PHYSIOLOGYNEWS

winter 2003 | number 53

Featuring:

A publication of the Physiological Society

This lock-keeper is turning a windlass to lift a paddle at the bottom of the lock-gate, and allow a barge to proceed through Flatford Lock on the River Stour in Suffolk. Constable exhibited this famous painting at the Royal Academy in 1824, and it is now on loan to the Carmen Thyssen-Bornemisza Collection in Madrid, Spain. This is analogous to the way that respiration rhythmically gates responsiveness of sympathetic-muscle and vagal-cardiac motoneurones and importantly orders autonomic outflow from the human central nervous system (see the article by Dwain Eckberg on pages 11–14).

John Constable, *The Lock*. Oil on canvas painted at Dedham in 1824.
Constable was born in East Bergholt, Suffolk on 11 June, 1776 and died in 1837.

The Society Dog

Published quarterly by the Physiological Society

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ISSN 1476-7996

The Physiological Society is registered in England as a company limited by guarantee, No 323575. Registered office: PO Box 11319, London WC1V 6YB. Registered Charity No 211585.

Prepared in the Publications Office by Charlene Gibbons, Lydia Grove and Linda Rimmer

Printed by The Lavenham Press Ltd

Cover photo

From an image supplied by Prem Kumar.
See page 2 for full details.

PHYSIOLOGYNEWS

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Action Points

Grants

Grant schemes have changed. For full information on Members' and Affiliates' travel grants, the Non-Society Symposia Grant Scheme, Postgraduate Support Fund information and the Vacation Studentship Scheme please visit:
http://www.physoc.org/grants

Membership applications

The final deadline for receipt of Full Membership application forms during 2003 is the last day of December

Change of address

Members should inform the Administration Office of any changes of address, telephone, fax or email addresses.

Changes can be emailed to: jgould@physoc.org or updated online at http://www.physoc.org

Forthcoming scientific meetings

Glasgow (29–31 March 2004)

Cardiff (6–7 July 2004)

Cork (1–3 September 2004)

London (13–16 December 2004)

Joint meeting with the Chilean Physiological Society

Bristol (20–22 July 2005)

Abstract submissions

Authors should submit their abstracts online. Full instructions will be available on the Society's website (http://www.physoc.org/) from the opening day of the abstract submission period.

Physiology News

Letters and articles and all other contributions for inclusion in the Spring 2004 issue, No. 54, should reach the Publications Office (lrimmer@physoc.org) by 5 January, 2004. Late copy can be included if space permits.

Suggestions for articles

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

Physiology News Online

Physiology News is now available on our website: http://www.physoc.org.

Guidelines for contributors

These guidelines are intended to assist [illegible]g their contributions and [illegible]subsequent editing process. [illegible]rial Group of *Physiology News* [illegible]o 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 readable and 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 final dénouement or conclusion.

Length of articles

This will be determined by the subject matter and agreed between the contributor and the commissioning editor. Articles will vary in length from 500 to 2,000 words.

Submission of articles

Authors should submit text in the form of a disk or emailed Word document. Use of disks reduces the risk of introduction of errors during re-typing. It is helpful to give brief details of the computer, operating system and software package(s) used.

Submission deadlines

Please contact the Executive Editor in the Publications Office (see Contents page for details) for submission deadlines. Late submissions may be deferred to a subsequent issue.

Illustrations and authors' photographs

Authors are encouraged to submit diagrams, drawings, photographs or other artwork to illustrate their articles or, if they cannot provide these themselves, to suggest appropriate illustrations. A photograph of the author(s) should also accompany submissions.Photographs may be colour or black and white, prints or transparencies or TIFF 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 2003*, http://www.physoc.org).

Cover figure

Three images were used to construct the front cover image. All were taken at the end of the second day of the Dublin meeting of the Physiological Society. The main image (Fig. 1) is a detail of the sculpture located in Trinity College, Dublin entitled 'Sphere with sphere' by Arnaldo Pomodoro. The next image required was of the Irish flag. This proved much harder to obtain than I had originally thought. The only one I could find was being used in the window of a gift shop (Fig. 2) but this was reversed as it was being hung from the inside of the shop and I was out on the street. The reflections from across the road were not going to help either and it really didn't have a 'flag' shape. The solution came in the unlikely form of the Italian Institute. The Italian flag hanging outside the Institute (Fig 3) was remarkably similar to the Irish one and so all that was required for the transformation was a couple of colour swatches selected from the green and orange sections of the Irish flag picture and the use of a colour range selection and a colour-blended fill using Photoshop® (Fig. 4). The final image (Fig. 5) was constructed by cropping the sculpture image to a square format to lose the uninteresting right hand side, lightening the shadow tones in the image using curves with a mask and applying a lot of saturation and sharpening before blending in the new flag using colour burn in layers. An increase in canvas size, a stroke in black and a further increase in canvas size made the simple border.

Prem Kumar

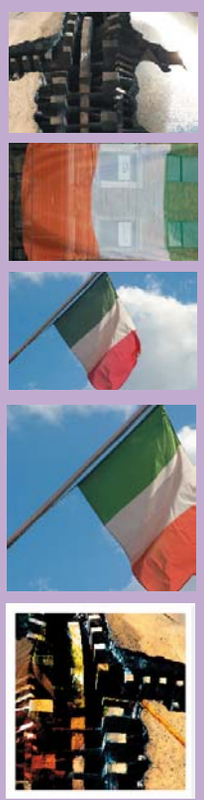


Figure 1 (top) through to figure 5 (bottom)

What is a university supposed to be?

Over the last few decades the British university system has been subjected to all sorts of financial pressures from central government and the pressures are beginning to tell on academic staff. We've all gone through abolition of the binary divide, teaching quality assessments and research assessment exercises, but with all the money that has been spent by departments and universities, I wonder if teaching and research in UK universities are any better now than they were before these exercises started.

If we consider the abolition of the binary divide and the formation of the post 1992 universities, one is left wondering exactly what has happened - I'm particularly uncertain as to what the original objective was, having spent 22 years in an old university and just over 3 years as a head of department in one of the new universities. Government insistence on increasing the participation rate (not a bad thing in itself), while decreasing the unit of resource and diminishing grant funding in real terms is leading to a redifferentiation of the system into those who want top up fees and those who do not, and also into those who do research and those who do not – all very divisive. And it gets worse, because we are also staring at locally agreed pay awards in different parts of the country; different attitudes to student fees in Scotland (but sadly not now in Wales). All this makes me wonder if the national university system will eventually fail, as groups of universities actively compete against each other in the open market.

I would not be so worried about competition between our universities if I thought that the business culture inflicted on them was run by competent individuals, but many senior administrators in the sector have little or no business acumen, although they may have an MBA or two among them! The result of the continuous pressure to take on more students is more and more teaching and administration for academics, gradually strangling their initiative and breaking the link between teaching and research.

Of course, the major problems for the sciences are that they are difficult (tough for poorer students trying to support themselves through university with a job) and expensive for departments and universities. But if less and less students do science degrees, what will eventually happen to our national knowledge base? Will we simply become a service economy? Who will staff our health service if there is no one left to teach the basics? These are political questions requiring long-term, political solutions. Unfortunately, politicians tend to think in the short-term, which makes the situation even more intractable, and what has not helped is the divided nature of the university system and the supine response of senior university administrators to government edicts. To sum up, successive governments have succeeded in dividing the system, but they have not improved it. Surely it's time for the power in individual universities to move towards a more equal balance between academics and administrators. Increased bureaucracy has reduced morale amongst many academics, although of course we are told that demonstrable 'outcomes' continue to be delivered!

Given all of the above, what is a university supposed to be? According to my rather old copy of Webster's New Collegiate Dictionary (1981), a university is defined as *'an institution of higher learning providing facilities for teaching and research and authorized to grant academic degrees'*. Probably few of us would disagree with that, but if only 10 or - if we are lucky - 20 British universities carry out serious research in the future, then what should the rest be called? Should they all be within the same system? Are we moving to a series of regionally based liberal arts colleges, delivering foundation degrees, rather than 'old-fashioned' universities?

Anonymous reviewing

It comes as no surprise that 82% of those reviewing articles for *The Journal of Physiology* are in favour of retaining anonymous reviewing (see article on page 37), although without knowing the exact question asked it is hard to draw firm conclusions. I restrain myself from mentioning turkeys and Christmas, because it is pleasing to note that many reviewers do not think that the present system is ideal and quite a lot felt that they should be allowed to add their names if they so wished. That seems a perfectly sensible viewpoint and could allow further evolutionary change, so come on *J Physiol*, give a positive lead and encourage reviewers to sign their referees' reports!

The future of voting for communications at Society meetings

Over the last few years the Physiological Society has made major changes to its structures and governance and has significantly modernised itself. Are we now at a point when voting to accept demonstrations, oral and poster communications should come to an end? Or do we wish to continue it? The powerful article by Dave Shirley and Matt Bailey in *Letters to the Editor* (page 42) should focus your attention. Let us know what you think – I'm sure that you will!

Bill Winlow

Welcome to Cambridge

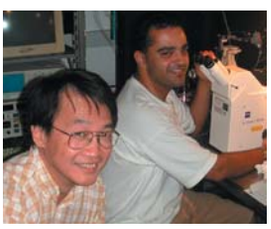
The Physiological Laboratory hosts the Society's meeting in December. Roger Thomas extends a warm welcome to a (probably) rather cold Cambridge

From the top: The Physiological Laboratory 1914 building; Plaque recording the Society of Drapers' gift; Roger Thomas outside the main entrance; Abby Fowden, acting Head of Physiology from January to August 2003 and co-director of the Division of Molecular and Developmental Physiology

This will be the second December meeting of the Society in Cambridge, and on behalf of the whole department, I wish you all a warm welcome. Of course, the Society used to meet here every summer, so this may even be the 100th Cambridge meeting. It will be held as usual mainly in or near the magnificent listed Physiological Laboratory, built by the Society of Drapers in 1914. Their presentation of the building to the University is commemorated by a fine plaque.

Since the Society last met here in July 2000 there have been many changes, but luckily Aileen Briggs, my secretary, and Alan Cattell, our Principal Assistant, are still here and will do most of the local organising. For those of you walking from the station may I recommend the shortcut via my College, Downing, which has an unpublicised gate behind the chapel into the Downing Site. This valuable land was sold to the University by the then very poor college late in the 19th century, and is now occupied by most of the biological departments as well as Geography, Earth Sciences and several others.

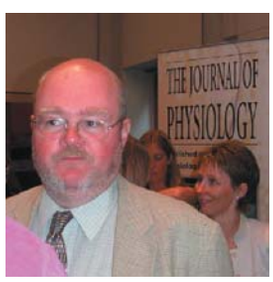
We teach various aspects of physiology, under the direction of Roger Carpenter, to over 1,200 students annually, in nine different courses. All have been revised, and some renamed, in the last few years, most notably those offered to science students. The University's first-year physiology course for scientists used to be much the same as our medical and veterinary course and was taught wholly by the Physiology Department. Now renamed Physiology of Organisms, it includes plant, fungal and even bacterial physiology, as well as more about non-mammalian animals. It is taught by members of the Departments of Plant Sciences and Zoology as well as

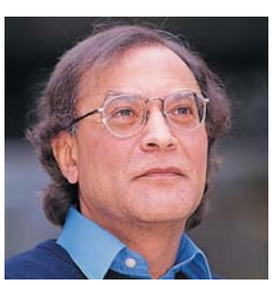


From the top: Roger Carpenter with eye-movement setup; Debbie Willoughby (Pharmacology) and Christof Schwiening conferring at a recent meeting at Babraham; Dino Guissani, Lister Fellow; Chris Huang and Richard Balasubramanian working on the confocal setup; Hugh Robinson; Bill Colledge

Physiology. This new course has proved very popular; it was taken by 235 students last year. All our courses, including our third year science course, contain a strong experimental element with practical classes using live tissues from frogs, crabs and other more expensive animals. Many procedures are performed on the students themselves, which cost us nothing except for disposable electrodes etc. A new second year neurobiology course has been created, taught by staff of five different departments. Consequently, our second-year physiology course now includes less neurophysiology. Indeed, the Faculty of Biology has decided that interdepartmental courses are good; single-department courses are bad. This means that much more teaching than before is at least nominally multi-departmental. The next step is to break down boundaries between departments altogether, or at least create larger ones to facilitate collaboration generally and make administration more cost-effective. Well, that is the idea.

Our research has recently been re-organised into three main divisions. The Division of Cell Physiology is headed by Chris Huang and me, and is focused primarily on the biophysics and physiology of membrane processes in both excitable and non-excitable cells. We use a variety of methods to explore intracellular ion regulation, notably fluorescent indicators and ion-sensitive microelectrodes. The division includes an MRC calcium co-operative centred on a confocal microscope. The Division of Molecular and Developmental Physiology covers the broadest range of interests, from molecular to perinatal physiology. It is headed by Azim Surani and Abby Fowden and concerns the mechanisms controlling mammalian development from the early embryo to the adult. The third division, headed by Andrew Crawford, is that of Sensory and Motor Physiology. It covers physiological mechanisms from the sense organ to the CNS and the motor response to sensory inputs. The senses studied are vision, audition and olfaction, and the motor responses range from eye movement to athletic performance. The division includes an MRC-funded Centre for the Neural Basis of Hearing led by Roy Patterson.

