeloma cells.[71]

By the mid-1980s, there was great interest in the potential use of monoclonal antibodies in man, and in particular their use in the control of transplant rejection and in the treatment of immunological malignancies and lymphoma. A major limitation was that humans would mount an antimurine immune response, so the production by genetic engineering techniques pioneered by Greg Winter in the MRC Laboratory of Molecular Biology (MRC LMB) of humanised antibodies was a breakthrough.[72] In these antibodies, the combining site of a mouse or rat antibody was genetically transplanted onto the framework of a human antibody, so that it could be repeatedly used in man with a greatly reduced chance of evoking an immune response.

Earlier, various workers, including Medawar *et al*, had used polyclonal antilymphocyte antibodies as immunosuppressant drugs in attempts to treat transplant rejection and multiple sclerosis (MS).[73–76] However, monoclonal antibodies, particularly humanised antibodies, offered clear advantages. Venture capitalists watched with great eagerness as biotechnology companies were spawned. However, much scepticism was shown by the major pharmaceutical companies who did not believe that monoclonal antibodies would find an enduring place in the pharmacopoeia. But by the 1980s it had become generally recognised, at least within the immunology community, that autoimmune diseases, collectively, represented a major public health problem for which new treatments were needed.

I believe the first patient with an autoimmune disease whose condition was seemingly cured by administration of monoclonal antibodies was treated in Cambridge as a result of research that moved in the currently more familiar direction from bench to bedside. The patient, a retired physician, with a 20-year history of refractory vasculitis unresponsive to various conventional cytotoxic and immunosuppressive drugs, was given a combination of monoclonal antibodies (anti-CD52 – Campath – and anti-CD4), based on a protocol devised for induction of immune tolerance in mice by Herman Waldmann. A sustained remission followed and there was no vasculitic relapse.[77] He died some years later of colon cancer. It seems that therapy had arrested the autoimmune response; whatever the mechanism, the outcome was remarkable. Campath is now in advanced trials in an important autoimmune disease, multiple sclerosis, in a collaboration between Alastair Compston and Herman Waldmann; the pilot

experiments were encouraging[78] and the outcome of these trials is eagerly awaited.

But even more unexpected was the dramatic benefit discovered by Maini and Feldmann at Charing Cross Hospital (Kennedy Institute) of the effects of monoclonal antibodies to tumour necrosis factor (TNF)-alpha. This work, for which Maini and Feldmann have lately received the Lasker Award, owed its origins to the finding of high levels of TNF-alpha in synovial tissue in rheumatoid arthritis (RA).[79,80] Although the potential of interfering with immunologically driven inflammation by inhibiting defined mediators had been the object of research for decades, few anticipated the scale of benefit that was found clinically by Maini *et al* and has now been confirmed by many others.

Antibody-based therapies now subtend a multibillion-dollar industry with more than 29 drugs in advanced clinical trials or approved for use in man, in conditions ranging from variant angina to B cell lymphoma.

Martin Lockwood sadly died in a sailing accident in 1999. He had been prescient in his conviction that monoclonal antibody therapy would make a major impact on the treatment of the systemic vasculidites. Today there is a flourishing European Collaboration researching the treatment of autoimmune vasculitis to which three of Martin's closest collaborators, Caroline Savage in Birmingham, Charles Pusey at Hammersmith and David Jayne in Cambridge, are major contributors.

Cambridge: academic medicine and the NHS

In the late 1980s, a small group of us (Patrick Sissons, Tim Cox, Martin Lockwood, Leszeck Borysiewicz, Tony Weetman, Stephen O'Rahilly, Krish Chatterjee and David Oliviera) were explanted from the RPMS to the Clinical School in Cambridge where I succeeded John Butterfield. The School, founded in 1976 in the wake of Lord Todd's Royal Commission on Medical Education, was small but had exceptional potential. Roy Calne had made Addenbrooke's Hospital famous with his pioneering work in liver transplantation; Calne and White had introduced cyclosporin in the late 1970s; and Terence English performed the first successful UK heart transplant at Papworth Hospital in 1979. Tom Sherwood, Professor of Radiology, with whom I had worked closely at the RPMS, was Clinical Dean.

