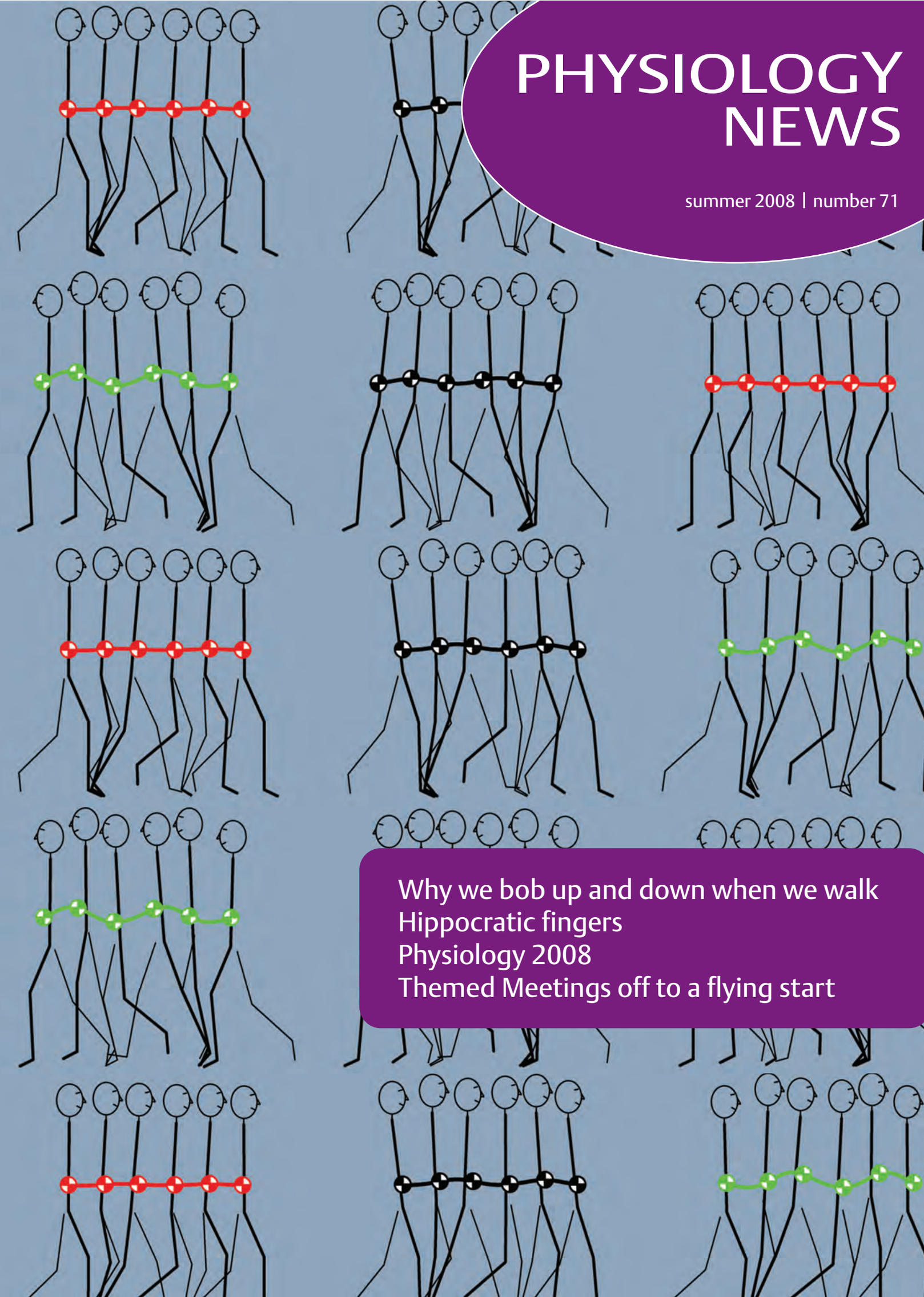


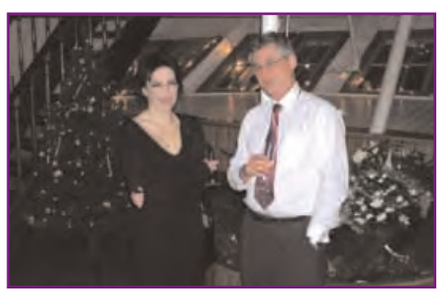
PHYSIOLOGY NEWS

summer 2008 | number 71

Why we bob up and down when we walk
Hippocratic fingers
Physiology 2008
Themed Meetings off to a flying start

Renal cortex: physiological basis of glomerular and tubular diseases

University of Bristol, UK
17–18 December 2007



University of BRISTOL

photos by David Marples

The Society's dog. 'Rudolf Magnus gave me to Charles Sherrington, who gave me to Henry Dale, who gave me to The Physiological Society in October 1942'

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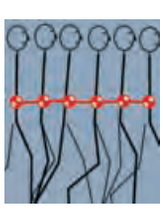

Front cover image from Massaad *et al.* (p. 25).

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Action points

Grants
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Physiology News

Deadlines

Letters and articles and all other contributions for inclusion in the Autumn 2008 issue, No. 72, should reach the Publications Office (lrimmer@physoc.org) by **11 July 2008**. Short news items and letters are encouraged, and can usually be included as late copy if space permits.

Suggestions for articles
Suggestions for future articles are welcome. Please contact either the Senior Publications Executive or a member of the Editorial Board of *Physiology News* (see contents page for details).

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Guidelines for contributors

These guidelines are intended to assist authors in writing their contributions and to reduce the editing process. The Editorial Board of *Physiology News* tries to ensure that all articles are written in a journalistic style so that they will have an immediate interest value for a wide readership and will be comprehensible to non-experts. In particular, scientific articles should give a good overview of a field rather than focus entirely on the authors' own research.

Format of articles
The main message or question posed should be introduced in the first paragraph. The background for the topic should then be established, leading up to the conclusion.

Length of articles
This will be determined by the subject.

Submission of articles
Authors should submit articles as a Word document attached to an email. Illustrations should be sent as separate attachments (see below) and not embedded in the text.

Illustrations and authors' photographs
Authors are encouraged to submit diagrams, drawings, photographs or other artwork with their articles and a photograph of the author(s) should accompany submissions. Illustrations and photographs may be colour or black and white, prints, transparencies or tiff/jpeg files with a **minimum resolution of 300 dpi**. Electronic colour figures should be saved in **CMYK mode**.

References
Authors are requested to keep the number of references to a minimum – preferably no more than two or three. Please cite all references in the style of *The Journal of Physiology* (see *Instructions to Authors* at http://jp.physoc.org).

Opinions expressed in articles and letters submitted by, or commissioned from, Members, Affiliates or outside bodies are not necessarily those of The Physiological Society.

In this issue

Welcome to the Summer 2008 *Physiology News*.

I am not sure how many times I have written as Editor 'This is our biggest ever issue'. While it is not something we particularly strive for, if good material is abundant, we may make the issue bigger. Or conversely, if we are a bit short of content, smaller. Obviously editors prefer 'abundant', and I like to think this is where we are with *Physiology News*. But complacency has to be avoided, and it bears re-stating that we rely on you to provide the content. So keep sending us your article ideas.

As ever, this issue includes plenty of science features, plus some old favourites (*Living History*), and some newer ones – e.g. our second ever *Soapbox*. Hopefully, something for every taste.

The issue also marks some anniversaries. It is 100 years since Student's *t*-test appeared, allowing statistical testing on small sample numbers, something of great significance for physiologists. And it is 130 years since the formal founding of the Cambridge Physiological Laboratory, site (slightly re-badged) of The Society's summer meeting. There can be few better places than Cambridge in July – weather permitting! – to look simultaneously into both the future, and the rich history and tradition, of our discipline.

Austin Elliott
Editor

Brain science, addiction and drugs
Students may need to be tested for brain- boosting drugs as they become more widely used by healthy people, says a report from the Academy of Medical Sciences (www.acmedsci.ac.uk)

The NIH mandate and The Physiological Society

From 7 April this year, the following US legislation became mandatory:

The Director of the National Institutes of Health shall require that all investigators funded by the NIH submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication: Provided, That the NIH shall implement the public access policy in a manner consistent with copyright law.

What this means for The Society is that 40% of the papers published in *The Journal of Physiology*, which earns over 80% of The Society's income, must be published on PubMed Central, which is freely available to all readers, a year after publication. In fact, as things stand at present, this is not a problem. *The Journal* makes all its content free to all readers on its two publisher sites 12 months after publication anyway. As part of the deal with the Wellcome Trust to scan the whole of the print archive, the final published version of *The Journal* is available on PubMed Central 12 months after it has been published on the other sites. Our other journal, *Experimental Physiology*, also provides free access via the Wiley-Blackwell Synergy and Highwire sites 12 months after publication and the journal's publisher has decided to implement a new free service that deposits accepted EP manuscripts to PMC on behalf of authors. So it seems that nothing substantial has changed and there is not too much to worry about.

However, there may be worrying longer term implications for The Society if the exclusivity of our journal content is eroded further.The patient's lobby in the US is powerful and active and will continue to press for earlier free access to primary research articles. For both of our journals earlier open access is already an option for authors, but we have to charge a fee for this service. NIH, unlike the Wellcome Trust or the Howard Hughes Foundation, has no mandate to pay for publication costs and there would be no support from researchers for using declining research funds to pay these costs.

In addition, librarians have started to think about cancelling subscriptions to journals whose content is freely available on other sites*. If the NIH succumbs to pressure and mandates publication on PMC 6 months after the official publication, will librarians continue to pay subscriptions for 6 months exclusive access to journal content? Will they demand reductions in the rates charged, leading to severe reductions in the income to The Society?

Some large weekly/fortnightly journals make their content free after 6 months, apparently without ill effect on their publishing income. *J Physiol* may be in the relatively secure position of being a fortnightly publication which is widely read and could probably continue to command a good subscription rate even if subscribers get only 6 months exclusive access to content. *Experimental Physiology*, however, is published in hard copy every 2 months and online every month. It might be more at risk of subscription cancellations. Another concern is that if subscription income is reduced, publishers will be less able to invest in the technology underpinning online publishing, with consequences for ease of access and secure archiving.

The Society works with Wiley-Blackwell to publish *J Physiol* and *Experimental Physiology* and this provides a measure of safety. Wiley-Blackwell are able to offer librarians 'bundles' of journals which they are likely to find attractive and purchase, even if much of the content were to become freely available 6 months after publication. So there is no need for any panic about our publishing income in the near future. But the world of scientific publishing is clearly undergoing major changes and we need to be prepared to respond to new situations that could threaten our journals and our Society. It is ironic that the NIH mandate, which is seen as a major achievement for the open access movement against the profits made by commercial publishers, may end up pushing small financially insecure but independent journals to seek the greater safety of working with large commercial publishers.

Michael Collis
Carol Huxley

*http://www.alpsp.org/ngen_public/article.asp?id=200&did=47&aid=157&st=&oaid=-1

Reference

Markwood P (2008). The NIH mandate – we're not in Kansas any more. *Learned Publishing* **21**, 83-84.

The NIH mandate – what it means for NIH-funded authors publishing in The Physiological Society journals.

Carol Huxley explains how authors funded by NIH who want to submit to *The Journal of Physiology* or *Experimental Physiology* are affected on p. 54.

Cardiac & Respiratory Physiology Themed Meeting

The Society's new series of Themed Meetings gets off to a flying start in Leeds

In March the University of Leeds was the venue for the first of the new series of 'themed' meetings. These are the latest stage of evolution in The Society's programme of meetings and represent a sort of hybrid between the traditional meeting with its mixture of oral communications, posters and invited lectures on diverse physiological topics and the very specialised 'focused' meetings of latter years. Essentially, a themed meeting revolves around a symposium related to the main theme, interspersed with free communications and posters on related topics.

In Leeds, the symposium *Determining control of the cardiovascular system in health and disease: from brain to blood vessel* provided the backbone for a Cardiac & Respiratory Physiology theme. Running over the two and a half days, 187 registrants were treated to exposées on five interconnected topics:

- Supraspinal control of autonomic outflow;
- Spinal circuitry controlling sympathetic nervous activity;
- Role of exercise in controlling auto--nomic function in health and disease;
- Effects of spinal cord injury on autonomic function;
- Autonomic control of blood vessels.

From cellular mechanisms to translational physiology

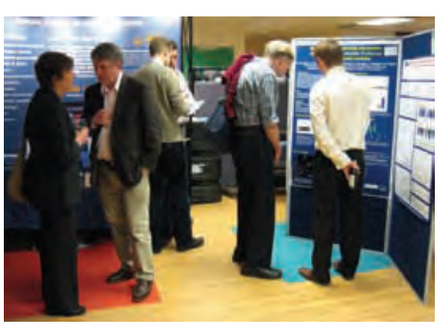
The international mix of speakers included both academic and clinical scientists. For each topic, the emphasis ranged from cellular mechanisms and the relationship of structure to function through to translational physiology and clinical medicine. 'Translational' science is something of a buzzword these days, especially amongst policy makers and grant-givers (see p. 45). But for cardiovascular and respiratory scientists, it is nothing new. They embraced the molecular age of physiology and utilized the reductionist approach to great effect but at the same time never abandoned *in vivo* experimentation and the integrative approach. This strategy has clearly paid dividends as witnessed by the many examples showing how the results of basic research are now being integrated into clinical medicine.

The meeting also included a memorial lecture for David Jordan. Dave's untimely death in 2007 represented a significant loss to the cardiovascular and respiratory community. Andy Ramage, his long term collaborator, celebrated Dave's contributions to physiology. In an entertaining and upbeat talk he showed how, with the help of a series of talented students and postdocs, their symbiotic physiological and pharmacological approach to classical *in vivo* electrophysiology had lead to significant advances in understanding the functional activity of cardiorespiratory control circuits.

Based on the views of all those I questioned both during and after the meeting, this first themed meeting was judged a great success. Sue and Jim Deuchars, who hosted the meeting with support from The Society's Events Team, must be congratulated both on the scientific programme and the organisation. The single lecture theatre (no theatre hopping or programme planning required here) opened directly onto a poster, trade exhibit, refreshment and registration area. The effect was to keep the theatre full all through the symposium and to promote a sort of cocktail party atmosphere round the posters that facilitated that important mix of scientific and social interaction that makes for a good meeting. Not to stray too far away from The Society's origins as a 19th century dining club for physiologists, a dinner was held at a local Italian restaurant. Here, the usual formal speeches were replaced with a lot of jokes and even more noise and a good time was had by all.

The next themed meeting will be held in Oxford from 9-11 September. Here the theme will be Metabolism and Endocrinology with a focused symposium on *Orchestration of metabolism in health and disease*. I hope this meeting will be as good as the first one.

Thelma Lovick



Wine and posters go hand in hand in Leeds (left); local organizer Sue Deuchars (right) with Heidi Adnum from The Society's Events Team (bottom left); Ida Llewellyn Smith gives the vote of thanks to Andy Ramage after the David Jordan Memorial Lecture (below).

K Michael Spyer's Festschrift at the Leeds Meeting, *Celebrating Spyer's science*, p. 52.

Welcome to Cambridge and PDN

Physiology, Development and Neuroscience extends a warm welcome to everyone attending the main meeting in Cambridge in July

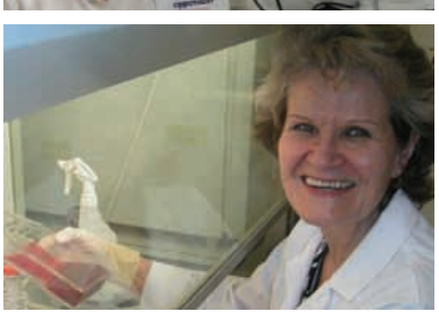
In the 5 years since The Society last met in Cambridge, where it used to meet annually, much has changed in both The Society and the Department of Physiology. The Society is now run by a team of administrators in London, and Physiology has merged with Anatomy to form the Department of Physiology, Development and Neuroscience, PDN for short. The idea of the merger was to energise research through enhanced synergy since many research groups in the two departments already had strong links. Another important consideration was the consolidation of teaching. The new department is responsible for over half the University's preclinical teaching. Two years after discussions began in 2003, all was agreed and PDN came into existence on 1 January 2006.

By mid-2008 over £5m will have been spent remodelling and modernising teaching and lab space for the old Physiological Laboratory and in the old Anatomy Building. The old Physiology tea-room has been converted into the Hodgkin-Huxley seminar room, and the whole department now shares one tea-room, near the new refurbished library in the old Physiological Laboratory.

Many research groups have moved buildings to facilitate collaboration, while the final-year teaching has been merged in the new space in Physiology. The administration and stores have all been consolidated in Anatomy. While Bill Harris, the Head of Department, is ex-Anatomy, several of the senior administrative posts are held by ex-Physiology people.

The department has over 60 active groups working on four major, overlapping research themes:

- **Cellular and Systems Physiology** consisting of 28 research groups primarily interested either in cell functioning or the way in which groups of cells co-ordinate their activities;
- **Developmental Biology and Reproduction** with 26 groups asking questions about how the myriad different cells are made, organised into tissues, arranged in the correct places and co-ordinated functionally to form an animal;
- **Neuroscience**, the largest theme, with 31 groups working at three 'levels' of analysis: cellular & molecular, systems and behavioural;
- **Form and Function** with 12 groups engaged in research on how tissues and organs are shaped and how the resultant architecture contributes to their specialised function.

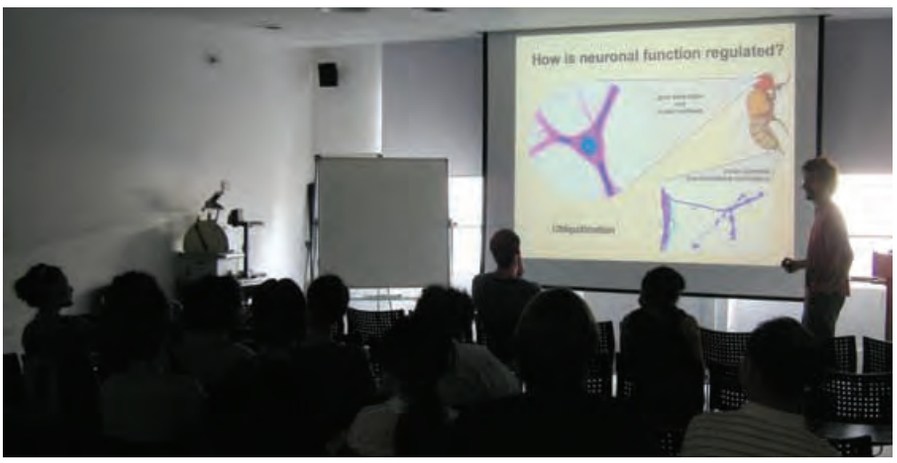


From top: Bill Harris and Roger Thomas soon after the merger was agreed; Abby Fowden and Alan Cattell discuss space issues; Geoff Cooke and Roger Keynes returning from the tea room; Andrew Murray measuring oxygen uptake by mitochondria; Teresa Tiffert with plasmodium-infected red blood cells.

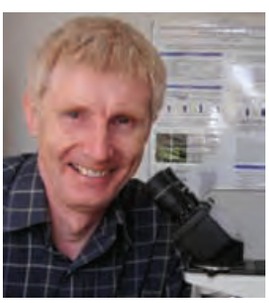
Since the merger, the department has appointed Andrea Brand to the Herchel Smith Chair of Molecular Biology, Andrew Murray as an RCUK Fellow, and several Career Development Awardees. Currently there are about 100 graduate students and 75 postdoctoral fellows.

PDN is the largest single provider of undergraduate teaching within the Faculty of Biology. The department contributes to the teaching of 19 undergraduate courses spanning two Triposes (Medical and Veterinary Sciences, and Natural Sciences). T

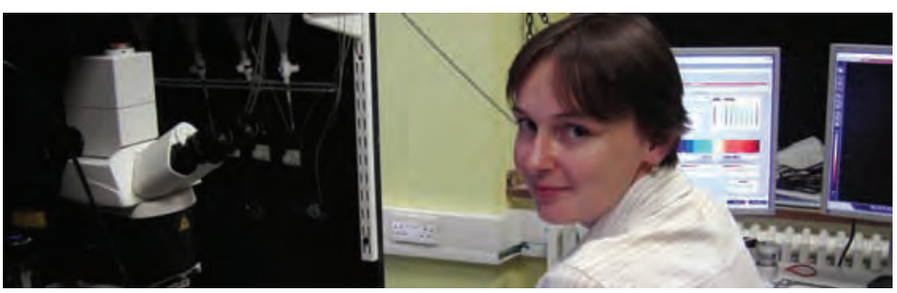
he teaching of physiology in the department has been put on the most solid foundation with the appointment of Matthew Mason as the University Physiologist — a post created in the merger to complement Joanne Wilton, the University Clinical Anatomist and David Bainbridge, the University Veterinary Anatomist.







Top row: Hodgkin-Huxley seminar room. 2nd row: Christof Schwiening (interrupted while installing a picospritzer), Richard Dyball and Ann Silver. 3rd row: Graham Burton (Trophoblast Research Centre), Roy Patterson (Centre for Neural Basis of Hearing) and Fiona Duncan (Secretary to the Department). 4th row: Roger Hardie, Francisco Suarez, Shona Brothwell, Marc Smith and Sue Jones. Above: Olga Larina measuring Ca and pH in Purkinje neurones.

Following an anonymous donation, the department recently established the Centre for Trophoblast Research*, headed by Graham Burton . The CTR is an exciting new initiative that aims to promote the study of placental biology, with special reference to the trophoblast. The trophoblast forms the interface between fetus and mother, and in addition to transport performs many diverse and important functions, including immunological, metabolic and endocrine roles. It is therefore crucial to a successful pregnancy, and hence the long-term health of the offspring. The Centre aims to provide an intellectual focus for researchers both within and outside Cambridge, to foster research collaborations, and to provide the highest international standard of teaching and training in both basic scientific and translational research.

PDN has also taken a lead in the formation of Cambridge Neuroscience†, a virtual centre of excellence connecting multidisciplinary neuroscience research and teaching across the University of Cambridge and affiliated Institutes, with a mission to increase our fundamental understanding of brain function and enhance quality of life. Of the 600 members of Cambridge Neuroscience, PDN has 94 members, the largest departmental contributor.

It is too early to judge the overall effect of the merger, but it is already clear that the new environment is very beneficial for research collaboration across disciplines, and has made life for all of us much more exciting and interesting.

Bill Harris
Roger Thomas
Department of Physiology, Development and Neuroscience, University of Cambridge.

*http://www.trophoblast.cam.ac.uk
† http://www.neuroscience.cam.ac.uk

For full details of Physiology 2008 see http://www.physiology2008.org

Hippocratic fingers

John Dickinson's final medical mystery looks at 'clubbing'

The Greek physician Hippocrates first observed in the 5th century BC that people who were ill with pus collecting in the chest often had 'clubbed' fingers. Such fingers are curved and warm, especially at their tips. There is increased thickness of the nail bed and of the soft tissues below it. In the clubbed finger in Fig.1 measurement A is greater than measurement B. The finger pulp is infiltrated with white blood cells. When clubbing is severe the fingers really look like small clubs. Finger-tip capillary blood vessels are dilated, the number of blood vessels and finger blood flow are increased. If clubbing has developed with pyogenic (pus-producing) lung disease, the finger tips return to their normal shape if the lung disease is successfully treated.

People with congenital cyanotic heart disease have clubbed fingers. Their cardiovascular system allows unoxygenated blue blood coming from the veins to be pumped straight into the aorta, without going through the lungs. The shunt is definitely the cause of clubbing because clubbing disappears if the shunt is surgically closed.

Generalised clubbing of fingers, often of toes as well, is also characteristic of long-standing sub-acute bacterial endocarditis, a blood-borne infection of the heart valves. It disappears after successful treatment of the infection. Localised clubbing, affecting only one upper or lower limb, has often been seen when there has been severe infection in an arterial graft in a large artery supplying that limb. In such cases the local infection has clearly caused the clubbing, which disappears when the infection is eradicated or the graft replaced.

It seems obvious either that as blood passes through the lungs of normal people a clubbing-producing factor is removed from the blood, or a clubbing-preventing factor is added.

Most authors have speculated that normal lungs remove a clubbing-producing factor. In her review in 1965 Jean Ginsburg wrote: 'It is tempting to speculate on the possibility that a vasoactive substance, produced by tissue metabolism, but normally destroyed during the passage of blood through the pulmonary capillaries, is released into the general circulation in amounts sufficient to influence vascular caliber. The problem at present is not the provision of a new theory, but a lack of precise data on biochemical and other changes in patients with clubbing or osteoarthropathy' (Ginsburg, 1965).

But a new theory was needed. Innumerable analyses of mixed venous and arterial blood had failed to find any soluble vasoactive or angiogenic material whose character or concentration was changed in an appropriate way during passage through the lungs. John Martin and his colleagues had been working on

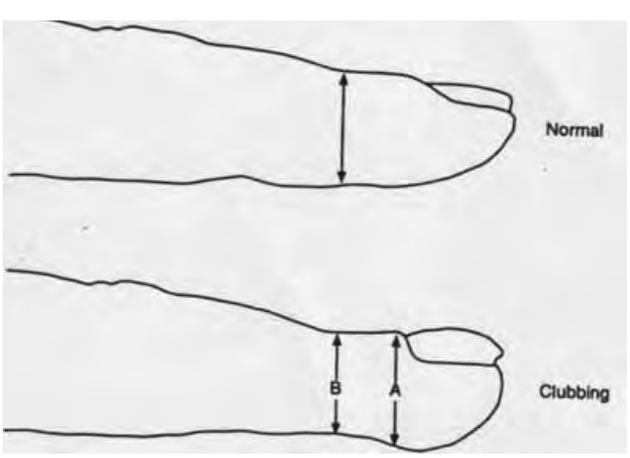


Figure 1. A normal and typical 'clubbed' finger, viewed from the side. Clubbing is present when measurement A (the thickness of the finger just proximal to the nail bed fold) is the same or greater than measurement B (the thickness at the distal joint).

John Dickinson caricatured by Richard Willson.

platelet physiology in the department of medicine at King's College Hospital. They had established that platelets are formed by the fragmentation of large multi-nucleate megakaryocytes from the bone marrow during their passage through the lungs (Trowbridge *et al*. 1982). Normal lung capillaries are narrow. Suspended particles of average diameter greater than 20–50 microns (three to seven times the diameter of a red blood cell) are too big to get through normal lung capillaries. Consequently only the denuded nuclei of megakaryocytes are seen in arterial blood, together with platelets or small platelet clumps. Megakaryocytes are often seen stuck in lung capillaries when lungs are examined after death. Megakaryocytes are typically plentiful in mixed venous blood but rare in systemic arterial blood, whereas platelets have the converse distribution.

John Martin and I are clinical doctors. We knew that clubbing could occur unilaterally, distal to the site of a brachial artery aneurysm. This suggested the possibility that platelet aggregates might collect in an arterial aneurysm, fall off and be swept along to the fingertips on that side in an axial stream. There was an

exciting 'eureka moment' when we realised that the factor responsible for clubbing might be particulate rather than soluble, if megakaryocytes or large platelet aggregates could get through the lungs without fragmenting (Dickinson & Martin, 1987).

We envisaged that 'platelet-derived growth factor' might be involved. One of its forms (-BB) stimulates the growth of new blood vessels and attracts white cells out of the bloodstream. It plays a part in inflammatory reactions. Thus the impaction of a megakaryocyte or a large clump of platelets in a finger tip might locally release high concentrations of this cytokine. Other powerful blood vessel growth promoters are 'vascular endothelial growth factor' and 'transforming growth factor beta-1', either of which might account for the pathological changes of clubbing - increased thickness of the nail bed and pulp, accumulation of excess tissue fluid and increased blood vessel growth. We do not at present know the relative importance of these various factors. All might be involved in producing the pathological changes of clubbing (Dickinson, 1993).

Although finger clubbing is not seen in normal adults or children, its study has renewed interest and generated new approaches to the physiology of platelets and growth factors.

Its study has explained many curious observations in clinical medicine. For example, clinical tests of the lung circulation have been made by injecting into an arm vein radioactive particles of different sizes (usually technetium-labelled macroaggregates of albumin). Such labelled particles can be followed to the lungs by detectors which can show whether the particles get through the lungs or stick there. In severe liver disease – often associated with finger clubbing – the pulmonary blood vessels are distended. They allow particles of 20–50 microns diameter to pass through.

Transplantation of a normal lung has been shown to eliminate clubbing, at the same time that average pulmonary blood vessel calibre returns to normal (Stoller *et al*. 1990).

A preliminary report by Stephen Fox and his colleagues has given strong pathological support to our hypothesis. At the time of necropsy they examined the nail beds of 24 unclubbed patients and of seven patients who had died with clubbing present. All five patients with notable clubbing had numerous tiny aggregated platelet clots (microthrombi) in their nail-bed capillaries. Two with mild or early clubbing had fewer platelet microthrombi. In only four out of 25 controls were 'very occasional platelet and whole blood microthrombi identified' (Fox *et al*. 1991). Dr Fox has kindly allowed me to mention two of his further (unpublished) observations on nail bed punch specimens taken during life from two patients with clubbing of unknown cause. In both of these numerous platelet microthrombi were identified in nail bed capillaries.

The intensive study and explanation for a well-known clinical phenomenon has helped to explain some apparent anomalies. For example, Martin and I have been able to explain why newborn babies – who have had a large right-to-left shunt all their lives – are not clubbed. Much of the fetal cardiac output goes straight to the placenta, where megakaryocytes fragment, suggesting that the placenta functions as a platelet-forming organ during intrauterine life, the lungs taking over this role after birth. Furthermore, impairment of platelet function is well recognised in the neonate. Platelets of the newborn have reduced glycogen content and platelet factor 3 availability, and have a 'defect in the release of their dense-body contents, most marked in response to adrenaline' (Wood, 1982). The rise in platelet thromboxane B2 levels shortly after birth suggests activation of the prostaglandin pathway at this time.

Teleologically it seems appropriate that fetal platelet dysfunction is only transient. Unrestrained and random growth stimulation from the impaction of megakaryocytes will stop immediately after birth. And at the same time that platelets mature and become capable of releasing their cytokine granules, pressures in the left heart rise above those on the right side and the shunt pathway shuts.

This and my two previous articles in *Physiology News* (**66**, 10–12 and **69**, 10–11), have given some examples of the ways in which clinical medicine can contribute to physiology.

Many more medical mysteries have been described from a physiological viewpoint in my recent book (Dickinson, 2005).

John Dickinson
Wolfson Institute of Preventive Medicine, London, UK

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Patently absurd?

Mike Taggart (below) argues that 'commercial potential' is a largely irrelevant and potentially damaging concept when applied to physiological research

Thanks in large part to the increasing prevalence of micro-management and the pressure to satisfy RAE-like bureaucratic criteria, 'Performance indicators' have become part of the academic lexicon. The appropriateness of many of these indicators for measuring the impact of academic research is hotly debated. One index that appears with increasing frequency in all sorts of R & D Performance Reviews should give extra cause for thought: this is commercialisation of one's research. A consequence is that it is no longer uncommon for researchers to consider their most exciting data in terms of suitability for registration of a patent as well as, or even rather than, submission to a scientific journal. At a university open meeting I attended last year regarding the ins and outs of the RAE, a questioner from the audience asked if patents were to be listed in the final submissions. 'Yes,' came the reply from the RAE lead 'but I suppose it depends if it is a good patent'.

This insistence upon research commercialisation is not just RAE-led, of course. It is a feature of funding application forms from many major grant-awarding bodies. It turns up here in many guises, like 'interaction with industry', 'translation to the marketplace', 'exploitation of research findings', etc. All are related in their wish to assess the potential to turn the research to financial profit. My foreboding is that ultimately if one's research topic has no identifiable short-term element of monetary gain then it may be deemed unworthy of support.

The pre-eminence of university Intellectual Property (IP) offices also illustrates the trend for exploring at every opportunity the potential routes from the laboratory to the commercial sector. Many vice-chancellors and principals view this

as a necessity for institutional survival within the modern business-style rules of engagement. The roots of this can be traced back to William Waldegrave's 1993 White Paper on Science and Technology that gave mention to government policy of funding research for 'wealth creation'. However, that this should now have become writ almost without challenge raises a serious point: should we be alarmed at the commercial pressures brought to bear on even the rationale for conducting research in academia?