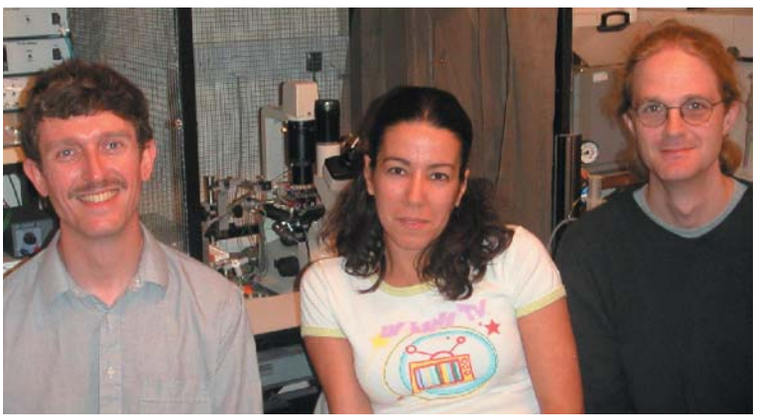




From the top: Andrew Crawford, director of the Division of Sensory and Motor Physiology; Ann Silver, invaluable and eagle-eyed editorial scrutineer; Stewart Sage; Azim Surani, co-director of the Division of Molecular and Developmental Physiology; Martyn Mahaut-Smith; Richard Dyball, Alexey Inyushkin and Antonio Gonzalez (Anatomy)

We are fortunate to host many retired physiologists, including James Fitzsimons, Horace Barlow, Andrew Huxley and, perhaps most valuable of all to the Society, Ann Silver. She seems to scrutinise a good fraction of our own PhD theses and some of the Society's important paperwork. It was she who noticed recently that the Society was breaking its own rules over elections to the Council. The Cambridge meeting will include an Extraordinary General Meeting to allow the Executive to have its way. Or not. Another member of the department who plays a major role in the Society's publications is Stewart Sage, now Chair of the Editorial Board of *The Journal of Physiology*.

As well as those in the department, there are many other members of the Society nearby, in other departments of the Faculty as well as further afield on the Addenbrooke's site and at Babraham. Indeed, two other departments (Pharmacology and Zoology) are headed by members of the Society. Many have played a role as a chair during previous meetings and will, I hope, do so again in December. I include photographs of some of them who were willing to pose for me at short notice. We already collaborate with many biologists in other departments, and such stimulating cross-departmental interactions are certainly one of the by-products of the college environment from which we and the students benefit so much. Another unusual aspect of life in Cambridge is that the university still allows University Teaching Officers to take sabbatical leave every 7 terms or years on full pay. Most people simply stop teaching or administering for the period of leave, but others go abroad.



Top left: Hugh Matthews,Salome Antolin and Johannes Reisert
Top right, Malcolm Burrows (Zoology);
Far left: Steve Edgley (Anatomy)
Left: Colin Taylor (Pharmacology).

I spent last year in Trieste working on mammalian neurones, leaving the department in the hands of two acting heads – the first, Trevor Lamb, was transported to Australia (although innocent) at the end of 2002, but the second, Abby Fowden, is thankfully still with us. I am particularly grateful to Abby, who led the department brilliantly through a very difficult period for the first seven months of 2003.

We are very effectively assisted by the administrative, secretarial, technical and maintenance staff of the Physiology Department, whose role is unobtrusive but crucial. The smooth running of the forthcoming meeting depends very much on their skill and dedication. They kindly try to keep me involved in some of the decision making, but they do all the hard work. We are all very fortunate to have such a staff.

Roger Thomas

Images of Dublin

A photographic record of the Society's meeting at Trinity College in July by Prem Kumar

Left, above: The entrance into Trinity College; Centre, top: Peter Murray, Paul McLoughlin, James Jones and David Jordan; Centre, bottom: Ronan O'Regan and his wife Deirdre; Right, top: The scrutineers need to know if this is SEM or SD; Right, bottom: Simon Green (left) and Stuart Warmington, Meeting organisers. More images of Dublin on page 10

Limits to human performance caused by muscle fatigue

David Allen and colleagues describe the mechanisms of fatigue in muscles and show how they differ in various types of activity

David Allen (top), Jan Lännergren (middle) and Håkan Westerblad

An elite athlete can run 100 m in 10s; naively one might imagine that the same athlete could run 1000 m in 10 x 10 = 100 s but in fact the world record is around 130 s. Similarly over 10,000 m the world record is not 1000 s but about 1600 s so again we find that over a longer distance performance declines. In this respect the human machine is very different to many other machines we are familiar with; for instance, once a car has accelerated to its maximum speed it can normally maintain that speed indefinitely until its supply of fuel runs out and then it simply stops; there is no period of gradually declining performance as the fuel is consumed as is seen with muscle performance. This difference between muscles and other machines suggests that there may be evolutionary advantages to a period of gradually declining performance; it might signal to the animal that fuel is starting to run out and that, for instance, an alternative strategy for escaping a predator might need to be considered.

In this article we show that the main cause of this decline in performance is located in the muscles and is associated with the consumption of energy sources. While energy sources decline, by-products tend to accumulate and some of these inhibit the contractile proteins and contribute to the reduced force. Moreover, one important cause of muscle fatigue is that calcium release, which normally triggers contraction, starts to fail in fatigued muscles.

Where is the site of fatigue?

Muscle contraction is a complex chain of events (Fig. 1). Voluntary contraction commences in the motor cortex and a volley of action potentials (i) activates the α-motor neuron (ii) which excites the muscle through the neuromuscular junction (iii). The muscle action potential is transmitted along the surface membrane (iv) and down the T-tubules (v). A voltage sensor in the T-tubules causes the opening of Ca^{2+} release channels in the sarcoplasmic reticulum (SR) (vi) causing a rise in myoplasmic $[Ca^{2+}]_i$. Ca^{2+} binds to troponin (vii), activating crossbridge cycling and force (viii) and relaxation requires the reuptake of Ca^{2+} into the SR (ix). To understand the mechanism of fatigue we need to identify the links in this chain which fail during repeated activity. One of the earliest investigations was the classic experiments of Merton (1954). The subject contracts a small muscle of the thumb repeatedly until the force produced is reduced to half, which takes a few minutes. Then the ulnar nerve, which supplies the muscle and lies just under the skin, is stimulated with external electrodes. If fatigue were in the brain or spinal cord, it should be possible to overcome it by stimulating the motor nerve – in practice, however, nerve stimulation usually has little effect on the force produced. To obtain this result the subject must be well motivated and indifferent to the pain of the fatigued muscle; obviously these conditions do not always apply particularly outside laboratory experiments. Thus, in well motivated subjects fatigue is mostly in the muscle itself and is conveniently studied in isolated muscles[1].

Why do muscles fatigue?

Muscles may fatigue for many different reasons. The CNS may fail to produce action potentials, the muscle action potential may fail because of ionic changes, energy stores can run out, reactive oxygen species may damage proteins, there may be structural damage to muscles, etc. The traditional explanation for fatigue is that the breakdown of muscle glycogen, a major store of energy inside muscle fibres, leads to

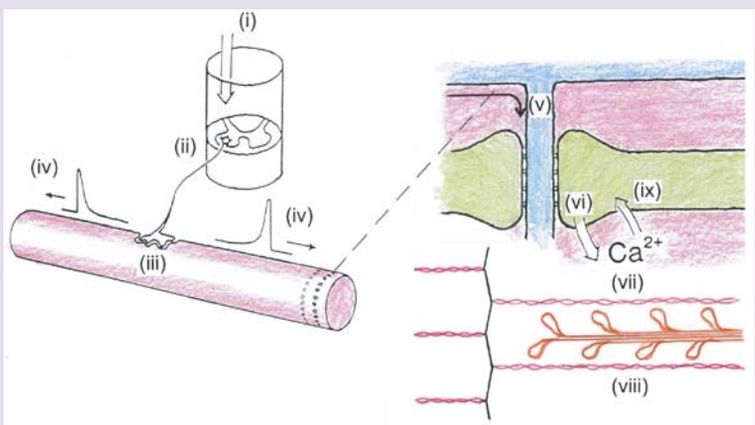


Figure 1. Diagram of the chain of events leading to muscle contraction. Numbers indicate processes described in the text. Modified from Westerblad *et al.* 1991

[1] For more discussion on mental fatigue see the article by Mads Dalsgaard on page 29

the accumulation of lactic acid which impairs muscle function. It is certainly true that in some types of fatigue intracellular lactic acid accumulates and that in experiments on isolated contractile proteins (skinned fibres) acidosis can inhibit the contractile proteins. However, there are other types of fatigue in which lactic acid does not accumulate and a recent finding is that when the effects of acidosis were examined at body temperature rather than at room temperature, the inhibitory effects were minimal. Thus the lactic acid theory of muscle fatigue is losing support, though this is not yet reflected in many text book accounts (Westerblad *et al.* 2002).

We need first to consider the energy sources inside muscle fibres. ATP is the universal source of energy inside cells and is used directly by the crossbridges and ion pumps. However, there is only enough ATP present to power a maximal contraction for 2–3 s. When a muscle is completely depleted of ATP, it cannot contract and becomes very stiff (rigor); if this were ever to occur in normal activity it would cause severe muscle damage and this may be one of the evolutionary pressures encouraging the development of fatigue. To avoid ATP depletion muscle has a variety of backup sources of energy and these are so effective that the bulk ATP concentration never falls below about 20 % of the resting level even in maximally fatigued muscles. To aid understanding we quote for each source of energy the period of time it could power maximal contraction recognising that this rarely occurs and that generally several pathways will be activated in parallel. An easily accessible backup source is phosphocreatine which directly recharges ATP and this supply lasts for 10–20 s. Another important source of energy is glycogen which is stored in the muscle and therefore readily accessible. Glycogen can be broken down anaerobically and used in this way would last only 2-3 min. The sources of energy so far mentioned do not require oxygen (anaerobic pathways) and are the only sources of energy in very brief maximal activities or when the oxygen supply is not available. Alternatively glycogen can be broken down aerobically. Although this process is markedly slower than the anaerobic breakdown, it produces enough ATP to keep the muscle contracting at a near maximal rate for 30–60 min. ATP can also be produced from the aerobic metabolism of fat stores, which are very large but can only be metabolised relatively slowly. Once the glycogen stores are depleted, muscles must rely on fat metabolism.

The 100 m sprint

The running speed during a 100 m sprint is much higher than during longer runs; the short duration means that fatigue is less of a problem. However, even during short sprints there is some fatigue and the maximum running speed in a 100 m sprint occurs after about 60 m. Why does the running speed decline during such a short sprint? The answer to this is not entirely clear. It is, however, clear that lactic acid has little to do with it. Relatively little lactic acid is formed during such a short activity. Instead most of the energy comes from breakdown of phosphocreatine. Breakdown of phosphocreatine *consumes* hydrogen ions so the net effect is that myoplasmic pH is not significantly altered during the sprint. One product of phosphocreatine breakdown is phosphate ions and these have been shown to depress muscle function: they reduce both Ca^{2+}-sensitivity of the contractile proteins (Fig. 1 vii) and the ability of the contractile proteins to produce force (Fig. 1 viii). Thus phosphate ion accumulation is probably an important contributor to fatigue during a 100 m sprint.

While phosphocreatine breakdown may contribute to fatigue by producing phosphate ions, it is also the fastest provider of ATP to cross-bridges and ion pumps which makes it possible for these to work at a high rate. Without a rapid ATP supply, ADP will accumulate and this will slow down crossbridge cycling and ion pumping and hence decrease the power output of the muscle. In recent years it has become increasingly popular for sprinters to ingest enormous amounts of creatine and this seems to have a small beneficial effect on the performance in sprint running – but for elite athletes even a very small improvement can make the difference between winning and losing. Excessive creatine intake results in increased levels of phosphocreatine in muscles so that the period of close to maximal performance is prolonged.

Continuous maximal contraction

A continuous maximal contraction is needed when lifting something very heavy, like a piano. Everyone will be aware of how rapidly fatigue can set in during such activities. In this situation the muscle machinery is going at full speed and energy is consumed at a rapid rate. In addition, the blood flow to the active muscle(s) is stopped during maximal contractions so that no delivery of oxygen (to support muscle contraction) or removal of metabolites or ions will occur. Thus severe fatigue develops within seconds and the muscle becomes rapidly weaker. Changes of the ionic distribution over the cell membrane probably contribute to this type of fatigue. Each action potential is associated with entry of sodium ions into the cell and exit of potassium ions from the cell; consequently potassium ions tend to accumulate outside of the fibres and this results in depolarization and impaired electrical activation of muscle cells. This extracellular accumulation of potassium is likely to be larger in the narrow lumen of the t-tubules (see Fig. 1) from which the potassium ions can only diffuse rather slowly. This leads to impaired propagation of action potentials into the deep parts of fibres and, as a consequence, reduced Ca^{2+} levels and contractile

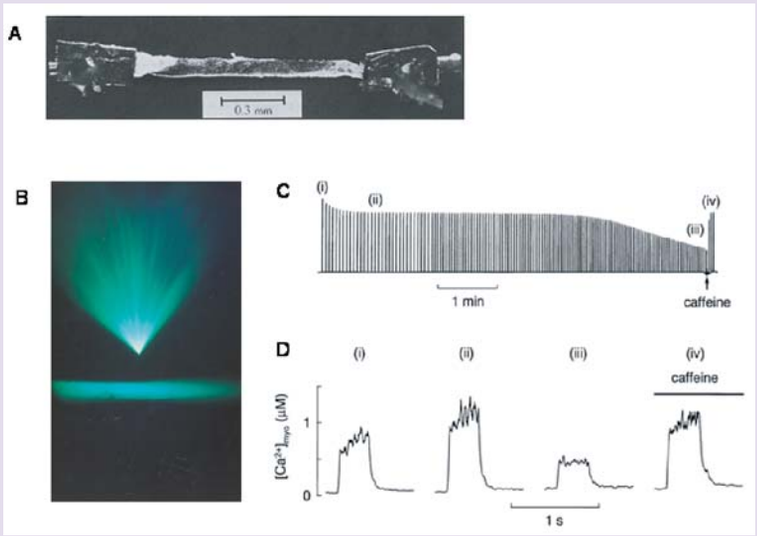


Figure 2. Single muscle fibre approach to studies of muscle fatigue. Panel A. Single fibre dissected from the mouse flexor brevis muscle. Note metal clips attach to the tendons provide connection to the force transducer. Panel B. Fluorescent image of a single fibre and a microelectrode just after the fibre has been pressure-injected with the calcium sensitive indicator indo-1. Panel C. Force record from a single fibre subjected to repeated tetani. When the force has fallen to about 30 %, application of caffeine causes a substantial recovery of force. Panel D. Intracellular calcium records from various stages of the force record in Panel C. Note substantial reduction in intracellular calcium when force was reduced to 30 % (record (iii)). Caffeine increased intracellular calcium to greater than the initial calcium signal and caused a substantial recovery of force.

activation in the central core of fibres (Westerblad *et al.* 1990).