But the overwhelming reason for moving to Cambridge was my conviction of the need for closer partnership between clinical and basic biomedical research. Cambridge provided an exceptional opportunity to do this. The historical excellence of medical science in Cambridge stemmed from the founding of the Department of Physiology by Michael Foster in the latter part of the nineteenth century.[81] But it was the proximity of the MRC Laboratory of Molecular Biology to the Clinical School on the Addenbrooke's site that was compelling. Collaborations were already developing between members of the LMB, particularly Cesar Milstein with Nick Hales in Clinical Biochemistry and Herman Waldmann in Pathology; and Peter Lachmann had returned to Cambridge to direct an MRC unit in 1976. Sydney Brenner and I had become friends in the early 1980s and Sydney joined the Department of Medicine where he transformed a talented group of young physicians into molecular biologists. Aaron Klug, who succeeded Sydney as Director of the LMB, was a great supporter, and Max Perutz, the founding Chairman of the LMB, was an inspirational figure with a keen interest in clinical medicine.

In what follows I do not wish to present the Cambridge developments in a parochial context, but more as an example of what can be achieved by common purpose between the NHS and universities and their associated research institutes. The Medical Director of the Regional Health Authority, Michael O'Brien, declared: 'East Anglia does not have the medical school it needs – we must ensure that it does'. His support was invaluable.

Unlike the Hammersmith Hospital, where the work of the Medical School dominated the activities of a small hospital, Addenbrooke's, a 1,000-bedded hospital, was and is the only provider of hospital care to a substantial population of East Anglia. We therefore sought wherever possible to link academic and service developments. A priority was the development of a culture of clinical research, a key to which is provision of advanced medical technology. For example, an early decision was to invest in imaging research which led to the development of the Wolfson Brain Imaging Centre: it seemed inconceivable that we could develop clinical neuroscience research without dedicated positron emission and magnetic resonance imaging technologies.

A further goal was to facilitate the growth of epidemiological research. Through the appointment to the Chair of Nick Day, already Director of the MRC Biostatistics Unit in Cambridge, we were able to put its work at the core of his Department's research programmes. Shortly afterwards, with the help of the NHS, we established the Institute of Public Health (IPH). There is a pleasing development in epidemiology: the gap is narrowing between the intensively investigated patient in a hospital setting and the study of large-scale populations. Medical technology, through the use of such techniques as automated biochemistry, genomic technology, imaging and endoscopy, allows research to be made on populations that not so long ago could only be carried out on small groups of patients in a clinical investigation unit. Nick Day's scientific contributions are reflected in his election to the Royal Society earlier this year.

We recognised the importance of partnership with the pharmaceutical industry, and early links facilitated by Tim Rink and later George Poste have formed the basis of a lasting relationship with SmithKline Beckman/Beecham (later GlaxoSmithKline). Private philanthropathy has been invaluable and I acknowledge enduring support from Raymond and Beverly Sackler and Sir Ka-shing Li. Such support provides freedom from the conservative tyranny of peer review and leverages developments that would otherwise be unattainable.

The investment over the past two decades is bearing fruit. We have new laboratories to enable clinical and non-clinical scientists to work side by side, a clinical research facility which has revitalised clinical research, and a laboratory for the emerging discipline of genomic epidemiology linked to the IPH. The School's scientific standing is high. Eight members of staff have been elected Fellows of the Royal Society since 1992.

But of paramount importance has been the culture change at Addenbrooke's NHS Trust where there is major commitment to academic medicine. I pay particular tribute to a succession of chief executive officers: John Ashbourne, Roy Male and Malcolm Stamp. In conjunction with the MRC and the University, the Trust (lately renamed Cambridge University Hospitals NHS Foundation Trust) has developed a plan to create a biomedical campus with the aim of improving services to patients, providing outstanding facilities for teaching and research, and maximising the wealth-generating opportunities the NHS offers.

Academy of Medical Sciences

This brings me to the recent work of the Academy of Medical Sciences (AMS): I pay particular tribute to John Bell for chairing the working party that produced the report, *Strengthening clinical research*.[82] This, in conjunction with the Bioscience Innovation and Growth Team (BIGT) report,[83] has had a major effect on government thinking: for the first time since it was created, NHS research and development is set to receive a substantial increase in funding.

This is not the occasion to discuss in detail the achievements of the recently formed Academy of Medical Sciences: it was created to fill a gap (some would say a chasm) between the Royal Society and the Medical Royal Colleges. Two of its outstanding achievements have been the Savill Report on medical careers[84] and the Bell Report on clinical research. The BIGT and AMS Reports emphasised the potential of the NHS as a vehicle for clinical research, enabling the more rapid development of therapies and diagnostics, and, by inclusion of patients in clinical trials, generally enhancing the quality of healthcare.