Patenting has, of course, occurred for centuries (first recorded, apparently, in 1449 for specialized stained glass windows destined for Eton College) and a 'good' patent one can assume to be an income-generating one. So it would be foolish in the present climate not to have an appreciation of the use of patents in the complete scientific process. Two arguments in favour run along the lines of:

- the investigator receives capital reward for their innovation;
- the host institution receives remuneration that can fund subsequent research.

A third, more fanciful, notion is that a patent enables the investigator to retain control of the research once it has entered the marketplace. It is also true that many academic researchers participate in research projects in a partnership with a commercial company, in which case they will already be familiar with the delicate juggling needed to marry the interests of academia (publish) and commerce (potential profit). Hopefully, this will have been amenably agreed with the IP contract at the outset, though agreeing (and reading?) these can be a headache.

However, there are obvious difficulties. When research is performed in a largely academic environment the data endpoint often arises as a result of project evolution – some of it predictable from experienced scientific judgement, some of it occurring unpredictably. Such is the beauty of studying unknowns. Rarely will the output be the direct result of having a starting intent to produce an invention. The latter term might jar the senses, but that is what a successful patent hangs on – an invention, or inventive step to a process, that would not in reasonable time be obvious to other experts in the field. My feeling is that for many physiologists it would be discomfiting to begin a research project with the objective of registering a patent (*ipso facto* seeking financial profit), for a number of practical and philosophical reasons.

First, there are several obstacles to the establishment of a 'good' patent. Even after the first experiments are complete, the effort to establish if a patent is worth submitting is substantial (time and cost to investigate prior art/knowledge and lodge the paper-work), as is the subsequent research to establish proof-of-concept before, finally, the even greater task of protecting any awarded patent against competitive infringement. How many patents posted by individual researchers through their university IP offices will those same institutes seek to legally protect if they are subsequently challenged by corporate interests? My guess is very few, and only if they are really 'good

James Watt (1736–1819). His vigorous defence of his condensing steam engine patents limited technical advances by competitors, arguably slowing the industrial revolution by up to two decades.

patents' (in effect, currently or foreseeably profitable).

Second, what of our career mentoring of the PhD students and post-docs involved in a research project that has a goal of submitting a patent application? It is possible that they will have been stymied from presenting their most worthwhile data at conferences, or publishing papers, to avoid untimely public disclosure. What do they show to their next prospective employer as evidence of scientific credibility? The issue even has complications for postgraduate degree examinations. A student I examined in a recent PhD viva would not divulge the molecular identity of an 'inhibitor X' used in experiments described in his thesis for fear of breaching commercial confidentiality. It was evident from our subsequent discussion that neither he nor his supervisor had thought this would be an impediment to passing the examination.

Third, an overt shift to an objective of research being 'wealth creation' runs the very real risk of squashing certain values that run through the core of academia – the ethos of education, enlightenment and intellectual endeavour for common social good, for example. One could even argue it is inimical to the long-held aspiration of The Physiological Society to have free and open communication of science, certainly unhindered by presumptions of future financial profit.

Finally, there remain other concerns for the impact on physiology and allied disciplines of a push to patent research findings. A patent might stall advances beneficial to society by impeding wider research participation in a timely topic; a patent pending could be used as an illustration of research output (e.g. RAE submissions) in place of peer-review scrutiny in a prominent scientific journal; efforts surrounding a patent submission could engender a non-collaborative spirit. These are not just hypotheticals; it is often mentioned, for example, that James Watt's vigorous patenting activity to 'protect' modifications of his condensing steam engine limited the technical advances of competitors thereby slowing the growth of the industrial revolution by up to two decades. Rather more gruesomely, having been disillusioned by the need to defend challenges to his patents for the industrial production of ammonia for agricultural use in the early 1900s, the chemist Fritz Haber channeled his subsequent efforts towards the establishment of poison gas munitions for use on the battlefields of the first World War.

Chemist and Nobel Laureate Fritz Haber (1868–1934). His disillusionment with his patenting struggles over the Haber process for ammonia production helped channel his later work towards the establishment of poison gas as a battlefield weapon.

Extreme examples perhaps – and it is noteworthy that they concern the physical sciences rather than physiology. This, and the earlier comment that a patent application must concern an invention, raises a broader question of the applicability of patent legislation to physiology or medical science research. The advice of the UK government IP Office (http://www.ipo.gov.uk) is surprisingly explicit. They state that a patentable invention must NOT simply be 'a scientific or mathematical discovery, theory or method', nor should it be 'a method of medical treatment or diagnosis'.

All of which brings us back again to the myriad guises of commercialisation that now appear on grant application forms. Are these questions and responses really a worthwhile determinant of where grant money should go? And if not, why are they there?

I will close with a recent reviewer's comment on a BBSRC grant proposal. When prompted to remark upon the significance of the research proposal for 'prosperity and quality of life', the referee wrote:

'How can one possibly tell? I decline the offer to indulge in the usual bullshitting. It looks like good work that could lead to better physiological knowledge and that is the basis of good medicine. What more can one ask?'

Quite.

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The Physiological Society Leeds Themed Meeting

Proceedings of the Cardiac & Respiratory Physiology Themed Meeting held in Leeds in March 2008 are now available online at http://www.physoc.org

Standing room only in Glasgow

It started with a phone call from David Saint inviting me to give the UK/Australian Physiological Society Visiting Lecture in 2007. After several milliseconds of ritual hesitation I agreed and then asked what was involved. The answer is a very typical academic mixture of loosely-defined tradition and back-of-the-envelope planning. The positive aspect is that you can do almost whatever you like within a few general guidelines.

The lecture alternates between the UK and Australia. One year an Australian is chosen who goes to the UK; the next year the reverse. In the former case the AuPS chooses the lecturer and pays the airfare, while the host country pays the travel and living expenses. The normal form is that the lecturer gives his lecture at a meeting of the host country Physiological Society and then visits a series of departments and repeats his lecture. In my case the formal first lecture was at a meeting of The Physiological Society in Manchester in September 2007, so the rest of my visit revolved around this fixed point.

A few months after the phone call I received an email from David Bennett, the International Events Coordinator of The Physiological Society (yes, they are grand enough to have paid administrative staff). We confirmed the Manchester meeting and he asked me where else I would like to go. I mentioned three or four institutions where I had lived, worked or played in an earlier life. A few months passed and I received an excel spreadsheet of about 20 institutions who had been contacted and requested my presence as a lecturer. The spreadsheet contained extracts from the letters from the institutions and the warmth of these invitations could be felt even from a spreadsheet. Like many scientists I suffer from a mild form of paranoia in which I feel that my ground-breaking research has not been fully appreciated by the rest of the world – one symptom of this condition is

David Allen, UK/Australian Visiting Lecturer in 2007, was presented with a certificate to mark the occasion.

that when I receive an invitation to talk about my research I find it very hard to say no. However, even I can see that giving the same lecture 21 times might pall, so I constructed an itinerary involving nine universities up and down the country constituting a grand tour of England and Scotland.

All seemed well with this plan but for two minor hiccups: David Bennett informed me that the UK Physiological Society had a limit on the expenses they would pay which, while generous, was not capable of funding a grand tour; secondly he asked me for my itinerary and I realized that responsibility for detailed planning might be mine. At this point I decided to hire a car and began to email everyone I knew on the path of the grand tour requesting a bed for the night.

Again friends and distant acquaintances rallied to the call and the financially-slimmed-down tour was intact.

Thus I arrived at Heathrow on a sunny morning in September and set off in my hire car for Manchester. Here I stayed with Austin Elliott, an ex-PhD student and frustrated literary talent, for the duration of the Manchester Physiological Society meeting. I gave lecture No. 1 to a large captive audience at a cardiac specialty meeting. Seemed to go down well and many old friends, including Brian Jewell, my PhD supervisor, and Otto Hutter, whose laboratory at University College I had inherited, were able to attend and provide welcoming talks.

The rest of the tour was a wonderful sequence of travelling, staying with friends and in the occasional hotel, giving my talk and being wined and dined by various hosts around the country. So I will just pick out some highlights to give the flavour.

Audiences were variable in size; always double figures (just) but definitely the highlight was Glasgow, where my friend and some-time post-doc Godfrey Smith had had the good sense to hire a very small lecture room which was filled to capacity leading to the proud boast of the title. Glasgow amazed me with opera – Car man (sic), intimate restaurants and delightful wine bars – the drunken crowds I remember

From left to right ; Mark Boyett (Manchester), Godfrey Smith (Glasgow), Stephen O'Neill (Manchester), Austin Elliott (Manchester), Clive Orchard (Bristol), David Eisner (Manchester), Otto Hutter (Glasgow Emeritus), Isabelle Baro (Nantes), Brian Jewell (Leeds Emeritus) and David Allen (Sydney).

from my youth seemed to have moved elsewhere. Godfrey is now the Professor of Physiology and runs a lab which made me feel proud of whatever contribution I made to his training. Langendorff-perfused hearts everywhere surrounded by sophisticated optical arrangements for recording action potentials on the surface; all home-made and controlled by complex electronics, purpose-built amplifiers and analyzing software.

A wonderful weekend in the Lake District staying with friends of friends in a 500 year old cottage. Perfect weather and a magical walk through Little Langdale and a visit to the local pub, the Old Dungeon Ghyll (quite unchanged from 40 years ago).

Visiting Dundee where new biomedical buildings seemed to stretch endlessly into the distance. Turns out they have done a deal with Big Pharma which contribute to new buildings and provide annual running costs; all part of a Faustian bargain which allows the drug company to have first look and negotiating rights at any new intellectual property.

Oxford. Lunch and walk in the University Parks with my son Ben, currently a computational protein chemist for a small start-up company in Cardiff. He loves it and the uncertainty of having no job in a couple of months unless he can persuade an investor with more money than sense to part with another $100,000 doesn't seem to trouble him. During my day in the Department of Physiology, Anatomy and Genetics (catchy names are all the rage) I was shown the cardiac NMR laboratory. Here they are developing a technique that improves the sensitivity of carbon compound detection by 10,000 fold (I hope I've got this right). All you need is a spare NMR machine (a million pounds will get you a second hand one) and the sample is exposed to an exotic magnetic pulse sequence and then has to be removed and injected rapidly into a heart in another NMR machine all within the 60 s or so before the exotic spin goes away.

And so to Heathrow and the long journey home. A kaleidoscope of memories of snippets of science, the beautiful English and Scottish country side and, above all, so many people who generously lent their time and friendship to make my visit enjoyable.

David Allen

Bosch Institute and School of Medical Sciences, University of Sydney, Australia

Brian Jewell gave The Society's vote of thanks to David Allen and writes:

I was very pleased indeed to be called out of retirement to co-chair the session because Mark (Boyett) and David (Allen) were both PhD students of mine.

The key point, I think, is that they were PhD students at UCL, as I had been in what I regard as a 'golden age'. We had two Nobel prize winners – A V Hill and Bernard Katz, soon to be joined by a third, Andrew Huxley – and this wealth of talent attracted a continual throughput of visiting scientists from around the world. When working in such an intellectually rich environment we acquire knowledge through our pores by a process akin to osmosis, and I am quite sure that they learned as much that way as they ever did from me.

There was an important additional factor for David and Mark in that my lab in the Physiology Department had previously been Otto Hutter's, with Denis Noble as his PhD student, so David and Mark were walking on hallowed ground and perhaps even breathing some of the high octane air left behind by its previous occupants.

We had in the Physiology Department a neurophysiologist called Jim Pascoe. Jim was not a Nobel laureate, but he was a memorable character who held court over coffee in the Starling Room and threw us many pearls of wisdom. One thing I remember him saying was that, for the continuing health of our subject, each of us has a responsibility to train at least one person who is as good as, and preferably better than, ourselves.

Notwithstanding the learning by osmosis, the walking on hallowed ground, and the breathing of high octane air that David and Mark enjoyed as PhD students, I would like to claim that I have fulfilled my obligation in this respect and it has given me enormous pleasure to see how well both of their careers have developed.

I would like now to focus my remarks on David for whom I am going to propose a vote of thanks on behalf of The Physiological Society. His choice of subject matter put me in mind very much of something A V Hill said in his Inaugural Lecture when he was appointed to the Jodrell Chair of Physiology at UCL in 1923.

Contrary to what you might think, I was not actually there on that occasion, but it included the following thoughts:

Medicine is continually demanding more information and help in the grievous and urgent problems which it has to solve – useful information, practical information, information which is likely to help heal men's minds and bodies. It is impossible not to be moved by this appeal, and in their hearts there are few physiologists who do not hope that their work may prove, in some sense and at some good time, of service to mankind in the maintenance of health, in the prevention of disease, and in the art of science and healing.

Those are fine words and worthy sentiments, especially as – and perhaps because – A V Hill was a mathematician by training, but what he then went on to say was this:

One's heart, however, is not always one's best guide; more useful in the end is the intellectual faith ... which urges Tom, Dick and Harry in their humble way to explore each his own little strange and miraculous phenomenon, whether in the organic or inorganic world.

David has had the satisfaction of doing both – exploring his own little strange and miraculous phenomena (I have in mind there, for example, his work with the jellyfish protein, aequorin) and, more recently, of applying the techniques of basic science to elucidating a clinical problem and greatly increasing our understanding of its aetiology. I am sure this has been immensely satisfying for David, whose career began in medicine.

His presentation was superb. It was a model of how science should be done and presented – the hypothesis established, a question framed, answered by suitable experiments, the hypothesis then revised or extended and tested again – and so on.

The strange origins of the Student's t-test

The centenary of the introduction of the Student's t-test may not be as auspicious an anniversary as some, but the Student distribution around which the t-test is based has had an impact on experimental design and sampling theory far in excess of the modest intentions of its originator, William Sealy Gosset (Fig. 1). In order to fully appreciate the impact of the Student's t-test on modern biostatistical analysis we must travel back over a century to assess the current statistical trends of the day.

At the onset of the 20th century statistical analysis was dominated by the concepts of populations and very large sample numbers, whose chief advocate was Karl Pearson. The central core of such analysis was the normal distribution, which was first derived by de Moivre in 1733 (de Moivre, 1738) to predict the outcome of games of chance, and later, based on the application of the distribution to astronomical analysis, expressed as a probability frequency distribution (equation 1) by Gauss:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{1}{2}\frac{(x-\mu)^2}{\sigma^2}} \quad (1)$$

where σ is the standard deviation, and μ is the mean. A large number of parameters/characteristics in biology are accurately described by a normal distribution (Fig. 2), which has the following characteristics:

- it is symmetrical about the centre, with this as the point containing the highest frequency;
- the mean, median and mode are the same;
- the inflexion of the curve is ± 1 standard deviation (SD) from the centre (mean);
- the tails asymptote towards zero;
- the means of large groups of samples (n > 120) from within a population described by a normal distribution, will also be normally distributed (note that this is not the case for smaller groups of samples, the catalyst that impelled Gosset to establish his 'Student's t' distribution).

Since biological parameters of populations are accurately described by a normal distribution, it follows that the properties of the normal distribution can be applied to these parameters. The key characteristic of a normal distribution is that the area under the curve, which equates to the proportion of data lying between two points, can be defined in terms of SDs relative to the mean. Thus 68% of the area is contained within ± 1 SD of the mean, 95% of the area is contained within ± 1.96 SD of the mean, and 99% of the area is contained within ± 2.56 SD of the mean (Fig. 2). This ability to accurately define the distribution of data relative to the SD led to the concept of confidence limits, where the probability that data will lie between distinct SD spans can be stated as a percentage. For example, there is a 95% probability that a data point for any normal distribution will lie between ± 1.96 SD of the mean. The normal distribution can also be used to make inferences about data from two sample groups. If the mean of one sample group lies between the 95% confidence limits of the other sample group, there is only a 5% chance that the two sample groups are not drawn from the same population. This is a key relationship, as we shall soon see.

Figure 1. William Sealy Gosset (1876–1937), pictured around 1908.

It is at this point that William Sealy Gosset enters the picture. Born in Canterbury in 1876, Gosset was educated at Winchester and Oxford, where he obtained a first in chemistry in 1897 and a first in mathematics in 1899. He was hired by the Guinness brewery in Dublin, in whose employ he spent the remainder of life, mainly at St James Gate in Dublin, and for the final 2 years at Park Royal, London. Gosset's academic background may seem at odds with his employment as a brewer, but Guinness realized around this time that in order to maintain its dominant market share as the biggest brewer in Ireland, it would have to introduce brewing on a

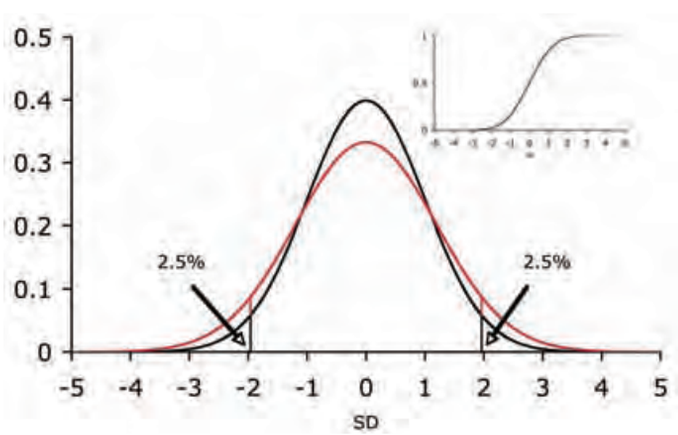

Figure 2 The normal and t distribution. The black line indicates a normal distribution with a mean of 0 and a SD of 1. The combined areas under the normal distribution bordered by the vertical black lines at ± 1.96 SD from the mean account for 5% of the area under the curve. The red line illustrates a t distribution, which is smaller and flatter than the normal distribution. An extension of the lines at ± 1.96 SD from the mean, meet the t distribution (red lines) encloses an area of greater than 5% of the area under the curve. Inset illustrates the cumulative distribution function for the normal distribution illustrated.

carefully controlled industrial scale. Such a venture would require rigorous quality control; hence the requirement for university trained chemists and statisticians. As any amateur brewer will attest, the brewing of beer has an element of the unknown, with success being not only dependent on the correct procedure, but also an element of luck! It was this reliance on luck for a successful product that Guinness sought to eliminate by scientific procedure. Beer, of course, is a combination of natural products; malted barley, hops and yeast, all mixed with water. These natural products share an inherent variability common to all agricultural products, whose quality is dependent not only upon crop variety, but also on climate, soil conditions, etc. Gosset's task as Apprentice Brewer was not only to assess the quality of these products, but also to do so in a cost effective manner. This necessitated using experiments with small sample numbers to draw conclusions that could be applied to the large scale brewing process. However, Gosset discovered that in using small samples the distribution of the means deviated from the normal distribution. He therefore could not use conventional statistical methods based upon a normal distribution to draw his conclusions.

In 1904 Gosset published an internal report entitled *The application of the 'Law of Error' to the work of the brewery*, where he described how '*the greater the number of observations of which means are taken, the smaller the* (*probable*) *error*'. Gosset also noted how, compared to a normal distribution, '*the curve which represents their frequency of error becomes taller and narrower*' as sample size decreases (Fig. 2, black line). The Guinness management realized the potential cost savings impact of the study and suggested Gosset consult with a professional mathematician.

Gosset therefore wrote to Karl Pearson at UCL, and they met on 12 July 1905 while Gosset was on holiday in England. This meeting led to an invitation for Gossett to visit Pearson's department at UCL in 1906/07 for a year, where he worked on his small samples problem. In 1908 Gosset published the fruit of his labours in a paper entitled *The probable error of a mean* in the journal *Biometrica* (Student, 1908), of which Pearson was Editor. However, Guinness had a policy of not publishing company data, and allowed Gosset to publish his observations on the strict understanding that he did so anonymously, and did not use any of the company's data. Gosset complied and published under the pseudonym 'Student' – the name under which he would publish 19 of his 21 publications. The name Student apparently came from the cover of a notebook Gosset used at the time – *The Student's Science Notebook* (Ziliak, 2008).

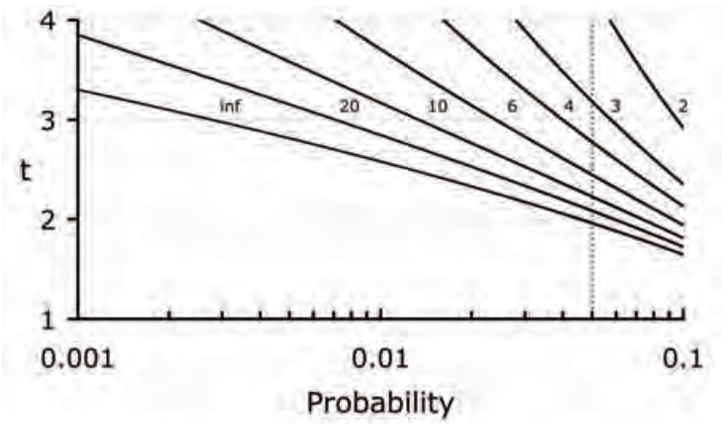

Figure 3. The value of t relative to the probability for an increasing value of n. At p = 0.05 (vertical dotted line) note how the value of t increases as the value of n decreases.

In his classic paper Gosset states that '*any series of experiments is only of value is so far as it enables us to form a judgment as to the statistical content of the population to which the experiment belongs*.' Or, stated another way – having n observations Gosset wanted to know within what limits the mean of the sampled *population* lay. In the paper Gosset partially derives the distribution of the error (termed z) and gives values of z from n = 4 to 10. This table is expressed as a cumulative distribution function (Fig. 2, inset) relative to n and z. The calculations required to generate the table of z values were very labour-intensive and took Gosset about 6 months to compute on a mechanical calculator, bringing to mind an equivalent labour carried out by Andrew Huxley in the mathematical reconstruction of the squid axon action potential (Hodgkin & Huxley, 1952). Gosset concluded the paper with several examples in which he computed the odds of a variety of scenarios occurring. To do this he simply divided the difference of the means by the standard deviation to calculate z, and then looked up the tables at the appropriate level of n. The cumulative distribution function was then converted to odds. The higher the value of z, then the greater the likelihood that the conclusions drawn from the test were correct. An essential component of the Student distribution is that, as the value of n decreases, so does the cumulative distribution function at the same value of z. Or, as we shall soon see, according to Fisher's argument the smaller the value of n the greater the value of t at the same probability (Fig. 3). This translated the familiar experimental scenario where the smaller the n value, the greater the difference between the two samples required in order to achieve any particular level of significance (Fig. 4). Such phrases as '*and in practical life such a high probability is in most matters considered a certainty*', '*but would occasion no surprise if the results were reversed by further experiments*' and '*would correspondingly moderate the certainty of his conclusion*' (Student,

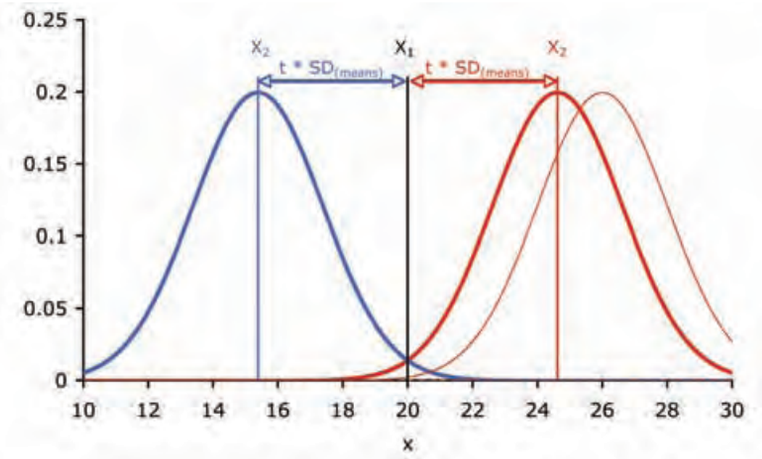

Figure 4. Graphical illustration of confidence limits and determination of significance between two groups. The mean of Group 1 is 20 (X_1), and the data have a SD of 2, and df of 8, with a resulting t value of 2.308. Thus the upper and lower 95 % confidence limits, respectively, are marked by the vertical red and blue lines at [20 – (2 * 2.308)] 15.384 and [20 + (2 * 2.308)] 24.384, respectively. If a second group of data have a mean value (X_2) of between 15.384 and 24.384, then this group of data are considered belonging to the same population as Group 1, as indicated by the normal distribution in thick red and blue lines. However, if the mean of Group 2 lies outside this confidence interval e.g. the thin red line normal distribution, then the group means are considered to be from different populations with a 95% degree of confidence.

1908), indicate the importance that Gosset put on the intuition of the individual experimenter or statistician in determining the meaning of the result. Gosset continued to use his table of z distribution in the course of his work, but otherwise it was ignored.

In 1912 Gosset communicated with a young statistician, Ronald Fisher, who would have an enormous impact on the acceptance of Student's distribution in day-to-day statistical analysis. Fisher is known today as one of the founding fathers of modern biostatistics, and he was not slow to appreciate Gosset's contribution. In 1917 Gosset published an extended table of z distribution for n = 2 to 30, stating of the table of z values in his 1908 paper '*I stopped at n = 4 because I had not realized that anyone would be foolish enough to work with probable errors deduced from a smaller number of observations*' (Student, 1917). Some years later In 1925 Fisher published a paper in which he fully derived the values of Student's distribution, his final distribution being equivalent to the form given by Gosset in 1908, and clearly showing it as a transformed normal distribution (Fisher, 1925). In this seminal paper Fisher also provided a worked example with two groups of unequal sample number. Fisher changed the nomenclature of the distribution from z, as it appeared in Gosset's original paper, to t, and changed the distribution from n to (n – 1) or degrees of freedom (df). On this amendment Gosset commented thus: '*When you only have quite small numbers, I think the formula we used* (*incorporating n – 1*) *is better, but if n be greater than 10 the difference is too small to be worth the extra trouble*' (Pearson, 1939). Pearson was skeptical, stating his contention that the number of samples should be large enough that n – 1 is indistinguishable from n. This reflected his continued resistance to the use of small sample numbers. However, Fisher must have exerted an influence at an early stage, as by 1917 Gosset was using (n – 1) in his extension of the table of z distributions (Student, 1917). Fisher also transformed the layout of Student's distribution to fit with his own agenda, namely to promote the use of probability as a determining factor in such calculations (Ziliak & McClosky, 2008). In Fisher's table the t value is expressed relative to p (probability) and df. Thus for a given df and desired probability the t value is located, and if this value is greater than the computed t value based on experimental data, then there is no significant difference in the data at that probability. This was certainly not how Gosset pictured his z distribution being used, but Fisher was an extremely eloquent and powerful advocate who rapidly came to dominate the field, and it is his form of the calculation that we use today (Rohlf & Sokal, 1995).

Gosset himself had no academic pretensions, and he comes across as a pragmatic man. He was dismissive about his mathematical ability, stating that the limits of his capabilities '*stopped at Maths, Mods [final examinations] at Oxford, consequently I have no faculty therein*' (Gosset, 1962). Gosset was clearly nobody's fool, but his mathematical skills were not on a par with Fisher or Pearson. That his derivation of t was incomplete and partly guessed bears out this point, but Fisher's fully derived t distribution of 1925 showed Gosset's intuition to be correct. Indeed Fisher comments '*any capable analyst could have shown him the demonstration he needed*' . Gosset's calculations frequently contained minor errors and he preferred to do his calculations on the backs of envelopes and scraps of paper (McMullen, 1939), an endearing amateurism only reinforced by comments such as '*In a similar tedious way we find*' during an extended derivation (Student, 1908). However, the t distribution allowed Gosset to proceed with his work for Guiness, and he was promoted to head experimental brewer and head of statistics, and finally in 1935 promoted to Head Brewer, a position he held until his death 2 years later.

This is a brief history of the introduction of Student's t distribution, but how is it applied in the Student's t–test? The first step in this calculation is to determine the value of t for the given df and level of probability which, for our purposes, we will take as 0.05 (5%). Given the t distribution we can then calculate

the confidence limits from the mean and SD of the sample groups (the t-test requires that both groups have the same variance and are normally distributed). Plotting these on a graph allows us to visually assess the data. If the mean of Group 2 falls outside of the upper and lower 95 % confidence limits (i.e. outside the confidence interval) of Group 1, then there is less than a 5% chance that the Group 2 data are from the same population as Group 1 (Fig. 4). It would be rather tedious to plot a graph of the data each time we wanted to compare two groups, but the graph can be reduced to a simple equation (equation 2).

$$t_s = \frac{|X_1 - X_2|}{SD_{(means)}} \qquad (2)$$

where X_1 and X_2 are the means of the two groups, and the vertical lines are an indication to subtract the smaller value from the larger. In this equation t_s is calculated based on the experimental data. If t_s exceeds the t value for the appropriate df and probability (i.e t_s > t) then the two groups are different at that level of probability.

It should be fairly easy to see from Fig. 4 the logic of the equation. The greater the value of t_s the more likely the two groups are to be drawn from different populations, irrespective of df. The greater the difference between X_1 and X_2, or the smaller the SD (means) the greater the value of t_s. There are numerous spreadsheet examples of t-test calculations, which are worth doing once to see the workings of the equation, but are superfluous for day-to-day calculations since spreadsheets programmes such as Microsoft Excel have built in t-test functions, and the above equation only yields a t_s value, not the exact p values, which cannot easily be calculated.

In conclusion, physiologists (and many other experimental scientists) owe Gosset a debt of gratitude for rendering obsolete the reliance on large sample sizes to determine differences between groups of data. The t-distribution allows experimenters the freedom to use small sample sizes in the confidence that they are not compromising the validity of any conclusions drawn: indeed the Home Office policy of Reduction, Refinement and Replacement with regard to animal experiments would be impossible without the t-distribution.

That Gosset, surely the unsung hero of 20th century statistics, made such an enormous advance in the field of statistics is almost certainly due to his application of theory to solve practical problems. In a letter to Fisher regarding his t distributions Gosset lamented that *'you are the only man that's ever likely to use them'*. A browse through any issue of *The Journal of Physiology* will serve to illustrate the ubiquity of Student's t test in the life sciences, and demonstrate just how unaware Gosset was of the revolutionary impact his work would have on all fields of biology and beyond.

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Sensory vestibular information and vertebrate motor behaviour

Strains of mutant mammals where the inner ear degenerates at birth can be used to study the effect of the lack of sensory vestibular input during development. Recent data show the maturation of the membrane properties of central vestibular neurons is delayed, but not impaired, by the absence of sensory vestibular information. However, the motor behaviour of adult animals remains strongly affected

Daniel Eugène (above, left), Nicolas Vibert (above) and Pierre-Paul Vidal (left).

The central vestibular neurones located within the brainstem receive most of the sensory input coming from the vestibular sensory organs of the inner ear labyrinth, which detect head motion. These neurones play a major role in the processing of vestibular, visual and proprioceptive information related to body motion. They transform this sensory information into motor commands used to stabilize gaze and posture. Central vestibular neurones include several groups of neurones that have distinct functional roles. For instance, the neurones of the medial vestibular nucleus (MVN) are mainly involved in gaze and posture stabilization within the horizontal plane, i.e. they control the left-right orientation of the eyes, head and front part of the body.

Over recent years, the neurones of the MVN, and to a lesser extent other vestibular neurones, have been studied both *in vivo* and *in vitro* in several mammalian species (Straka *et al.* 2005). Within each functional group, the membrane properties of individual vestibular neurones would shape their sensitivity and response dynamics. For instance, *in vitro* studies indicated that the MVN contains two major subtypes of neurones (type A and type B neurones), which differ in their spike and after-hyperpolarization shapes, as well as in the profile of their repolarization during inter-spike intervals (Fig. 1). This distinction has important consequences for their response dynamics (Straka *et al.* 2005). The presence of different subtypes of vestibular neurones that display distinct membrane properties and response dynamics seems to be a ubiquitous feature, since the vestibular neurones in chick and frog also subdivide into populations with different electrophysiological properties (Straka *et al.* 2005).

Studies in chick, mouse and rat have shown that central vestibular neurones are electrophysiologically immature at birth, and undergo a gradual process of post-natal maturation of their membrane properties. They are therefore a good model to study to what extent during development the activity of sensory afferents is necessary for the maturation of the neurones that receive and process sensory information in the central nervous system. The question is whether the signal carried by sensory vestibular neurones, which gives information on the head movement of the pups, is necessary for the maturation of the membrane properties of central vestibular neurons.