The 5 km race

Events of this sort last 10 or so minutes and are performed at quite close to the maximum capacity of the muscles and involve both the aerobic and the anaerobic ATP pathways. In isolated muscle fibres stimulated until fatigue there is a prominent failure of Ca^{2+} release from the SR (see Fig. 2D). It is easy to show that this decline of Ca^{2+} release is important in the failing muscle performance because caffeine can increase SR Ca^{2+} release and overcome (temporarily) much of the decline of force (Fig. 2C & D). Fortunately, this effect of caffeine occurs at concentrations about 1000 times greater than achieved in muscles after normal ingestion of tea or coffee. The exact cause of the failure of Ca^{2+} release is not known but it probably involves a metabolic component because it is accelerated by inhibiting oxidative phosphorylation. A recent hypothesis is that SR Ca^{2+} release may fail because Ca^{2+} and phosphate inside the SR exceed the solubility product of Ca^{2+} phosphate and precipitate, thereby reducing the free Ca^{2+} available for release. This mechanism only operates because myoplasmic phosphate rises substantially during fatigue and is capable of entering the SR (for review see Allen & Westerblad, 2001)

Running longer distances

Anyone who has tried running a marathon will be aware of the overwhelming muscle weakness experienced in the final stages of the race. This type of fatigue develops quite suddenly, known as 'hitting the wall', and correlates with the near final depletion of glycogen in muscles. However, the precise reason why glycogen-depleted muscles feel weak remains something of a mystery. Muscle biopsies show that at the end of a marathon glycogen is depleted but ATP is only marginally reduced and studies on isolated muscle proteins show that this small reduction of ATP does not affect contractile performance. Lactic acid accumulation is minimal under these circumstances, because lactic acid can leave the cell after a few minutes, and phosphate accumulation is also only moderate. Instead the main factor seems to be failure of calcium release inside the cells which is associated with the glycogen depletion. New insights into this have recently come from skinned fibre experiments which also show a failure of Ca^{2+} release around the time the glycogen is depleted (Stephenson *et al.* 1999). However, in these experiments both ATP and PCr were present in the solutions applied to the skinned muscle and should prevent any metabolic consequences of glycogen depletion. Instead it has been suggested that glycogen may exert a structural role, especially since some of the most labile glycogen in muscle is located at T-tubular/SR junction. Because glycogen is so central to muscle performance, many diets and training procotols have been devised which increase muscle glycogen and such diets can cause a pronounced improvement in performance for activities lasting 1–2 hours.

Recovery from fatigue

Recovery from fatigue has been found to be complex, with both fast and slower components. The faster component is due to reversal of the metabolic changes which caused fatigue in the first place; for example, wash-out of lactic acid and restoration of the phosphocreatine store which will take up the excess of phosphate ions. These processes are relatively fast and are completed in minutes. There remains a second component of fatigue which recovers much more slowly, taking several days for muscles to regain their normal capacity. In real life we will experience this as 'heavy' legs: we can perform almost normally but this requires markedly more mental effort (i.e. the CNS has to produce action potentials at a higher frequency to obtain the same force).

Experiments on isolated muscles suggest that this delayed recovery is also caused by reduced Ca^{2+} release (Westerblad *et al.* 2000). The action potential is normal and the SR is normally loaded with Ca^{2+} but the coupling between the action potential and Ca^{2+} release is damaged. One suggestion is that some Ca^{2+} activated process might damage the proteins involved in Ca^{2+} release.

This delayed recovery is likely to be an important cause of overtraining, which is seen in many sports. Many athletes and coaches believe that the more you train, the better you perform. However, there is a limit to the beneficial effects of training. With too much training the slow phase of recovery is never completed and performance can start to decline. Some athletes respond to this decline by training even more and a vicious cycle develops. Nevertheless, there is wide recognition of this problem and it is one of the factors which underlies the practise of 'tapering' of training adopted by most athletes before big events.

In conclusion, the answer to the question 'What causes muscle fatigue?' is 'It depends on the type of activity'. While this answer is hardly inspiring during a dinner conversation, it is perfectly logical considering the numerous parallel processes that operate close to their maximum during intense muscle contractions.

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References

Allen, D. G. & Westerblad, H. (2001). Role of phosphate and calcium stores in muscle fatigue. *J Physiol* **536**, 657-665.

Merton, P. A. (1954). Voluntary strength and fatigue. *J.Physiol.(Lond.)* **123**, 553-564.

Stephenson, D. G., Nguyen, L. T., & Stephenson, G. M. M. (1999). Glycogen content and excitation-contraction coupling in mechanically skinned muscle fibres of the cane toad. *Journal of Physiology* **519**, 177-187.

Westerblad, H., Allen, D. G., & Lannergren, J. (2002). Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News Physiol Sci.* **17**, 17-21.

Westerblad, H., Bruton, J. D., Allen, D. G., & Lannergren, J. (2000). Functional significance of Ca^{2+} in long-lasting fatigue of skeletal muscle. *Eur.J Appl.Physiol* **83**, 166-174.

Westerblad, H., Lee, J. A., Lamb, A. G., Bolsover, S. R., & Allen, D. G. (1990). Spatial gradients of intracellular calcium in skeletal muscle during fatigue. *Pflügers Archiv European Journal of Physiology* **415**, 734-740.

Westerblad, H., Lee, J. A., Lännergren, J., & Allen, D. G. (1991). Cellular mechanisms of fatigue in skeletal muscle. *American Journal of Physiology* **261**, C195-209.

...more images of Dublin

Clockwise from top left:
Jochen Prehn, Brian Harvey and Chris Bell
Statue of Burke outside Trinity College
Danny McQueen and Cecil Kidd
James Joyce overlooks the GPO building
Lutz Pott with Marie-Cecile Wellner-Kienitz from the Ruhr University, Bochum
The Craic on O'Connell Street

Breathing powerfully modulates human vagal and sympathetic motoneurone responsiveness

Fluctuations of vagal and sympathetic motoneurone membrane potentials alter their susceptibility to stimulatory inputs and thereby order the timing of efferent neural outflow from the human central nervous system, writes Dwain Eckberg

Dwain Eckberg

In 1733 Stephen Hales, a vicar in the London borough of Richmond upon Thames, published his direct observations on fluctuations of the level of blood in a glass tube inserted into a crural artery of a mare 'tied down alive on her back' (Hales, 1733):

'*When it was its full height, it would rise and fall at and after each pulse two, three, or four inches, and have there for a time the same vibrations up and down at and after each pulse, as it had when it was at its full height; to which it would arise again, after forty or fifty pulses.*'

Study of haemodynamic rhythms continues and, in recent decades, has been extended to those of conscious humans, who can be evaluated safely with noninvasive methods, and whose rhythms can be explicated by elegant, computer-based algorithms. Respiratory-frequency rhythms are of peculiar importance in humans, because human subjects bring to the laboratory a unique capability – they can control their breathing precisely. This article, which is based on a more extensive recent review (Eckberg, 2003), treats human autonomic rhythms at breathing frequencies, as manifestations of ongoing neurophysiological processes.

Breathing orders autonomic neural outflows

Figure 1 illustrates how breathing modulates the intervals between heart beats (the 'R-R intervals', whose abrupt changes reflect the ebb and flow of vagal-cardiac nerve activity). In this simple nine minute experiment, the volunteer was asked to breathe at steadily decreasing frequencies, between 15 and three breaths min^{-1}.

Respiratory excursions of R-R intervals (middle panel) were small at the most rapid breathing rate (left), increased as breathing slowed (centre), and then decreased at the slowest breathing rate (right). The bottom panel is a horizontal section of a sliding fast Fourier transformation moved from the beginning of the period (bottom) to the end (top). R-R interval spectral power importantly tracked breathing frequency (diagonal black-to-orange swath). Low-frequency rhythms, (bottom panel, extreme right), were present throughout the recording,

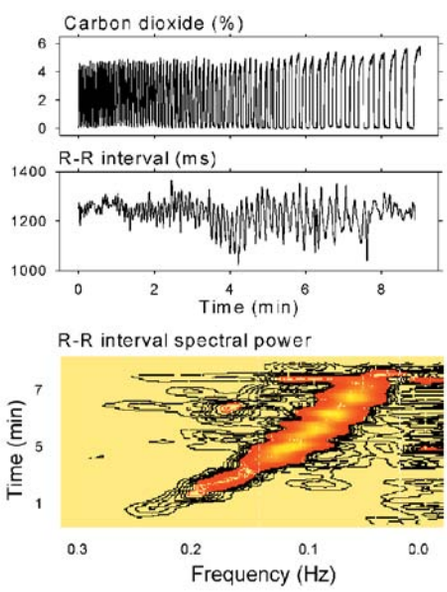
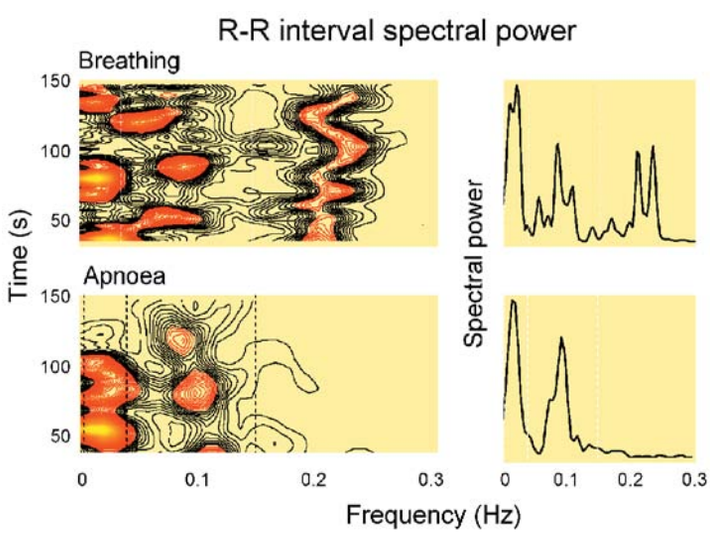


Figure 1. Responses of one supine subject to ramped breathing, between 15 and 3 breaths min^{-1}. End-tidal carbon dioxide (top panel), taken as an index of breathing, increased slightly. Respiratory R-R interval excursions (respiratory sinus arrhythmia, middle panel) were small at the extremes of breathing frequency, and large at moderate breathing rates. The horizontal section of the sliding fast Fourier transformation (bottom panel) indicates that R-R interval spectral power tracks breathing frequency (diagonal black to yellow swath). These data indicate that breathing rate importantly determines R-R interval fluctuations.

Figure 2. Sliding fast Fourier transformations (left) and power spectra (right) obtained from another subject during uncontrolled breathing (upper panels) and inspiratory apnoea (recorded after two min of hyperventilation with 100% oxygen, lower panels). The vertical wavy black to orange pattern on the right side of the left upper panel illustrates how R-R interval spectral power tracks small spontaneous fluctuations of breathing frequency. During apnoea, spectral power was absent at former breathing frequencies (right in each lower panel). This suggests that it is breathing itself that is responsible for R-R interval respiratory-frequency fluctuations; they are not driven by another oscillator. Adapted from Badra et al. (2001).



and appeared to be uninfluenced by breathing.

In another experiment (Badra *et al.* 2001), volunteers hyperventilated for two min whilst breathing 100% oxygen, and then held their breaths for as long as they could. Figure 2 shows measurements obtained from one volunteer during uncontrolled breathing and apnoea. The left panels depict horizontal sections through the subject's sliding R-R interval power spectra, and the right panels depict single power spectra made from the entire three min time series. During uncontrolled breathing, respiratory-frequency R-R interval fluctuations tracked spontaneous breathing frequency fluctuations (left upper panel, right). Spectral power for the entire three min period documented strong periodicity at the breathing frequency, about 0.2 Hz, or one breath every five s (right upper panel, right).

During apnoea, fluctuations at former breathing frequencies were absent (lower panels, right). Therefore, these results suggest that it is breathing itself that is responsible for respiratory-frequency fluctuations of vagal-cardiac nerve activity; they are not driven by some other oscillator which, in turn, modulates respiration.