The Bell Report also emphasised the need for what it termed 'experimental medicine', the detailed study of well characterised groups of patients by state-of-the-art techniques, encompassing the latest imaging modalities and appropriate biochemical, genomic and proteomic technologies. For the biotechnology and pharmaceutical sector, experimental medicine can facilitate target choice and accelerate drug development. Experimental medicine is an area where the physician scientist is pivotal, and both the pharmaceutical and clinical academic communities are deeply concerned that the infrastructure for this type of medical research needs to be greatly strengthened.

The regulatory environment also provides considerable obstacles: there is growing concern that the accumulation of well-intentioned legislation with specific objectives, such as the Data Protection Act, is in danger in aggregate of not acting in society's interest. The proposed Human Tissue Bill has been a startling example of how ill-considered legislation might bring vital medical research to a halt. This is part of the more general problem of ensuring dialogue between scientists and the public so that research issues are fully understood and the public fully involved – as indeed was the case in preparing the way for the legislation that allowed human embryonal stem-cell research to proceed in the UK. But in general our society has yet to resolve fully the question of balancing public benefit with the rights of the individual.

It has long been recognised that substantial barriers exist that inhibit those with the talents and potential to make major contributions through clinical research. This issue has now been recognised at a high level in government. I am pleased that Mark Walport, himself a distinguished clinical scientist and Director of the Wellcome Trust, is now chair of the Academic Careers Subcommittee of Modernising Medical Careers and the UK Clinical Research Collaboration, which I hope will bring this matter to a long-overdue solution.

It was in part the seemingly intractable problem of combining research with postgraduate professional training that caused us to set up the Cambridge MB/PhD programme in 1990. This programme, led by Tim Cox, provides exceptional students with an opportunity to do research before graduating in medicine. The programme has now reached the stage where it is clear that many of its graduates are committed to academic careers. Importantly, it provides these gifted students with an opportunity to engage in research when they are young.

Exceptional opportunities: the role of the clinical investigator

Over the past 25 years, many people, including very influential leaders of Western medicine, have expressed concern that the clinical investigator is an imperilled species.[85-86] How does this accord with the theme of this lecture? First, I must acknowledge the success of basic biomedical research and its contribution to medicine. Some see the recent emphasis being paid to so-called translational research as a threat to the successful biomedical enterprise. But it is not a question of clinical versus basic researchers. There is complementarity, at best synergy, between them: the techniques and experimental systems developed mainly by basic scientists and their application to define normal biological processes are given vital direction and sometimes radically reorientated by clinical observation. Further, the successful application of basic research by clinical researchers fuels the case for more investment (by government, the charitable public and industry) in medical research – basic and clinical.

From the perspective of a clinical researcher, it is surprising that there is not a greater spirit of optimism, considering that research tools are now of a power hitherto inconceivable: within weeks of the SARS outbreak the responsible corona virus was identified and fully sequenced (compared with a period of years for the same to be achieved for HIV); brain imaging is capable of elucidating the neuronal basis of normal cognitive function and is revolutionising research in psychiatry; gene array technology is creating a new paradigm for cancer therapy.

So why do clinical academics feel so threatened? Clinical research is inherently difficult. Our scientific training teaches us to formulate hypotheses and set up experiments to test them, rigorously controlling variables so as to minimise confounding factors. Humans are genetically heterogeneous and when they are ill there are many confounding variables. The reality of much clinical research is that the starting point is a series of observations in which the astute clinician notices something

exceptional or, more usually, has technology which is being harnessed for the first time in a clinical setting.

As a member of peer review research committees, I have often witnessed the destructive criticism of clinical research applications, usually by clinical peers with a seemingly infinite capacity to identify problems over which the hapless would-be researcher has no control. Basic biomedical scientists witnessing these deliberations can be forgiven for concluding that the only respectable clinical research methodology is the randomised controlled trial. 'Research is the art of the soluble' (Medawar), so gifted clinical investigators flee to the laboratory where they are in control of their experiment by, for example, experimenting on mice genetically constituted to order. (Ironically, it remains extremely difficult and expensive to study in mice physiological and disease processes readily accessible in humans.) And in the laboratory, disease-orientated research is increasingly becoming the domain of non-medically qualified research workers – who require a shorter period of university education and can devote themselves fully to medical research without the distractions of clinical practice. The clinical research worker may be seen as expensive, not wholly engaged and addressing problems that are difficult and messy. Funding agencies faced with the choice of rigorous proposals for basic biomedical science versus research on patients behave predictably, but in so doing may forget that it is the complex real-life problems that need resolution.