To address that question, we (Eugène *et al.* 2007) used a strain of vestibular-deficient mutant mice known as the KCNE1$^{-/-}$ mutant mice (Vetter *et al.* 1996). In these knockout mice, the KCNE1 potassium channel gene is inactivated, which leads to a selective degeneration of all sensory cells of the inner ear just after birth, while sparing the central nervous system (Warth & Barhanin, 2002). As a result, the KCNE1$^{-/-}$ mutant mice are deaf, their vestibular organs cannot encode head movements and the sensory vestibular afferents do not transmit any input. However, the central vestibular neurons look intact.

In adult normal animals, bilateral suppression of sensory vestibular input by destruction of the two inner ear labyrinths does not modify the resting posture, but these animals show a quasi-constant head bobbing behaviour (de Waele *et al.* 1989). The KCNE1$^{-/-}$ mutant mice display similar deficits (Fig. 2*B*), but show in addition a permanent "waltzer"

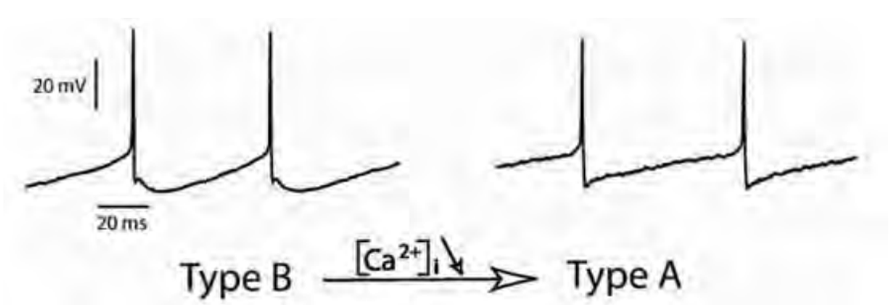

Figure 1. Action potential profiles of the two major neuronal types in the medial vestibular nucleus (MVN). The type B MVN neurone (left) is mainly characterized by a double after-hyperpolarization. The type A MVN neurone (right) exhibits a single, deep after-hyperpolarization. Several membrane properties of type A and B neurones depend on the intracellular free calcium concentration ($[Ca^{2+}]_i$), but the fall in this value would transform type B neurones into type A neurones.

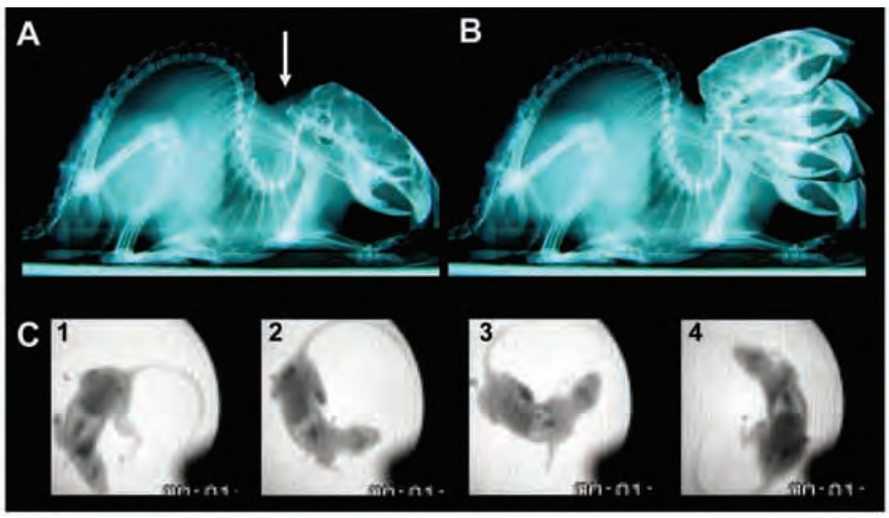

Figure 2. Body postures of the vestibular-deficient KCNE1$^{-/-}$ mutant mouse *A*, mouse at rest. X-rays show that the skeletal configuration is stereotypical, with a clear S-shaped resting posture similar to normal animals. Note the vertical cervical column (white arrow). *B*, mutant mouse showing head bobbing. Four superimposed X-ray photographs illustrate the head oscillations in the sagittal plan (10-30° at 3-5 Hz). *C*, circling behaviour of a mutant mouse. The mouse performed up to almost 3 complete turns per second during short episodes of spontaneous circling.

phenotype (Vidal *et al.* 2004), i.e. their locomotion is frequently interrupted by episodes of rapid circling in a preferred direction (Fig. 2*C*). This suggests that suppression of sensory vestibular input induces the circling behaviour, but only when happening during a 'sensitive period' around birth. These mutant mice offer a unique opportunity to examine how central vestibular neurons mature in the absence of sensory input from the vestibular sensors, and to determine whether modifications of these neurones are responsible for this behaviour.

In juvenile KCNE1$^{-/-}$ mutant mice, the mutation resulted in a strong decrease in the expression of the calcium-binding proteins calbindin, calretinin and parvalbumin (which regulate the intracellular free calcium (Ca^{2+}) concentration of neurones) within the MVN (Eugène *et al.* 2007). This decrease was associated with modifications of the membrane properties of MVN neurones, which provoked an 80% increase of the spontaneous discharge rate of type B neurones. These modifications were most likely related to an increased intracellular free Ca^{2+} concentration. Indeed, decreasing the intracellular free Ca^{2+} concentration within the recording pipette resulted in a decrease of the spontaneous discharge rate of type B MVN neurones back to control levels. In control mice with normal inner ear hair cells and no behavioural symptoms, a low Ca^{2+} concentration provoked an increase in the relative proportion of type A compared to type B neurones (Fig. 1).

In adult mice, in contrast, there was almost no difference between the membrane properties of MVN neurons in KCNE1$^{-/-}$ mutant versus control mice. The expression levels of calbindin and calretinin were still lower in adult homozygous mutant animals than in normal adult mice, but the amount of these proteins expressed in MVN was much closer to normal than in juvenile mice.

Altogether, these data demonstrate that suppression of sensory vestibular inputs during a 'sensitive period'around birth induces the circling/waltzing behaviour that characterizes numerous strains of mutant mammals, but that this behaviour is not due to persistent abnormalities of the membrane properties of central vestibular neurons. Indeed, maturation of the membrane properties of central vestibular neurons is delayed, but not impaired by the absence of sensory vestibular information, which suggests that the membrane properties of these neurons are at least in part genetically coded. Our data suggest that modulation of the expression levels of calcium-binding proteins in central vestibular neurons could play a major functional role. First, it could be involved in the recovery of the resting discharge of central vestibular neurons that follows an acute deafferentation of these cells in adult animals by infectious or traumatic lesions of the labyrinth, i.e. in the so-called vestibular compensation process (see Straka *et al.* 2005). Second, regulation of the intracellular free Ca^{2+} concentration might be used to modify the relative proportions of type A and type B MVN neurons according to functional demands.

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Nucleotide release and airway epithelial physiology

Nucleotide release provides extracellular communication in poorly innervated tissues. In airway epithelia, synchronous release of nucleotides and mucins ensures efficient lung innate defence

Silvia Kreda (above, left), Richard Boucher (above) and Eduardo Lazarowski (left).

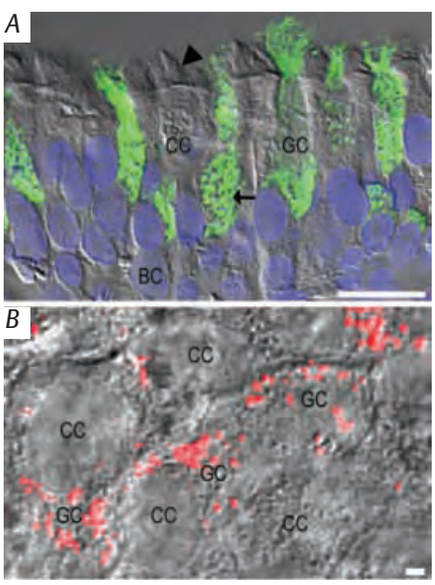

Figure 1. Organisation of the superficial airway epithelium in human large airways. *A*, Confocal microscopy image of a histological section of human bronchial epithelium. The picture represents the overlay of the differential interference contrast (DIC) (grey, cell shape), FITC (green, mucin), and 4,6-diamidino-2-phenylindole (DAPI) (blue, nuclei) channels. Ciliated cells (CC) exhibit lumenal cilia (arrowhead), basal cells (BC) are at the bottom of the epithelial layer, and goblet cells (GC) express secretory granules that are stained with an antibody against mucin MUC5AC (arrow) as previously described (Kreda *et al.*, 2005). Bar, 20 μm. *B*, Confocal microscopy picture depicting a live primary human bronchial epithelial cell culture loaded with the FM 4-64 probe to label secretory granules (goblet cells). The image corresponds to a xy (*en face*) optical section within the sub-apical region of the cells and is the overlay of the DIC (grey, cell shape) and FM 4-64 (red, mucin granules) channels. Bar, 2 μm.

The airway epithelium is a complex tissue comprising at least three distinct cell types (ciliated, goblet, and basal cells; Fig. 1*A*). How does this complex epithelium, which is poorly innervated and exhibits little intercellular coupling, coordinate its activities? The answer is: nucleotide release.

Nucleotides, of which adenine-triphosphate (ATP) is the best known, not only are part of our nucleic acids, phosphorylation and reducing reactions, and signal transduction molecules, but also act as pharmacological ligands for purinergic receptors. Remarkably, extracellular ATP has been detected in the majority of non-excitatory tissues, including most epithelia, endothelia, smooth muscle, fibroblasts, astrocytes and blood cells. The significance of nucleotides as extracellular signalling molecules is emphasised by the ubiquitous distribution of several families of ectoenzymes that catalyze nucleotide breakdown and inter-conversion. Thus, not just ATP, but also its products of ectoenzyme activity (e.g. ADP, AMP and adenosine), are present in the extracellular milieu. Together, they elicit a varied array of physiological responses downstream from at least 19 different nucleotide-activated (purinergic) cell surface receptors (Lazarowski *et al.* 2003). In the lung, nucleotides and their metabolites are present in the airway surface liquid (ASL), a thin layer composed mainly of water, electrolytes and gel-forming mucins, that bathes the epithelial surface. ASL nucleotides regulate lung innate defence activities. Purinergic receptors on ciliated cells regulate ciliary beating and ASL volume via ion and water transport, and on goblet cells, purinoreceptors modulate mucin secretion. These activities are finely tuned to allow effective mucociliary clearance, an important element of the lung innate defence.

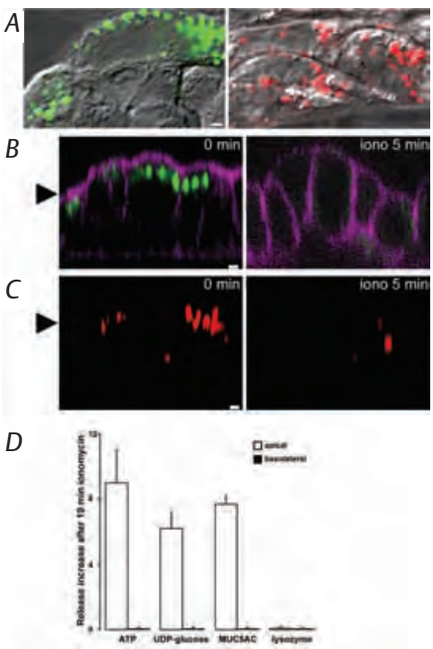

Figure 2. Coordinated release of nucleotides and mucins from goblet cells. *A*, Confocal microscopy xy (*en face*) images of Calu-3 cell monolayers expressing 1 μm granules labelled with MUC5AC (green) antibodies in fixed cells (left panel) and FM 1-43 probe (red) in live cells (right panel). Note the similarity between the fluorescently-labelled granules in Calu-3 cells and goblet cells of human bronchial cultures in Fig. 1*B*. *B*, Confocal microscopy xz images depicting sub-apical localisation of MUC5AC immunostained granules (green) in fixed Calu-3 cells (left panel). Ca^{2+} ionophore ionomycin treatment (iono) promoted exocytotic release of mucins, with MUC5AC granules disappearing from the cells (right panel). Actin cytoskeleton was labelled with fluorescent phalloidin (purple) to identify cell boundaries. *C*, To study real-time exocytosis of mucin granules, the probe FM 1-43 was employed to label Calu-3 cell secretory granules. The image depicts a xz scanning of live Calu-3 cells displaying sub-apical localisation of FM 1-43 labelled granules (red) before (left panel) but not after (right panel) 5 min of ionomycin treatment (most granule-associated FM 1-43 fluorescence was lost upon exocytosis and insertion into the plasma membrane). Bars, 2 μm; arrowheads indicate the position of the apical plasma membrane. *D*, Calu-3 cells challenged with ionomycin stimulated apical but not basolateral release of ATP, UDP-glucose, and mucin MUC5AC. In contrast, the release of lysozyme was not affected by Ca^{2+}-stimulated exocytosis. Data are represented as the mean ± SEM (n= 3-5) and depict the increase in the extracellular bath of each molecule after 10 min of (10 μM) ionomycin treatment compared to time=0. Experimental details are described elsewhere (Kreda *et al.* 2007).

The balance between liquid secretion (ion and water transports), mucin secretion (granule exocytosis), and ASL transport (ciliary beating) ensures the rapid removal of inhaled foreign materials. The 'imbalance' of these epithelial defence activities leads to severe lung disease. COPD, cystic fibrosis and primary ciliary dyskinesia are examples of obstructive lung disease originated by lumenal mucus accumulation, ASL volume depletion, and ineffective cilia beating, respectively (Knowles & Boucher, 2002).

Purinergic regulation of native defence activities occurs chiefly on the lumenal surface of epithelial cells. Indeed, the activity of releasing nucleotides into the ASL resides within the epithelial cells themselves. Only in the last 10 years has it been understood that cells can regulate the release of ATP and other nucleotides (i.e. nucleotide-sugars and uridine nucleotides) by mechanisms that do not involve cell-lysis (Lazarowski *et al.* 2003; Kreda *et al.* 2007). In the airways, ATP is released in response to a variety of stimuli, and likely reflects the histological complexity of this epithelium. Thus, there is no consensus about the mechanism(s) of nucleotide release, and a combination of both conductive and exocytotic pathways may be responsible for epithelial nucleotide secretion. A conductive mechanism would involve a cell surface channel or transporter that release nucleotides directly from the cytoplasm. An exocytotic mechanism would require the trafficking of vesicles to the cell surface. These vesicles could contain nucleotides and/or conductive molecules to be released into the extracellular milieu or inserted into the plasma membrane, respectively. However, with the exception of purinergic neurons and a few non-excitatory cell types (e.g. platelets), nucleotide-containing granules are not apparent in most non-excitatory cell types, including epithelial cells.

Recent work by our and other groups (Kreda *et al.* 2007; Tatur *et al.* 2007),

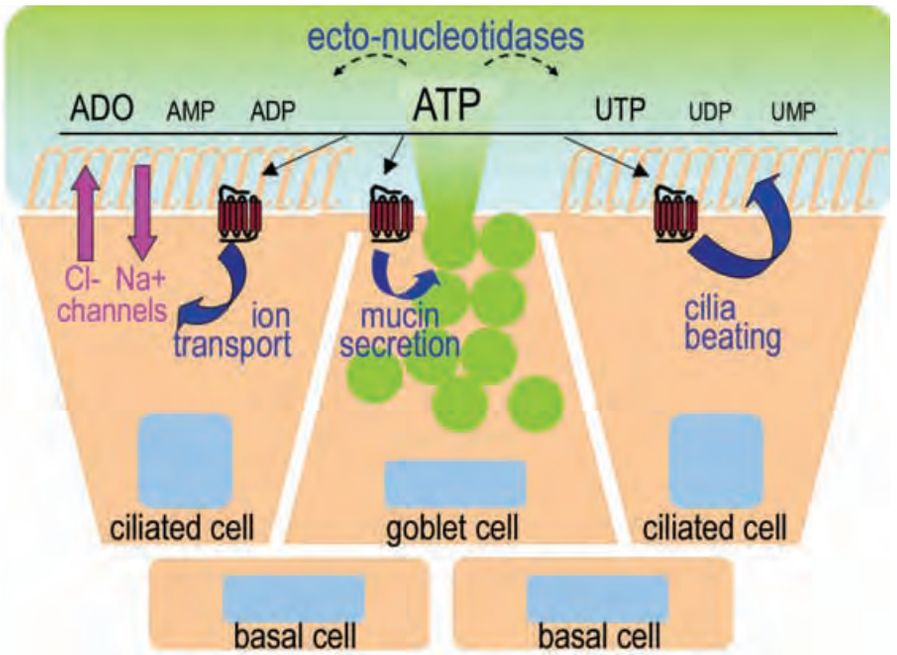

Figure 3. Model depicting nucleotide release and regulation of mucociliary clearance activities in human airway superficial epithelium. ATP and other nucleotides, e.g. UTP, and UDP-sugars (not shown) are released by epithelial cells into the ASL. Ecto-nucleotidases present in the epithelial surface catalyse the hydrolysis and inter-conversion of ATP into adenosine and other nucleotides. Purinergic regulation is achieved mainly by lumenal ATP and adenosine and to a lesser extent by UTP and UDP via activation of several cell surface G-protein coupled receptors (in red). ATP and UTP bind to purinergic $P2Y_2$ receptors that promote stimulation of cilia beating (ciliated cells), mucin secretion (goblet cells), and inhibition of ENAC activity (ciliated cells). The transport of water into lumen and, therefore, the volume of ASL is governed mainly by the transport of Cl^- and Na^+ chiefly via CFTR and ENAC (in purple), respectively. Adenosine, the product of ATP hydrolysis, binds to purinergic A_{2b} receptors on ciliated cells and promotes cystic fibrosis transmembrane conductance regulator (CFTR) channel activity, which in turn inhibits ENAC. Cl^- secretion is thus promoted and water moves into the ASL. UDP binds to purinergic $P2Y_6$ receptors on ciliated cells and promotes cilia beating although, $P2Y_6$ receptors are less abundant than $P2Y_2$ receptors. UDP-glucose, which is also secreted with ATP, binds to purinergic $P2Y_{14}$ receptors expressed on inflammatory cells, but its role on airway epithelial physiology is not well defined. In our model (Kreda *et al.* 2007), one mechanism of nucleotide release involves the synchronous exocytotic secretion of nucleotides (ATP and UDP-glucose) with gel-forming mucins from goblet cells. Nucleotides thus released ensure regulation of ion/water transport and ciliary beating activities on neighbouring cells necessary to hydrate and disperse newly secreted mucins into the ASL, resulting in efficient mucociliary clearance.

suggests that nucleotide release from airway epithelial cells does encompass exocytosis of nucleotide-containing vesicles. Airway epithelial goblet cells express gel-forming mucin secretory granules (Fig. 1*B* and 2*A*), which are released onto the lumenal surface by Ca^{2+}-dependent exocytosis. Goblet cells and neurons employ a similar exocytotic machinery to secrete mucin granules and synaptic vesicles, respectively. The fact that ATP and uridine-diphosphate-glucose (UDP-glc) are concentrated within the endo-plasmic reticulum and Golgi, where they facilitate glycosylation, phosphorylation and folding of secretory proteins, suggests that nucleotides could be stored within mucin secretory granules during granule assembling and 'hitch hike' with mucins for the 'exocytotic ride' to the airway lumen. Notably, sputum specimens from cystic fibrosis individuals exhibiting airway mucin hypersecretion contain remarkably high levels of nucleotides relative to healthy subjects (Lazarowski *et al*. unpublished).

Using biochemical and confocal microscopy approaches, we recently determined that ATP and UDP-glc are released lumenally from goblet cell lines by receptor-activated, Ca^{2+}-

dependent exocytosis (Fig. 2; Kreda *et al.* 2007). The kinetics of ATP release and mucin-granule secretion were similar and triggered by identical stimuli. Moreover, quinacrine, which labels ATP-rich granules in secretory cells, accumulated in the granules of airway goblet cells. These quinacrine granules were released concomitantly with ATP and gel-forming mucin granules into the lumenal surface by Ca^{2+}-dependent exocytosis (Kreda *et al.* 2007).

In summary, our latest data suggest that a pool of nucleotides is released simultaneously with mucins by Ca^{2+}-dependent exocytosis. Although the possibility that nucleotides are concentrated within mucin granules remains to be confirmed, the finding that nucleotides and mucins are being 'coordinately' secreted is relevant to ASL regulation. Mucins are condensed in granules and upon secretion they expand greatly as a gel and disperse into the ASL. Ion and water transport activities are, therefore, necessary for mucin hydration and dispersion; however, these activities are absent from goblet cells. We hypothesise that ATP is coordinately released with mucins and, therefore, it can signal in a paracrine fashion ciliated cells to increase ion and water transport (and cilia beating) to hydrate and disperse secreting mucins in the ASL (Fig. 3). Regulated nucleotide release is the key to coordination of these epithelial activities. Good coordination, good breathing, good health.

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Women utilize lipid as fuel more than men during exercise – is there a paradox?

Individuals interested in maintaining body weight at a healthy level, as well as individuals seeking to lose body fat, typically look to exercise and dietary modifications to achieve a situation in which daily fat oxidation matches or exceeds daily dietary fat intake. With regard to body fat loss goals in modern society, perhaps it is unfortunate that the evolution of skeletal muscle metabolism over millions of years led to a metabolic preference for carbohydrate oxidation during vigorous muscle contraction. That is to say, when exercise intensity is high, carbohydrate is the predominant fuel in exercising humans. However, during sustained exercise bouts of moderate intensity, the rate of body fat mobilization (rate of lipolysis as measured by the release of glycerol and fatty acids from triacylglycerol) increases to provide fuel energy sources for working muscles and other tissues (Friedlander *et al*. 1998, 1999). Fat oxidation during exercise is classically considered to be the major way in which regular physical activity can aid in efforts to lose body fat. However, work by Henderson *et al*. (2007) (Fig. 1) brings insight that body fat mobilization and oxidation during the postexercise recovery period is also of great importance, and in some cases of greater importance than the fat oxidation that occurred during the exercise session.

Factors that affect exercise fat oxidation may affect body fat mass. For years it has been known that gender is one such factor with a significant impact upon fat metabolism during exercise (Friedlander *et al*. 1998, 1999; Tarnopolsky *et al*. 1990). Although for both women and men carbohydrate is essentially the only fuel at or above the peak aerobic capacity (VO_2peak), in comparison to men, women are typically found to derive a significantly larger proportion of energy from lipid during exercise intensities in the moderate range. The 'crossover' concept (Brooks & Mercier, 1994) describes the effect of relative exercise intensity (expressed as % VO_2peak) upon fuel selection during exercise. As described by the 'crossover' concept, fat oxidation can make up a considerable proportion of exercise energy expenditure (EEE) if the intensity is low to moderate, but not if the exercise is quite challenging (e.g. ≥ 75% VO_2peak). Importantly though, at moderate intensities, women are able to derive

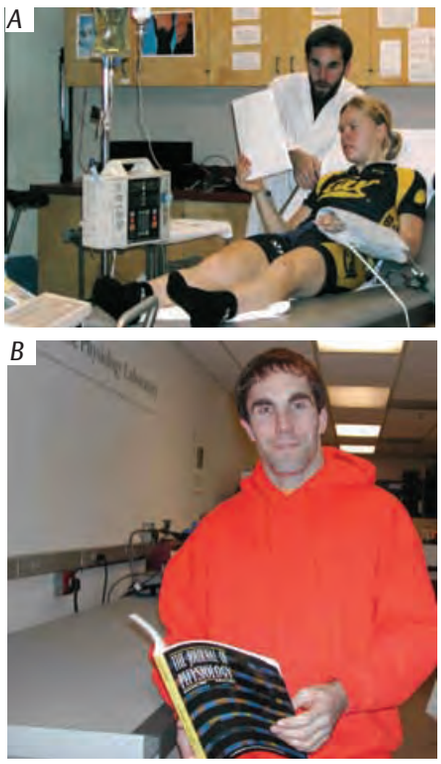


Figure 1. Brooks' Lab at UC, Berkeley. *A*, Greg Henderson shows the movie menu to subject ZZ01. Sterile tracer sources for ^{13}C-palmitate (soft bag) and a combination of D5-glycerol and D2-glucose (inverted bottle) are shown at the top left. The infusion pump is below; tracers are delivered to the subject's right arm and 'arterialized' blood is sampled for a heated left arm hand vein. The subject will rest for 90 min for tracer equilibration, exercise for either 60 min (at 65% O_2peak) or 90 min at 45% O_2peak), and recover for 3 hr. Respiratory gas exchange will be determined throughout and also the next day. *B*, Greg Henderson reading the results of his efforts showing relatively greater lipid mobilization and oxidation during exercise in women as well as more rapid recovery of parameters of lipid and glucose metabolism in women compared to men after exercise.

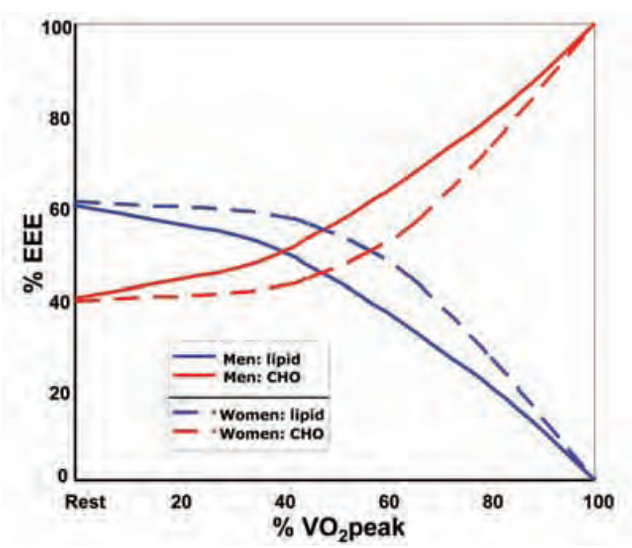
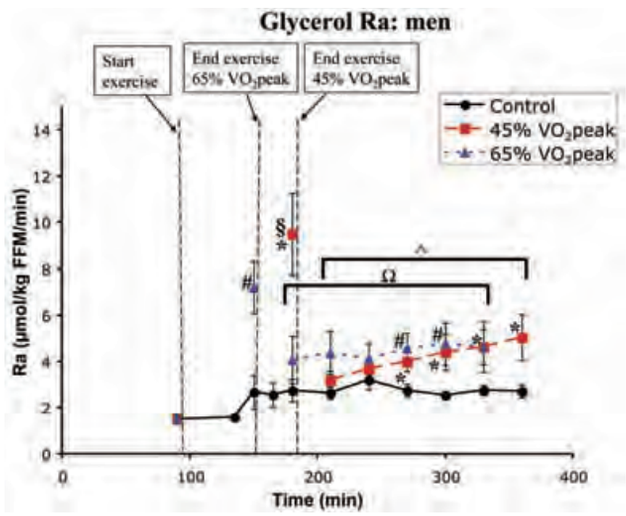


Figure 2. The 'crossover' concept. Energy substrate partitioning (% energy from CHO and fat) as a function of relative exercise intensity as given by % VO_2peak. In a postabsorptive state, humans depend mostly on lipid energy sources during rest and mild intensity exercise. However, as relative exercise intensity increases from mild through moderate and on to hard intensity, energy from CHO oxidation rises where as fat oxidation declines. Crossover from fat to CHO dependence occurs in the range of 45 to 65% VO_2peak. In exercising women crossover typically occurs at a higher % VO_2peak than in men. However, as found by Henderson *et al*. during recovery from exercise women return to the typical pattern of energy-substrate partitioning more rapidly than do men who display elevated lipid mobilization and oxidation in recovery (adapted from Brooks & Mercer, 1994).

a larger proportion of energy from fat than men, and therefore, while the 'crossover' concept applies to both genders in the same qualitative manner, separate curves for men and women represent the relationship between relative exercise intensity and substrate oxidation rates (Fig. 2). Again, to refer back to the classical thought that exercise fat oxidation causes body fat loss, one might naturally assume that exercise must facilitate weight loss more in women than men. However, this assumption would not be correct, and quite the opposite appears to be true. That is, paradoxically, women actually achieve less fat loss than men in exercise programmes (Donnelly & Smith, 2005) even though they oxidize relatively more fat during exercise bouts (Friedlander *et al*. 1998, 1999; Tarnopolsky *et al*. 1990).

Exercise is a powerful tool to alter pathways of lipid mobilization and fatty acid partitioning between storage and oxidation, and it is of interest to understand how behavioural choices can alter the rate of lipid oxidation. While it has been known that during moderate intensity exercise the rate of lipid oxidation increases, particularly in women, less is known about how energy substrate mobilization during exercise integrates with that which occurs for the remainder of the day, and the following day, after the exercise session comes to completion and daily activities are resumed. In order to better understand the importance of the postexercise recovery period upon pathways of lipid metabolism and substrate oxidation, we have recently combined stable isotope tracer methodology with indirect calorimetry in both male and female study participants (Henderson *et al*. 2007). Each volunteer was studied under three different conditions with metabolite tracer infusion and blood and breath sampling: (1) before, during and 3 hours after bicycle exercise at 45% VO_2peak; (2) before, during and 3 hours after bicycle exercise at 65% VO_2peak (equal energy expenditure as first condition); and (3) in a sedentary condition involving no exercise. In each of the three conditions, the participants were also studied the next morning by breath collection for additional measurement of whole body lipid and carbohydrate oxidation rates. Despite relatively less fat oxidation during exercise in men, lipid mobilization was elevated more in men (Fig. 3*A*) than in women after exercise (Fig. 3*B*). Furthermore, the lipid oxidation rate was elevated more in men after exercise, even including approximately 21 hours after exercise on the following day. It has now become apparent that the gender difference in exercise lipid metabolism may actually reverse in the postexercise recovery period such that the total impact of exercise upon lipid metabolism may actually be larger in men than women. These recent results resolve the long-standing paradox associated with the fact that women oxidize fat more than men during exercise, but that women who exercise retain more body fat than do men who exercise similarly.

A



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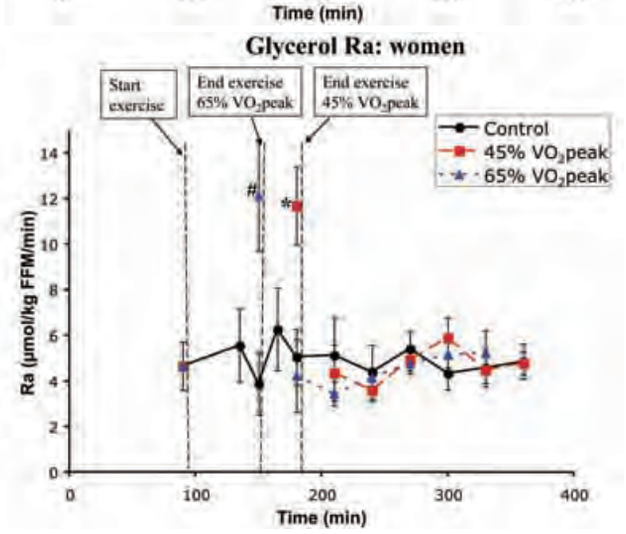

Figure 3. Lipid mobilization. Glycerol kinetics during and after exercise in men, *A*, and women, *B* . Glycerol rate of appearance (Ra) represents the rate of lipolysis (triacylglycerol mobilization). Values are means ± SE. Men, n = 10; women, n = 8. Time (min), duration elapsed since beginning tracer infusion. FFM, fat free mass; *45% VO_2peak trial significantly different from corresponding time points in control trial, $P < 0.05$; #65% VO_2peak trial significantly different from corresponding time points in control trial, $P < 0.05$; §45% VO_2peak trial significantly different from corresponding time points in 65% VO_2peak trial, $P < 0.05$; ^average post-exercise (30 to 180 min post-exercise) in 45% VO_2peak trial significantly different from corresponding average in control trial, $P < 0.05$; Ωaverage postexercise (30 to 180 min postexercise) in 65% VO_2peak trial significantly different from corresponding average in control trial, $P < 0.05$ (from Henderson *et al*. 2007; Fig. 4*A-B*).