Breathing gates vagal and sympathetic motoneurone responsiveness

In 1976, Lopes and Palmer advanced the provocative notion that breathing somehow 'gates' the responsiveness of vagal-cardiac motoneurones to stimulation. My colleagues and I (Eckberg & Orshan, 1977; Eckberg *et al.* 1980) followed up on studies conducted earlier in animals and altered baroreceptor input by changing pressure in a neck chamber. When suction is applied to the neck, pressure within the carotid arteries pushes against a vacuum, the carotid arteries and the carotid arterial baroreceptors stretch, vagal-cardiac nerve activity increases, and the heart rate slows. Neck suction is perceived as rising arterial pressure, and neck

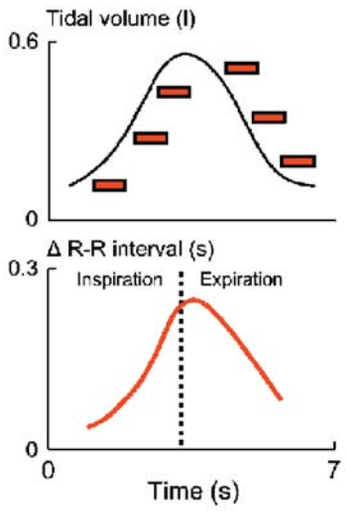


Figure 3. Average lengthening provoked by 0.6 s neck suction pulses applied at different times in the breathing cycle (upper panel), in six healthy volunteers. These static measurements document dynamic changes of the susceptibility of vagal-cardiac motoneurones to stimulatory inputs during the breathing cycle. Adapted from Eckberg *et al.* (1980).

pressure (which compresses carotid arteries) is perceived as falling pressure.

Figure 3 depicts average responses of six healthy young volunteers to brief (less than one s) neck suction, applied at six different times in the breathing cycle. The upper panel shows subjects' average tidal volume, and the timing of each neck suction pulse. The lower panel shows average R-R interval responses to neck suction, plotted according to the timing of neck suction. The static measurements depicted in the lower panel of Fig. 3 illustrate dynamic neurophysiological changes occurring within the central nervous system during each breath. They show that vagal responsiveness to presumed identical surges of incoming arterial baroreceptor traffic is not uniform over time. Vagal-cardiac motoneurones are minimally responsive when baroreceptor stimuli arrive in early inspiration and late expiration (extreme left and right), and are maximally responsive when baroreceptor stimuli arrive in late inspiration and early expiration (centre).

In a subsequent study (Eckberg *et al.*1985), we documented similar phasic responsiveness of sympathetic-muscle motoneurones to reductions of baroreceptor input (neck pressure), during the breathing cycle. Therefore, responsiveness of both medullary vagal-cardiac and spinal sympathetic-muscle motoneurones varies continuously during quiet breathing in healthy human subjects.

This discussion focuses on how respiration gates autonomic motoneurone responses to very brief changes of baroreceptor input; other research shows that respiration also gates autonomic activity at different steady-state levels of baroreceptor stimulation (Eckberg *et al.* 1988).

These data introduce a new concept to the issue of respiratory gating: the magnitude of efferent autonomic neural fluctuations secondary to gating is determined by the magnitude of stimulation of motoneurones. Respiratory fluctuations are large when the level of stimulation is large (as with low pressure and sympathetic activity, or high pressure and vagal activity). An obvious corollary of this is that when the level of stimulation is very low, respiratory gating of motoneurones may be absent.

These data do not answer the question: 'how is respiratory gating of autonomic activity expressed when levels of motoneurone stimulation are extremely high?' Anrep *et al.*1936) measured peak minus valley R-R interval fluctuations in spontaneously breathing dogs over a wide range of arterial pressures. They reported that inspiratory-expiratory R-R interval differences vary parabolically over the arterial pressure range: at very high (as well as at very low) arterial pressure levels, respiratory gating of R-R intervals is absent.

We (Eckberg & Orshan, 1977) performed a related experiment in humans. We found (as discussed above) that vagally-mediated R-R interval lengthening provoked by moderate levels of neck suction is greater when stimuli are applied in expiration than inspiration. However, R-R interval lengthening provoked

by intense neck suction is equal when stimuli are applied in expiration and inspiration. In another experiment (Cooke *et al.* 1999), we increased muscle sympathetic nerve activity physiologically, with graded passive upright tilt, and measured the amounts of muscle sympathetic nerve activity occurring during inspiration and expiration. The results indicate that increasing levels of sympathetic motoneurone stimulation lead to decreasing inspiratory -expiratory differences of sympathetic outflow. Therefore, human respiratory gating of both vagal-cardiac and sympathetic-muscle motoneurones is finite, and can be overcome by high levels of motoneurone stimulation.

These results speak to the complexity of respiratory gating of autonomic motoneurone firing. The gate is not binary, open or closed; the level of gating is continuously variable (Fig. 3). The gate is not simply shut in inspiration and open in expiration; the gate is open widely in late inspiration, and nearly shut in late expiration. Finally, respiratory gating does not exist independent of the level of the stimulation whose effects are gated; gating is driven by the level of motoneurone stimulation, and can be wholly absent when that level is low or high. This last concept is illustrated schematically in Fig. 4. In this drawing, respiratory gating is represented by a lock in a canal. The respiratory gate opens and closes independent of the level of stimulation (which is represented by the height of the water before the gate). This drawing illustrates the fact that periodic fluctuations of the water level downstream from the gate depend critically on the level of water upstream from the gate. In an elegant study performed in conscious cats, Gilbey and coworkers (1984) explored the electrophysiological basis for respiratory grouping of vagal-cardiac motoneurone firing. They showed that although ambiguous nucleus neurones receive baroreceptor inputs with fixed latencies after arterial pulses, vagal-cardiac motoneurone responsiveness

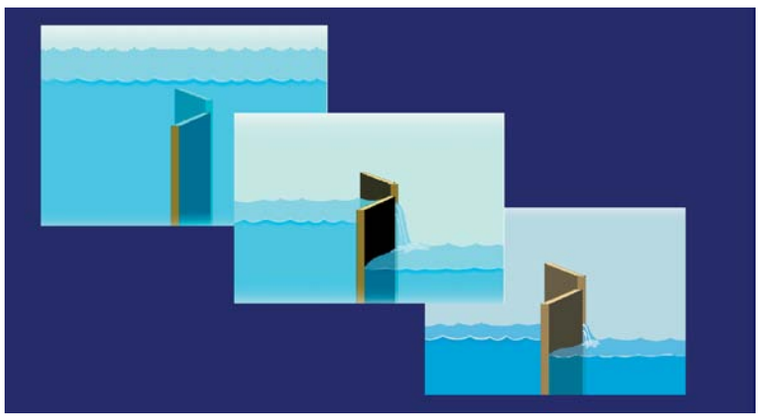


Figure 4. Schematic illustration of the dependence of respiratory gating of autonomic activity on the level of motoneurone stimulation (indicated by the height of water before the lock). The gate opens and closes without regard to the level of upstream stimulation. Downstream fluctuations are small when the upstream level of water is high or low. Adapted from Eckberg (2003).

depends on other membrane potential fluctuations occurring in synchrony with respiration. The ability of vagal-cardiac motoneurones to fire in response to excitant amino acids fluctuates in parallel with these respiratory fluctuations of vagal-cardiac motoneurone membrane potentials.

Respiratory gating expressions in resting humans

Sinus arrhythmia, the rhythmic heart rate changes that occur during quiet breathing, reflects fluctuating levels of vagus traffic to the sinoatrial node, and is mediated by respiratory gating of vagal- cardiac motoneurone responsiveness to stimulation. We recently documented another expression of respiratory motoneurone gating (Rothlisberger *et al.* 2003). At rest, systolic pressures and R-R intervals episodically rise and fall together. These parallel changes are regarded as expressions of ongoing baroreflex physiology.

Although such 'baroreflex sequences' occur at frequencies lower than breathing frequencies (Badra *et al.* 2001), we considered that they may, nonetheless, be influenced by breathing (Rothlisberger *et al.* 2003). Figure 5 shows signal-averaged (on early expiration) muscle sympathetic nerve activity (upper panel) and parallel sequences of increasing ('up' sequences) or decreasing ('down' sequences) systolic pressures and R-R intervals. The lower panel indicates clearly that respiration modulates the timing of both up and down baroreflex sequences. It is likely that the timing of both up and down baroreflex sequences is determined by respiratory gating of muscle sympathetic nerve activity. Sympathetic bursts occur primarily in expiration and, after a short latency, trigger sequential arterial pressure elevations and baroreflex- mediated R-R interval lengthening. As

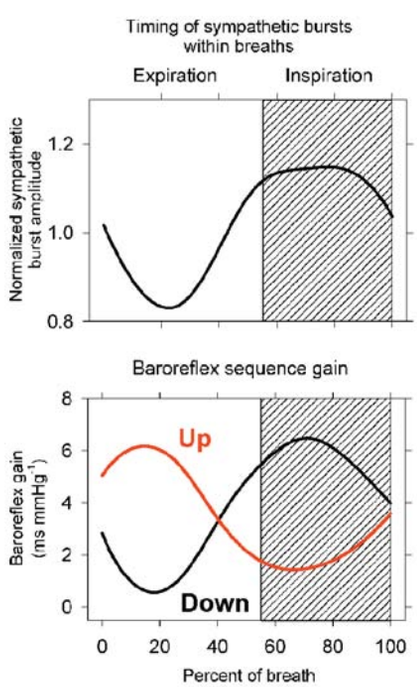


Figure 5. Average muscle sympathetic nerve activity and spontaneous up and down baroreflex sequences, triggered on early expiration. Spontaneous baroreflex sequences occurred deterministically during the breathing cycle. These data indicate that breathing orders the timing of muscle sympathetic bursts, and presumably as a consequence, the timing of up and down baroreflex sequences. Adapted from Rothlisberger *et al.* (2003).

responses to sympathetic bursts wear off, up sequences are supplanted by down sequences.

Conclusions

In healthy resting human subjects, breathing gates responsiveness of both vagal- cardiac and sympathetic-muscle motoneurones, such that responsiveness is maximal in late inspiration and early expiration, and minimal in early inspiration and late expiration. Fluctuations of efferent autonomic neural outflow secondary to respiratory activity depend critically on the level of stimulation of autonomic motoneurones. At low levels of stimulation (as with high arterial pressure and sympathetic activity, or low arterial pressure and vagal activity), respiratory gating is minimal. Conversely, at high levels of stimulation (as with low arterial pressure and sympathetic activity, or high arterial pressure and vagal activity) respiratory gating may be absent, because respiratory influences have been overcome. At usual arterial pressures, respiratory activity orders R-R interval fluctuations (respiratory sinus arrhythmia), the timing of muscle sympathetic bursts, and thereby, the timing of spontaneous (vagal) baroreflex sequences. It may be that the greatest significance of respiratory gating of autonomic motoneurone responsiveness lies in its utility as a tool to understand otherwise obscure human neurophysiological mechanisms.

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Acknowledgments

The research described in this review was supported by longstanding grants and contracts from the National Institutes of Health, the Department of Veterans' Affairs, and the National Aeronautics and Space Administration.

References

Anrep GV, Pascual W & Rossler R (1936). Respiratory variations of the heart rate. 1- The reflex mechanism of the respiratory arrhythmia. *Proc Roy Soc Lond Ser B -Biol Sci* **1198**,191-217.

Badra LJ, Cooke WH, Hoag JB, Crossman M, Kuusela TA, Tahvanainen KUO & Eckberg DL (2001). Respiratory modulation of human autonomic rhythms. *Am J Physiol* **280**, H2674-H2688.

Cooke WH, Hoag JB, Crossman M, Kuusela TA, Tahvanainen KUO & Eckberg DL (1999). Human responses to upright tilt: a window on central autonomic integration. *J Physiol* **517**, 617-628.

Eckberg DL (2003). The human respiratory gate. *J Physiol* **548**, 339-352.

Eckberg DL, Rea RF, Andersson OK, Hedner T, Pernow J, Lundberg JM & Wallin BG (1988). Baroreflex modulation of sympathetic activity and sympathetic neurotransmitters in humans. *Acta Physiol Scand* **133**,
221-231.

Eckberg DL, Kifle YT & Roberts VL (1980). Phase relationship between normal human respiration and baroreflex responsiveness. *J Physiol* **304**, 489-502.

Eckberg DL, Nerhed C & Wallin BG (1985). Respiratory modulation of muscle sympathetic and vagal cardiac outflow in man. *J Physiol* **365**, 181-196.

Eckberg DL & Orshan CR (1977). Respiratory and baroreceptor reflex interactions in man. *J Clin Invest* **59**, 780-785.

Gilbey MP, Jordan D, Richter DW & Spyer KM (1984). Synaptic mechanisms involved in the inspiratory modulation of vagal cardio-inhibitory neurones in the cat. *J Physiol* **356**, 65-78.

Hales S (1733). Statical Essays: Containing Haemastaticks; or, An Account of some Hydraulick and Hydrostatical Experiments made on the Blood and Blood- Vessels of Animals. W Innys, R Man by & T Woodward, London.

ILopes OU & Palmer JF (1976). Proposed respiratory 'gating' mechanism for cardiac slowing. *Nature* **264**,454-456.

Rothlisberger BW, Badra LJ, Hoag JB, Cooke WH, Kuusela TA, Tahvanainen KUO & Eckberg DL (2003). Spontaneous 'baroreflex sequences' occur as deterministic functions of breathing phase. *Clin Physiol Funct Imag* (In Press).