The challenge of complexity: the need for new methodologies

At the beginning of this Oration, I referred to Bell, Davidson and Scarborough's textbook. In the Foreword, written by RC Garry, Michael Foster was quoted:

> *We may speak of an organism as a complex structure, but we must strive to realize that what we mean by that is a complex whirl, an intricate dance, of which what we call biophysical activity, biochemical reactions, histological structure and gross configuration are, so to speak, the figures.*

Garry added:

> *Often an imperfect performance reveals the intricacy of a dance more clearly than the most polished execution.*

Much of what I have presented is where an imperfect performance has revealed the role of an individual defective dancer. And in the examples of seemingly complex medical problems I have chosen, the defective dancer has been exposed, and in so doing the structure of the dance has indeed become better understood – a satisfying state of affairs.

But to take the analogy further: what if the poor performance is due to the combined effects of multiple small errors by the dancers, perhaps because they are dancing to unfamiliar music? Suddenly we are dealing with a problem of great complexity. And to return to medicine, a challenge of great complexity is indeed posed by the major public health problems of our time, for our genetic constitution relates uneasily to the changed environment in which most of us exist – the problem of diseases of maladaptation, the theme of David Weatherall's splendid Harveian Oration.[87] Examples are obesity and diabetes, high blood pressure, allergy and autoimmunity.

I shall use the example of insulin-dependent diabetes, a disease caused by the autoimmune destruction of islet cells. Early hopes that predisposition to this disorder was due to a relatively small set of HLA genes were soon dashed. John Todd's paper in *Nature* last year elucidated the small contribution of one immunoregulatory gene, that coding for CTLA-4.[88] This publication involved more than 10,000 subjects in 16 centres, 53 authors, who included geneticists (of mice and men), immunologists, computational biologists and specialist statistical geneticists, supported by a five-year programme grant costing more than £20 million!

Problems of this complexity pose an exceptional challenge and need collaboration on a scale comparable to that developed by particle physicists. Biological complexity is now being addressed in leading universities under titles such as systems biology or integrative biology, with computational biology as its principal methodology but requiring widespread collaboration. In his Harveian Oration, *Great ideas in biology*,[89] Paul Nurse discussed ways in which the complex molecular interactions of the cell might be transformed into logical informational structures and processes more familiar to those in disciplines such as mathematics and physics. In my own university, we have made or are about to make appointments linking the departments of mathematics, physics and engineering with biology and medicine. Collaborations are being established to make sense of data being generated on an unprecedented scale by techniques such as gene arrays and the various 'omics'; this in turn is driving the development of new statistical and computational methodologies. There are also vast largely untapped datasets in the patient records, to which in due course will be added the output of initiatives in genetic epidemiology such as the UK Biobank. Addressing these questions requires major resources, extensive collaboration and long-term commitment; and in particular removal of cultural barriers or funding mechanisms that discourage interdisciplinary and inter-institutional activity.

Where does this leave the physician scientist? My response is, centre stage. The medically qualified research worker has one outstanding advantage: there is no system of university education which teaches human biology as effectively as a medical school does. But we need to do better by present and future generations of medical students. Of concern is the tendency for teaching and research to become distinct activities: research is increasingly conducted in purpose-built laboratories which are not embedded in the hospital, entry to which is restricted (not least because of animal terrorists). Medical students, who by-and-large are exceptionally gifted, can reasonably expect to have contact with the best researchers in their medical schools, as I did nearly half a century ago. But the competitive pressure of research and of the Research Assessment Exercise means that many university staff believe they cannot afford to spend time teaching. MB/PhD programmes are rare in the UK and Europe. Sadly, the professionalising of medical education attracts a cadre of teachers who often have little substantive research experience. Last, but by no means least, we seem to be losing the traditional

belief that NHS consultants should have a substantial commitment to research before being considered for a post in a teaching hospital.

But I remain optimistic. The UK government is committed to substantial investment in the science base over the next decade. Medical science is one of the country's great academic strengths; we have powerful and research-intensive pharmaceutical, biotechnology and healthcare industries; and the role of the NHS in medical research is recognised. John Reid, the Secretary of State for Health, has declared:

> *For us, science and research constitute a front-line service, as they too reduce distress and pain and save lives.*[90]

The outcome is the UK Clinical Research Collaboration which is now being established with a substantial budget, and is a development which is being watched with great interest internationally.

But I must leave the last words to my distinguished predecessor, Clifford Allbutt, Regius Professor of Physic 1892–1925. I quote from his Harveian Oration (1900):

> *It was in Padua that medicine, long degraded and disguised, was now to prove her lineage as the mother of natural science, and the truth of the saying of Hippocrates, that to know the nature of man one must know the nature of all things.*[91]

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The full text of the Harveian Oration on which this paper is based is available from the Publications Department of the Royal College of Physicians. The lecture is also published in The Lancet.