The 'crossover' concept describes the effect of relative exercise intensity in men and women upon fuel utilization. Men and women respond similarly to exercise in terms of the balance of fuel selection, but women utilize more lipid in the moderate intensity range (Fig. 2). Dependence on carbohydrate derived fuels (glycogen, glucose, lactate) leads to depletion of those energy sources, and during the post-exercise recovery period metabolism is altered, especially in men in whom lipid is used to conserve carbohydrate. Extending the study of exercise metabolism to include the postexercise period helped resolve an apparent paradox in the literature. Considering both the exercise and recovery periods, it is now possible to understand why compared to men, women are more capable of conserving fat and total body mass in response to exercise stress.

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Why do we bob up and down while walking? The mystery of Antiquity walking shadow finally deciphered

You may find it trivial to move up and down while walking, but this motion is a masterpiece of 4 cm shaped by 7 million years of marriage between smoothness and bounce. This vertical movement, first noticed by Aristotle and now tracked by satellites, is particularly beneficial for loading our muscles to perform a little work efficiently. A lesson on how optimization is a relative success story, and not perfection, is described here

You are watching TV, when you suddenly notice a mass of brainy heads bobbing up and down in a walking crowd. Walking accounts for 20% of their energy budget and brainy heads account for another 20%. So why are these 'expensive' heads moving further? Puzzled, you take a stroll while carefully holding a cup full of boiling coffee, and here with each step the coffee bobs up and spills out of your cup. Unconsciously, you flex your legs to walk smoothly; but why does this bobbing occur when you have the ability to walk flat?

Interestingly, this vertical bobbing was noticed 2400 years ago by Aristotle, who had a habit of walking around the lyceum while talking. He curiously stated in the first known written reference to walking analysis in 350 BC: '*If a man were to walk parallel to a wall in sunshine, the line described (by the shadow of his head) would be not straight but zigzag, becoming lower as he bends, and higher when he stands and lifts himself up* '. Ironically, even his school was named later *Peripatetic*, meaning to walk up and down the shaded walks. Hence, could that be but a shadow?

Why do we bob up and down?

'*... man, the only erect animal ...*' Aristotle again hinted, and indeed it is all in our legs. Humans walk on straight legs. Consequently, our body's centre of mass (CM) rises as the supporting leg becomes vertical and descends again so as to arc upwards with each step (Fig. 1). This vertical movement enables humans to save energy by reducing the muscle work because we slow down as we rise and speed up as we fall, thus passively recovering kinetic energy into gravitational potential energy and back again as in an inverted pendulum (Cavagna, 1963). In straight walking, the line of action of our weight passes close to the leg joints, which also decreases the need for large forces in our leg muscles.

Human walking is unique so even the apes – our closest relatives – bend their legs when walking on two legs, which reduces the rise of the CM (i.e. flat gait) (Alexander, 1992). This is, however, paramount for arboreal locomotion because it enhances stability on branches. Accordingly,

Firas Massaad (above, left), Thierry Lejeune (above) and Christine Detrembleur (left).

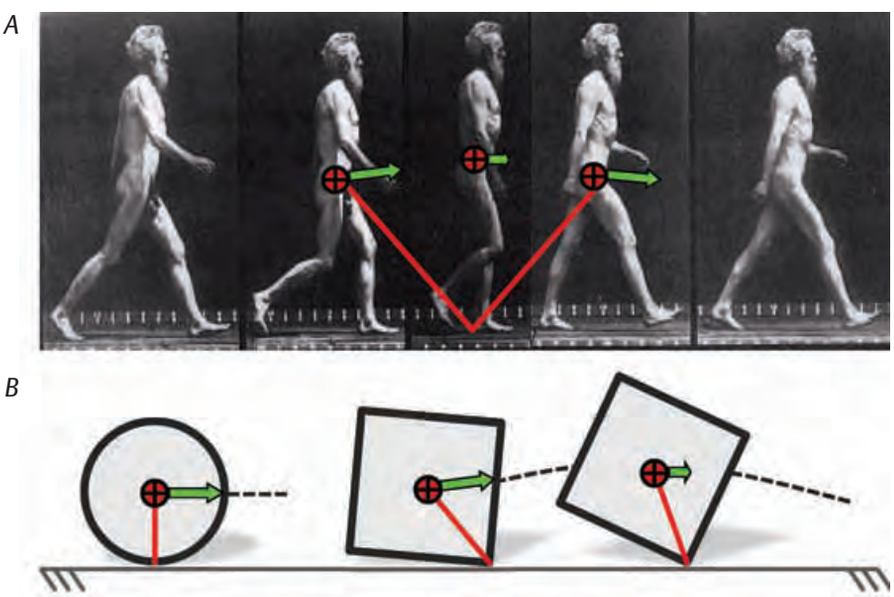

Figure 1. Pendulum-like style of walking. *A*, Sequences by Eadweard Muybridge, the father of the motion picture, of himself walking in 1887. Indeed, by freezing time we can clearly notice his vertical head bobbing (about 3-4 cm at a common walking speed ~4 km h^{-1}) during a step (from left to right foot contact). Sticks represent the inverted pendulum model of his CM displacement (crossed circle), located approximately midway and just above the hip joints. The CM reaches its lowest point when both feet are on the ground then vaults over the supporting leg so as to move forward along a series of arcs of circles. The CM forward velocity (green arrow) slows as he moves upward, and kinetic energy is converted into gravitational potential energy, which is partially recovered as he falls forward and downward after crossing the vertical. *B*, The wheel is considered as the most efficient instrument of motion because its CM remains horizontal. Velocity of the wheel CM can thus be kept constant because it is perpendicular to a line connecting the CM with the ground. The locomotory legged apparatus, as a 'square wheel,' inherently implies vertical displacement and forward speed changes of the CM responsible for much of the mechanical work to walk (adapted from Cavagna,1978).

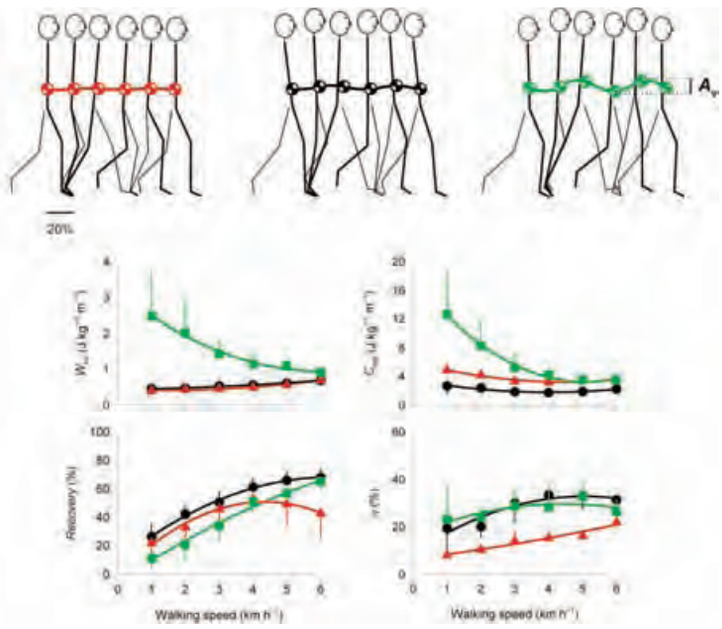

Figure 2. Flat and bouncy walking. *A*, The stick figures show the segments' positions (right limb in thick lines) every 20% of the walking cycle (between successive right foot contacts) for a female (21 years old, 1.6 m height) walking at 4 km h^{-1} in flat, normal, and bouncy styles from left to right respectively. The hourglass circle depicts the CM displacement in flat (red curve), normal (black curve), and bouncy walking (green curve). A_v represents the amplitude of her vertical CM displacement. To walk flat, she spontaneously adopted a flexed-legged pattern of the supporting leg. *B*, Left panels, from top to bottom, show the total mechanical work provided by muscles to walk and the percent of pendulum-like recovery of mechanical energy versus walking speed for six subjects in normal (black circle), flat (red triangle), and bouncy walking (green square). Each data point represents mean values, vertical bars indicate one standard deviation from the means, and lines are least squares regressions. Right panels, from top to bottom, depict the net energy cost, and the muscle efficiency versus walking speed.

scientists postulated that our common ancestors with apes, approximately 7 million years ago (Ma), may also have walked flat as the first transition to terrestrial bipedality (Schmitt, 2003). However, recent computer modelling suggested that the australopithecines (represented by the 3.2 million year old 'Lucy') already walked straight and moved up and down. Therefore, our bobbing is more ancient than we previously thought.

We make war so that science may live in peace

Our CM kept out of the limelight until after the Second World War, when masses of people were lumbering with stiff straight legs, bobbing extensively and were out of breath. Suddenly, the realisation dawned that although relatively straight legs may save energy, the straighter we walk, the more we bob and the more energy we consume. Hence, a landmark article postulated that if humans walked flat like the CM of a wheel (Fig. 1), muscle work and energy consumption would be minimized because bouncy walking with excessive vertical bobbing would waste energy to redirect and raise the CM against gravity (Saunders *et al*. 1953). However, recent human experiments showed that flat walking costs more energy than normal walking (Ortega & Farley, 2005). Recent breakthrough in computer modelling also concluded that work requirements would be minimized by normal bobbing (Srinivasan & Ruina, 2006).

Why is our natural bobbing optimal?

Therefore, we asked healthy adults to walk on a treadmill (1 to 6 km h^{-1}) while they were provided with their CM displacement feedback (Massaad *et al*. 2007). For each speed, they walked normally, with minimum vertical CM displacement (flat walking), and with maximum displacement (bouncy walking). We measured the total mechanical work provided by their muscles (W_{tot}), their oxygen consumption as energy cost (C_{net}), their muscle efficiency ($\eta=W_{tot}/C_{net}$), and the EMG activity for their leg muscles .We also calculated their vertical CM amplitude (A_v) and the '*recovery*' percent of mechanical energy exchanged between gravitational potential and kinetic energies of the CM.

The subjects were able to decrease A_v in flat walking. However, W_{tot} was normal, C_{net} nearly doubled with a sharp decrease in η (Fig. 2). In bouncy walking, W_{tot} and C_{net} dramatically increased but η remained normal. In both flat and bouncy walking, the recovery decreased and muscle co-contraction timing increased (i.e. when antagonistic muscles are working against each other).

The optimal combination of ~3 cm in A_v at ~4 km h^{-1} minimized C_{net}, corresponding to normal human walking. In bouncy walking, C_{net} increased slightly with a quite large increase of A_v from the optimum, whereas in flat walking, C_{net} was highly sensitive to reductions of A_v (Fig. 3).

Wasteful flat, exuberant bouncy

Thus, the energy cost increased in both flat and bouncy walking. This increase was unexpectedly sharpest in flat walking where muscles provided little work but wasted energy. In bouncy walking, muscle provided extra work but conserved energy. Indeed, walking flat is like an experienced old man driving a rusty car using too much fuel although he drives straight, while bouncy walking is like a reckless teenager with a well-oiled car using the necessary fuel, but going through exuberant zigzags.

Despite the prediction of Srinivasan & Ruina's model that normal walking

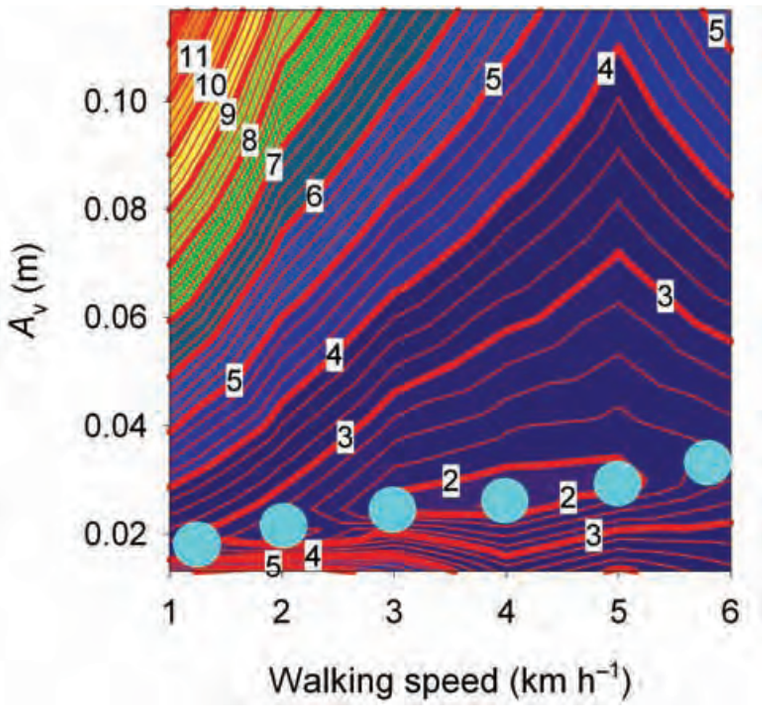

Figure 3. Optimization of human bipedalism. Contours show the energy cost C_{net} (in J kg^{-1} m^{-1}) against the amplitude of vertical CM displacement A_v and walking speed. Each contour indicates a higher cost than the one inside. A central contour surrounds the minimum cost region that looks like a valley on a map. The mean C_{net} for our subjects in normal walking (cyan circles) are plotted at the corresponding speed and vertical CM displacement. C_{net} was more sensitive to reductions of A_v in flat walking than the increase of A_v in bouncy walking. For instance, at around 4 km h^{-1} the subjects were able to reduce A_v by nearly half (from about 0.025 to 0.015 m) which nearly doubled their C_{net} (from about 1.7 to 3.2 J kg^{-1} m^{-1}); however, they were able to increase A_v up to ~3-4 times higher (from about 0.025 to 0.088 m), but their C_{net} only doubled (from about 1.7 to 4.2 J kg^{-1} m^{-1}).

would minimize work, we found no significant difference in work requirements between flat and normal walking. However, our muscles are inefficiently working in flat walking in opposition to bouncy walking. Therefore, not only do humans bob up and down in normal walking to save energy via a pendulum-like mechanism, as commonly thought since the 1960s, but they also load their muscles more efficiently this way.

Efficient locomotion is when most of the metabolic energy input is transformed into mechanical work. Flat walking like a wheel (the most efficient tool of motion) is inefficient probably because energy losses may have occurred due to muscle co-contraction and the need for high muscle forces due to flexed legs. However, in bouncy walking, the muscle co-contraction did not affect muscle efficiency. Hence, it seems that efficiency determinants remain a mystery because it is not yet possible to measure the force and the energy consumed by individual muscles during walking. Would another war resolve this mystery?

Efficiency was settled first

Even after probably 7 million years of ancestry with apes, we are still close to a flat walking (Fig. 3). This suggests that reducing our bobbing is indeed important, but to certain limits. Nature may ultimately have chosen the best compromise between safe flat locomotion that requires little work and bouncy 'risky' locomotion that turns walking into a 'series of falls' but improves muscle efficiency.

Straight legs are efficient. This was probably a primary criterion likely achieved if the short 'Lucy' already walked straight. The increase in leg length seen in H. erectus 1.8 Ma would have made it possible to cover longer distances more effectively at high speeds. Thus, nature may have maximized muscle efficiency (rusty to well-oiled) millions of years before reducing energy consumption at higher speed which was probably vital. This recalls Peter Drucker's famous quotes: '*Efficiency is doing better what is already being done*' ... '*Efficiency is doing things right; effectiveness is doing the right things*'. He was obviously not the first father of management!

Carefree of age, our CM is heading the future

Mankind specialization is basic as vertical bobbing appeared since quadrupedal terrestrial locomotion emerged ~400 Ma (i.e. since legs were adopted). However, early sprawling vertebrates like salamanders lumbered and bobbed excessively than cursorial animals that move gracefully like dogs; hence, our perception of lumbering exists (Reilly *et al*. 2006).

Modern humans, however, take advantage of both flat and bobbing walking. They shortcut millions of years of evolution in a few seconds to carefully hold their beloved coffee and walk flat to achieve impressive walking speeds in competition. Our natural bobbing is also used to generate electricity to power portable devices and is now tracked by satellites to assess human walking in daily life.

Recent humanoid robots also rival human walking by bobbing with simple control of actuators while some state-of-the-art robots still use sophisticated motor control to walk flat with excessive energy consumption. This echoes what we have found as physiological determinants of optimal bipedalism.

In conclusion, our results showed that our natural bobbing loads our muscles efficiently. This may have a direct implication in robotics to walk flat no longer and rehabilitation of pathological gait to bob excessively

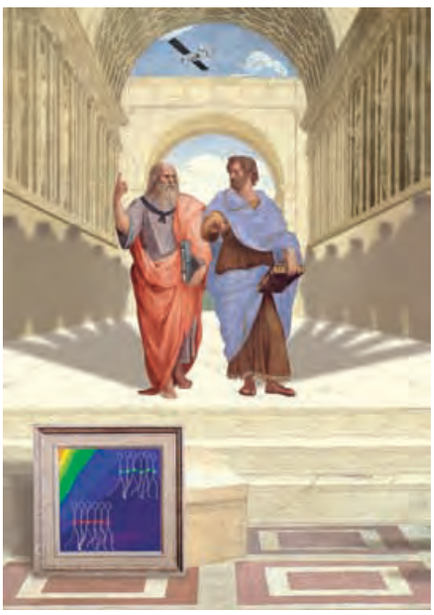

Figure 4. Antiquity walking shadows. Figure adapted from 'the school of Athens,' one of the most famous paintings by the Italian renaissance artist Raphael, painted in 1511. You can see Aristotle on the right talking with his mentor Plato while they walk up and down the shaded colonnades at the Greek 'university'. Plato is pointing upwards to the source of higher inspiration, the realm of ideas 'Look to the heavens if you want to know.' Aristotle, on the other hand, is gesturing downwards, towards the starting point of all the natural sciences 'Look around you if you want to know.' Indeed, Plato's ideas notably the myth of the cave shadows (that humans are like men sitting in a cave seeing shadows on the wall, as symbolised by Aristotle's shadow) would have inspired his student Aristotle to step outside and walk in the sun. Aristotle, by looking around him, noticed the bobbing of our head shadow while walking in the sun (as symbolised by Plato's shadow). Only 2400 years later, we were eventually able to understand the physiological mystery of their head bobbing in the painting laying down the stairs, whereas satellites are now tracking our up and down movement to assess walking in daily life (figure conceived by Firas Massaad and designed by Marianne Roegiers [marianne@ccmproductions.be]).

no longer either. You may now wonder how disastrous it would be for future humans to walk while holding their coffee if they were heading towards further head bobbing. However for now, 2400 years after Aristotle, we can eventually assert that our bounciness is not but a walking shadow (Fig. 4). Still, like Shakespeare, we say '*Life is but a walking shadow*.'

Acknowledgements

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Exercise for liver health

Sedentary life-style, together with irregular rhythm of life and high energy diet, are nightmares to social and financial policymakers today

Physical inactivity simultaneously with increased body adiposity are significant risk factors for the development of metabolic syndrome, i.e. hypertension, type 2 diabetes and cardiovascular diseases which are today the leading causes of death especially in industrialized countries. This ticking time bomb has awakened physicians and authorities to write exercise prescriptions and underline the importance of physically active life-style and keep-fit training. Regular exercise training, in its different forms, is achievable for everyone, cheap, and an effective health preventor when performed regularly.

Abnormalities in lipid metabolism are usually among the first symptoms expressing unhealthy way of life. Impaired free fatty acid (FFA) metabolism is associated with reduced lipid oxidation and increased body adiposity. Impaired lipid oxidation leads to a higher concentration of circulating FFAs in the blood and a higher FFA supply for tissue uptake. Increased tissue FFA uptake, especially in the skeletal muscle and liver, inhibits the glucose metabolism and further creates a predisposition for impaired glucose tolerance (Arner, 2002).

Regular exercise training is known to improve impaired glucose tolerance and lipid metabolism. Although the positive effects of increased physical activity on FFA metabolism are well known in metabolic disorders, the results in skeletal muscle (Jansson & Kaijser, 1987; Turcotte *et al.* 1992; Bergman *et al.* 1999) and myocardium (Heiss *et al.* 1976; Turpeinen *et al.* 1996) in healthy young adults are contradictory. This inconsistency in study results may partly be explained by the different methods and physio-logical state used. To our knowledge no previous investigations studying the effects of exercise training on hepatic FFA uptake exists.

The liver is one of the most versatile and important internal organ in the body. It transforms food to nutrients, maintains normal blood glucose concentration, acts as a buffer against toxic substances, storages and releases nutrients and vitamins, and excretes hormones. In a fasting state, liver mainly utilizes FFAs and amino acids as fuels. In the whole body lipid metabolism liver converts carbohydrates into FFAs, consumes, stores, and releases lipids as part of lipoproteins and thus, controls blood lipid levels. As whole body lipid metabolism is linked to hepatic function, impaired lipid metabolism i.e. increased concentration of circulating FFA increases hepatic lipid accumulation and further hepatic insulin resistance and type 2 diabetes.

Jarna Hannukainen (above, left), Pirjo Nuutila (above) and Kari Kalliokoski (left) .

Studies investigating the effect of physical activity on liver in general are rare and the effects of physical activity on hepatic FFA uptake are largely unknown. In rats and elderly humans exercise training has been shown to increase liver oxidative metabolism (Mauriz *et al.* 2000). In rats, exercise training has been suggested to reduce liver triglyceride production and in rats and humans to increase high density lipoprotein cholesterol production (Gorski *et al.* 1990; Ghanbari-Niaki *et al.* 2007). Interestingly, exercise training has also been shown to change hepatic gene expression. In obese mice model exercise training normalized 38 of the 62 hepatic gene transcripts altered due to high fat diet. These included hepatic genes which relate to tissue defense or detoxification (Lee *et al.* 2006).

Exercise training-induced enhancement in insulin sensitivity has been previously shown to be due to improvements in peripheral insulin sensitivity. However, increased body

A

B

C

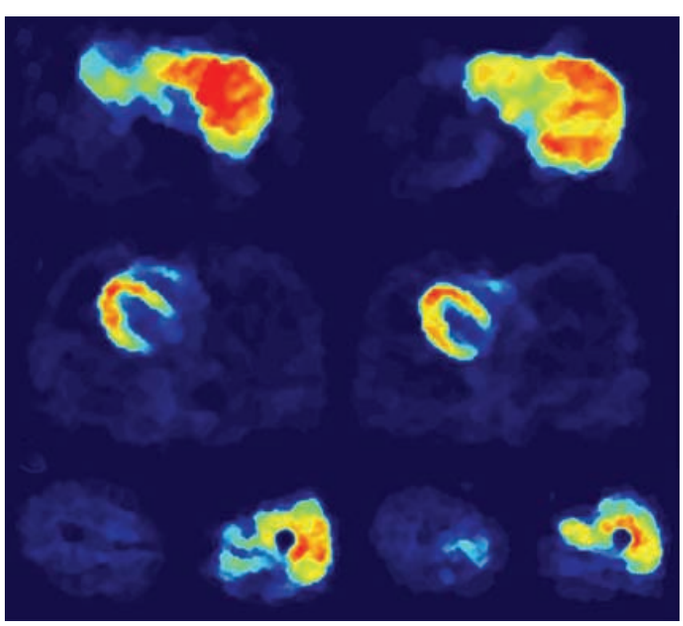

Figure 1. Positron emission tomography images (PET) of free fatty acid uptake in different tissues in healthy monozygotic twins discordant for physical activity and aerobic fitness. PET images of *A*, the liver; *B*, the heart; and *C*, the femoral region (right leg is exercising and left leg resting) in the less (left side) and more active (right side) twins.

adiposity with increased circulation of FFA and triacylglycerol in muscle and liver ensue insulin resistance in both of these tissues. Recently it was shown that exercise training also improves hepatic insulin sensitivity in sedentary men due to endogenous hepatic glucose production (Shojaee-Moradie *et al.* 2007).

An increased circulation of FFA predispose to hepatic fat accumulation and the pathogenesis of non-alcoholic fatty liver disease (NAFLD) which may lead to significant liver-related morbidity and mortality. At the moment no established therapy for NAFLD exists and current treatment relies on life-style modifications, i.e. reducing metabolic risk factors by weight loss through diet and regular exercise training (Mishra & Younossi, 2007). Recently physically active lifestyle was found to associate with lower intrahepatic fat content in healthy humans (Perseghin *et al.* 2007).

We have been interested on hepatic function and the effects of physically active lifestyle on liver metabolism. We are especially interested to know which are the specific mechanisms how exercise training influence on hepatic FFA uptake. Is the positive influence of exercise just secondary via decreased body adiposity? Or does exercise training also have primary effects on tissue FFA uptake? In our recent study we investigated the effect of moderately increased physical activity and fitness on tissue FFA uptake with healthy non-obese young adult male monozygotic twins discordant for physical activity and aerobic fitness (Hannukainen *et al.* 2007). Using positron emission tomography and [^{18}F]FTHA we found that hepatic FFA uptake was ~30 % lower in the more active compared to the less active group (Fig. 1). However, hepatic FFA uptake was not associated with the amount of physical activity or with fitness state, but correlated significantly with the whole-body fat percentage. Although both the more and less active twins had normal weight, the more active twins had 10 % lower whole-body fat percentage. This was mainly due to the lower amount of abdominal subcutaneous fat, although the difference in visceral fat mass between groups was statistically more significant. When the difference in the whole body fat percentage was taken into account in statistical analyses (ANCOVA), the difference in hepatic FFA uptake between the groups decreased ($p = 0.08$) suggesting that hepatic FFA uptake is at least partly influenced by body adiposity. The lower hepatic FFA uptake in the more active twins is in agreement with the suggestion that with decreased body adiposity, especially in the intra-abdominal area, the rate of adipose tissue lipolysis is lower, thus decreasing the FFA load to the liver (Arner, 2002).

Although hepatic FFA uptake was lower in the more active twins, we did not found any differences in myocardial or skeletal muscle FFA uptake at rest or during exercise between the groups. Thus, as the liver is the most versatile organ and covers most of its energy demand by oxidizing FFA, it is reasonable to presume that it is more sensitive to even small changes in the availability of substrates compared to skeletal muscle and the myocardium.

It is very likely that physically active lifestyle is a significant preventor for hepatic fat accumulation. However, at the moment no exercise training intervention exists to prove this assumption, which is mostly due to limited availability of appropriate study methods. Thus, intervention studies are needed to prove the positive influences of exercise training on liver health.

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The relatively high Ca^{2+} flux in Ca^{2+} sparks could be due to the Ca-binding protein calsequestrin in the sarcoplasmic reticulum

Twenty times more Ca^{2+} is reported to come from calsequestrin than the pool of free Ca^{2+} in the sarcoplasmic reticulum (SR) during voltage-activated SR Ca^{2+} release in skeletal muscle, thus highlighting its function as a very strong buffer of the global $[Ca^{2+}]_{SR}$. Calsequestrin may also serve an important function of preventing a local decrease in $[Ca^{2+}]_{SR}$ near the mouth of an open SR Ca^{2+} release channel thereby enhancing the Ca^{2+} flux through the channel

Contraction in skeletal muscle is initiated by an action potential that propagates into the centre of the fibre along invaginations of the surface membrane called T-tubules (Fig. 1). Activation of transmembrane proteins in the T-system – known as dihydropyridine receptors (DHPRs), or voltage sensors – somehow opens ryanodine receptors (RyRs) or Ca^{2+} release channels in the closely apposed SR membrane, probably via the physical link between the two proteins. Ca^{2+} released into the myoplasm activates the contractile proteins thereby causing the muscle to contract. In the junctional region, the Ca^{2+} release channels form a closely packed array in which every other channel is linked to a voltage sensor and the others are uncoupled. One important question in the field has been whether or not Ca^{2+} released from an open channel can activate neighbouring channels (uncoupled or coupled) under physiological conditions via a positive feedback mechanism known as Ca^{2+}-induced Ca^{2+} release (CICR). This article reviews some recent evidence supporting this idea. It then turns to a recent study from our laboratory concerning the buffering of $[Ca^{2+}]$ within the SR ($[Ca^{2+}]_{SR}$) by calsequestrin. The possibility is then raised that local buffering of $[Ca^{2+}]_{SR}$ by calsequestrin near open SR Ca^{2+} release channels could account for the apparent evidence for CICR.

With the introduction of confocal microscopy in recent years, it became possible to measure spatially-distinct, elementary Ca^{2+} release events termed Ca^{2+} sparks using fluorescent Ca^{2+} indicators introduced into the myoplasm. Since

Paul Pape (left) and Karine Fénelon.

the density of Ca^{2+} sparks increases with increasing membrane voltage, each Ca^{2+} spark is presumably initiated with the stochastic activation of a voltage-sensor protein in the T-system and its associated SR Ca^{2+} release channel. The possibility of CICR was recently evaluated by comparing the flux of Ca^{2+} ions in Ca^{2+} sparks to that through single, isolated Ca^{2+} release channels reconstituted into artificial lipid bilayers. In a thorough analysis of Ca^{2+} spark data from frog fast-twitch skeletal muscle, Baylor *et al*. (2002) determined that an average spark produces a peak Ca^{2+} current of 2.5 pA. Based on two estimates of Ca^{2+} currents through isolated SR Ca^{2+} release channels in bilayer experiments of 2 pA and 0.5 pA, Baylor *et al*. (2002) concluded that Ca^{2+} sparks are composed of 1-5 SR Ca^{2+} release channels. One reason that the lower single-channel estimate of 0.5 pA by Kettlun *et al*. (2003) is probably more appropriate is that it was measured under relatively physiological conditions. This would imply that the Ca^{2+} sparks are composed of 5 SR Ca^{2+} release channels on average – one activated by its associated voltage sensor and the other 4 by CICR. Another possible interpretation, presented below, is that the Ca^{2+} flux is larger in Ca^{2+} sparks since calsequestrin – acting as a source of Ca^{2+} – was present, whereas calsequestrin was absent in the bilayer experiments.

MacLennan & Wong (1971) discovered calsequestrin and proposed that it acts to buffer luminal $[Ca^{2+}]$ since it has such a high capacity for binding Ca. Volpe and Simon (1991) challenged this idea with their estimate that only ¼ of released Ca^{2+} comes from calsequestrin and the rest comes from the pool of free Ca^{2+} in the SR.

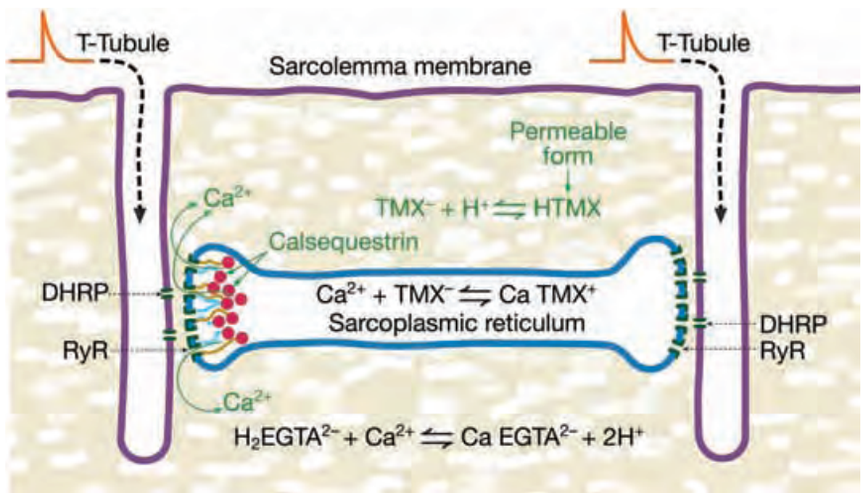


Figure 1. Schematic of SR Ca^{2+} release and methods used to study the role of calsequestrin in skeletal muscle. Calsequestrin is linked to the Ca^{2+} release channel (RyR) by the chain-like proteins triadin and junctin (orange and blue strands).