Kidney development – consequences of disruption

Strong evidence now indicates that kidney development can be disrupted by an adverse intrauterine environment at a very early, preglomerular stage, and this may programme hypertension in the adult

Karen Moritz, Miodrag Dodic and Marelyn Wintour

In a recent article in *The Journal of Physiology* (Wintour *et al.* 2003) we showed that adult sheep which had developed hypertension as a result of a brief (two day) exposure to a synthetic glucocorticoid (dexamethasone) very early in development (20% of gestation) had a 40% reduction in the number of nephrons (filtering units). As nephron number is determined before birth in the sheep, as in the human, and as there was no evidence of increased destruction of nephrons in the hypertensive sheep, it was concluded that these sheep had been born with a reduced nephron number. This now seems to be quite likely as other groups have reported, recently, a reduction in nephron number in the perinatal period, in lambs which were treated with dexamethasone at mid-gestation (Figueroa *et al.* 2003) or subjected to undernourishment at an early stage of development (Langley-Evans *et al.* 2003).

There are several very interesting factors which arise from these studies. The first is that hypertension may be a consequence of perturbation of kidney development in utero, or in the period of active nephrogenesis (reviewed in Moritz *et al.* 2003), whereas hypertension does not necessarily result when a kidney is removed after all the nephrons have been formed. Studies in humans, rats, mice and sheep show that hypertension does result when a reduced number of nephrons occurs either by genetic influences, or by experimental removal of one kidney during the developmental period. It is assumed that when reduction in nephron number occurs during the development period, some compensatory changes occur in the remaining kidney, such as a permanent increase in the capacity to retain salt and water, and potentially changes in the expression of a number of genes, which lead to the elevation in blood pressure in the

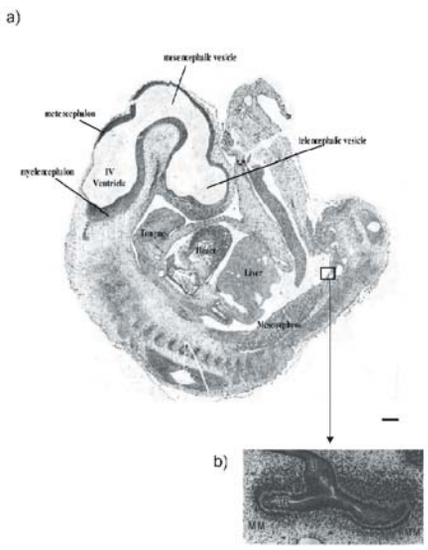

Figure. A saggital section of the ovine embryo at 28 days of gestation: (a) illustrating the immaturity of the kidney and brain of the ovine fetus at the time of glucocorticoid treatment. Scale bar = 2.5 micro metres; (b) enlargement of the caudal end of the mesonephros to show the ureteric bud (U) which has undergone the first branching (UB) and is surrounded by metanephric mesenchyme (MM).
Reprinted from Dodic *et al.* (2002). *Trends in Endocrinology & Metabolism* **13**, 403-408, with permission from Elsevier

adult. These changes are not seen in the second kidney when one kidney is removed in the adult. In other words, it is not simply a reduction in nephron number which induces hypertension, but the combination of too few nephrons and some compensatory changes.

The second remarkable feature of the findings is that the 'critical period' in which the kidney is vulnerable to environmental factors, such as excess glucocorticoid hormones, is so early in development. In both the sheep and the rat (Wintour *et al*. 2003; Ortiz *et al*. 2002) the decrease in final number of nephrons occurred when the treatment was applied before the first nephron was fully formed. This period is early in long-gestation species (human, sheep) but comparatively late in rodents (Moritz & Wintour, 1999). As shown in the Fig. the ovine fetus, at the time of exposure to dexamethasone, had large temporary kidneys (the mesonephroi) and was at the earliest stage of the beginnings of development of the permanent kidney (metanephros). The equivalent time in human development would be 5–6 weeks (Moritz & Wintour,1999).

The third important implication suggested by the findings is that any factor which can disrupt nephrogenesis might also have the same consequence of programming high blood pressure in the adult offspring. There are some factors (mild vitamin A deficiency; raised blood glucose, due to type 1 or 2 diabetes which are also known to be able to reduce nephron number in the developing fetus (Moritz *et al*. 2003). These are far more likely to be encountered by the pregnant woman than is excess exposure to glucocorticoid at an early stage. It is estimated that there are 250 million people with mild vitamin A deficiency, of whom a substantial number are women in the child-bearing years (Nelson, 2003; Christian, 2003). Whilst vitamin A treatment might not show a significant effect on parameters such as infant mortality, the long-term effects on the incidence of hypertension and renal failure have not yet been tested. There are also hundreds of millions of adults with diabetes, mostly type 2, of whom a substantial number are women in their reproductive years (Nelson, 2003). Exposure to a diabetic environment, in utero, in humans, has been shown to be associated with an increased incidence of impaired glucose tolerance, and a defective insulin secretory response in adult offspring (Sobngwi *et al*. 2003). The problems can thus become self-perpetuating – an unhealthy intra-uterine environment leads to unhealthy mothers. The exact mechanisms by which the kidney development is so affected warrants intensive investigation.

The incidence of hypertension in people over the age of 45 years is 25%. Many of these have no genetic predisposition , or current lifestyle 'risk factors'. For many indigenous populations (Australian Aborigines, Pima Indians, African Americans) there are high rates of renal failure (Hoy *et al.* 2003). It is possible that, in some cases, both hypertension and renal failure result from disrupted nephrogenesis during fetal development. Thus it is of critical importance for future health of the population to improve the health of all pregnant women.

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References

Christian P (2003). Micronutrients and reproductive health issues: an international perspective. *J Nutr* **133,** 1969S-1973S.

Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K & Bertram JF (2003). A stereological study of glomerular number and volume: preliminary findings in a multiracial study of kidneys at autopsy. *Kidney Int* **63,** S31-S37.

Langley-Evans SC, Fahey A & Buttery PJ (2003). Early gestation is a critical period in the nutrirional programming of nephron number in sheep. *Pediatr Res* **53,** 30A, 402.

Figueroa JP, Massmann GA, Rose JC & Zhang J (2003). Single maternal glucocorticoid administration at 80 dyas of gestation reduces fetal sheep nephron number at term. *PediatrRes* ***53,*** 15A, 115.

Moritz KM & Wintour EM (1999). Functional Development of the meso- and metanephros *Pediatr Nephrol* **13,** 171-178.

Moritz KM, Dodic M & Wintour EM (2003) Kidney Development and the fetal programming of adult disease. *BioEssays* **25,** 212-220.

Nelson RG (2003). Intrauterine determinants of diabetic kidney disease in disadvantaged populations . *Kidney Int* **63,**S13-S16.

Ortiz LA, Quan A, Weinberg A & Baum M (2001). Effect of prenatal dexamethasone on rat rena development. *Kidney Int* **59,**1663-1669.

Sobngwi E, Boudou P, Mauvis-Jarvis F, Leblanc H, Velho G, Vexiau P, Porcher R, Hadjadj S, Pratley R, Tartaranni PA, Calvo F & Gautier J-F (2003). Effect of a diabetic environment in utero on predisposition to type 2 diabetes. *Lancet* **361,** 1861-1865.

Wintour EM, Moritz KM, Johnson K, Ricardo S, Samuel CS & Dodic M (2003). Reduced nephron number in adult sheep, hypertensive as a result of prenatal glucocorticoid treatment. *J Physiol* **549,** 929-935.

A new window on sympathetic neuromuscular transmission

Seeing ATP and noradrenaline at work in small arteries and vas deferens. W Gil Wier explains

W Gil Wier

The sympathetic nervous system regulates blood pressure in part by controlling the diameter of muscular resistance arteries. Nevertheless, the mechanisms controlling release of the 'triad' of sympathetic neuro-transmitters (NA, ATP and neuro-peptide Y, or NPY) are largely unknown. And, perhaps surprisingly to those not in the field, even the post-junctional effects of two of these, ATP and NPY, are still obscure. Recently, however, a new 'window' on sympathetic neuromuscular transmission has been opened, through the combination of an old experimental technique, electrical stimulation of sympathetically innervated whole tissues (arteries or vas deferens), with simultaneous high resolution confocal imaging of pre-and post-junctional [Ca^{2+}] transients.

Sympathetic neuromuscular transmission

Sympathetic motor neurons are good examples of the new principle of 'multiplicity of neurotransmitters' (see Stjarne, 1999), by which the old 'ergic' classification of neurons (Dale's Principle) has been rendered obsolete. At the varicosities present along the terminals of these nerves (Fig. 1) not one, but multiple neuro-transmitters are 'co-released': nor-adrenaline (NA), ATP, and NPY. In small arteries and vas deferens, it is likely that NA and ATP are not always released in parallel. Details of co-transmitter release are sketchy at best, but Stjarne (2001) has advanced the provocative hypothesis that NA and ATP are present in two types of small synaptic vesicles; a 'big' quantum type containing a relatively much larger amount of ATP compared to NA than the other, 'small' quantum type. It is certainly true, in small arteries, that the effects of ATP predominate at lower frequencies of nerve activity, while NA predominates at higher frequencies. After release, ATP binds primarily to ionotropic (ion-channel) purinergic receptors (probably homomeric $P2X_1$) on smooth muscle cells, activating inward current, including entry of Ca^{2+}, and produces the excitatory junction potential (EJP) (see Ralevic & Burnstock, 2003 for review of purinergic signalling). On mouse mesenteric arteries at least, other purinergic receptors of the metabotropic type, such as $P2Y_6$, may also be present (Vial & Evans, 2002), but these will be activated only by pyrimidine nucleotides (released from endothelial cells or platelets). On smooth muscle, NA binds mainly to α_1-adrenoceptors and activates the well-known signaling cascade involving production of 1,4,5-$InsP_3$, activation of receptor activated channels (ROCs), activation of protein kinase C and other events (see Wier & Morgan, 2003, for review). These events culminate in increases in intracellular [Ca^{2+}], activation of Ca-calmodulin dependent myosin light-chain kinase (MLCK), as well as modulation of myosin light-chain phosphatase (MLCP) activity. As with all synapses, the spatio-temporal dynamics of transmitter action at sympathetic varicosities are influenced by diffusion, uptake and enzymatic degradation of transmitter, and receptor desensitization. Through pre-synaptic α_2-adrenoceptors, NA inhibits its own

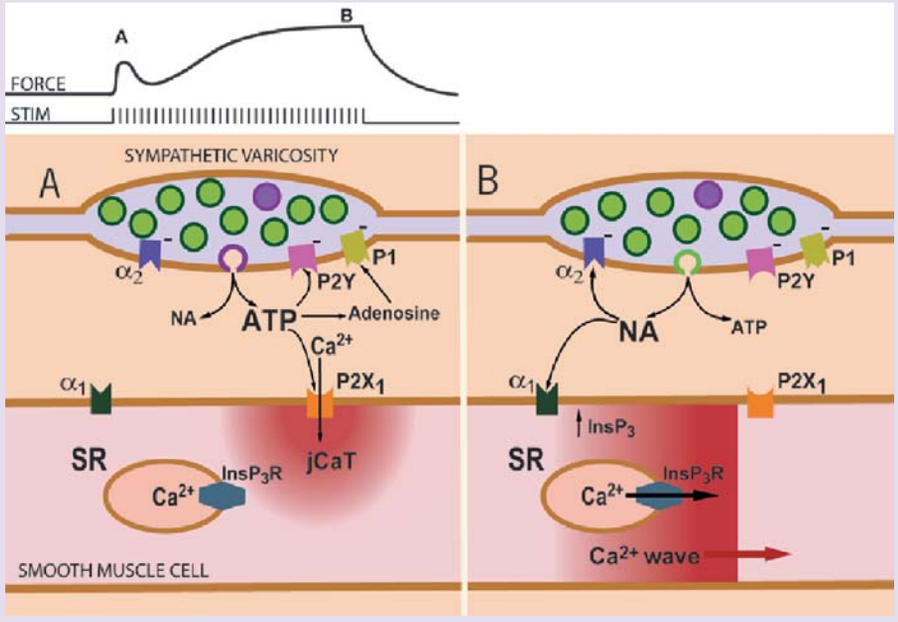

Figure 1. Real and hypothetical events of sympathetic neuromuscular transmission in a small artery. (A) Early during a train of nerve fibre action potentials, smooth muscle contraction is activated mainly by post-junctional Ca^{2+} transients ('jCaTs') induced by neurally released ATP. JCaTs are localized to the post-junctional region, and arise from Ca^{2+} that has entered via P2X receptors. At this time, sympathetic varicosities may release mainly small vesicles that contain a relatively high concentration of ATP (*viz.* the relatively few 'big' quanta proposed by Stjarne, 2001) (B) Later during a train of nerve fibre action potentials, jCaTs are rare, and contraction is activated by Ca^{2+} waves that arise from sarcoplasmic reticulum (SR). Ca^{2+} release from SR is activated by $InsP_3$, produced after binding of NA to α_1-adrenoceptors. At this time, sympathetic varicosities may release small synaptic vesicles (the more numerous 'small' quanta, green) that contain a relatively high concentration of NA.

release, as does ATP though pre-synaptic P_1 (adenosine) and P2Y receptors.