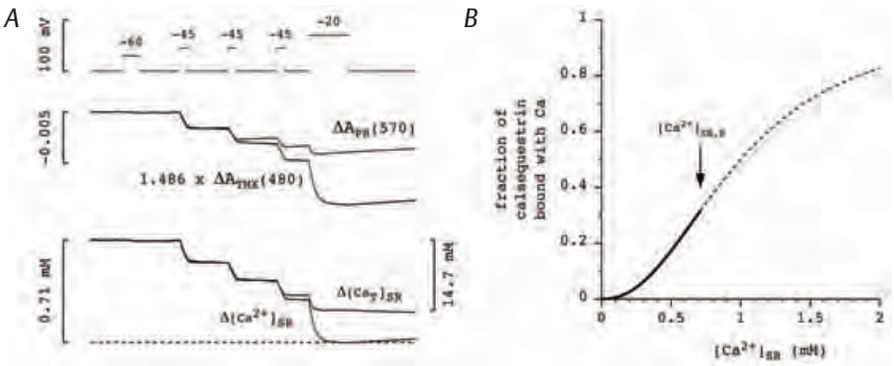


Figure 2. Free and total Ca^{2+} in the SR and the Ca-calsequestrin binding reaction. The top trace in panel *A* shows the voltage trace with voltage-clamp pulses from the baseline potential of -90 mV to the voltages indicated in mV. The trace labelled $\Delta A_{PR}(570)$ is the associated phenol red-related absorbance change at 570 nm measured at the same time. The corresponding TMX signal at 480 nm ($\Delta A_{TMX}(480)$) was scaled by 1.486, thereby showing that the start of the signals have approximately the same time courses. These signals correspond to those shown in Fig. 2*B* of Pape *et al*. (2007) except that they are from the same stimulation shown in their Figs. 3-5. The bottom pair of traces are $[Ca_T]_{SR}$ and $[Ca^{2+}]_{SR}$ derived from the $\Delta A_{PR}(570)$ and $\Delta A_{TMX}(480)$ signals, respectively. These traces are the same as those shown in Fig. 5A of Pape *et al*. (2007). The bottoms of the calibration bars are the zero levels indicating that essentially all of the Ca^{2+} was released from the SR. The tops of the calibration bars indicate the resting levels of free and total Ca, 0.71 mM and 14.7 mM, respectively. Panel *B* plots the Ca-calsequestrin binding curve given by the Hill equation for cooperative binding:

$$f_{CSQ} = \frac{[Ca^{2+}]_{SR}^{n}}{[Ca^{2+}]_{SR}^{n} + K_{D,CSQ}^{n}}$$

where f_{CSQ} is the fraction of Ca-binding sites on calsequestrin bound with Ca, n is the Hill coefficient and $K_{D,CSQ}$ is the dissociation constant for Ca binding assumed to be 1 mM. The value for n was 2.26 in this case. See text and Pape *et al*. (2007) for details.

(Calsequestrin is considered to be the source even though a Ca^{2+} ion has to unbind from calsequestrin and enter the pool of free Ca^{2+} before being released.) We recently reported on experiments in isolated fast-twitch skeletal muscle fibres aimed at resolving the Ca^{2+} buffering properties of calsequestrin in frog fast-twitch skeletal muscle (Pape *et al*. 2007). These experiments involved the simultaneous measurement of two optical signals: one to measure the total amount of Ca in the SR (denoted $[Ca_T]_{SR}$) and the other to give $[Ca^{2+}]_{SR}$. $[Ca_T]_{SR}$ was measured with the EGTA/phenol red method which requires a relatively large concentration of the Ca^{2+} buffer EGTA and a pH indicator such as phenol red. Essentially all of the Ca^{2+} released into the myoplasm is rapidly captured by EGTA, which releases two protons for each Ca^{2+} bond (Fig.1), producing a pH change detected with phenol red. $[Ca^{2+}]_{SR}$ was measured with one of the original Ca^{2+} indicators, tetramethylmurexide (TMX). TMX's ability to enter and, thereby, monitor $[Ca^{2+}]$ in an internal compartment is most likely due to the membrane permeability of the uncharged, protonated form of TMX expected to be present in trace amounts. Owing to its low affinity for Ca^{2+} (K_D = 2.6 mM) and the presence of 20 mM EGTA in the myoplasm, the CaTMX signal from the myoplasm should be negligible compared to that from the SR. The middle traces in Fig. 2*A* show the absorbance signals (ΔA) from the two indicators measured in response to the voltage-clamp stimulation shown at the top. With a reasonable assumption for the resting value of $[Ca^{2+}]_{SR}$ (denoted $[Ca^{2+}]_{SR,R}$) these signals yielded the superimposed $[Ca_T]_{SR}$ and $[Ca^{2+}]_{SR}$ signals shown at the bottom of Fig. 2*A*. As indicated by the calibration bars, the total amount of Ca in the SR at the start (14.7 mM) was much greater than the resting level of $[Ca^{2+}]_{SR}$ (0.71 mM). This is attributable to the strong buffering by calsequestrin. An important thing to note is that the signals superimpose initially whereas the $[Ca^{2+}]_{SR}$ decreases much more relative to the $[Ca_T]_{SR}$ later on. This indicates that the buffering action of calsequestrin is greater at the start of the stimulation when there is a physiological amount of Ca in the SR and that this ability to buffer $[Ca^{2+}]_{SR}$ decreases as the SR Ca content decreases. This feature is evident in the Ca-calsequestrin binding curve derived from these results and shown in Fig. 2*B*. The binding curve is highly cooperative with a Hill coefficient of 2.26 in this case and about 3 on average. The slope of the curve is maximal at the resting value of $[Ca^{2+}]_{SR}$ (shown by the arrow) indicating that the buffering by calsequestrin is maximal at physiological $[Ca^{2+}]_{SR}$. In summary, calsequestrin rapidly buffers released Ca^{2+} with greater than 20 times on average more Ca^{2+} coming from calsequestrin as opposed to the free pool. This result clearly indicates that calsequestrin serves to buffer the global or spatially-averaged $[Ca^{2+}]_{SR}$. We turn now to the possibility that it may also serve to prevent a local decrease in $[Ca^{2+}]_{SR}$ near the mouth of an open SR Ca^{2+} release channel. Such a local depletion is depicted in Fig. 3*A*.

A starting point for assessing the possible contribution of calsequestrin is to consider what happens if calsequestrin is not present. As done previously for other ions (cf. Hille, 1968), the following analysis gives the diffusion-limited Ca^{2+} flux by considering an open Ca^{2+} release channel as a point sink located in a semi-infinite medium. $[Ca^{2+}]_{SR}$ as a function of r from the mouth of the channel should rapidly (within μ secs) approach the steady-state condition given by

$$[Ca^{2+}]_{SR}(r) = [Ca^{2+}]_{SR}(\infty) - \frac{\phi}{2\pi D_{Ca} r} \qquad (1)$$

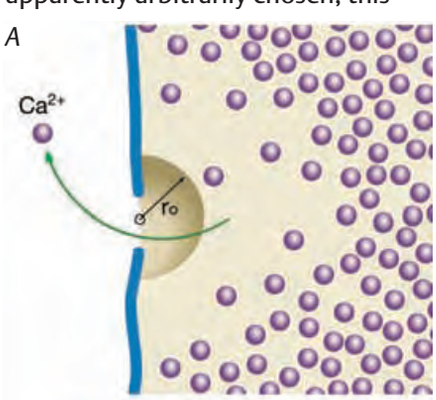
where $[Ca^{2+}]_{SR}(\infty)$ is the concentration far from the channel, D_{Ca} is the diffusion constant for Ca^{2+}, and ϕ is

the flux of Ca^{2+} ions through the channel. (Note that this agrees with Fick's Law since D_{Ca} times the surface of a hemisphere of radius r ($2\pi r^2$) times the gradient ($d[Ca^{2+}]_{SR}/dr$) is equal to the flux, ϕ.) Since $[Ca^{2+}]_{SR}$ cannot be negative – as would be the case if r is very close to zero – a distance r_0 is introduced at which $[Ca^{2+}]_{SR}$ equals zero at the diffusion-limited, maximum flux (ϕ_{max}). r_0 is the 'radius of the channel mouth' in Peskoff and Bers (1988). With this definition,

$$\phi_{max} = 2\pi r_0 D_{Ca} [Ca^{2+}]_{SR}(\infty) \qquad (2)$$

we now calculate the value of r_0 corresponding to a current of 0.5 pA, the best estimate of the single-channel current above, obtained in bilayers experiments. Using Faraday's constant (10^5 coulombs/equivalent), 0.5 pA corresponds to a flux of 2.5 x 10^{-18} moles/sec. With a value for D_{Ca} of 3 x 10^{-6} cm^2/sec and $[Ca^{2+}]_{SR}(\infty)$ given by 1 mM (see Pape *et al*. 2007 for these parameters), the corresponding value of r_0 is 13.8 Å. The diffusion gradient from equation (1) for this case – the middle and thicker curve plotted in Fig. 3*B* – indicates a significant amount of local depletion for distances within 100 Å from the channel. If the value for r_0 of 13.8 Å is reasonable, this would indicate that the 0.5 pA single-channel current could be diffusion-limited. Hille (1968) considered a value of r_0 for Na channels of 3 Å. Although apparently arbitrarily chosen, this value seems reasonable since the Pauling radius for Na is 0.95 Å. Since Ca has a similar radius, 0.99 Å, the same value of r_0 might apply for the SR Ca^{2+} release channel. As indicated by the left-most curve in Fig. 3*B*, the maximum current (I_{max}) for this case is only 0.11 pA. This suggests that the single channel current of 0.5 pA might actually be larger than the diffusion-limited value. Likewise, the single-channel current of 2 pA above gives a value for r_0 of 55 Å which seems much too large (see right-most curve in Fig. 3*B*) and, thus, further supports the idea above that the 0.5 pA value is more appropriate. It is possible that the single-channel current is greater than the diffusion-limited maximum owing to the presence of a net negative charge near the mouth of the channel that results from the local depletion of Ca^{2+}. However, the maximum enhancement from this effect is only about 2-fold (cf Peskoff & Bers, 1988). Moreover, it should be significantly less in this case since the single-channel current is relatively low and since the Ca^{2+} release channels are significantly permeable to K^+ and Mg^{2+} ions so that counter-ion movements of these ions into the SR would tend to reduce the net negative charge. These considerations indicate that the value of 0.5 pA is near, if not significantly above, the maximum single-channel current sustainable by diffusion.

A quantitative assessment with calsequestrin present is beyond the scope of this article. However, since calsequestrin has such a high Ca-binding capacity and fast kinetics, it should be able to supply Ca^{2+} ions thereby minimizing any local depletion of $[Ca^{2+}]_{SR}$. This could help explain the 5-fold greater current in Ca^{2+} sparks above compared to the single-channel current (2.5 pA vs. 0.5 pA) since only the former was determined with calsequestrin present. In summary, calsequestrin strongly buffers the spatially-averaged $[Ca^{2+}]_{SR}$. It may also serve to support relatively high single-channel Ca^{2+} fluxes by buffering local $[Ca^{2+}]_{SR}$ near the mouth of the SR Ca^{2+} release channel. Calsequestrin should also serve to enhance the Ca^{2+} flux by introducing a negative voltage near the mouth of the channel due to negative charges on calsequestrin created when Ca^{2+} ions leave their binding sites.



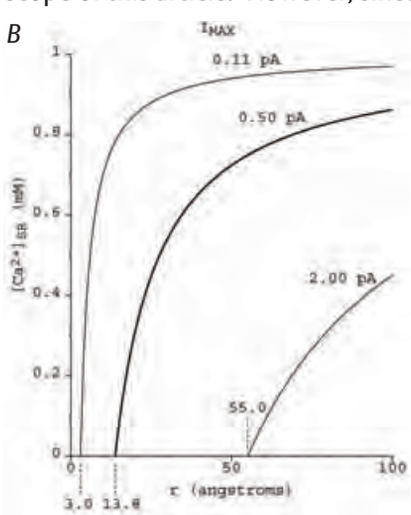


Figure 3*A* (above) and 3*B* (right). Local depletion of $[Ca^{2+}]_{SR}$ See text for details.

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Taste receptors and glucose absorption in the small intestine

Natural sugars and artificial sweeteners activate taste receptors in the gastrointestinal tract to triple sugar absorption through the apical GLUT2 pathway and also to increase incretin secretion within minutes. George Kellett highlights the underlying mechanisms and looks at the dietary consequences and clinical opportunities

George Kellett in his office in 2005.

Taste receptors and glucose sensing

Intestinal brush cells share morphological similarities with lingual taste cells and express the G protein α-gustducin, a key signalling protein in taste reception (Hofer *et al.* 1996). This discovery prompted a decade of speculation that nutrient sensing mechanisms may exist in intestine analogous to those for taste reception. Such speculation has finally become fact, prompted by the cloning of lingual sweet taste receptors.

The T1R family of taste receptors comprises three members, namely, T1R1, T1R2 and T1R3, which act as functional heterodimers. T1R1 + T1R3 in rodent act as a broad spectrum amino acid detector; in human they sense the umami or 'delicious' taste of monosodium glutamate in Chinese food. T1R2 + T1R3, on the other hand, detect sweet taste. Following cloning, transcripts for T1R2, T1R3, α-gustducin and also for members of the T2R family, which recognise bitter taste, were found in intestine. The hunt for a function was on.

Intestinal sugar absorption by SGLT1 and apical GLUT2

In the classical model, the Na^+/glucose cotransporter SGLT1, a low capacity, low K_m transporter, acts between meals as an effective scavenger by driving secondary active cotransport of glucose from the lumen (1–2 mM) uphill to the plasma (~ 4mM). Glucose is then transported into the blood by the high capacity, high K_m facilitative transporter GLUT2, which is present normally only in the basolateral membrane of the absorptive cell (enterocyte).

However, SGLT1 becomes saturated at concentrations below those of 30-200 mM generated at the apical membrane after a meal. My lab therefore proposed the apical GLUT2 model (Kellett & Helliwell, 2000), in which SGLT1 exerts a key regulatory role by initiating rapid insertion of GLUT2 from intracellular vesicles into the apical membrane (Fig. 1). GLUT2 then provides the necessary additional transport capacity at high glucose concentrations; moreover, as glucose at the apical membrane increases, additional GLUT2 is inserted to cope with increased load. Finally, as glucose is absorbed, GLUT2 traffics rapidly away from the membrane.

Calcium and taste signals control apical GLUT2

During digestion, apical insertion of GLUT2 is dependent on a large, glucose-induced Ca^{2+} influx through the non-classical L-type channel $Ca_v1.3$ (Fig. 1), resulting from apical membrane depolarisation by SGLT1 (Mace *et al.*, 2007b; Morgan *et al.* 2007). However, Ca^{2+} absorption is maximal at 20 mM glucose, but there is no increase in GLUT2 insertion. We therefore concluded there must be a second, downstream signal prompting GLUT2 insertion at higher glucose concentrations (>30 mM).

Taste receptors are activated by natural sugars at high concentrations, signalling through α-gustducin to activate PLC β2 and hence PKC βII, which promotes apical GLUT2 insertion. We noted that the sugar concentration range for activation coincided with that of 30-100 mM for apical GLUT2 insertion. In contrast, artificial sweeteners such as sucralose achieve a similar response at 1–2 mM. Sucralose is useful for intestinal studies, since it is neither absorbed nor metabolised and can only act through T1R2 + T1R3. We therefore quickly confirmed that taste

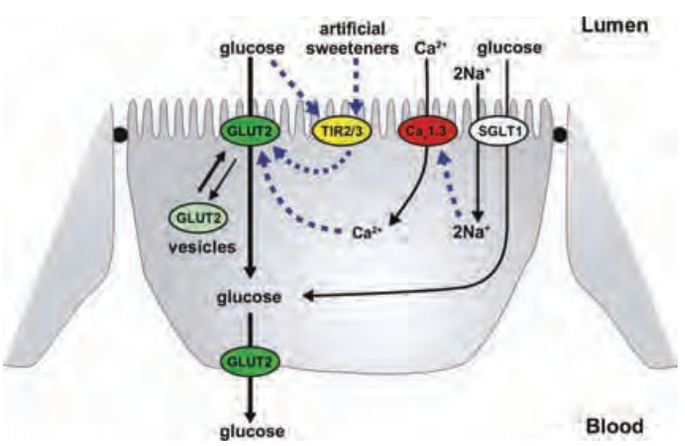

Figure 1. Regulation of apical GLUT2 in enterocytes after a meal
After a meal, glucose concentrations at the apical membrane are high. Membrane depolarisation by glucose transport through SGLT1 increases the influx of Ca^{2+} through $Ca_v1.3$ and induces cytoskeletal rearrangement to permit GLUT2 translocation from intracellular vesicles to the apical membrane. For translocation to be initiated, however, a second signal must be provided by glucose binding to sweet taste receptors. Artificial sweeteners bind to taste receptors synergistically with glucose.

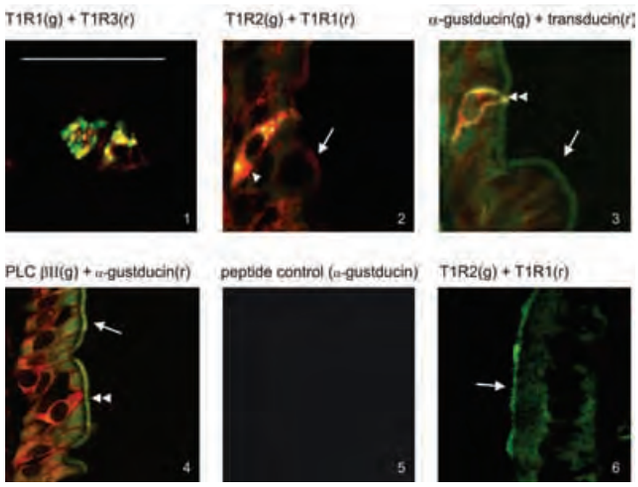

Figure 2. Colocalisation of taste reception molecules in rat jejunum
Merged images of dual-labelled taste reception molecules; colour code, green (g), red (r). Image 1: Paneth cells located at the very bottom of the crypt. Images 2-4, 6: single arrow, enterocyte brush-border membrane, single arrow-head, solitary chemosensory cells (SCC); double arrow-head, SCC tip in brush-border membrane. Scale bar 50 μm. Note that the peptide control for a-gustducin (image 5) is totally negative and indistinguishable from those for other components, because spectral unmixing techniques were used to subtract all background contributions. Images 1-3, 6 are reproduced and adapted with permission from Mace *et al* (2007a).

receptors provide the second signal (Fig. 1) by showing that, in perfused rat jejunum *in vivo*, 1 mM sucralose doubled the rate of absorption of 20 mM glucose within minutes by increasing apical GLUT2 and associated absorption 3-fold (Mace *et al.* 2007a); on this time scale, SGLT1 was unaffected. Sucralose acts through the same PLC β2-dependent pathway as high glucose.

The presence of T1R1, 2 & 3, α-gustducin, transducin and PLC β2 throughout rat jejunum was demonstrated by Western blotting of apical membrane vesicles, which revealed that, like apical GLUT2, all taste reception components undergo rapid trafficking. As anticipated, immunocytochemistry demonstrated the presence of taste receptors, G-proteins and PLC β2 in SCC, used here as a general term to include brush, bipolar and enteroendocrine cells (Fig. 2). They were also found in Paneth cells and in jejunal enterocytes, which have all the necessary calcium and taste sensing machinery to account for increased glucose absorption (Fig. 1).

Margolskee and colleagues have since discovered that taste receptors in duodenal enteroendocrine cells can mediate glucose-induced secretion of incretins GIP (glucose-dependent insulinotropic peptide) and GLP-1 (glucagon-like peptide-1) from K and L cells respectively (Jang *et al.* 2007). Moreover, sucralose increases SGLT1 mRNA, protein and active glucose absorption of mice on a low carbohydrate diet for four weeks. Secretory and SGLT1 effects are both blocked in T1R3 or α-gustducin knockout mice (Margolskee *et al.* 2007). Nevertheless, a taste-sensing role for enterocytes is emphasised by the observation that fructose-induced increases in SGLT1 mRNA and protein are dependent on T1R3 in a clonal enterocytic Caco-2 human cell line, which expresses transcripts for T1R3 and α-gustducin and also T1R2 and T1R3 protein in the plasma membrane (Le Gall *et al.* 2007).

Diet, artificial sweeteners and health

The Western world is currently facing an epidemic of obesity and diabetes. It is generally helpful to reduce dietary calorie intake by the use of artificial non-caloric sweeteners; indeed, that is one reason why they are to be found worldwide in thousands of diet products, especially processed foods and fizzy drinks. However, artificial sweeteners bind to taste receptors synergistically with natural sugars to increase sugar absorption and incretin secretion within minutes. We therefore need to understand the long-term consequences for health of complex, but common dietary situations in which natural sugars and artificial sweeteners are consumed together, like eating hamburger and chips with a thirst-quenching fizzy diet drink. A reassessment of how artificial sweeteners can best be used to nutritional advantage may be necessary.

Equally, it might prove possible to use artificial sweeteners clinically to increase the secretion of incretins GIP and GLP-1 and improve insulin sensitivity. Such an approach would provide an alternative to the development of GLP-1 receptor agonists or GLP-1 analogues for the treatment of diabetes and obesity. Similarly, total parenteral nutrition (TPN) is often used in the management of infants suffering from malabsorption or other gastrointestinal disorders, because they cannot feed enterally. TPN, however, results in mucosal atrophy that can be ameliorated by supplementation with GLP-2 (Cottrell *et al.* 2006). Could artificial sweeteners provide a similar positive effect and enhance or restore the mechanism of absorption?

Conclusion

The discoveries outlined represent no more than a snapshot of a rapidly changing literature. Even so, they represent a sea change in our understanding of intestinal sugar absorption and invite exciting possibilities. Multiple, redundant regulatory pathways surely exist, as befits a single organ that mediates the input side of homeostasis. Understanding their integration and how one nutrient affects absorption of another will become fundamental areas of research. Moreover, clinical trials designed on the basis of new knowledge of apical GLUT2 and incretin secretion are required to

assess the dietary impact of artificial sweeteners and to investigate new opportunities for beneficial dietary or pharmaceutical intervention. The future of intestinal research is regulation.

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Skills plan for bioscience

Research by the Sector Skills Council for Engineering, Manufacturing & Technology (SEMTA) has revealed that two out of five UK bioscience companies have hard-to-fill vacancies and one in five organisations admit skills gaps in their current workforce.

There are concerns that the skills gap will lead to the loss of Britain's status as one of the world's leading centres for bioscience research and development. UK bioscience revenues alone are worth £3.3 billion per annum, and the sector employs 55000 people.

In response to the skills shortfall, a range of bioscience companies, trade associations, government, unions, professional bodies, qualification authorities, awarding bodies and education/training providers, working with SEMTA, have produced an industry standard set of competencies that form the basis for job design, recruitment, training and performance assessment. The new standards will also provide a framework for organisations to fill key vacancies by up-skilling their current bioscience workforce.

The 10 year skills action plan for the sector was launched by the Rt Hon John Denham MP, Secretary of State for Innovation, Universities and Skills at the House of Commons on 6 February.

Speaking at the launch, John Denham said 'This sector-wide commitment to skills will do much to enhance the UK's position as a leading centre for research and development. I am particularly pleased that the plan emphasises partnership between employers and education and training providers. This will be instrumental both in promoting the sector to young people and in equipping graduates with the high-level skills they need to contribute to the success of our bioscience industries.'

Sir Alan Jones, Chair of SEMTA: 'Collectively tackling skills priorities will bring about the step-change needed to safeguard a great future for the UK bioscience industry.'

The Bioscience Sector Skills Agreement is available at: www.semta.org.uk

The unit of absolute certainty

At one Physiological Society Dinner B Delisle Burns pointed out that few physiologists have given their names to apparatus or units. He contrasted this with eponymous surgeons who live on in perpetuity simply for adding an extra tooth to a pair of forceps. He then suggested some new, physiologist-based units, including the particularly apt 'Feldberg' as the unit of absolute certainty. Pedro Guertzenstein, one of Feldberg's co-workers, and I had tried to pinpoint precisely the area in the ventral brain stem involved in the maintenance of arterial blood pressure (see Feldberg & Guertzenstein, 1972). Various experiments allowed us to localise this area to the parvicellular part of the lateral reticular nucleus but we were cagey about its dimensions, using phrases like 'about' and 'not wider than' (Guertzenstein & Silver, 1974). Not so Feldberg who, in one of his Sherrington Lectures, firmly says 'not larger than 1.5 mm^2'.

Ann Silver

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A chemical engineering perspective of the gut

The flow patterns existing within the intestine are a function of wall motility and the flow properties of digesta, and are likely to affect the efficiency of nutrient uptake. Spatiotemporal mapping of intestinal motility and chemical engineering techniques are being used to evaluate the physiology of gastrointestinal mixing

The form and function of the gut are likely to be limited by the same fluid dynamic constraints that limit the efficiency of chemical reactors (Penry & Jumars, 1986). However, the interplay between contractile events in the wall of the intestine and the rheological properties of digesta and their influence on the efficiency of mixing and absorption are poorly understood. The use of quantitative techniques to assess mixing that are based on those developed by chemical engineers in conjunction with simultaneous longitudinal and radial high resolution spatiotemporal mapping are starting to unravel the complex dynamics of mixing in the living gut.

The efficient absorption of nutrients requires the movement of nutrients from the lumen to the interior of the enterocyte. Two processes contribute to this movement: radial mixing (i.e. forced convection) and the slower molecular diffusion (Edwards *et al.* 1988). Regardless of the absorptive efficiency of the enterocyte, the rate of uptake of a nutrient from the lumen will be compromised if the radial mixing is insufficient to maintain high nutrient concentrations adjacent to the enterocyte. Radial mixing is best achieved within the lumen of the gut by the establishment of turbulent flow. Nutrient transport may be slower within the mucus layer as it may be influenced by molecular diffusion, and hence, a nutrient concentration gradient will exist between the periphery of the lumen and the surface of the enterocyte.

Patrick Janssen (left) and Roger Lentle.

The pattern of flow of a liquid such as water through a simple tube is generally laminar at velocities similar to the average rate of flow through the gut. Laminar patterns of flow are characterised by a concentric series cylinders of fluid flow of increasing velocity from the wall to the centre of the lumen which travel in parallel and therefore are not conducive to efficient radial mixing. The disruption of this laminar pattern requires elements of the liquid to have sufficient kinetic energy, the level of which depends upon the relative magnitudes of the viscous and inertial forces exerted on it, i.e. the Reynolds number (Re). Hence, in laminar flow, Re is low and viscous forces predominate. When Re is high, inertial forces dominate and elements of fluid will have sufficient kinetic energy to form turbulent eddies each of widely differing length and duration.

While it is possible to predict the extent of turbulence and radial mixing of material in simple tubular conduits using the rheometric characteristics of digesta, the modelling of flow in the intestine is rendered complex by its gross and microscopic morphologies, and the timing and form of the various types of contractile activity. However, the type of flow regime that prevails in a reactor system can be inferred from the concentration profile of a pulse of dye marker after it has traversed that system. We applied this technique to measure the extent of mixing in living isolated segments of small intestine (Janssen *et al.* 2007).

Active segments perfused with low viscosity solutions displayed a pattern of marker distribution characteristic of strong turbulence with chaotic vortices of widely different lengths and durations (Fig. 1). This indicated that efficient radial mixing could be readily established; a finding that differed from that predicted by modelling the flow of a watery liquid through a simple conduit. When segments were perfused with a guar gum solution with similar flow properties to those of distal small intestinal digesta i.e. a

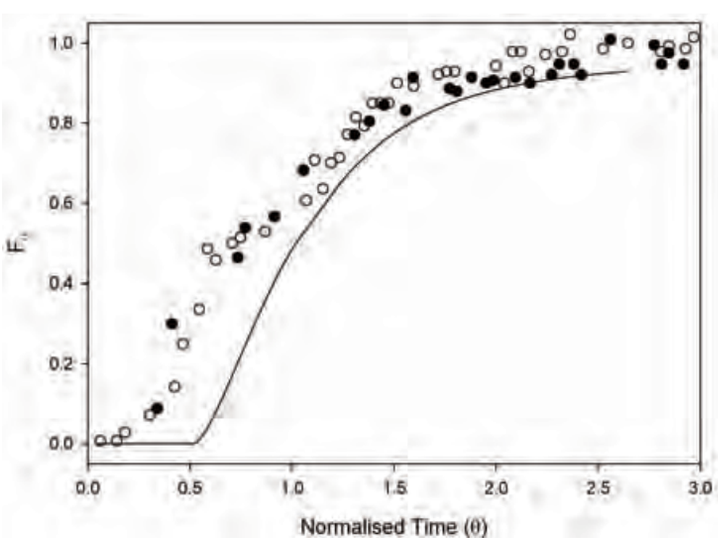

Figure 1. Changes in dye marker concentration when a low viscosity solution is perfused through segments of possum ileum. The graph shows two replicates (○, •) from active ileal segments and one from an inactive segment (solid line). The x-axis represents time scaled by the average residence time and the y-axis represents the probabilty of fluid having that residence time.

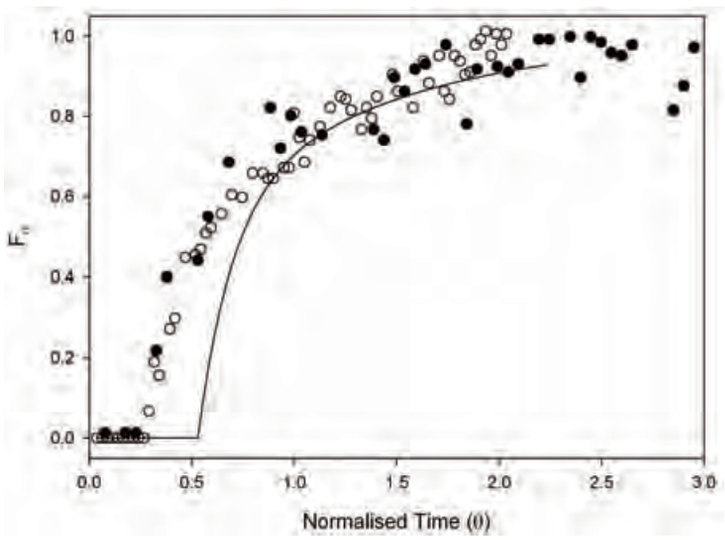

Figure 2. Changes in dye marker concentration when a high viscosity guar gum solution is perfused through segments of possum ileum. The graph shows two replicates (o, •) from active ileal segments and one from an inactive segment (solid line).

high apparent viscosity that is reduced by shear (pseudoplasticity), the marker distribution indicated a laminar mixing regime (Fig. 2). This laminar mixing regime was less effective than turbulence at generating mixing in both longitudinal and radial directions. Hence, while radial mixing is widespread in watery digesta such as may enter the proximal gut, it is less evident in more viscous pseudoplastic digesta such as is found in the more distal small intestine, hence the latter may require longer transit time to enable adequate nutrient absorption to occur.

This work indicates the importance of motility in determining the flow regime and efficiency of nutrient uptake in the intestine. The study of motility in isolated sections of intestine has been furthered by the development of a spatiotemporal mapping technique that condenses circular muscle motility from a sequence of images onto a 2-dimensional map (Hennig *et al.* 1999). Our group has developed a mapping technique based on cross-correlating sequential images that also allows the visualisation of longitudinal muscle motility (Lentle *et al.* 2007b). These techniques were used to show that the turbulence obtained with low viscosity perfusates was due to highly oscillatory flow within the segment. While the average velocity of flow in the segment was low, contractile activity was sufficient to locally increase flow rates and fluid inertia to a level that brought about widespread turbulence. The relative magnitude, the phase relationship and the interaction between patterns on the circular and longitudinal maps has been used to characterise differing contractile patterns in the proximal colon of the rabbit and to infer separate levels of organisation of interstitial cells of Cajal (Lentle *et al.* 2008) (Table 1).

Further work is under way using dye markers to characterise the mixing initiated by these differing contractile activities.

Aside from providing a greater understanding of the physiology of gastrointestinal transit and mixing, these findings have significant implications for nutritionists and pharmacologists. A detailed understanding of the effects of the rheometric properties of food ingredients on the efficiency of mixing and absorption will aid in the development foods that are designed to impair or promote the absorption of macronutrients, or in the targeting of pharmaceutical agents at the distal gut mucosa. Moreover, they afford sensitive *ex-vivo* models that are convenient for determining the effects of pharmaceuticals that are known to influence motility and mixing.

Table 1. Characteristics of motility patterns in the haustrated rabbit colon

Motility Activity	Propagation Direction	Propagation Speed (mm/s)	Hexamethonium sensitive	Associated ICC
Haustral progression	Aborad	0.13 ± 0.02	Yes	Submucosal
Ripple contractions	Mainly orad	1.4-8.5	No	Submucosal
Mass peristalsis	Aborad	8-21	Yes	Intramuscular
Fast phasic	Mainly aborad	30 ± 6	No	Auerbach's plexus

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Force-frequency relation and myofilament Ca^{2+} sensitivity

In the failing heart alterations in Ca^{2+} sensitivity of the myofilaments contribute to the decrease in pump function when heart rate increases, for instance during exercise. An altered balance between kinase and phosphatase activity, which influences phosphorylation and thereby function of the myofilament protein troponin I appears to be responsible

In a number of mammalian species, including man, an increase in heart rate results in an enhanced pump function of the heart. The positive pressure-frequency relationship was first described by Bowditch in the isolated frog heart (Bowditch, 1871) and is also known as the *Treppe* or *staircase* phenomenon. In the failing heart, where pump function is impaired, a blunted or negative pressure-frequency or force-frequency relation is observed (Gwathmey *et al.* 1990). Fig. 1 illustrates a positive and a negative Treppe in isolated healthy and failing rat cardiac muscles, respectively. The negative force-frequency relation will markedly impede the exercise capacity of the heart, one of the defining symptoms of patients with heart failure.

The positive force-frequency relation has been attributed to an increased influx of Ca^{2+} ions per unit of time in myocardial cells. This results in an increase in Ca^{2+} content of the sarcoplasmic reticulum and thereby in an increase in the amplitude of the intracellular Ca^{2+} transient during contraction. During heart failure the intracellular Ca^{2+} content is reduced, in a frequency-dependent manner by alterations in the Ca^{2+}- and Na^{+}-ion homeostasis.

However, not only the amount of Ca^{2+}, but also the sensitivity of the contractile myofilaments for Ca^{2+} determines the magnitude of force developed by the cardiomyocyte. Myofilament Ca^{2+} sensitivity is modulated by the phosphorylation levels of the regulatory thin and thick myofilament proteins, troponin I (TnI) and myosin light chain 2 (MLC2) (e.g. van der Velden *et al.* 2003). Beta-adrenergic stimulation during exercise accelerates heart rate, but also activates the classical second messenger cAMP dependent

Ger Stienen (left), Regis Lamberts and Jolanda van der Velden.

pathway, resulting in activation of protein kinase A (PKA). PKA phosphorylates, amongst others, serines 23 and 24 of the inhibitory subunit of the troponin complex TnI. This addition of phosphate groups, i.e. negative charge, causes a reduction in Ca^{2+} sensitivity of the myofilaments and thus a reduction in peak force during contraction (systole). Phosphorylation of TnI is also implicated in an acceleration of cellular relaxation and thereby promotes filling of the ventricles with blood during the resting (diastolic) phase of the cycle. Intriguingly, there have been some reports suggesting that an increase in pacing frequency is accompanied by an increase in myofilament Ca^{2+}-sensitivity.

During heart failure Ca^{2+} sensitivity was found to be increased in human (van der Velden *et al* 2003) and rat myocardium (Lamberts *et al.* 2007), though this is not an universal finding. The increased Ca^{2+} sensitivity in heart failure has been attributed to a reduced PKA-mediated phosphorylation of TnI as a result of downregulation and/or desensitisation of the β-adrenergic receptors. This could modulate a putative effect of contractile protein phosphorylation on Ca^{2+} sensitivity of the myofilaments.

Therefore we addressed the following question: Do alterations in Ca^{2+} sensitivity, by a change in phosphorylation of the myofilament contractile proteins, contribute to the force-frequency relation in healthy hearts and is this also the case in failing hearts?

The first clues were obtained from force and intracellular Ca^{2+} transient measurements in rat cardiac trabeculae. These results revealed a parallel increase in force and amplitude of the Ca^{2+} transient in healthy control rats, but a decline in force in trabeculae from failing

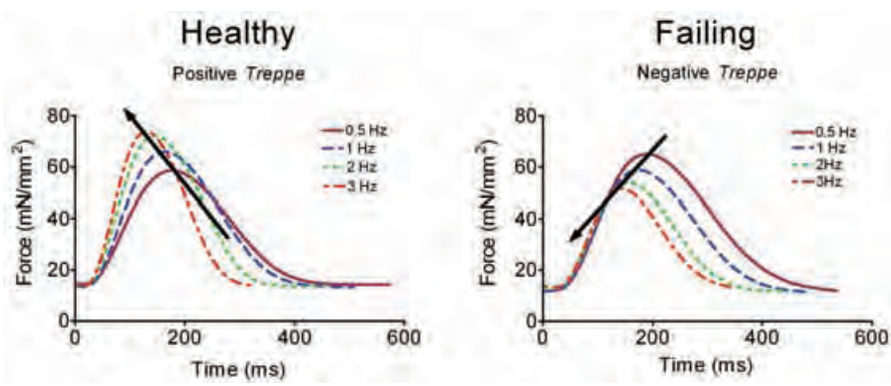

Figure 1. An example of a positive force-frequency relation (positive *Treppe*) found in right ventricular trabeculae from healthy rats, and of a negative force-frequency relation (negative *Treppe*) from cardiac trabeculae from monocrotaline treated failing rats (27°C, 1 mM external Ca^{2+}).

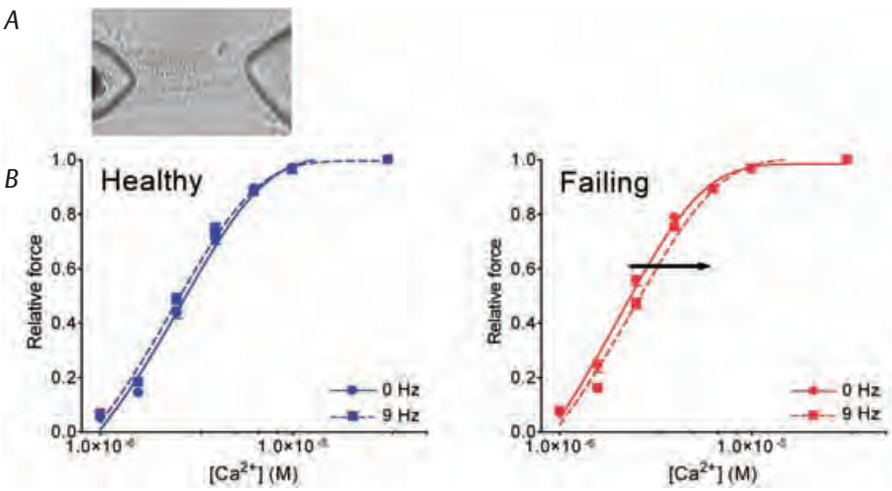

Figure 2. Single permeabilised cardiomyocytes, *A*, from healthy and failing quiescent (0 Hz) or stimulated (9 Hz) hearts, isometric force-Ca^{2+} relations, *B*, were studied. In the failing cardiomyocytes the force-Ca^{2+} curve shifted significantly to the right with an increase in frequency, indicating a decreased Ca^{2+} sensitivity with frequency. In healthy cardiomyocytes there was no significant change in the force-Ca^{2+} relation.

hearts without a change in the amplitude of the Ca^{2+} transient. This suggested that changes in myofilament Ca^{2+} sensitivity have occurred.

Subsequently, we studied myofilament Ca^{2+} sensitivity in permeabilised cardiomyocytes, isolated from quiescent and electrically paced hearts (9 Hz) perfused in a Langendorff set-up (Fig. 2). In the control cells, the force-Ca^{2+} relations were practically identical, indicating that the Ca^{2+} sensitivity of the contractile apparatus remained unaltered. In cardiomyocytes from failing rat hearts, the force-Ca^{2+} relation shifted to the right, indicating a decrease in Ca^{2+} sensitivity with pacing frequency. Determination of the phosphorylation levels of TnI and MLC2 by one and two dimensional gel electrophoresis revealed that the phosphorylation levels of both proteins remained constant in healthy hearts but that both

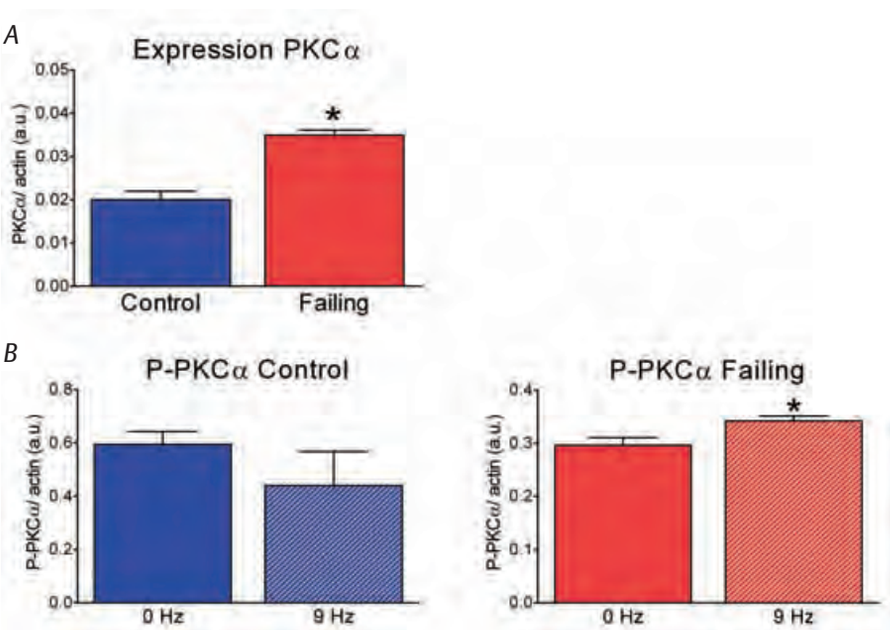

Figure 3. *A*, Increased expression of PKCα in failing hearts compared to control. *B*, Phosphorylation of PKCα is frequency-dependent in failing hearts, but not in control. Abbreviations used: PKCα = protein kinase C alpha, P-PKCα = phosphorylated PKCα.

increased with pacing frequency in failing hearts (Lamberts *et al.* 2007). Thus, in the healthy heart, the increase in Ca^{2+} influx per unit of time is primarily responsible for the positive force-frequency relation. However, in the failing heart, frequency-dependent Ca^{2+} desensitisation of the myofilaments together with alterations in Ca^{2+} homeostasis result in a negative force-frequency relation. This frequency-dependent Ca^{2+} desensitisation can be attributed to a frequency-dependent increase in phosphorylation of the actin-myofilament protein TnI, because MLC2 phosphorylation would have the opposite effect.

The next question is: how does stimulation frequency influence TnI phosphorylation? The answer to this question should reside in an altered balance of kinase or phosphatase activity. In particular, the Ca^{2+}-dependent kinases or phosphatases might be involved because the time-averaged intracellular Ca^{2+} concentration still increases with pacing frequency in both groups. Hence the main candidates would be the classical Ca^{2+}-dependent isoforms of protein kinase C (PKC) and the Ca^{2+}-dependent phosphatase calcineurin (PP2B). PKCα and PP2B are up regulated in heart failure (Braz *et al.* 2004) and thus their contributions may be more conspicuous in failing than in healthy hearts. In agreement with these findings, we recently showed an increased protein expression of one of the Ca^{2+} dependent isoform, PKCα, in failing rat hearts. In addition, a modest but significant increase in the phosphorylation level of PKCa with frequency in the failing hearts was found (Fig. 3) (Lamberts *et al.* 2007).

In summary, in the failing heart alterations in Ca^{2+} sensitivity of the myofilaments contribute to its negative force-frequency relation. The frequency-dependent changes in phosphorylation levels of PKC-phosphorylatable sites of TnI originate from an altered balance in

kinase and phosphatase activity, once more indicating the importance of kinase/phosphatase activity in regulation of cardiac function.

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Launch of Cardiff Neurosciences Centre

On 25 January 2008, Cardiff University (CU) held an internal interdisciplinary neuroscience conference, *New opportunities for neuroscience at Cardiff* , to celebrate the launch of Cardiff's Neurosciences Centre (CNC). Over 80 basic and clinical neuroscientists from across the different schools of CU attended this inaugural event. The participants received 16 presentations in four themed sessions entitled *Neuroscience imaging and signal processing*; *Functional genetics and gene| environ - ment interplay*; *Neural systems, behaviour and cognition*; *Opportunities for the neuroscientists of the future*. The presentations provided an overview of the range of neuroscience research taking place at CU, the research techniques available, the opportunities for collaborative neuroscience research and updates concerning current and future neuroscience events.

A notable highlight of the day was John Aggleton's presentation detailing the recent £4m funding from the Wellcome Trust secured by CNC scientists from the School of Biosciences, Medicine and Psychology to establish a 4 year PhD programme in integrative neuroscience. This award covers a 6 year period, and will fund a total of 30 students, with the first intake of five students in October.

The meeting underlined the breadth, as well as the strength, of neurosciences in Cardiff, with attendees coming from a wide range of disciplines (Schools of Biosciences, Psychology, Medicine, Optometry, Pharmacy, Chemistry, Engineering, Social Sciences and English). As with most such meetings, the opportunities for informal interaction were as highly valued as the formal sessions, and by the end of the day several new interdisciplinary groups were in the early stages of being formed. A number of participants commented that Cardiff neurosciences seems to have reached a critical point in its development and seems poised to expand and move forward into new interdisciplinary areas.

The director of CNC for the first year is Mike Owen from the School of Medicine. In his words 'This is an exciting time for neuroscience research at Cardiff, and we are grateful for the support of the Vice Chancellor in establishing this initiative. There are tremendous strengths in neuroscience in Cardiff, but we are convinced that

Cardiff Neurosciences Centre Steering Group – John Aggleton (left) with Kevin Fox and Director Mike Owen. For abstracts and presentations from the launch and further information visit http://www.cardiff.ac.uk/cnc or contact Vanessa Davies at cnc@cardiff.ac.uk.

we can do even better if we develop more cross-school and interdisciplinary links. A major goal of our centre will be to encourage the translation of basic research into improved physical and mental health, and we are well placed to do this following the merger of CU with University of Wales College of Medicine'.

CNC is a virtual centre aimed at bringing together neuroscience expertise from across the various schools of CU, and also the Cardiff and Vale NHS trust. There is a wide range of neuroscience interests in Cardiff, including both basic and clinical research. Currently, over 130 senior researchers are active members of CNC, with areas of interest covering molecular and cellular neuroscience, neuronal development, neurophysiol- ogy, synaptic plasticity and learning and memory, neurodegeneration, neuroimaging, psychiatric genetics, behavioural neuroscience, visual neuroscience, cognitive neuroscience and perception. The activities organised bythe CNC (seminars, conferences, workshops, special interest meetings, newsletters and identification of grant opportunities) will encourage more networking, create new channels of communica- tion, facilitate new ideas, help secure more large multi-disciplinary grant funding, and most of all promote interdisciplinary research and teaching.

Vanessa Davies
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Feeding signals to the hungry mind

Obesity and its related disorders such as type II diabetes, hypertension and heart disease are amongst the most immediate and challenging of today's health-care concerns. In the UK, the average body mass index (BMI) is now 27, the healthy range being 18-25, and nearly a quarter of all adults are classed as 'obese'. Most worrying is the dramatic manner in which these measures have worsened over the last 10-15 years, especially amongst our children – the UK now has the largest increase in childhood obesity in Europe, bringing it on a par with the USA.

Against this backdrop it was therefore timely that the topic of this year's GL Brown Prize Lecture was how the brain controls body energy homeostasis and the factors that lead to an imbalance between energy intake and expenditure. The prize was awarded to Nina Balthasar of Bristol University whose lecture Feeding signals to the hungry mind received its first airing at Cardiff University, School of Biosciences on 6 December 2007. Around 100, mainly young, people packed into the Seminar Room of the New Life Sciences Building to hear the lecture.

Nina commenced by reiterating the stark facts and then proceeded to explain the central role played by the brain and its prominent two way connection with several peripheral tissues, including the gut, in controlling body weight. She then went onto present some key results from her own research which involves using genetic techniques combined with systems level phenotypic analyses to probe the neural circuits that regulate food intake and energy expenditure.

She first focused on leptin receptors (lepRs) and explained how a specific deletion of these receptors from POMC and/or AgRP neurons in the hypothalamus leads to mild obesity. This indicates that whilst lepRs on these neurons are clearly involved in

G L Brown Prize lecturer Nina Balthasar.

leptin's regulation of body weight homeostasis, they are not solely responsible. This suggests that other leptin targets that reside in other areas of the hypothalamus, but also outside the hypothalamus, are also important.

Her attention then switched to melanocortin 4 receptors (MC4Rs). A detailed understanding of MC4Rs is critical to tackling the obesity problem because whilst deficiencies in leptin signalling can be addressed with recombinant leptin, there are no treatments for similar problems with melanocortin signalling. MC4Rs are present in many areas of the CNS, any of which might be involved in melanocortin's regulation of energy homeostasis. However, because lesions of the paraventricular hypothalamus (PVH), an area rich in MC4Rs, cause serious obesity this has been considered as a key area where melanocortin may act in this capacity. Nina showed that obesity in mice lacking MC4Rs can be partially reversed by specifically re-expressing MC4Rs in the PVH. Interestingly, this reversal seems to be due to a complete rescue to normal food intake, however reduced energy expenditure is unaffected. An important future issue is therefore identifying the location of the MC4Rs that control energy expenditure.

The lecture generated a lively discussion. Issues raised ranged from not losing sight of the role of mitochondria in energy expenditure to the use of inducible knockouts (KO) as a desirable approach to answering some of the important outstanding questions, particularly which effects are developmental versus effects in the adult. With regard to the use of inducible KOs, Nina was quick to agree but also pointed out that the use of either doxycycline or tamoxifen were anathema to anyone working in endocrinology. That brought to a close a thoroughly enjoyable lecture and one which for both scientific and health-related reasons is likely to stick in the memory.

Stuart Hughes
University of Cardiff, UK

The GL Brown Prize Lecture was instituted in 1975 in memory of Professor Sir George Lindor Brown and is a peripatetic lecture aimed at a younger audience in order to stimulate an interest in physiology. Nina Balthasar's lecture is likely to be published in *Experimental Physiology* early in 2009.

Sir George Lindor Brown, 1903-1971 (*photo coutesy of Godfrey Argent Studio*)

Research Defence Society

The *Winning the debate* symposium, organised by the Research Defence Society (RDS) on 21 February, was designed to bring together representatives of all communities working in, or supporting, animal research. It was a well-attended and engaging meeting, held at the London Wellcome Collection to

highlight the vast progress made in the communication of research in the past 20 years and where it can move forward in the future. Research scientists from academia and industry, animal technicians, journalists, science communicators, politicians and representatives from charitable organisations including medical charities and the RSPCA came together to discuss the changing public opinions of science. The increased public support is a result of better communication, especially since the introduction of new legislation on domestic extremism in 2004, which has given scientists the protection to speak out. The symposium was structured with six invited speakers, each followed by a questions and discussion session with the audience. The meeting aimed to highlight the best ways groups could be involved in promoting the message about the positive reasons to use animals in research, whilst still ensuring improving and refining experiments for the welfare of animals.

The animal research issue in the UK has been a very controversial topic for a long time. Because of the climate enforced by animal rights extremists in the past 20 years, a closed door approach to scientific research resulted in public mistrust of the scientific community. Lack of openness gave the impression that there was something to hide. This provided an easy platform for extremists to obtain public support. Seeing propaganda about animal research displayed in every high street, videos about the 'cruelty' of vivisection sent to schools, and very little counteracting debate from the scientists for fear of attacks meant public opinion could be tarred by the misinformed anti-vivisection message. Many scientists, including Dominic Wells (Imperial College, London) and Colin Blakemore, (Chief Executive of the MRC), who gave talks at the symposium, were often at the negative receiving end of animal rights campaigns but helped to pioneer the change in the attitudes towards science and research by standing up for their work and highlighting the medical progress made through it. Simon Festing (Director, RDS) supported this in his comment 'get the message out with good science'. The results that lead to medical progress justify the research involved. Colin Blakemore, Dominic Wells and Simon Festing all stated the need to give a balanced, accurate account and allow people to make up their own minds. Festing highlighted the Government pledge to support research and those involved in It and the responsibility of researchers to educate the public and gain their support.

Assistant Chief Constable Anton Setchel (National Co-ordinator for Domestic Extremism) talked about the successful changes in law and policing initiated in 2004 to protect researchers from extremist activities. He described the successful reduction in extremist activity since its introduction and stated that it is now safe to speak out and be open about research.

Animal rights extremist tactics have altered in the past decade and they have moved from random and indiscriminate attacks to targeted campaigns on individual groups, often not only the animal researchers themselves. Two key victories for the extremists had to be the cancellation of the new Cambridge Neuroscience Centre and the closure of the guinea pig breeding farm after they dug up the remains of a relative of a family who bred guinea pigs for research. Festing mentioned the negative impact such attacks had on the extremists' campaign, going too far in the public's view. Blakemore highlighted a massive sign of the change in public opinion to research and a step towards 'winning the debate' - a pro-research tabloid headline about the loss of medical progress as a result of the halting of Oxford laboratory building works in response to extremist pressures. These changes reflect the hard work and effort by a lot of scientists to communicate and justify their work and it has been worth it.

Wells spoke of how and to whom we should be talking about our research. Large amounts of science are public-funded and so it is our duty to let the public know the good we do. His key points requiring action were to:
1) improve education of the public (a number of people in the audience thought schools were the best place to target);
2) innovate and refine research protocols to keep research and welfare standards high, maintaining public trust; and
3) educate the future generation of biomedical researchers. In communicating science to the public we must discuss and justify the research, highlight both the pros and the cons, be proactive and get good stories out there.

Mark Henderson (Science Editor, *Times*) gave the media position on reporting research - the stories they print must sell papers and it is up to scientists to pitch them at a favourable angle to give journalists something to use. The press can provide a useful outreach to the public by writing research success stories whilst highlighting the use of animal research somewhere in the story.

As well as scientists, other groups have an active involvement with animal research. Medical research charities fund work that uses animals yet they do not always use their status in society to support it. The medical charity's role in reinforcing their support of the work they fund was covered by Betty McBride (Policy and Communications Director, British Heart Foundation [BHF]). She highlighted ways in which the BHF has taken steps to acknowledge their support of some critical work involving animals. She encouraged other charities to do the same. At the moment, the BHF is one of the only medical research charities adopting this 'open' approach. As for what the 'open' approach is, the BHF is 'transparent' about their support of research; they state it in their leaflets, have a press statement and train staff about it, but they do not 'shout about it from the

rooftops'. Charities are in a difficult position as they rely on donors for their success. Holding their head up and stating support for this controversial topic has the potential to reduce supporter numbers. However, Betty McBride feels that honesty from the start is less damaging than later exposure by extremist groups through negative press coverage. A representative from Sparks (Sport Aiding Medical Research for Kids) said that they had lost two nominations for awards because they acknowledged that they support some research involving the use of animals. If awarding bodies have this attitude very few, if any, medical research charities would qualify.

Ulltimately, the concensus of the meeting was that research and its' communication and place in the public eye has increased greatly; however, still more needs to be done. Not all scientists are willing or happy to communicate because of the haunting past, but it is a necessity now and also the best time ever in terms of security and public support. As in Wells' view, if we cannot stand up for our research and defend its importance then should we be doing it at all?

Fiona Randall

Oxford Pro-Test Rally

Laurie Pycroft and Tipu Aziz (top, right) lead several hundred marchers in Oxford, including Society Members, in support of animal research in science in February.

Evan Harris MP (centre, right) spoke in strong support and was unfazed by the one vocal animal rights activist who turned up (centre, left) – and was swiftly dealt with by the police presence.

The timing of the march was important, as some Oxford bioscientists have felt nervous about the possible animal rights response to the imminent opening of their new laboratory.

Liz Bell

(photos by Liz Bell)

MRC showcases latest breakthroughs in neuroscience and mental health

MRC Medical Research Council Leading science for better health

In these days of scarce funding for research, particularly by Government agencies, it is interesting to know who is getting the money and what they are doing with it. Periodically, the Medical Research Council (MRC) holds showcase events that highlight different aspects of its research funding portfolio. Neuroscience was the focus of the latest event, held in Bristol in February. Over the 2 days, formal presentations from 13 invited MRC-funded speakers were interspersed with poster sessions. More than 100 posters of MRC funded work were also on display and, importantly, plenty of time was allowed for discussion and networking during the poster sessions.

The showcase revolved around the themes of neuroimaging, neurodegeneration targets in disease and neurobiology. Neuroimaging is developing into a powerful research and diagnostic tool. Ed Bullmore Cambridge) described its potential for development as a patient screen to predict outcomes of drug treatment or susceptibility to psychiatric disease, whilst Adrian Owen (Cambridge) showed how real time fMRI might be used as a behavioural 'proxy' which could be more quantitative than cognitive assessment in diagnosis. Paul Grasby (Imperial) described how PET scanning might be used to reveal differences in neurotransmitter binding characteristics in normal and diseased states.

Moving the focus to disease and proteomics, Sarah Tabrizi (UCL) considered transmissible spongiform encephalopathies, showing how protein misfolding may occur and how protease catalytic function is inhibited by prions. An anti-protein folding approach to treatment of neuromuscular disorders was also highlighted by Mike Hanna (UCL), who showed how mutations of voltage gated ion channels could lead to hyperkalaemic paralysis in dystrophic patients. Nigel Hooper (Leeds) then described how prion protein can inhibit proteolytic processing by amyloid precursor protein, leading to the fascinating concept of using prion protein to prevent the development of Alzheimer's disease.

In the targets for disease session Pierluigi Nicotera (Leicester) talked about differential sensitivity of dendritic compared to somatic mitochondria with respect to the toxic effects of Ca^{++} efflux and influx in excitotoxic cell death and showed how localised synaptic damage can be the trigger for initiating widespread degenerative changes. The molecular characteristics of the role of mitochondrial protease in cell death and disease was described by Miguel Martins (Leicester) who showed how an accumulation of unfolded proteins may contribute to the degenerative state in Parkinson's disease. Mental gymnastics were then needed to focus on Matt Jones' (Bristol) interesting presentation on the application of coherence analysis to multiunit recording at up to 120 sites in the brain during behavioural testing to probe decision making and other aspects of cognitive function using conscious rats as disease models of schizophrenia and Down's syndrome.

The final session on neurobiology covered an equally broad spectrum from the behaviour of human infants to genetic screening in invertebrates. Maria Fitzgerald (UCL) described how the results of her elegant studies on mechanisms underlying pain processing in neonatal rats have been translated into the clinical setting, where they are being used to improve pain management in paediatric medicine. Thelma Lovick's (Birmingham) work on neural processing in the female brain showed how natural cyclical changes in female steroid hormone levels induce upregulation of $GABA_A$ receptor subunit gene expression in brain circuits known to be associated with anxiety-like behaviour. She showed how these findings translate to oestrous cycle-linked changes in the behaviour of the animal that may be relevant to the development of premenstrual dysphorias in women. Changing tack completely, Michael Hastings (Cambridge) described the use of molecular genetic analysis of transcriptional feedback loops in understanding the role of cellular pacemakers. Stephen Nurrish (UCL) closed the session by showing how behaviour of *C. elegans*, with its limited gene pool, can be used as a simple animal model to screen genes that control the role of diacyl glycerol in regulation of priming the loading of the synaptic vesicle.

Hugh Gurling (UCL) pitches an idea to Industry.

What are the MRC's priorities for neuroscience in the future? Chris Watkins from the MRC's Research Management Group pointed to translational research: the 'bidirectional transfer of knowledge between basic work with that of the person', an area in which funding is soon set to double. He also emphasized the need for *in vivo* skills, the erosion of which has been lamented for a decade or more, not least by the pharmaceutical industry. The MRC has just established a £3million Pilot Industry Collaboration Award Scheme. At the showcase event representatives from industry were given the opportunity to describe what they look for in a collaboration and academic hopefuls looking for industrial collaborators got the chance to pitch their ideas. What does industry want? The message was loud and clear. They are looking for innovation and insight and are interested in inventive, cutting edge work that they can turn into new medicines that make them lots of money. They want new techniques, particularly new animal models that are good predictors of human disease. And they also want short term use of techniques that are already up and running in academic research labs. However, full economic costing is pricing UK universities out of the market and much work is already being outsourced to countries such as India and China.

There is no doubt that the range of expertise and innovation in MRC-funded neuroscience research in the UK at the present time is impressive. But the MRC, like other major funding agencies, has been able to support around only 10% of the proposals it receives, which means that for a lot of the time a significant proportion of our best research brains are focused on chasing money rather than chasing answers. I left this meeting very excited about UK neuroscience, but at the same time I couldn't help thinking what might have been if the financial climate had been even a few degrees warmer.

Thelma Lovick

Undergraduate membership of The Society

In order to support the next generation of physiologists, and encourage life-long participation in the activities of The Society, last year we introduced a new membership category for undergraduates. Following the successful pilot in 2007, we are pleased to introduce 77 undergraduate members and the formation of four undergraduate societies. Whilst individual membership allows undergraduates to benefit from full access to the new web site, networking opportunities and eligibility to apply for grants, we encourage students to join as a group and set up their own undergraduate society.

Establishing an undergraduate society enables each member to benefit from a £5 discount – making the fee just £10 per member. In addition, societies are eligible to apply for funding to support their activities, such as those reported by the societies at Oxford and Huddersfield Universities on the Education pages in this issue.

Existing benefits of undergraduate membership are outlined below, and we hope to build on these in 2008.

Students are responsible for their own society, coordinating their application and subsequent activities, but should be overseen by a Member or Affiliate of The Society.

Becoming a member

If you are an undergraduate student and are interested in joining, or are a current Member and are keen to encourage your students to do so, the following steps will guide you through the process of making and submitting an application.

- If you are a student, identify a Member of The Physiological Society in your department who is willing to provide you with support and guidance. If you are a Member, identify a student who is willing to co-ordinate an application for membership and encourage fellow students to join;
- Identify all students who study physiology as part of their degree course (remember this spans many disciplines including medicine, biochemistry, pharmacology etc.) and invite them to an informal meeting;
- Each prospective member should download and complete an application form from The Physiological Society web site;
- The student co-ordinating the application should gather the completed applications and payment together, and pass to The Society representative who should verify the applications and submit;
- Completed applications for membership should be submitted by The Physiological Society representative. If your university does not have a representative, please contact us.

If you would like further advice on how to become an undergraduate member or on how to establish a Society, please contact me at imagre@physoc.org.

Irrum Magre

Benefits of joining The Physiological Society as an undergraduate member

- Hard copy and online access to Physiology News;
- Funding for seminar schemes;
- Vacation Studentships – eligibility to apply for up to £1200 to support you whilst working in a research lab over the summer holidays;
- Eligibility for undergraduate prizes in physiology;
- Careers conferences/advice;
- Opportunity to write for Physiology News;
- Discounted meeting attendance.

Water world

Call it a Sherlock Holmes pulling a James Bond trick or compare it to a Quixotic charge on the wind mills, physiologists past their prime often love to resurface and try a few potshots at the younger generation. Some of them in high spirits may even describe them as upstarts. Fortunately, learned societies and journals recognize the needs of these senior citizens and provide opportunities in the form of state-of-the-art lectures, over and above views, perspectives and prefatory chapters. I often wondered how the sleuth from Baker Street would have reacted to 007's girls, gadgets and gimmicks! But I am sure he would have relied on his powers of deduction and unique reasoning, which have endeared him to everyone. Reading about water transport in the recent issue of *Physiology News* (Zeuthen, 2007), I could not resist the urge to take a plunge. Before testing the waters, I thought I should read A to Z on the subject. (I mean from Agre's pores to Zeuthen's oocytes) However, I found that the role of aquaporins in the gut is yet to be firmly established. Hence I reversed the gear and started from the rear. Through ingenious techniques, Zeuthen's group could pick up the flow of water along with glucose as the sugar enters through the sodium glucose co-transporter that was expressed in the oocyte. Of course, the water gets in, swells it up and that's what they measured in the first place! But the water that enters the enterocytes in this manner during the process of intestinal absorption needs to get out through the basolateral membrane to gain access to the circulation. Zeuthen (2007) speculates the presence of other cotransporters in this part of the cell membrane, which may facilitate the exit. Does the metabolism play any role in water transport? Work on everted gut sacs has shown that stepping up their metabolism either by raising the phosphate concentration of incubating medium or by enclosing a little noradrenaline in the sac, leads to an increase in water transport. When glucose is included in the phosphate rich media, water transport increased even more in the proximal intestine, accompanied by an increased release of lactic acid. Glucose uptake by the sacs in high and low phosphate did not differ. Further, inhibitors of glycolysis, prevented the action of noradrenaline.(Mary & Rao,1989; 1991) Therefore glucose metabolism does seem to enhance the water transport. How does it do it? Larsen, the strong proponent of para cellular route of water transport, believes (Larsen 2002) that the sodium pump elevates the concentration of the cation in the lateral space between the enterocytes drawing in more water. Zeuthen advocating a transcellular route hypothesizes operation of channels in the basolateral membrane, some of which may require energy directly or indirectly (Zeuthen2007). Metabolism can thus aid these processes and increase water transport.