Observing sympathetic neuromuscular transmission

Classically, the release of ATP has been detected electrically, as excitatory junctional currents (EJCs) recorded with loose-patch electrodes. These electrodes are ~ 12 μm in diameter and thus may encompass several sympathetic varicosities. With this technique, it can also be difficult to distinguish small EJCs arising beneath the electrode from large EJCs arising one cell layer deeper in the wall (Bennett *et al*. 2001). Perhaps surprisingly for this relatively mature field, a new technique has emerged recently for observing purinergic sympathetic neuromuscular transmission, with temporal and spatial resolution sufficient to resolve events at single sympathetic varicosities. As so often since the early 1990s, the combination of fluorescent Ca^{2+} indicators with confocal microscopy has provided the new observations. Cunnane and his colleagues in Oxford (Brain *et al*. 2002) were investigating pre-synaptic Ca^{2+} signaling in mouse vas deferens and found, unexpectedly, their fluorescent Ca^{2+} indicator (dextran-linked, low affinity Oregon Green) also in a subset of smooth muscle cells. By whatever means the indicator might have gotten there, it revealed localized smooth muscle Ca^{2+} transients, adjacent to sympathetic varicosities, after nerve stimulation. The Ca^{2+} transients were termed 'neuroeffector Ca^{2+} transients' (NCTs). At about the same time, we (Lamont & Wier, 2002) were asking the question, 'Does NA elicit propagating Ca^{2+} waves in SMC during neurogenic contractions of small arteries, just as it does (Zang *et al.* 2001) during bath-application of NA?' Unexpectedly, we observed brief, spatially localized Ca^{2+} transients that occurred in the vicinity of nerve fibres, before the adrenergic Ca^{2+} waves began. These localized Ca^{2+} transients were completely resistant to blockade of α_1-adrenoceptors, L-type Ca^{2+} channels or ryanodine receptors. They were abolished, however, by the purinergic receptor blocker suramin. Thus, they certainly represented Ca^{2+} that had entered the cell through post-junctional purinergic receptors (probably $P2X_1$) activated by neurally released ATP. As a manifestation of the excitatory junction current (EJC) we termed them 'junctional Ca^{2+} transients' or 'jCaTs'. It seems clear that NCTs and jCaTs represent very similar phenomena but in vas deferens and arteries, respectively.

Opening the window

Synaptic physiologists can expect to use jCaTs to examine function of an individual sympathetic varicosity at an unprecedented level of detail. For example, the ability to monitor pre- and post-junctional activity for long periods at a single varicosity should provide the best estimates yet of the probability of transmitter release at a single varicosity. It may also provide new information on quantal content, by carefully observing amplitude distributions of jCaTs at single varicosities, and reveal differences amongst individual varicosities.

Students of smooth muscle contraction can also expect to benefit. Briefly, an initial transient component of the neurogenic contractions of small arteries appears to be activated by jCaTs (Fig. 1A), while a larger, maintained component of the contraction appears to be associated with asynchronous propagating Ca^{2+} waves (Fig. 1B), similar to those elicited by exogeneous α_1-adrenoceptor agonists, such as phenylephrine (PE) (Lamont *et al*. 2003).

Clearly, bath-application of ATP (in particular), as typically done in the past, does not mimic the events involved in contractile activation by sympathetic nerves. Most importantly, the pattern of 'tonic' sympathetic nerve activity to small arteries consists of intermittent bursts of action potentials. Given the frequency dependence of ATP and NA release (and/or their effects) this should provide a spatio-temporal pattern of activation within the artery wall that is very markedly different than that achieved with simple bath-application of NA or ATP. Furthermore, the sympathetic co-transmitters act synergistically. The most recent data indicate that: 'All three co-transmitters contribute significantly to vascular responses and their contribution varies markedly with impulse numbers' (Bradley *et al*. 2003). Optical indicators of sympathetic neuromuscular transmission, such as jCaTs and NCTs now provide another view into this complex picture.

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References

Bennett MR, Farnell L, Gibson WG, Lin YQ & Blair DH (2001). Quantal and non-quantal current and potential fields around individual sympathetic varicosities on release of ATP. *Biophys J* **80**, 1311-1328.

Bradley E, Law A, Bell D & Johnson C (2003). Effects of varying impulse number on cotransmitter contributions to sympathetic vasoconstriction in rat tail artery. *Am J Physiol Heart Circ Physiol* **284**, H2007-H2014.

Brain KL, Jackson VM, Trout SJ & Cunnane TC (2002). Intermittent ATP release from nerve terminals elicits focal smooth muscle Ca^{2+} transients in mouse vas deferens. *J Physiol* **549**, 849-862.

Lamont C, Vainorius E & Wier WG (2003) Purinergic and adrenergic Ca^{2+} transients during neurogenic contractions of rat mesenteric small arteries. *J Physiol* **549**, 801-808.

Lamont C & Wier WG (2002). Evoked and spontaneous purinergic junctional Ca^{2+} transients in rat small arteries. *Circ Res* 91, 454-456.

Ralevic V & Burnstock G (2003). Involvement of purinergic signaling in cardiovascular diseases. *Drug News Perspect* **16**, 133-140.

Stjarne L (1999). Catecholaminergic neurotransmission: flagship of all neurobiology. *Acta Physiol Scand* **166**, 251-259.

Stjarne L (2001). Novel dual 'small' vesicle model of ATP- and noradrenaline-mediated sympathetic neuromuscular transmission. *Autonomic Neuroscience: Basic and Clinical* **87**, 16-36.

Vial C & Evans RJ (2002). $P2X_1$ Receptor-deficient mice establish the native P2X receptor and a $P2Y_6$-like receptor in arteries. *Mol Pharmacol* **62**, 1438-1445.

Wier WG & Morgan KG (2003). α_1-Adrenergic signaling mechanisms in contraction of resistance arteries. *Reviews of Physiol Biochem Pharmacol* (Published On-Line, 20 July 2003). DOI: 10.1007/s10254-003-0019-8

Zang W-J, Balke CW & Wier WG (2001). Graded $\alpha_1$adrenoceptor activation of arteries involves recruitment of smooth muscle cells to produce 'all or none' Ca^{2+} signals. *Cell Calcium* **29**, 327-334.

Brain glycogen re-awakened

Why concern ourselves with an apparently inconsequential compound, asks Angus Brown

Angus Brown

Glycogen is the main carbohydrate storage depot in the body. It is a highly branched polysaccharide of D-glucose into which excess glucose is stored, and from which glucose (or equivalent) is rapidly liberated upon demand. Glycogen is an advantageous form in which to store excess intracellular glucose, as it reduces osmotic forces on the cell, requires no ATP for initiation of metabolism, is rapidly metabolised and, unlike fatty acids, can yield ATP under anaerobic conditions. The main stores of glycogen are in the liver and skeletal muscle, but its function is dependent upon location. Liver glycogen is released as glucose into the systemic circulation in response to falling blood glucose levels, thus maintaining normoglycaemia and benefiting the entire organism. Muscle glycogen, however, is used as a local energy source solely by skeletal muscle cells during muscle contraction.

Glycogen is also present in the brain, where it is located predominately in astrocytes, but its function is unknown. It is widely accepted that brain glycogen can only support function for a few minutes in the absence of glucose, thus a role as a viable energy reserve has been universally dismissed. This assumption appears valid when one considers the amount of glycogen contained in each tissue. Resting skeletal muscle contains about 400g of glycogen (1–2% of tissue weight), well fed liver contains about 100g of glycogen (6–8% of tissue weight), but current estimates of brain glycogen are a meagre 0.5–1.5g (0.1% of tissue weight). Why concern ourselves with an apparently inconsequential compound? As ever the answer lies in the fine detail. It is the central dogma of brain energy metabolism that the brain does not contain any significant energy reserves, and is thus entirely dependent on the systemic circulation to deliver a constant, uninterrupted supply of glucose in excess of demand. Glucose is not the only bloodborne metabolite, but the limiting permeability of the blood brain barrier excludes potential metabolites from the brain. However, a variety of *in vitro* brain preparations, in which bloodborne delivery of metabolites is circumvented, can survive on non-glucose metabolites. These include, but are not limited to, the sugars mannose and fructose, the monocarboxylates lactate and pyruvate, and the ketone bodies β-hydroxybutyrate and acetoacetate. Glucose-derived metabolites generated *within* the brain parenchyma are therefore potential substrates for oxidation. The question of non-glucose metabolites serving as nutrients for neurones is highly controversial (Chih *et al*. 2001; Vannucci & Simpson, 2001). The diplomatic view is that it is inconceivable/highly likely* that the brain utilises glucose-derived metabolites, thus a prominent role for glycogen in supporting brain function is inconceivable/a realistic possibility* (* delete accordingly).

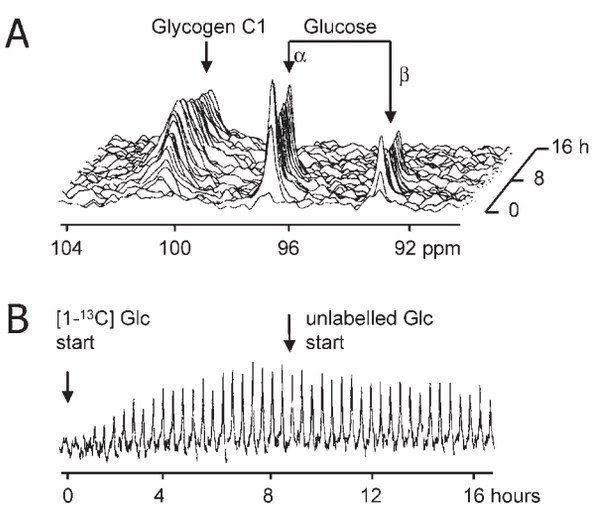

Figure 1. Incorporation of systemically injected 1-^{13}C labelled glucose into brain glycogen in an anaesthetised rat. (A) ^{13}C spectra recorded over the entire 16 hour experimental period. 1-^{13}C glucose infusion was started at 0 hours, switched to unlabelled glucose at 8.5 hours and the net accumulation of glycogen label, and detection of labelled glucose illustrated. (B) A plot of glycogen C1 resonance at 100.5 ppm measured over time, taken from the same experiment as in A (from Choi *et al*. 1999)

Glycogen and pathological conditions

A consequence of the discovery of mammalian insulin in the early 1920s was the introduction of insulin-induced systemic hypoglycaemia in the 1930s as a clinical therapy to alleviate symptoms of schizophrenia, a practice brutally depicted in the recent film *A Beautiful Mind*. Such clinical therapy prompted inquiries as to the effect of limiting glucose delivery on brain function and biochemistry. In adult dogs insulin-induced hypoglycaemia led to decreases in brain glycogen initially in areas with the highest metabolic demand (Himwich, 1959), correlating brain glycogen metabolism with function. (In the mammalian brain there is clear evidence that astrocytic glycogen is metabolised to lactate, which is shuttled out of the astrocyte into the narrow extracellular space,

then transported into neural elements where it is oxidatively metabolized, see Fig. 2). This ability of glycogen to support brain function for limited periods in the absence of glucose has since been demonstrated in the mouse optic nerve, where glycogen content at the onset of aglycaemia determined the duration of function during subsequent aglycaemia (Brown *et al*. 2003). NMR spectroscopic detection of 1-^{13}C glucose incorporation into brain glycogen has been used to study the effects of hypoglycaemia in rat, for the first time allowing continual *in vivo* measurement of mammalian brain glycogen. These studies confirmed a hypoglycaemia-related decrease in brain glycogen, but only after brain glucose had fallen to zero (Choi *et al*. 2003). However, iatrogenic hypoglycaemia is a result of insulin therapy, and the rarity of spontaneous systemic hypoglycaemia suggests that brain glycogen's primary role is *not* as an emergency energy reserve.

Glycogen and physiological conditions

A clue to the function of brain glycogen during physiological activity emerged from the Soviet Union over 50 years ago, with studies demonstrating that brain glycogen accumulated during sleep, decreased in a region-specific manner during sleep deprivation, and was mobilised upon waking, suggesting the awake brain degrades glycogen. The effects of general anaesthetics on brain glycogen provided supportive evidence. Under *in vivo* conditions sustained general anaesthesia resulted in elevated brain glycogen content, but general anaesthetics had no effect on glycogen levels of pure astrocyte cultures. Although a broad picture emerged of the conscious, awake brain constantly metabolising glycogen, what remained unclear was the specific conditions/stimuli that promoted brain glycogen degradation.

Sensory stimulation of rat face and vibrissae resulted in decreased glycogen in the sensorimotor cortex contralateral to the stimulus side, suggesting that sudden increases in focal energy demand are met by increased localised glycogenolysis (Swanson *et al*. 1992). Glycogen in the mouse optic nerve declined during intense physiological activity, and depletion of glycogen, or block of lactate transport, reduced function during periods of high intensity stimulus, even in the presence of normoglycaemic glucose. Glycogen therefore acts as an energy *buffer*, providing supplemental energy metabolite in the form of lactate during periods of increased tissue energy demand, when ambient normoglycaemic glucose is unable to meet immediate energy requirements (Brown *et al.* 2003). Thus, the following definition of a role for brain glycogen has emerged – to rapidly provide supplemental energy metabolite to support function during transient, localised increases in neuronal energy demand.

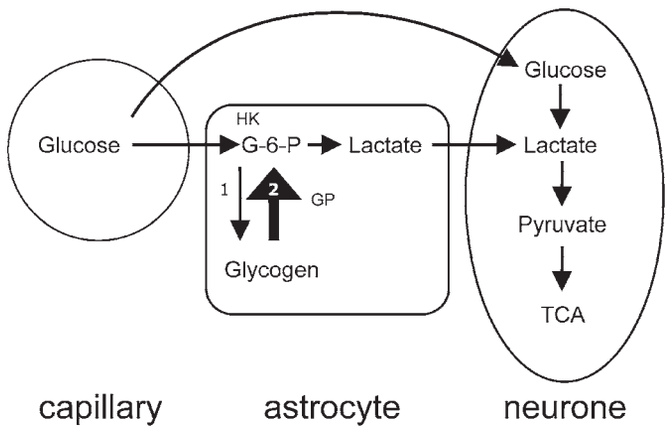

Figure 2. A model illustrating proposed glycogen metabolism during periods of increased tissue energy demand. Glucose is taken up by astrocytes and phosphorylated to glucose-6-phosphate (G-6-P) by hexokinase (HK). G-6-P is the initial substrate for glycolysis and is metabolised to lactate. G-6-P is also the initial substrate for glycogenesis when glucose is in excess (1). During periods of increased neuronal energy demand glycogen in degraded ultimately to G-6-P by glycogen phosphorylase (2), thus degradation of existing glycogen is a more energetically efficient way to rapidly deliver lactate to neurones than increased uptake and phosphorylation of glucose. It is hypothesised that glycogen can provide energy for finite periods of time to support localised brain function during periods of increased tissue energy demand. Neurones may also take up glucose directly (Chih *et al.*, 2001)

The future

Interest in brain glycogen has been re-awakened, encouraged by techniques that can measure *in vivo* brain glycogen for the first time, and evidence of a physiological role for brain glycogen. Key questions remaining unanswered include:

- How does the neurone signal to the astrocyte to initiate glycogen degradation?
- Does insulin regulate brain glycogen and, if so, is brain glycogen affected in type-1 diabetes?
- Is regional glycogen content correlated with specific brain activity?

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References

Brown AM, Baltan Tekkök S & Ransom BR (2003). Glycogen regulation and functional role in mouse white matter. *J Physiol* **549**, 501-512.

Chih C, Lipton P & Roberts EL (2001). Do active cerebral neurons really use lactate rather than glucose? *Trends Neurosci* **24**, 573-578.

Choi IY, Seaquist ER & Gruetter R (2003). Effect of hypoglycemia on brain glycogen metabolism *in vivo*. *J Neurosci Res* **72**, 25-32.

Choi IY, Tkac I, Ugurbil, K & Gruetter R (1999). Noninvasive measurements of [1-^{13}C]glycogen concentrations and metabolism in rat brain *in vivo*. *J Neurochem* **73**, 1300-1308.

Himwich WA (1959). Biochemical changes in the brain occurring during insulin hypoglycaemia. In *Insulin Treatment in Psychiatry*. Ed. Rinkel M & Himwich, HE, pp. **85-105**. Philosophical Library, New York

Swanson RA, Morton MM, Sagar SM & Sharp FR (1992). Sensory stimulation induces local cerebral glycogenolysis: demonstration by autoradiography. *Neuroscience* **51**, 451-461.

Vannucci SJ & Simpson IA (2001). Synopsis of the workshop entitled "Is lactate a nutrient for neurons" held at the Brain Energy Meeting in Trondheim, Norway. *J Neurosci Res* **66**, 821-823.

Investigating schemes for control of human movement

Mark Hinder and Theodore Milner's recent investigations favour control by formation of an internal dynamics model over equilibrium point control


Mark Hinder (left) and Theodore Milner

Intrinsic muscle properties have for a long time been known to stabilize limb posture and movement. Specifically, muscles act like springs, producing restoring forces when the position or trajectory of a limb is perturbed. Limb posture represents an equilibrium between the muscle forces and external forces acting on the limb. It was proposed that limb movement could be controlled by shifting this equilibrium point from one position to another. Two versions of what is commonly referred to as the equilibrium point hypothesis (EPH) were formulated – the λ-model and the α-model. The λ-model was based on the hypothesis that a fixed voluntary motor command represented a set of equilibrium positions called an invariant characteristic. Changing external torque would cause the joint angle to shift along the invariant characteristic until equilibrium was once again established (Feldman, 1966a, b; Feldman & Astrayan, 1965). A different invariant characteristic could be selected by changing the voluntary motor command, initiating movement to some new desired position. The voluntary motor command which selected the equilibrium position was referred to as the reciprocal command while the command which determined the stability of the equilibrium position, by changing the joint stiffness, was referred to as the co-contraction command. Over time, other features were added to the λ-model, such as velocity sensitivity. However, none of these features affect the essential tenet that the same final position should be achieved if the steady state forces are not altered, a property which we will refer to as equifinality. The α-model arose from observations of deafferented monkeys trained to move their heads or arms to specific target positions (Bizzi and Polit, 1978). Transient perturbations to the position of the head or arm were shown to have no effect on the ability to achieve the intended target position, leading to the hypothesis that the final equilibrium position was completely specified by the level of activation in agonist and antagonist muscles, another form of equifinality.

Although the simplicity of the EPH is very appealing, given that no inverse dynamics calculations are necessary, it seems unlikely that it can explain the large repertoire of human motor behaviour, particularly rapid adaptation to novel environmental dynamics. An alternative view that has gained much popularity is the concept of an internal model, which transforms desired motor behaviour into specific motor commands based on knowledge of mechanical properties of the limbs and environmental dynamics acquired through experience (Kawato, 1999). Support for this idea has been acquired by examining the after effects of adaptation to novel dynamic environments (Shadmehr & Mussa-Ivaldi, 1994; Lackner & Dizio, 1994; Conditt *et al.* 1997). After subjects have become proficient in compensating for novel dynamics, unexpected alteration of the dynamics results in perturbed trajectories that reflect the forces learned during adaptation. However, these studies have not been specifically designed to pit the hypothesis of internal model formation against EP control. Consequently, proponents of the EPH have argued that the results are not, in fact, incompatible with the EPH (Feldman *et al.* 1998).

We recently reported new evidence

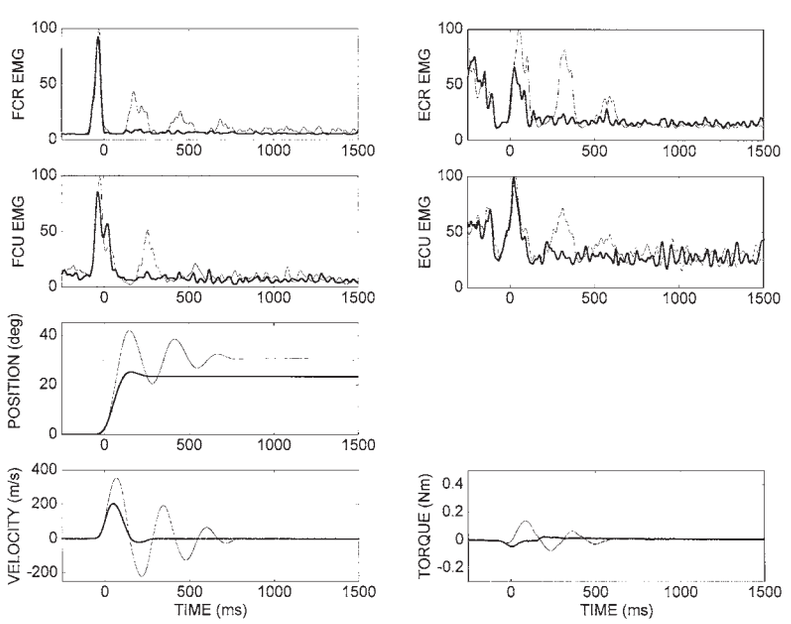

Figure 1. Comparisons of EMG (normalized to peak), position and velocity for perturbation trials (thick lines) with 100% reduction in assisting torque and the corresponding pre-perturbation trials (thin lines) for a single subject. Data were averaged for the 10 trials under each condition. In the perturbation trials, the final position fell substantially short of the target. The patterns of feedforward muscle activation, associated with movements to the target, were similar under the two conditions. The target is represented on the position plot as a dashed horizontal line.

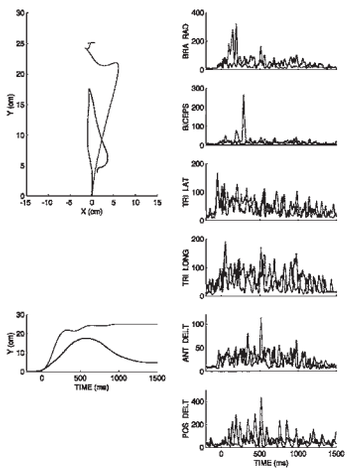

Figure 2. Position and EMG for a horizontal arm movement after adapting to a force field, which assisted movement in proportion to velocity (thin line). On the next movement in the same direction, the force field was unexpectedly inactivated (thick line). The movement reversed direction prior to reaching the target and stopped closer to the initial position than to the target position. This occurred despite an apparent reduction in the EMG of antagonist muscles (brachioradialis: BRA RAD, biceps and posterior deltoid: POS DELT) There did not appear to be any difference in the activity of agonist muscles (triceps lateralis: TRI LAT, triceps longus: TRI LONG and anterior deltoid: ANT DELT).

that supports the formation of an internal model of the required task dynamics but not EP control (Hinder & Milner 2003). We designed an experiment where equifinality would not be predicted by internal model formation. Briefly, we considered a single degree-of-freedom wrist flexion movement in which subjects moved to a target while assisted by a velocity dependent torque. Following extensive learning, the strength of the assisting torque was unexpectedly reduced by 25, 50, 75 or 100% on randomly selected trials, prior to movement onset. Subjects consistently stopped short of the target on these perturbed trials (Fig. 1). The undershoot was directly proportional to the reduction in the assisting torque. EMG analysis suggested that subjects generated the same feedforward motor commands whether or not the movement was perturbed. Consequently, the muscle torque was insufficient to drive the limb to the intended final position on the perturbed trials without the full assisting torque.

This result is entirely consistent with formation of an internal model, but would not be predicted under the EPH. Under the EPH, the central nervous system specifies an equilibrium trajectory, which is effected by a continuous shift in the instantaneous equilibrium position. Ghafouri and Feldman (2001) recently claimed that this trajectory reaches its final position at approximately the time of the peak movement velocity. This would imply that the unexpected increase in load should have produced an increase in agonist muscle force sufficient to drive the wrist to the intended final position. Although we have only shown that the EPH does not hold under one specific condition, we plan to further investigate the generality of our finding. In particular, we believe that equifinality is more likely to be achieved if unexpected changes in dynamics assist movement or if stretch reflex gain is high.

The results of a similar experiment, examining reaching movements in the horizontal plane, also suggest that equifinality may not hold when an internal model has been formed (unpublished observations). In this experiment, subjects adapted to a force which assisted motion regardless of movement direction (negative damping). For most directions subjects adapted by increasing the stiffness of the arm by means of co-contraction. In these cases, equifinality was achieved when the assisting force was unexpectedly eliminated. However, for forward reaches, which involved the least co-contraction, several subjects actually reversed direction and stopped closer to the start than the target position (Fig. 2). This suggests that not only was the agonist muscle force no longer adequate to fully extend the arm, but the antagonist muscle force that had been used to oppose the assisting force and decelerate the arm, was now sufficient to reverse the movement direction.

We expect proponents of the EPH to continue to raise objections to such counter examples, although formulations of the EPH, which can account for adaptation to novel dynamics, require that increasingly complex control signals be learned (Gribble & Ostry, 2000). Furthermore, a recent theory of internal model formation, which includes impedance control (co-contraction commands), is able to circumvent the need for complex inverse dynamics calculations (Franklin *et al.* 2003). It may only be a matter of time before the EPH gives way to the rapidly evolving concepts of internal model formation.

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References

Asatryan DG & Feldman AG (1965). Functional tuning of the nervous system with control of movement or maintenance of a steady posture-I. Mechanographic analysis of the work of the joint or execution of a postural task. *Biophys* **10**, 925-935.

Bizzi E & Polit A (1978). Processes controlling arm movements in monkeys. *Science* **201**, 1235-1237.

Conditt MA, Gandolfo F, Mussa-Ivaldi FA (1997). The motor system does not learn the dynamics of the arm by rote memorization of past experience. *J Neurophysiol* **78**, 554-560.

Dizio P & Lackner JR (2001). Coriolis-force-induced trajectory and endpoint deviations in the reaching movements of labyrinthine-defective subjects. *J Neurophysiol* **85**, 784-789.

Feldman AG, Ostry DJ, Levin MF, Gribble PL & Mitnitski AB (1998). Recent tests of the equilibrium-point hypothesis (lambda model). *Motor Control* **2**,189-205.

Feldman AG (1966a). Functional tuning of the nervous system with control of movement or maintenance of a steady posture-II. Controllable parameters of the muscle. *Biophys* **11**, 565-578.

Feldman AG (1966b). Functional tuning of the nervous system with control of movement or maintenance of a steady posture-III. Mechanographic analysis of execution by man of the simplest of motor tasks. *Biophys* **11**, 766-775.

Franklin DW, Osu R, Burdet E, Kawato M & Milner TE (2003). Adaptation to stable and unstable dynamics as achieved by combined impedance control and inverse dynamics model. *J Neurophysiol* (in press)

Ghafouri M & Feldman AG (2001). The timing of control signals underlying fast point-to-point arm movements. *Exp Brain Res* **137**, 411-423.

Gribble PL & Ostry DJ (2000). Compensation for loads during arm movements using equilibrium-point control. *Exp Brain Res* **135**, 474-782.

Hinder MR & Milner TE (2003). The case of an internal dynamics model versus equilibrium point control in human movement. *J Physiol* **549**, 953-963.

Kawato M (1999). Internal models for motor control and trajectory planning. *Cur Opin Neurobiol* **9**, 718-727.

Lackner JR & Dizio P (1994). Rapid adaptation to coriolis force perturbations of arm trajectory. *J. Neurophysiol* **72**, 299-313.

Shadmehr R & Mussa-Ivaldi FA (1994). Adaptive representation of dynamics during learning of a motor task. *J Neurosci* **14**, 3208-3224.

MGF: a local growth factor or a local tissue repair factor?