Will increased glucose metabolism promote uptake of glucose and water at the apical membrane? We could not demonstrate an increase in glucose uptake under these conditions in the everted sacs. Therefore it is unlikely that enhanced water transport has occurred through this transcellular route. However, we can not rule out the passage of water through other transporters in the apical membrane that have been switched on by glucose metabolism. Glucose entry itself may trigger the operation of NHE (Turner & Black, 2001) which may be further facilitated by the protons generated by metabolism (Thwaites *et al*. 1999). But does it allow water to go through it? It may or nay, of course.

J Prakasa Rao
Department of Physiology, Kasturba Medical College, Manipal, India

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Jogging

Whilst many may think David Miller's argument (*Physiology News* **70**, 53) is biased towards the car (we are invited by him to be surprised), the truth may be even more unbelievable (see http://www.timesonline.co.uk/tol/news/uk/science/article2195538.ece).

The crux seems to be the greenhouse gas production involved in supplying fuels to cars and people. Petrol production involves much lower greenhouse gas production than the dairy/beef industry – but I don't know whether I believe that research ...

Of course, one can add an almost infinite number of elements into the calculation in an attempt to show that one transport is better than another. Cold engines on short trips are inefficient, pollution and lack of exercise lead to more illnesses – but that also leads to the CO_2 benefit of shorter lives!

Christof J Schwiening
Department of Physiology, University of Cambridge, UK

Sixth Form Workshops
The Physiological Society has funds available to run interactive workshops for 6th form students with an interest in biology. The workshops provide students, who will soon be considering their degree and career options, with an opportunity to try experiments similar to those that they may run as part of an undergraduate course. In addition, as these events are usually held in university departments, the sessions provide an insight into university life. These events have proven to be a powerful form of advertising for both physiology and the participating university. If you would be interested in running an event of this kind, or have any ideas for future workshops, contact Chrissy Stokes (cstokes@physoc.org).

Chrissy Stokes

This issue of *Physiology News* provides me with an excellent opportunity to introduce myself as the new Head of Education and Membership Services, replacing Donna Brown.

Studying for a PhD in physiology at Bristol University, I was first introduced to The Society as an Affiliate Member and later became co-organiser of a Young Physiologists' Symposium. I thoroughly enjoyed the organisational aspect of this experience, and decided to pursue a career in scientific communication following completion of my studies – first as an assistant editor at BioMed Central and later as a science programme officer in the physiological sciences funding stream at the Wellcome Trust.

Since joining The Society in December, I have had the opportunity to draw on my experiences to promote physiology, related career opportunities and the activities of The Society. One of the main areas I am keen to focus attention on in 2008 is our Undergraduate Associate membership category, and we are currently working to develop the benefits for these Members. Undergraduates are the scientists of the future and The Society recognizes the importance of encouraging and supporting them.

Several undergraduate societies have already been established at institutes throughout the UK, and some of their recent activities are outlined in this section. If you are interested in establishing an undergraduate society, or would like to encourage your students to do so, read the details on p. 46.

Christabel Stokes

Researchers in Residence

Walking up that familiar long, sloping hill to Longdendale Community Language College I was transported back 15 years to my old school days. However, this time I wasn't there as a student but as an ambassador for Researchers in Residence, a scheme that places PhD and postdoctoral researchers in secondary schools with the aim of making science more relevant and exciting for young people.

As I turned the corner and saw the building I was filled with apprehension. Would my old teachers see me as a scientist or as the same naïve school girl I once was? Could I, a second year PhD student, really do science justice? All of those fears faded away when I was greeted by the familiar beaming smile of Brian Parker (no relation!), the Head Teacher. Within minutes I was taken to the staff room and reacquainted with my old teachers and introduced to new faces, one of whom was Ali Abubakar, Head of Science.

Ali and I decided that, for the week I was there, I would attend science lessons and give each class a presentation. I had prepared a presentation in advance and began with two questions:

- What did you expect me, a scientist, to look like?
- Do you use science in your everyday life?

The answers were consistent:

- Male with grey, curly hair and glasses, wearing a white lab coat with lots of pens in the top pocket and carrying test tubes with bubbling, coloured liquid in! I know we can all think of a colleague that fits that description but, in my experience, it does not describe the general scientist cohort.
- At first the answer was no. But with a little prompting it soon became apparent that they used science all the time without realising it, from the shampoo they use to wash their hair to the mp3 players and mobile phones they all had.

The rest of the presentation was focused on what life is like for me as a scientist researching mesenchymal stem cells, with the main aim of regenerating bone. They all knew what cells were but, interestingly, thought stem cells made up the stem of a plant, very logical thinking! I went on to explain that I wanted the stem cells to make bone. It was the summer of 2006 and Wayne Rooney had broken his metatarsal just before the Football World Cup. I explained that in order for him to play his foot needed to heal and it did this by recruiting stem cells to make new bone. I am pleased to report that the questions that followed were in genuine interest of science and once even snowballed into a whole class debate as to whether parents have the right to choose the hair, eye colour, etc. of their unborn children.

In today's society, young people are often portrayed in a bad light but I was proud of, and encouraged by, the positive response I received and was happy that I could give something back to the school that first inspired my love of science. I would thoroughly recommend this scheme.

Siân Parker
Tissue Injury and Repair Group, University of Manchester, UK

Life after physiology

On Saturday 26 January, the Oxford University undergraduate associates of The Physiological Society held a careers evening, *Life after physiology*. The premise for the event was to invite alumni of the physiology course to speak with us about their career choices and experiences. As the vast majority of university-wide careers fairs mention nothing about scientific research, many undergraduates may not consider it as an option. Our aim was to give students a non-biased view of life in research from people with similar backgounds to their own. Furthermore, for those already interested in research, there was useful insight into PhD application procedures and how to secure funding.

The seven speakers ranged from PhD students to principal investigators, giving us a cross-sectional view of a research career. After the main event there was further opportunity for students to chat with the guests over an enjoyable dinner at a local curry house.

I feel the great success of the evening was mainly thanks to the speakers' honesty - without agenda; they did not sugar-coat research life but instead spoke with passion about their work and gave invaluable advice (a refreshing contrast from the recruitment strategies used by many corporate graduate employers!).

Considering the success of the evening, we hope this will become an annual tradition and several of the speakers have already agreed to return next year.

Rachael Houlton
President, Oxford Physiology Society

Cations in physiological signalling

Keeping up with a broad, multi-faceted subject like physiology is a serious challenge, so PhD students from the University of Manchester organised a 2 day, Young Physiologists' Symposium, *Cations in physiological signalling*, in March 2008. After many months of organising, around 100 delegates (mainly PhD students and first post-doctoral researchers with a few final year undergraduates) came from across Europe to take part in four academic sessions which focused on the role of cations in:

- Cardiac physiology;
- Vascular physiology;
- Neurology;
- Signalling in non-excitable tissues .

Alan North (University of Manchester), Ole Kemi (University of Glasgow), Daniella Riccardi (University of Cardiff), Hugh Pearson (University of Leeds) and Arthur Weston (University of Manchester) gave plenary lectures interspersed with short talks from 16 young physiologists.

Judging by the number of questions that the excellent talks provoked, the symposium definitely succeeded in its main aim, which was to provide an informal environment for young physiologists to discuss their scientific interests and gain experience of presenting their work. This aim continued to the poster session (sponsored by ICON) in which 49 posters were presented at a dinner and drinks reception in the dramatic setting of the mammals gallery of Manchester Museum.

The symposium ended with a 'careers lunch' (sponsored by STEMnet), where delegates were able to chat to successful scientists who have taken interesting and varied paths in their lines of work.

The organising committee thoroughly enjoyed the symposium and were delighted with the feedback. In the words of one delegate: 'Really enjoyed the conference, good talks, well organised and hope it's done again in the future. Good to present in a less intimidating atmosphere'.

Alexandra Hughes
University of Manchester

World record breaking DNA model

As part of National Science & Engineering Week, the under-graduate society at the University of Huddersfield worked with a group of 6th form students to create the longest ever model of a DNA gene (above).

The model, an exact replica of the insulin gene, measured 16 meters in length – almost as long as a cricket pitch – and was verified as 'accurate' and meeting the world record requirements by legendary cricket umpire Dickie Bird, who also fixed the last piece in place.

Jeremy Hopwood, science lecturer and event organiser, explained: 'The aim was to build an exact copy of the insulin gene; our model was 16 meters in length and comprised over 1118 base pairs. The previous record stood at a mere 300 base pairs. The biggest difference was that the previous record breaker was a random sequence. Our students were following the exact gene sequence for insulin.'

The Huddersfield DNA Day started with an inspirational introductory talk focusing on the genetics of diabetes, followed by a short presentation about the model building. The mixed team of students then joined forces to complete the world record model at approximately 2 p.m.

Developing PHILTER – the **PH**ysiologists' **I**mage **L**ibrary & **TE**aching **R**esource

The Physiological Society is developing PHILTER, a source of fresh images and animations to enliven lecture and seminar presentations. PHILTER will provide access to PowerPoint sequences, movie loops and self-contained programs. We hope researchers and teachers alike will find it an attractive platform to showcase their work to a wider audience.

Resources will be categorised by theme, the first of which to be illustrated will concentrate on the 'core' features of systems physiology common to most 1st and 2nd year courses for biomedical professional courses and science students. PHILTER will provide a dynamic route for the latest research findings to enhance and inform. Apart from the intrinsic didactic qualities this approach offers, it gives a vital platform for undergraduates to appreciate that even fundamental physiological concepts are continually researched and refreshed.

PHILTER will be accessible from The Society's new web site, and resources will appear under the aegis of The Society, marking the standard that contributors have achieved. A discussion forum will be available and we hope to initiate a Wikipedia-style feature for Members to contribute and revise online. After the initial launch and running-in period, most of the resource will be accessible freely to all users. However, some items will remain accessible only to Society Members (via their personal login) and others (e.g. featuring explicit animal work or human subjects) will be subjected to further restriction.

The PHILTER team seeks contributions from single images to full PowerPoint lectures. Programs simulating and demonstrating physiological processes and principles, or movies of classic experiments are welcome. Material can be reformatted if necessary; please enquire if you need advice. Submissions will be possible online once PHILTER is fully implemented.

Details of copyright release arrangements will be available soon. We are very happy to answer questions on all aspects of PHILTER.

Submissions can be sent to David Miller (Coordinator for PHILTER), c/o Chrissy Stokes (cstokes@physoc.org) or to The Physiological Society, Peer House, Verulam Street, London WC1X 8LZ .

Visit The Society's education site at www.physoc.org for details of events and available resources for all stages of education.

If you are interested in contributing to any of The Society's events, or have ideas for new activities, please email education@physoc.org

What next?

Anita Roopun, right, considers the options once a PhD is in the bag

You've passed your viva, drunkenly made an Oscar-worthy thank you speech to your lab mates and changed the title on your Visa card ... so what next?

The majority of students who finish their PhD and decide on a scientific career fall into three main categories: those that veer towards industry, those who definitely want to be an academic, and the many that say 'I never want to see another damned pipette/lab-coat/antibody again'. Often even falling into the latter category as I did, one can soon develop amnesia and decide an academic life beckons. I have worked as a postdoc for over 2 years now and have not regretted this decision.

The definition of a postdoc is very labile and the job can mean you do anything, from being an experienced extra pair of hands to someone who has complete research independence. This means the type of position you take is somewhat dictated by your employer. Research on your employer is obviously important, and since I already knew the attitude of the lab I joined, I decided to give up my perpetual student status (and 10% discount at the cinema) and start my first 'proper' job. Although there was a natural progression from my PhD to a postdoc, the two positions held distinct dynamics. Many postdocs are not only expected to churn out data and papers, but are encouraged to take up new responsibilities. In my case, I became more involved in collaborating with other groups and presenting data at conferences, as well as teaching.

One of the points that made me decide to pursue a postdoc is that you have so much more freedom in the direction of your research than most PhD students and those working in industry. The post should

provide a balance of being sufficiently involved with students, grants and papers, but not so much so that it becomes a burden. A postdoc is a prerequisite to life in academia and, I suppose, should provide a taster of what may be to come.

Lecturing and mentoring under-graduate students was a new experience of my job, and I have just completed my first couple of lectures. Although pretty daunting and time-consuming, I did enjoy it and found it rewarding - as well as having an excuse to buy a 'look-more-intelligent' shirt and boots.

Working abroad for long or short time periods is actively encouraged, and is critical for networking and gaining new skills, as well as providing the opportunity to travel. Although I merely moved universities in the UK, some people pounced at the opportunity to move further afield - PhD students from our lab have emigrated to Japan, Australia and America and not looked back.

However, becoming a postdoc is not all merriment and freedom; there is obviously a lot of hard work required. Most postdocs are expected to work a minimum 50 hour week, which obviously requires a high level of motivation. Additionally, life in academia is ruthless; if you produce too few papers it is hard to get the next postdoc position let alone a fellowship, and even if your average impact factor is into double figures, your job is more often than not in the fate of a successful grant.

Nevertheless, becoming a postdoc is not like working in an 'ivory tower' - in fact, it can be quite the opposite. In my experience, postdoc-ing has been hard work, rewarding and worthwhile. Even if it is does not suit everyone, it could never be considered a wasted few years, as you are sure to have acquired a range of skills and experiences suited for any job - whether it be scientific or not.

Anita Roopun
Neural Networks Group, Institute for Neuroscience, Newcastle University, Newcastle upon Tyne, UK

Introducing a new SIG

The Society is delighted to introduce a new special interest group (SIG) to complement the 22 existing groups. The Health and Wellbeing Physiology SIG will provide a focus for Members interested in studying the benefits of maintaining health and fitness.

SIGs have long been established within The Society with the aim of promoting networking and collaboration amongst Members with common research interests.

Discussion within and between SIGs is encouraged by The Society, and has recently been facilitated with the introduction of online forums. To ensure efficient communication between SIG members and the Meetings Committee, convenors have been identified for each group. Convenors also act to co-ordinate activities of a SIG, and ensure representation of the group at meetings.

Using a matrix based on SIGs, The Society has recently identified six over-arching 'themes', which will provide the driver for scientific meetings. Themed meetings replace Focused meetings, incorporating a number of SIGs and providing a catalyst for broader discussion amongst distinct yet related groups.

A full list of SIGs can be found in the Members' area of our web site: www.physoc.org.

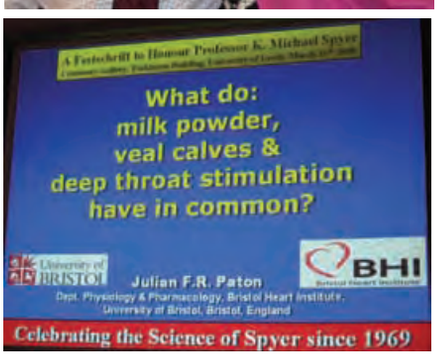
Experimental Physiology

Celebrating Spyer's science

Festschrift speakers (from left): Robin McAllen, Michael Gilbey, Susan Deuchars, James Schwaber, Michael Spyer, Jim Deuchars, Alexander Gourine, John Coote, Brunello Ghelarducci, David Paterson and Julian Paton. Mike and Chris Spyer (below).

The artic coolness and slate grey skies hanging over the city of Leeds on the afternoon of Sunday 16 March were far removed from the minds of those attending K Michael Spyer's Festschrift. Within the Centenary Gallery of the magnificent Parkinson Building of Leeds University was a warm and friendly atmosphere enriched by Spyer's immediate family, friends and colleagues. In total, 70 people amassed to be entertained by Spyer's symphony. Colleagues spoke from his earliest days at the University of Birmingham to the present day. It struck up with tales about the arrival of the young Spyer at Birmingham and his associations with Sydney Hilton. We heard excerpts about his fellowship in Pisa, Italy, and how he learnt to source his experimental preparations with the use of a Fiat motor car and a large sack. Innovation in those days was key, but so was a good biological question of which Spyer had many. Spyer pioneered so much good science: hypothalamic control of cardiovascular reflexes; vestibular affects on blood pressure; medullary control of cardiac vagal activity – some thing which today is used as a prognostic indicator for cardiovascular disease; central coupling between breathing and heart rate and arterial pressure via the sympathetic nervous system. Not a stone appeared unturned – even the cerebellum was brought into the limelight of cardiovascular control. We heard of a romance in the Spyer laboratory and, of course, his beloved Tottenham Hotspur. It was a historic afternoon full of reminiscing of the good old times with Spyer, the successes, the triumphs, and the emotions. Spyer is an outstanding British physiologist who has inspired, influenced and motivated so many individuals. His accomplishments are massive and his aspirations live on. His work has been outreaching both nationally in terms of ensuring that the funding agencies remained on track for supporting basic cardiovascular science, but also overseas where he has influenced significantly scientific research activity in Italy, Portugal and the USA. The afternoon ended with words from the man himself: in true fashion he advised that doing experiments is key and generates hypotheses; innovative ideas come from those in research groups – a critical mass is pivotal and collaboration essential. He reflected that the afternoon was in one way like living through one's own obituary. But, he agreed, he would not have missed it for his life!

Julian F R Paton
David J Paterson



Speakers
John Coote (Birminham)
Jim Deuchars (Leeds)
Susan Deuchars (Leeds)
Brunello Ghelarducci (Pisa, Italy)
Michael Gilbey (London)
Alexander Gourine (London)
Robin McAllen (Melbourne, Australia)
David Paterson (Oxford)
Julian Paton (Bristol)
James Schwaber (Philadelphia, PA, USA)

Messages sent from former colleagues
Geoffrey Burnstock, David Bradley, Roger Dampney, Ged Goldsmith, Martyn Harris, Sydney Hilton, Diethelm Richter, Janusz Lipski, Steven Mifflin, Clifford Saper, Luis Silva-Carvalho and John Widdicombe

New Editors

Ken Baldwin

Ken received his PhD in Exercise Physiology at the University of Iowa under the direction of Charles M Tipton. Following postdoctoral training at Washington University in St Louis, under the mentorship of John Holloszy, in 1973 he joined the faculty of the Department of Physiology and Biophysics in the School of Medicine at the University of California, Irvine where he currently holds the rank of Professor, Above Scale. Baldwin's research is focused on the general theme of striated muscle plasticity. His research specifically addresses mechanisms regulating muscle growth, atrophy and shifts in muscle fiber phenotype using a variety of animal models.

Peying Fong

Peying Fong currently is Assistant Professor in the Department of Anatomy and Physiology at Kansas State University. She earned her baccalaureate at Yale University and her PhD at the University of California, San Francisco. Peying conducted

Chair David Paterson promotes *Experimental Physiology* in New Zealand.

postdoctoral studies at both the University of California, Berkeley, and at the Center for Molecular Biology, Hamburg, Germany. There, in the laboratory of Thomas J Jentsch, she expanded her repertoire of approaches to include molecular techniques for studying the CLC family of voltage-gated chloride channels. Since then, Peying has integrated ion channel structure-function analysis into many aspects of her research. Peying's current research interests fall within the general area of epithelial ion transport in health and in diseases as diverse as cystic fibrosis, polycystic kidney disease and Pendred syndrome.





New EP Editors (clockwise from top left): Ken Baldwin, Peying Fong, Steve Harridge, David Murphy and Wayne Giles.

Wayne Giles

Wayne is a Professor of Medicine and Kinesiology at the University of Calgary. His research involves studies of cardiac pacemaker mechanisms and of cell-cell interactions in the mammalian myocardium. Cellular physiology methods are utilized and attempts are made to develop detailed mathematical models of action potential mechanisms in myocytes from selected regions of the heart. The possibility that fibroblasts or myofibroblasts have significant paracrine and autocrine effects in the heart is a current research focus.

Steve Harridge

Steve received his PhD from the University of Birmingham and then travelled to Scandinavia to do his post-doctoral research with Bengt Saltin at the Karolinska Institute in Stockholm and then at the Copenhagen Muscle Research Centre. He joined the Royal Free Hospital School of Medicine as a Lecturer before spending seven years in the Department of Physiology at University College London. He is currently Professor of Human & Applied Physiology in the Division Applied Biomedical Research at King's College London. His research takes a multidisciplinary approach to human skeletal muscle function and adaptation.

David Murphy

David received his PhD in tumour virology from Imperial College, London. It was during his time as a postdoc in Brigid Hogan's lab at the MRC National Institute for Medical Research that, thanks to a serendipitous encounter with Geoff Raisman, he discovered neuroendocrinology in general, and the vasopressin system in particular. The rest of his career, firstly as a Senior Scientist at the Institute of Molecular and Cell Biology in Singapore, and latterly as Professor of Experimental Medicine at the University of Bristol, has been devoted to the application of molecular genetic and genomic technologies towards a better understanding of the function and regulation of this key physiological model. David is of the opinion that collaboration, transcending the boundaries of disciplines and nations, is the key that will unlock the secrets of genome. Only by sharing ideas, data and resources can we develop the multidisciplinary experimental approaches that will determine the functional relevance of molecular and intracellular processes at the systems level. David is thus working with scientists across the globe on the functional genomics of neuroendocrine systems.

May Themed Issue

Recent advances in the renin-angiotensin system: ACE2 and (pro) renin receptor. Edited by Mohan K Raizada and Julian F R Paton.

The May issue of *Experimental Physiology* is dedicated to the discussion of recent advances in the field of the renin-angiotensin system. It contains review articles and peer-reviewed original papers on two newest members of the system: angiotensin converting enzyme 2 (ACE2) and (pro) renin receptor. This issue provides an up-to date discussion on their involvement in cardiovascular disease (CVD). We hope that it will be helpful to both basic and clinical researchers in providing a state-of-the-art status of this fast emerging field and their implications in cardiovascular physiology and pathophysiology.

The NIH mandate

What it means for NIH-funded authors publishing in The Physiological Society journals

Since 7 April 2008 the NIH has made it mandatory for funded researchers to submit their accepted papers – the final peer-reviewed manuscript – to PubMed Central (PMC) no later than 12 months after the official publication date. How does this affect authors funded by NIH who want to submit to *The Journal of Physiology* or *Experimental Physiology*?

Since both journals make all their content free after 12 months, the mandate will not conflict with subscription arrangements and there will be no requirement to pay an open access fee. There is also no conflict with copyright agreements. In addition both journals will submit to PMC on behalf of the author, although they have different mechanisms for achieving this.

For *J Physiol*, as a result of the Wellcome Trust initiative to digitise the print archive, all content is published on PMC 12 months after publication on the official sites (Synergy and HighWire). The final published version of all papers is submitted by Wiley-Blackwell to PMC and is acceptable in place of the accepted peer-reviewed manuscript. NIH-funded authors who publish in *J Physiol* therefore automatically comply with the mandate once their paper is accepted.

Experimental Physiology does not have all its content published on PMC and therefore the final peer-reviewed manuscripts of NIH-funded papers published in EP need tobe submitted to PMC no later than 12 months after publication. The journal's publisher, Wiley-Blackwell, will implement a new free service that deposits accepted manuscripts to PMC on behalf of authors, after an embargo period of 12 months. They are working with the NIH to establish a robust process to implement deposition and, like many other publishers and societies, seek to persuade NIH to link out of PMC to the final published version on the journal sites.

So long as the NIH does not reduce the allowable maximum delay between official publication and submission to PMC any further, NIH-funded authors can continue to publish in The Society's journals secure in the knowledge that they are compliant with the mandate.

Carol Huxley

Society President Ole Petersen receives his CBE from The Queen at Buckingham Palace on 7 May. © BCA Film.

Tax relief on subscriptions

Did you know that you may be able to claim tax relief on your Physiological Society subscriptions? Under Section 21 of the Income and Corporation Taxes Act (1998), subscriptions paid to The Society are allowable as a fee or subscription to professional bodies or learned societies.

The Inland Revenue publishes a booklet listing all qualifying organisations (www.hmrc.gov.uk/list3/list3.pdf). It may be possible to backdate your tax claim for up to 6 years. To enquire about a claim please contact your tax office (your employer will give you the address). This applies to UK taxpayers only.

Casey Early

Change in governance structure

At the EGM held on 17 March at the University of Leeds, the proposal by Council to introduce a new governance structure was approved unanimously.

This will now be introduced from the AGM in July 2008, bringing The Society into line with the governance structures of most other learned societies.

The current roles of 'President' and 'Chair of the Executive Committee' will be subsumed into one, to be called 'President'. Both the Council and the Executive committee will be chaired by the President. The Executive committee will now consist of the President of The Society, the deputy President, a Treasurer, a Meetings Secretary and three other members who will normally chair other committees of The Society.

Simon Kellas

BIOSCIENCES FEDERATION

I am quite often asked why the BSF doesn't 'do something' about the career structure for research bio-scientists. More often than not the questioner is thinking only about the public sector and especially the career structure for postdocs in universities. I answer by asking what exactly the questioner thinks the BSF could do – the response is usually rather vague.

Action can only follow an analysis of the problem. In many ways the situation is well understood, but it does require stating. In the public sector, the modern biology that has raised so many expectations is usually conducted by large teams funded by significant amounts of external money. The team may consist of one tenured senior member of the academic staff, perhaps a more junior member of the academic staff and maybe a dozen people on fixed term contracts. In most institutions there will be few, if any, opportunities for short term staff to join the faculty.

However, they may not all wish to become a university academic. The majority may be postdocs but they will have a range of career aspirations. Some postdocs will have a predominantly technical role. They fill positions that used to be occupied by staff that had completed vocational training, which may have culminated in an HNC, and who became treasured technicians with a tenured post. These positions have largely disappeared. And with them stability in essential expertise: sometimes, probably too often, promising areas of research are closed down because a postdoc leaves and his or her critical skills cannot be replaced.

Other postdocs do not aspire to become team leaders. They have seen the pressure that arises when teams are maintained on grants and want a different 'work life balance'. Although they may want nothing more than a 'first lieutenant' role, many in this cohort are truly excellent scientists. When I was in Bristol, there were tenured University posts of Research Associate and Senior Research Associate. These positions also have largely disappeared.

Finally, some postdocs are truly driven by their research and strongly promote their work at meetings and elsewhere. They are conscious of citation metrics and identify the route for a research career. Many of this smaller cohort succeed.

All of this is well known. I write about it briefly not to indicate a yearning for a golden age (which it was not!), but to emphasise that there are different career paths in research for public sector bioscientists and that separate structures are needed for each. But that is only the beginning, honesty is also needed. How many group leaders really state explicitly that a postdoc is in effect a technician? How many think that their responsibility is discharged by finding another postdoc position for someone who would be better off doing something else – perhaps running a pub!? How many are truly delighted when the ambitious, successful postdoc begins to overshadow them? How many suggest that their postdocs should join a contract research organisation and not think about being an international star? How many acknowledge openly that the biosciences cannot continually expand and therefore all postdocs will not get jobs in the area?

So what does 'do something about careers' actually mean? Certainly I believe it is possible to 'do something'. Whilst at Babraham we created two career paths for postdocs: one for potential team leaders and one for team players. Entry to both paths was very competitive. The potential team leaders were funded by the Institute for 2 years and had to get a significant grant within this time – preferably a prestigious personal Fellowship. Astonishingly, virtually all were successful. There was no promise of a tenured post but all became very much better equipped to find one. The team players had to have needed generic skills and the ability to refresh them: they also had to be excellent scientists. This very successfully opened a much needed career path for some and provided stability in the essential expertise that the organisation needed. But, of course, this cost money and is not something that many other organi-sations were/are prepared to do.

Let us focus on the last sentence for a moment. Babraham was (is) not cash rich. The Institute decided to reduce the scope of its activities in order to improve the scale. The issue of 'scale and scope' is not systematically addressed in this country. Universities do not have to teach all subjects, or indeed undertake research in any. Some universities do not hesitate to reorganise schools and close subject areas to improve the structure and financial strength of the organisation. Perhaps the argument should be made more strongly that human capital is the greatest asset of all and that 'scale and scope' issues apply very strongly to staff at all levels.

So what can the BSF do? Currently we are engaged in working with others on identifying skills shortages – both current and anticipated, both vocational and generic. This work holds promise of important outcomes. But I would be delighted if we could also look at the career question in a potentially constructive way in order to make generic recommendations. Please write to me if you have a view about the constructive way forward.

Richard Dyer
Chief Executive Officer

The Physiological Society Benevolent Fund

The Fund exists to help anyone who 'by the nature of their employment can be considered to have contributed to the advance of physiology' and who is in 'necessitous circumstances'. There are many ways in which the Fund can help – grants (or loans) towards funeral expenses, emergency travel, medical costs, childcare arrangements, relocation costs and unexpected bills. Spread the word to anyone that may need support. Full details from Liz Bell (ebell@physoc.org).

Microarrays. Just say no. They will mess with your mind

The inevitable result of having a carefully thought out and insightful scientific hypothesis is that you occasionally end up looking a bit stupid. Best go fishing and make the hypothesis up after. Isaac Walton

I am a recovering microarray addict. I became addicted to gene chips 3 years ago.

It started innocently enough: some senior academics suggested that using microarrays would make me feel and look smarter and would give me a certain scientific je ne sais quoi. Armed with a microarray and this strange Latin street slang I could get any paper I wanted into a high ranking journal. '*The upper echelons of British science all use these, why don't you try one*', said one 'respectable business associate' of a street gang called the Royal Society. So I started using microarrays secretly in a government laboratory. At night I would apply cDNA randomly to microarrays and generate gigabytes of meaningless information, it gave me a sense of exhilaration and opened my eyes to a whole new plane of consciousness. There were web sites and whenever I felt down and stupid in a scientific meeting I would say meaningful things like '*can't you use improved pathway analysis for over represented genes*'? and people would look at me as if I had said something deeply profound.

Things progressed and I soon invited sinister looking microarray dealers in to the lab to sell gene chips to PhD students, store microarrays and stash gene analysis software. In turn the dealers filled the laboratory up with silly looking plastic and silicon squares and gigabytes of totally scientifically irrelevant information.

Then things got really out of hand and my whole life took a downward spiral. I didn't realise that gene chips cost £500 each and I soon realised that I had a £1000 a day microarray gene chip habit. I then started to develop a number of behavioural problems associated with my new lifestyle. One chief side effect of repeated exposure to microarrays is that you can develop *Microarray Induced Obsessive Compulsive Disorder*. This has two main symptoms – the first is an inability to answer any question whatsoever without reference to microarray data, and the other is that you develop a phobia towards any *in vivo* work. Things got infinitely worse: I couldn't perform sexually without thinking about how many of my 44000 genes (in replicates of 11), were switched on, or indeed off. I even drowned the family cat when I realised nobody had actually developed a cat microarray.

As a result of my obsessions and inappropriate behaviour, my laboratory became an unfit place to work. When biochemists, *in vivo* scientists or electro physiologists came into the lab, I used to thrust microarrays into their hands and say '*man this makes your results look so much more real*'.

I finally developed *Microarray Induced Psychosis*. I thought I was sane and that the rest of the world was ill!

I watched the TV series *Rome* and began to sacrifice ever increasing numbers of small rodents to Affymetrix, the god of microarrays.

The woman in Tesco's eyes glazed over when I said '*have you ever stopped to consider which of the 44000 genes of the mouse genome are switched on when a mouse is exposed to a 2 for 1 offer and how you could hierarchically cluster those genes. You haven't have, you! You are ill*!' She called security.

Things hit rock bottom. The consumable budget ran out and I was left with a pile of chips with no resale value and three hard drives full of indecipherable data. When the microarray dealers realised the lab was bankrupt, they refused all contact and cut me dead. '*Just a few microarrays*', I said to one '*until we get some more money, please just to tide me over*'; he threatened me and slammed the phone down.

I lost my job and entered a treatment programme. At the treatment centre I met several other scientists whose lives had been destroyed after they had become addicted to fashionable scientific techniques. I met Dave who couldn't remember the 1980s after his brain had become overloaded by a mixture of acid house music and monoclonal antibodies. And Mike who had ended up in prison after dabbling with double-stranded RNA interference techniques.

The psychotherapist at the treatment centre explained that we had addictive personalities and that we should stay away from scientists who repeatedly used trendy or fashionable scientific words. Following some months of therapy I came to realise they were dangerous and could mess with your mind.

I left science soon after and returned to my first love, medieval history, and am now writing a thesis entitled *Subliminal proto-feminist Islamic imagery in the Norman codpiece* which I am hoping Channel 4 will eventually turn into a four part mini-series. But there is just one thing that would give any Norman codpiece a certain medieval *je ne sais quoi*. A microarray.

You could do all sorts of experiments: you could grind up a medieval codpiece, extract the RNA and work out which genes were switched on or off. You could even expose transgenic mice to Norman codpieces of differing designs, and cluster analyse their genomic response. If I applied, do you think the MRC would give me a grant? I thought it might fall into one of their interdisciplinary categories: how about *Multidisciplinary synergies in the post-genomic age, hierarchical gene clustering and the Norman codpiece*?

Please, I want to work with microarrays again.

Dr Keith Cormorant is unwell

Peter Raymond Lewis

1924–2007

Peter Lewis died on 17 December 2007 aged 83 after coping valiantly with Parkinson's disease for over 15 years. He was elected a Member of The Society in 1954, serving on the Editorial Board of *The Journal of Physiology* from 1975 to 1977. Peter's research career reflected his wide interests. He went up to Exeter College, Oxford with an open scholarship to read chemistry, but his subsequent D Phil thesis on the kinetics of bacterial growth led him into biology and ultimately to neuroscience.

In 1948 Peter joined the Cambridge Physiological Laboratory where, financed by a grant from the Rockefeller Foundation, he worked with Richard Keynes on the net movements of sodium and potassium in squid nerves during nervous activity. This was the first effective application of radio activation analysis to biological problems. He then became interested in the role of the CNS in the maintenance of diurnal rhythms in man, a question that had been little studied. In collaboration with Mary Lobban he made two MRC-funded expeditions to Spitzbergen where, in the perpetual summer daylight, their subjects lived on strictly controlled 'days' of 21, 22 or 27 hours. While the fluctuations in body temperature adopted a new rhythm quite rapidly, the effect on peak output of water, sodium and potassium was less consistent leading to the conclusion that more than one mechanism may be involved in controlling diurnal rhythms.

After 4 years as a University Demonstrator, Peter was appointed an Assistant Director of Research in the Department of Anatomy where, with his talent for innovation, he had an enormous formative influence on colleagues and students and, mainly with Charles Shute, he began the important work on cholinesterases that became the core of his research career. His chemical background enabled him to introduce improvements to the histochemical method developed by George Koelle. He also adapted the technique for use in electron microscopy, the thiocholine substrate being far more specific than thiolacetate that had been used for EM histochemistry hitherto. A major study of the distribution of cholinesterase-containing fibres in the rat brain resulted in two landmark papers in *Brain* in 1967. Peter the chemist was always more cautious than Charles the anatomist, in equating cholinesterase-containing with cholinergic. In a collaborative study, I was recruited to measure the level of choline-acetyltransferase proximal and distal to a lesion in the fimbria. The increase in the histochemical reaction for acetycholinesterase proximal to the lesion, and its disappearance distally, was mirrored by the changes in cholineacetyl-transferase. This strengthened the evidence that the cholinesterase-containing fibres in the fimbria did indeed represent a cholinergic input to the hippocampus, probably from the ascending reticular formation. Degeneration of this ascending cholinergic pathway is now widely believed to underlie Alzheimer's disease. In 1970 Peter moved back to The Physiological Laboratory where Charles had been appointed Professor of Histology on the retirement of E N Wilmer.

While the bulk of Peter's work concerned the anatomy of the cholinergic system in the CNS, his research topics included spectral sensitivity curves, monoamines, placental esterases and weeping lubrication in mammalian joints. He will, however, be remembered for more than his range of scientific interests. Cambridge students, whether medics, vets or natural scientists, benefited from his enthusiasm and skills as a lecturer, demonstrator and supervisor. His breadth of knowledge meant that he contributed to the teaching of biochemistry, cytology and neurology as well as anatomy and physiology. During the early part of his retirement he continued to demonstrate to histology classes often using his Parkinsonian symptoms, and their control, as a teaching aid.

In May 2007 a society was founded in his college, Corpus Christi (where he was a Fellow from 1959 till his death), to bring together all its medical alumni and students (preclinical and clinical). Despite his worsening health Peter played a major role in establishing the society which, by unanimous decision, has been named the Lewis Society of Medicine. A founding member described him as an invaluable source of scientific and general wisdom, while one of his erstwhile technicians described him as a perfect gentleman. These words neatly sum up Peter the scientist and Peter the man. Our sympathy goes to his wife Joyce and children Anne and Mark, and to his friends worldwide.

Ann Silver

Gertrude Falk

1925–2008

Gertrude came from the University of Washington in Seattle to work with Paul Fatt in the Biophysics Department at UCL in 1961, on a Guggenheim Fellowship. Although her PhD work at Rochester was on diuresis in the rat, she then became one of the early microelectrode electrophysiologists. She had worked as a postdoc with Gerrard in Chicago and studied a wide range of smooth and striated muscle types. She and Paul Fatt tackled the question of the puzzlingly high capacitance of muscle – this was before it was established that the membranes of the transverse tubules were

continuous with the surface membrane. They used two electrode recording techniques that required an in-depth understanding of the electrical properties involved ('real' biophysics). They reached the conclusion that the 'internal' membranes accounted for the high capacitance (*Proc R Soc Lond B Biol Sci* [1964]. **160**, 69-123) about the same time as the electron microscopy revealed the structure.

Gertrude continued to collaborate with Paul for some years, turning their techniques to electrical studies of rod outer segments (chosen as a tissue that did not move). It is worth remembering that when they started to work on retina very little was known about phototransduction. They were among the first to look at the cellular biophysics of the problem.

Gertrude's interests in the synaptic connections and function of the retina started with a theoretical paper (as well as two extensive and scholarly handbook chapters) that she and Paul wrote in 1974. Jonathan Ashmore began working with her as a post-doc at that time and claims that he only got the job because he could solve cable equations analytically – which must have struck a chord, as Gertrude recounted that in her student days in the USA she was well-nigh a national champion at doing integrations in her head. The joint work became a lively collaboration and produced a small clutch of *Nature* papers.

Gertrude Falk pictured in 1995 by Martin Rosenberg.

This was then carried forward over many years by Gertrude and Richard Shiells: shortly before she retired they discovered that the rod-ON-bipolar cell synapse depended upon a metabotropic glutamate (mGLUR6) receptor cascade. It was a critical scientific combination, with Richard's experimental skills complementing Gertrude's encyclopaedic knowledge of the literature, old and new.

She continued to teach occasionally for many years beyond her retirement, and to come to the Starling Room to indulge her great conversational skills and challenging opinions until just a couple of months ago. Her great sense of humour and ready amusement at the oddities of life and people was always tempered by her warm and generous spirit.

Gertrude had a fierce sense of justice and ready sympathy for the underdog. She was a loyal and kind friend to anyone in need; typically this was shown not in mere words of protest, but was translated into action. Her indifference to conventions is well illustrated by the occasion when, drinking coffee in the men's staff common room, at that time still segregated, she responded calmly to the Beadle summoned to escort her out: 'well, I am certainly going to finish my coffee first', and did so at her leisure.

Gertrude and Paul Fatt were married for a period, and had one daughter. Although they later divorced, their relationship remained amicable. Her illness was sudden and, perhaps mercifully, quite short since the thing that saddened her most during this, aside from the prospect of not seeing her two grandchildren grow up, was the likely loss of her memory and her intellect.

Jonathan Ashmore
Lynn Bindman
Tony Gardner-Medwin
Sally Page

Christopher Bell

1941–2008

Chris Bell died at home in Melbourne, less than a year after his retirement from the Chair in Physiology in the School of Medicine at Trinity College Dublin. He was 66. Although he spent his academic life in departments of physiology, he was equally at home in the company of pharmacologists and, indeed, was the first winner of the Sandoz Prize (now called the Novartis Prize) of the British Pharmacological Society. He was an active member of The Physiological Society, the British Pharmacological Society and the Australian Physiological and Pharmacological Society. Chris served the APPS over many years as a member of Council (twice), Treasurer, National Secretary and CEO, this commitment being recognised by election to Honorary Membership in 2007. He also contributed to the updating of the Australian code of practice for animals used for scientific purposes. Chris was an editor for many international journals and completed his second term on the Editorial Board of the *British Journal of Pharmacology* only a few months before he retired from Trinity.

Chris grew up in what was then the rural outskirts of Melbourne in a wooden cottage on a smallholding on the banks of the Yarra river. His parents were part of a diffuse community of artists centred around Eltham since the early 1900s. His

mother ran the house and garden and painted; his father worked in design. Originally taught by his mother at home, Chris later walked, 2 miles each way, over the Greensborough hills to school. With this rural background, Chris was keen from an early age to find out more about the culture and history of the wider world. His interest in nature led him to take zoology at Melbourne University where he was one of Geoffrey Burnstock's first PhD students. Chris's contemporaries in this laboratory included Max Bennett, Graeme Campbell and Terry Bennett. He was subsequently awarded a National Heart Foundation Overseas Research Fellowship to work at two of the best biomedical research laboratories in the UK, first with Marthe Vogt at Babraham, where he met Ann Silver and Denis and Gretel Sharman, and then with John Vane, at the Royal College of Surgeons in London. During this time, he was studying the catecholaminergic innervation of peripheral tissues, particularly those of the reproductive system. Neither lab was for the merely competent post doc: Chris was fully able to meet the high standards demanded and to retain the scientific respect and friendship of both his supervisors. This early recognition of his research ability was formally marked by the award of the Sandoz Prize in 1972.

Chris returned to the Department of Physiology in Melbourne and soon built up an international reputation in vascular physiology, first on the control of the uterine circulation and then on vasodilator nerves, particularly those using dopamine in the renal vascular bed. He was awarded a DSc in 1980, just about 10 years after his PhD. He also found time to write a number of popular books on physiology, including one aimed at schoolchildren. In the 1990s, he became increasingly involved in the computerisation of teaching physiology and devised a number of programmes particularly for practical classes.

In 1995, he was appointed to the Chair in Physiology in Trinity College Dublin where he continued his research into the Otago strain of genetically hypertensive (GH) rats. These animals have a genetic neurotrophic defect leading to a perinatal loss of NPY-containing sympathetic neurones and, perhaps consequently, an abnormal distribution of CGRP- and substance P-containing neurones. Intriguingly, in these GH rats, expression of the immediate early gene, *c-jun* was a marker for the apoptotic neurons. He also started a new research programme, in human cardiovascular physiology, with emphasis on the effects of exercise.

In Dublin, his research effort was inevitably diluted as he sought to invigorate the Physiology Department in Trinity College. During his tenure, he ensured the survival of the Department and of physiology as an academic discipline, in spite of the waves of 're-organisation' that swept through Trinity (as in most UK medical schools) and, at the same time, he fostered active scholarship, learning and research in the Department. Over these years, with the support of his colleagues, his leadership was essential to the success of this endeavour. His commitment to teaching both medical and physiology students led him to revise and renew both courses at Trinity College and to initiate and foster two new courses, on exercise physiology and neuroscience. Chris also established and maintained a highly effective teaching collaboration with Hungarian physiologists, through Zoltan Szelenyi at the Medical School in Pecs, with exchange visits by faculty and medical students.

But Chris was much more than an excellent scientist and an inspiring teacher. He had an abiding interest in Wellington and the Napoleonic Wars. When at Babraham, he and Denis Sharman assessed how far a musket ball would actually go with the powder they had in those days. Loud bangs, much smoke and quite a lot of singed hair but I don't think those experiments were ever published! This interest was able to flourish when he moved to Dublin (where he wanted to buy Wellington's house) and included visits to Waterloo and many of the battlefields of the Peninsular War. All of this was underpinned by an extensive library – the newest book would end up on a guest's bedside table together with something from P G Wodehouse, whose works, like those of Arthur Ransome, were a favourite relaxation. Chris had an extensive collection of classic films on tape and this enthusiasm was backed by a huge *Encyclopaedia of Film*, frequently consulted to settle points of ignorance or dispute.

All these interests had to be fitted around his serious domestic concerns – house, food and wine – all undertaken with the same mixture of enthusiasm and painstaking research. He loved old houses and restored several Victorian houses in Melbourne with meticulous attention to detail; for instance, making sure the patterns used for the plaster decoration were those available at the time the house had been built. In Dublin, I remember driving around with him in search of a door knocker or some such trivial (to me) detail that was of the correct style for the house. And it wasn't enough to be 'Georgian', it had to be correct, down to the nearest 10 years. Although this seemed somewhat obsessive, the house looked fantastic at the end of it all. In wine and food, his knowledge and tastes were extensive. He would set up a Sunday lunch around half a dozen different Gewurtztraminers because we had been discussing these one day in the lab. The lunches were not just about wine but about food too. He was an adventurous and accomplished cook and always with style – but he would leave the puddings, thankfully and successfully, to his wife, Christine.

Despite at times appearing somewhat forbidding and unapproachable, Chris will be remembered by his many friends and students, as a man of generosity and kindness, tempering his intellectual rigour with humour, and totally

committed to the welfare of his students. For us, his life was, indeed, a life ended untimely.

Y S Bakhle

(*I am grateful to Chris' friends and colleagues for their help in writing this obituary*).

Veronica Campbell adds:

Chris Bell was appointed to the Chair of Physiology in Trinity College Dublin in 1995 and under his wise and experienced hand, the Department expanded research activity and postgraduate training in exercise physiology and neuroscience. He was deeply involved with the Irish Medical Council's reorganisation of medical education, which continues to bear fruit, and he held the position of Director of Preclinical Studies in Trinity for a number of years. His keen interest in undergraduate education is reflected by the publication in 2005 of *Case-based medical physiology* written with Trefor Morgan and Cecil Kidd. He challenged our students to express their full intellectual potential using a tough, yet endearing, paternalistic manner. His relationship with staff over his 12 year tenure developed into one of 'optimal homeostasis'. Neophytes, in particular, benefited from his experience and ability to ignite their potential.

An excellent photographic portrait of Chris now hangs in the Department. This most recent addition to the line of occupants of the Chair of Physiology at Trinity College, captures the attention of even a casual observer, as it is the only portrait with a smiling face. This is a poignant daily reminder of the man we knew and respected.

A student's perspective from **Saoirse O'Sullivan**

I joined the Department of Physiology as a Junior Sophister student, the year after Chris was appointed to the Chair at Trinity. Chris was an intimidating figure and not all students took to his 'interrogative' form of teaching. His was the only class where everyone studied *before* entering, and we were all the better for it! I enjoyed Chris's course on cardiovascular physiology so much that I went on to complete my Senior Sophister research project with him, during which time he persuaded me to carry on to a PhD (in fact, he may have filled in the forms for me!). That was typical of Chris, always thinking ahead for his students. I was only in the first year of my PhD when he asked where I was thinking of going for my post-doc! Even after I left the Department and moved across the water, Chris continued to mentor me and advise me on my career progression, and it is without doubt that I would not be where I am today without Chris's constant support and guidance. Chris's technique was a perfect balance of guidance and letting you find your own way. Indeed, I feel privileged to have been guided by such a supervisor and now, as I am just about to embark on the supervision of my first PhD student, I hope that I can follow in his footsteps.

Other than nurturing my academic progression, I also have Chris to thank for the following, for which I am eternally grateful: an unusually expensive taste in white wine, a serious appreciation for olives, having dined in some excellent restaurants and an aspiration to live in a beautiful big old house. He is greatly missed.

William Keatinge

1931–2008

William Richard Keatinge (Bill) was born on the 18 May 1931. He was educated at Rugby School and subsequently studied medicine at Pembroke College, Cambridge and St Thomas's Hospital in London. He completed his National Service with the Navy in Cambridge (1956-58) before taking up the post of Director of Studies in Medicine and Junior Fellow at Pembroke College Cambridge (1958-60). He subsequently spent 1/2 years in San Francisco as a Fulbright Scholar before returning to England to an MRC post and Fellowship at Pembroke College, Oxford (1961-68).

Bill joined the Department of Physiology at the London Hospital Medical College (LHMC) as Reader in Physiology on 1 January 1969 and was promoted to a Personal Chair in Physiology in July 1970. Bill was appointed Head of the Department of Physiology in October 1981. Following the merger of the Basic Medical Sciences Departments from LHMC and St Bartholomew's Hospital Medical College (Barts) in 1990 with Queen Mary and Westfield College (QMW) Bill became Head of Physiology in the joint school. He held this post until his retirement in 1995 when he became an Emeritus Professor.

Bill ran an active and successful research group, which was highly rated and supported by the MRC for many years, with a series of project and programme grants. Among his many publications are important articles on survival in cold water and local mechanisms controlling blood vessels. He had many international collaborations, developed especially close links with Russia and led a large EU Eurowinter grant which co-ordinated research in eight European countries. Following the break up of the Soviet Union it became possible for him to extend his Eurowinter project to Siberia. He made full use of the advantages of being part of a multi-faculty environment at Queen Mary, University of London, by forging a link with the Russian Department and learning to speak the language well enough to be understood on his visits to the new Russia. He wrote many chapters in textbooks and journals of both physiology and medicine, principally on temperature regulation and the control of blood vessels.

Bill served as Preclinical Dean at the LHMC, at a time of considerable

change and uncertainty. The merger of the preclinical departments at the LHMC, with those at Barts and the move to QMW eventually happened but only after discussions lasting over 20 years and many false dawns. A new building at QMW and a new curriculum were initiated at the time of the merger in 1990 and Bill had played a leading role in designing and implementing these changes.

In the following year Bill was elected Dean of the Faculty of Basic Medical Sciences at QMW. He took on the reins of authority at yet another difficult time for the Faculty when student numbers were being rapidly increased and staff appointments reduced. In 1994 Bill's term as Dean ended and he was content to leave administration behind to concentrate on his research, which was of paramount importance to him. He retired in 1995 but continued to attract grant funding and pursue his research interests. He retained an office and facilities at Queen Mary and concentrated on environmental problems such as global warming and the fact that cold caused far more deaths than heat. He was frequently the expert, interviewed by the media, at times of extreme cold and during heat waves.

Bill also made substantial contributions to the teaching of human physiology to undergraduates and postgraduates throughout his career. He organised the whole body and nutrition module in the Integrated Curriculum introduced in 1990 at QMW for the large combined intake of medical students at the new Mile End site.

Bill was first married to Annette Hegarty who predeceased him and with whom he had three children, Richard, Claire and Mary. He subsequently found happiness again with Lynette Nelson who became his second wife. His children and Lynette survive him. Although he became ill several years ago he faced his illness with considerable courage and dignity. He continued to work until a few weeks before his death and completed the write-up of his final projects in a distinguished research career. He will be greatly missed not only by his family but also by his many friends and colleagues.

Bill was elected to membership of The Society in 1968, served on the Committee from 1977 to 1981 and was a member of the Editorial Board of *The Journal of Physiology* from 1979 to 1986.

Margaret Bird

Speed, Ecstasy, Ritalin

The science of amphetamines
By Lesley Iversen. Oxford University Press. 222 pp, £12.95 (paperback)
ISBN 978-0-19-853090-9

Amphetamines are commonly used both medicinally and recreationally in our society. There are numerous descriptions about how they work and how safe or dangerous they are. In this book, Leslie Iverson has provided a nicely structured and easy to read review of the science of amphetamines alongside their medicinal applications and their recreational uses that would be equally readable by an informed scientist or lay reader. He gives a balanced description of the good, the bad and the ugly of amphetamines, from the positive clinical results seen in some patients with attention deficit hyperactivity disorder (ADHD) to the very sinister methamphetamine (crystal meths) addiction epidemic that is sweeping some parts of the world. The author himself has set out in this book to highlight the 'good and evil' of these drugs without personal opinion. Many amphetamines, including ecstasy, are Schedule 1 (Classification A) which classifies them as dangerous narcotics. Their use is also illegal. Medically, these drugs do not always have such dangerous effects as propaganda says; however, because of the illegal and classification status, they are often portrayed in this way as a deterrent from their use. The chapters stand alone, making it a useful text for delving into for specific information. The book begins with an introduction to the chemistry of amphetamines and their affects on the brain which sets the scene for the applications of amphetamines discussed in later chapters. He describes the medical uses of amphetamines and gives a very interesting account of their rise and fall in terms of therapeutic effects to many different diseases followed by the discovery of negative side effects, including addiction and toxicity. The two following chapters describe the uses as performance enhancers and as substances of abuse and a plethora of scientific reasoning for both are provided. Chapter 6 highlights how research into amphetamine-induced psychosis has been beneficial in identifying brain mechanisms underlying schizophrenia where psychotic behaviours are similar. The final three chapters give a balanced case for amphetamines, their potential dangers and also their useful efficacy in many clinical situations. Positive additions to this book are the short personal experiences of patients being treated with amphetamines and also abusers of amphetamine that are scattered throughout the text. They help the reader to understand the scientific explanations by reading individual experiences. Overall, this book is a stimulating and intellectual read, with bundles of good science weaved into an informative story which would be useful to anyone interested in the uses and abuses of amphetamines.

Fiona Randall

Technicians

The Royal Society has published a themed issue of *Notes and Records* entitled *Technicians*. Two of the papers included – *Working with C Sherrington* (an interview with Mr T J Surman) and *Working with Cambridge physiologists* (an interview with Clive Hood) – may be of particular interest to Society Members. *Notes and Records* subscribers can access the issue at http://publishing.royalsociety.org. Print copies (£16) are available from Portland Customer Services, Commerce Way, Colchester CO2 8HP (+44 (0) 1206 796351, sales@portland-services.com).

100 years ago in J Physiol

On the Munchi arrow poison and strophanthin. George Ralph Mines (1908). *J Physiol* **37**, 37–49.
George Ralph Mines (1886–1914) died tragically in mysterious circumstances before his 30th birthday. However, his place in physiology, and particularly in medicine, is secure. In less than a decade of work Mines published 13 papers in *J Physiol*, of which the most famous is the 12th, *On dynamic equilibrium in the heart* (Mines, 1913).

Mines came to Cambridge in 1904 as an undergraduate, and published almost all his papers from the Physiological Laboratory. The paper on the Munchi arrow poison appeared when he was only 22, 2 years before he was elected to The Physiological Society in 1910, and represents part of his work as what we now identify as a PhD student. Mines is credited on the paper as 'BA, Scholar of Sidney Sussex College, Cambridge.' He had taken his undergraduate degree the previous year, and had published his first paper before graduating with Keith Lucas (Lucas & Mines, 1907) , also to die sadly young. Mines was a contemporary and friend of A V Hill – the two men were the same age, and were elected to The Society the same year – who helped Mines with mathematical ideas. The Cambridge Physiological Laboratory of the time was dominated by the contrasting personalities of J N Langley and W H Gaskell, and Mines' paper begins with an acknowledgement of Langley's influence:

'In the course of some experiments on the behaviour of skeletal muscles in saline solutions, Professor Langley suggested that I should examine the influence of the Munchi arrow poison ...'

An earlier study from Cambridge by Frohlich (1905) gives more detail of the source of the poison, conjuring up a vanished world of colonial adventure:

'The arrow poison [was] presented to the Physiological Laboratory, Cambridge, by Major Alder Burdon, at the time Assistant Resident, Lower Benue Province, Northern Nigeria. The arrows were taken in action against the Baikorana clan on the river Ome, March, 1900. Major Alder Burdon describes the poison, when freshly prepared, as being nearly always fatal to a man in about half an hour.'

George Ralph Mines, pictured around 1912.

Mines discusses possible ingredients:

'Lewin (in his treatise on arrow poisons) states that the arrow poison of the " Muntschi tribe in Haussa land' is greatly dreaded ... Several plants are said to be used in its preparation, together with snake venom and some part of a poisonous fish found in the Benue river ... It is well known that the seeds of *Strophanthis hispidus* are employed as an arrow poison in many parts of Africa ... It is said that some tribes attempt to make their weapons still more deadly by placing the poisoned arrow-heads in the flesh of putrefying corpses.'

In the paper Mines tests the poison systematically on the heart, visceral smooth muscle, spinal reflexes and skeletal muscles of frogs. The most sensitive system proves to be the heart, where the poison produces arrthythmias, ventricular alternans, contracture and ultimately arrest. Mines also shows that low doses produce positive inotropic effects.

The paper is very modern in appearance, and contrasts sharply in this regard with the study of Frohlich from the same laboratory three years earlier. Indeed, the cardiac force traces could easily have appeared seventy or eighty years later in essentially similar form. The writing is also a model of clarity. Mines notes:

'It is clear from these experiments that the aqueous extract of the Munchi arrow poison contains a toxic substance which in its action on the heart exactly resembles strophanthin.'

Strophanthus – the seeds are poison.

And summing up:

'Evidence is offered for the belief that the chief toxic ingredient of the Munchi arrow poison ... is the glucoside strophanthin.'

Mines worked in Cambridge until the end of 1913, interrupted only by brief spells working at the marine stations in Roscoff and Plymouth. He then moved to Canada, first to the University of Toronto, and then on to become Professor and Chair of Physiology at McGill aged just 29 in Autumn 1914.

Mines' lasting fame stems from two papers; in the first (Mines, 1913) he hypothesized and described the concept of circus-movement reentry of current in the heart. The second, published in a Canadian journal (Mines, 1914) and neglected until its rediscovery decades later, identified the 'vulnerable period', a time window within the cardiac cycle when arrhythmic currents or electrical stimuli can provoke ventricular fibrillation. Mines' work on these two topics was far ahead of its time, and his diagrams of circuit currents regularly fool even experienced doctors, who often assume they date from around the late 1930s.

A few months following his appointment at McGill, Mines was found unconscious in his lab on a Saturday evening, connected to some physiological equipment. He was rushed to hospital, but died later that night. Based on a lecture in which Mines had referred to physiologists like Henry Head who had used themselves as experimental subjects, it was speculated that he had been conducting experiments on himself; some have suggested he might have fallen victim to a self-induced cardiac arrhythmia. For more on Mines' life, the article by DeSilva (1997) is a good starting point.

Austin Elliott

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6th International Scientific Conference for Medical Students in GCC Countries
18–21 January 2009

The 6th International Scientific Conference for Medical Students in the GCC Countries will be held in the Faculty of Medicine & Health Sciences (pictured above), United Arab Emirates University, Al Ain from 18–21 January 2009.

The conference will be an international event attracting the participation of undergraduate and postgraduate researchers working in the field of medicine. The event rotates around the GCC countries and was last held in Al Ain in 2004 when it attracted several eminent keynote speakers and more than 800 delegates from 35 countries. Keep an eye on the developing website at http://www.gcc6mconf.uaeu.ac.ae

Matsue, Japan
3rd International Symposium on Physiology and Pharmacology of Temperature Regulation
23–26 July 2009
http://www.med.shimane-u.ac.jp

SECC, Glasgow, UK
SEBatGlasgow2009 (SEB Annual Main Meeting 2009)
28 June–1 July 2009
www.sebiology.org/meetings

For full details of these and other non-Society events visit
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2008

Cambridge, UK (14–16 July)
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Shanghai, China (12–16 September)
International Workshop on *Latest advances in ion channel techniques applied to physiological problems*

Beijing, China (20–22 October)
Joint International Meeting of The Physiological Society with the Chinese Association for Physiological Sciences and the Canadian, Australian and American Physiological Societies
http://www.beijingphys2008.org

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Vascular & Smooth Muscle Physiology Themed Meeting with a Focused Symposium on *Vascular responses to mechanical stress: cellular crosstalk and integration*

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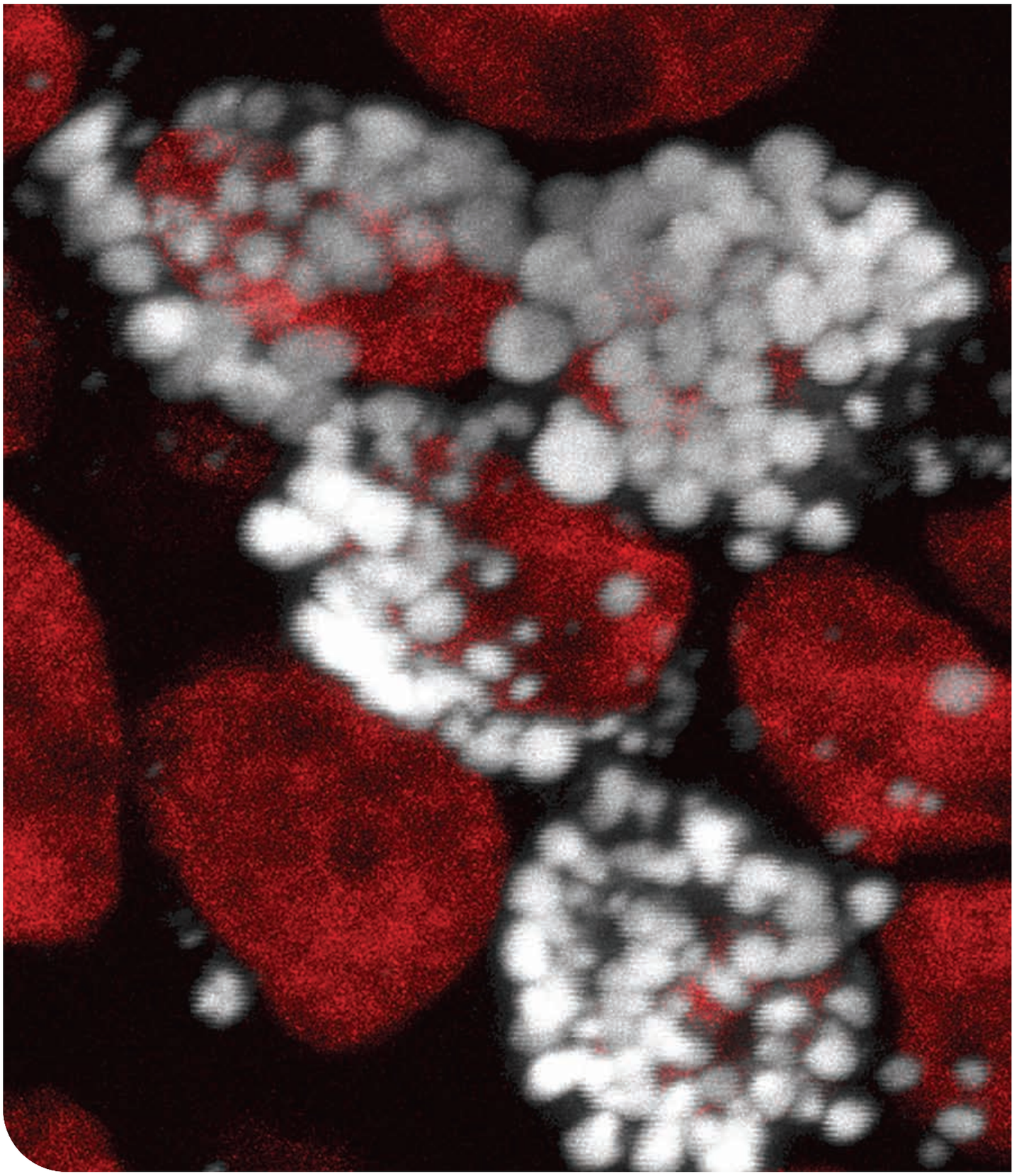

Mucin granules in Calu-3 cells. The image represents a 3-D reconstruction of an en-face confocal microscopy scanning of airway epithelial Calu-3 cells. Mucin granules, which are identified with MUC5AC antibodies (white), are organised apically from the nuclei (red) (Kreda *et al*. p. 19).

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