Combining physiological and molecular biology methods have indicated how a factor expressed by stressed muscle induces local muscle fibre repair and adaptation

Geoff Goldspink

Mechano Growth Factor (MGF) is derived from the insulin-like growth factor (IGF-I) but its sequence differs from the systemic IGF-I produced by the liver. MGF is expressed by mechanically overloaded muscle and is involved in tissue repair and adaptation. It is expressed as a pulse following muscle damage and is apparently involved in the activation of muscle satellite (stem) cells. These donate nuclei to the muscle fibres that are required for repair and for the hypertrophy processes which may have similar regulatory mechanisms.

Cloning of MGF

About 10 years ago our group set about cloning the growth factor(s) involved in the local regulation of muscle mass using a technique known as differential display. For this purpose we needed to have an animal model in which we could make a muscle grow rapidly. Previous work had shown that if the tibialis anterior in the mature rabbit was electrically stimulated whilst held in the stretched position by plaster cast immobilisation it increased in mass by 35% within seven days (Goldspink *et al.* 1992). The RNA in the stretch/stimulated muscles increased considerably but most of this was ribosomal RNA. Using various oligonucleotide primers and the RT-PCR technique, it was possible to detect RNA transcript that was only expressed in the stretched/stimulated muscle and not in resting control muscles. This was cloned and sequenced and it became evident that it was derived from the insulin-like growth factor gene by alternative splicing. The terminology of the IGF-I splice variants is a problem when attempting to apply it to non hepatic tissues (Hameed *et al.* 2003a). Therefore we named this newly discovered splice variant mechano growth factor (MGF) as it is expressed in response to mechanical stimulation (Yang *et al.* 1996, McKoy *et al.* 1999) and as it has a different downstream sequence to the liver type of IGF-I (Figure).

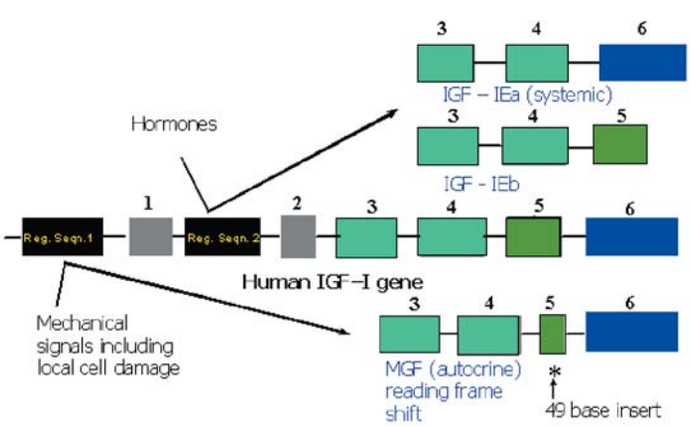

Figure. Shows the way in which the IGF-gene is spliced in muscle as a result of exercise and/or muscle damage (Regulatory Sequence 1) and hormones (Regulatory Sequence 2). In human muscle a 49 base insert changes the reading frame in MGF resulting in a different carboxy peptide. Hormones upregulate the expression of the IGF-I gene particularly the IGF-IEa. Muscle also expresses an IGF-IEb form but its function is not known. The IGF-receptor domain is included in all these splice variants and is encoded by exons 3 and 4.

Different products depending on the splicing of the IGF-I gene

The systemic type of IGF-I (IGF-IEa) is expressed constitutively by many tissues, including skeletal muscle, but the mRNA of MGF is not detectable or barely detectable unless the muscle is exercised and/or damaged. The sequence of MGF has a 52 base insert in the rat and 49 base insert in the human in exon 5. As these inserts are not multiples of three, the 3´ downstream sequence is different in MGF to that of IGF-IEa. This reading frame shift has important functional consequences as the carboxy peptide is involved in the recognition of the binding proteins that stabilize and target these growth factors. Also, in the case of MGF, this part of the peptide acts as a separate growth factor involved in initiating muscle satellite (stem) cell activation in addition to its IGF-I receptor domain which increases protein synthesis.

As well as having a different carboxy peptide sequence, MGF expression kinetics are different to those of IGF-IEa as shown by Gregory Adam's group in the USA who found that MGF is expressed earlier than IGF-IEa in response to exercise. Hill & Goldspink (2003) showed that following muscle damage MGF is produced as a pulse lasting a day or so followed by the splicing of the IGF-I gene towards IGF-IEa production that continues for a longer time.

Local damage repair and/or hypertrophy?

Intramuscular injection of its cDNA inserted into a plasmid vector demonstrated that MGF is a potent inducer of muscle hypertrophy. This resulted in a 25% increase in the fibre cross-sectional area of the injected muscle within two weeks (Goldspink, 2001). Similar experiments have also

been carried out using liver IGF-IEa cDNA in viral constructs which resulted in a 25% increase in muscle mass, but this took over four months to develop (Musaro *et al*. 2001). *In vivo* experiments in which muscles of the rat were subjected to mechanical damage or injection of a myotoxic agent also demonstrated (Hill & Goldspink, 2003) that MGF precedes muscle satellite (stem) cell activation. This is in accord with the finding that when skeletal muscle cells in culture, were either transfected with the MGF cDNA or were treated with the MGF carboxy peptide they increased in number but stayed as monocleated myoblasts Yang & Goldspink, 2002). It appears that MGF plays a dual role in inducing satellite cell activation as well as protein synthesis and this is probably why it is much more potent than the liver type or IGF-IEa for inducing rapid muscle hypertrophy.

As muscle repair and hypertrophy appear to involve the same signalling, the question still needs to be answered as to whether local damage is a prerequisite for muscle hypertrophy? Therefore the saying 'no gain without pain' may have a physiological basis.

Failure to maintain muscle loss in ageing and disease

Muscle loss (sarcopenia) is one of the most obvious effects of ageing. The work of Owino *et al*. (2001) indicated that muscles in old rats when surgically overloaded were much less able to express MGF than those in younger animals. Hameed et al. (2003b) reported that this was the case for elderly male volunteers as compared to muscles of young men. None of the other parameters measured showed a marked age-related decline although it has previously been known that circulating growth hormone levels in the over 70s are much lower than those in teenagers (Rudman *et al.* 1981). Growth hormone is known to upregulate the IGF-I gene. With Michael Kjaer's group in Copenhagen (Hameed *et al*. 2003c) we have recently found that administration of growth hormone combined with resistance exercise considerably improved MGF levels and increased cross-sectional area of the muscles in the elderly. Muscle loss is a major problem in certain hereditary diseases. In dystrophic muscles it seems that there is an inability to produce MGF in response to mechanical overload (Goldspink *et al.* 1996). The systemic type of IGF-IEa is also produced by muscle, including dystrophic muscle, but MGF is apparently required to ensure muscle fibre repair and survival. Therefore the role of the IGF-I splice variants including MGF seems to be very relevant to understanding the aetiology and the development of possible treatment in these muscle wasting conditions including sarcopenia.

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References

Adams GR (2002) Exercise effects on muscle insulin-signalling and action. Invited Review. Autocrine/paracrine IGF-I and skeletal muscle adaptation. *J Appl Physiol* **93**, 1159-1167.

Goldspink G, Scutt A, Loughna P, Wells D, Jaenicke T & Gerlach G-F (1992). Gene expression in skeletal muscle in response to mechanical signals. *Am J Physiol* **262**, R326-R363.

Goldspink G, Yang SY, Skarli M & Vrbova G (1996). Local growth regulation is associated with an isoform of IGF-I that is expressed in normal muscles but not in dystrophic muscles. *J Physiol* **495**, 162.

Goldspink G (2001). *Method of treating muscular disorders*. United States Patent No. US 6,221,842 B1.

Hameed M, Orrell RW, Cobbold M, Goldspink G & Harridge SDR (2003a) Clarification. *J Physiol* **549**, 995.

Hameed M, Orrell RW, Cobbold M, Goldspink G & Harridge SDR (2003b). Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *J Physiol* **547**, 247-254.

Hameed M, Lange KHW, Andersen JL, Schjerling P, Kjaer M, Harridge SDR & Goldspink G (2003c). The effect of recombinant human growth hormone and resistance training on IGF-I mRNA expression in the muscles of elderly men. *J Physiol*, in press.

Hill M & Goldspink G (2003). Expression and splicing of the insulin-like growth factor gene in rodent muscle is associated with muscle satellite (stem) cell activation following local tissue damage. *J Physiol* 549, 409-418.

McKoy G, Ashley W, Mander J, Yang SY, Williams N, Russell B & Goldspink G (1999). Expression of insulin-like growth factor-I splice variant and structural genes in rabbit skeletal muscle induced by stretch and stimulation. *J Physiol* **516**, 583-592.

Musaro A, McCullagh K, Paul A, Houghton I, Dobrowolny G, Molinaro M, Barton ER, Sweeney HL & Rosenthal N (2001). Localized IGF-I transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet* **27**, 195-200.

Owino V, Yang SY & Golsdpink G (2001). Age-related loss of skeletal muscle function and the inability to express the autocrine form of insulin-like growth factor-1 (MGF) in response to mechanical overload. *FEBS Lett* **506**, 259-263.

Rudman D, Kutner MH, Rogers CM, Lubin MF, Fleming GA & Bain RP (1981). Impaired growth hormone secretion in the adult population: relation to age and adiposity. *J Clin Invest* **67**, 1361-1369.

Yang SY & Goldspink G (2002). Different roles of the IGF-IEc peptide (MGF) and mature IGF-I in myoblast proliferation and differentiation. *FEBS Lett* **522**, 156-160.

Yang SY, Alnaqeeb M, Simpson H & Goldspink G (1996). Cloning and characterisation of an IGF-I isoform expressed in skeletal muscle subjected to stretch. *J Muscle Res Cell Motil* **17**, 487-495.

Austin Elliott, pictured left 'in deep thought' (his words), contemplates his future as the new Editor of *Physiology News* from January 2004. To ease his path, unsolicited articles are always welcome for consideration by the Editorial Group.

Please send any suggestions to lrimmer@physoc.org or to any members of the Editorial Group (see Contents page for details). As noted elsewhere, articles submitted under the 'Unbelievable' column banner, and subsequently published, will attract a small fee.

Finally, any members interested in joining the Editorial Group should contact Austin at:

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How mutations in myosin affect the heart

Half of all cases of familial hypertrophic cardiomyopathy are associated with a mutation in myosin. Here, Sarah Calaghan and Michelle Peckham discuss whether it is a loss or a gain of function of mutant myosin that results in hypertrophy of the heart

Sarah Calaghan (top) and Michelle Peckham

For every 500 people reading this article, one person will have a disease known as familial hypertrophic cardiomyopathy (FHC) in which the heart is enlarged without an obvious cause, such as hypertension. It is now known that FHC is due to mutations in one of several proteins found in the cardiac muscle cell, and for a person with FHC there is a 50% chance that this mutation is in the contractile protein myosin. In this article we discuss the reasons that mutations in myosin cause enlargement of the heart (hypertrophy). Although many people with FHC have no symptoms of disease, hypertrophy places them at risk of fatal disturbances in cardiac rhythm. Indeed, FHC attracts a great deal of attention because it is the most common cause of sudden cardiac death in the young.

An introduction to myosin

Myosin is found in the thick filaments of cardiac, skeletal, and smooth muscle. It is a protein that has two globular heads about 16nm long attached to a tail that is about 160nm long (Fig. 1A). The tails pack together to form the backbone of the thick filament, and the heads stick out from the filament to interact with actin

It is the interaction of myosin in the thick filaments with actin in the thin filaments that causes filament sliding, and hence muscle contraction. This interaction is driven as a result of ATP hydrolysis by myosin. After myosin hydrolyses ATP to ADP and phosphate, it binds to actin and actin accelerates the release of phosphate. Phosphate release is closely coupled to the power stroke, in which myosin heads pull on the actin filament to produce force or movement. With the arrival of numerous structures for myosin since the early 1990s, we are now starting to understand how the structure of myosin might change during the power stroke (Houdusse & Sweeney, 2001). Release of phosphate is closely coupled to the closing of the actin binding cleft as myosin forms a tight hold on actin, and to a tilting or bending of the lever which is connected to the myosin filament. The tilting of the lever is generated through part of the myosin called the converter domain (Fig. 1B), which connects the ATP pocket to the lever, and the lever rotates around a pivot found just before the converter. At the junction between the converter and the lever a few residues have a relatively unstable conformation forming a pliant region which allows the lever to tilt independently of converter movement.

In FHC, over 50 mutant alleles have been described in the β-myosin heavy chain gene; most mutations are located in the actin- and ATP-binding sites in the motor domain, in the converter domain and in the lever arm.

Myosin in the heart

In the adult human heart, two isoforms of myosin are expressed. The predominant isoform is β-myosin heavy chain, but a small amount of α-myosin heavy chain is also present. These 2 isoforms can be distinguished on the basis of the speed with which they hydrolyse ATP; the α isoform has 2-3 times the actin-activated ATPase activity of the β-isoform. Myosin isoform expression is plastic and can change throughout life, as a result of disease, or merely as a consequence of getting older.

How do myosin mutations cause hypertrophy?

We know that mutations in myosin have negative consequences for the heart, yet, surprisingly, it is still not clear how the mutations result in cardiac hypertrophy. For example, sarcomeric disarray is known to occur in muscle that contains mutant myosin. Does this mean that mutant myosin is not incorporated normally into the sarcomere, causing aberrations of contractility that lead to hypertrophy? This possibility has been excluded because it has been shown that changes in contractility precede sarcomeric disarray in muscle expressing mutant myosin. It is now thought that it is changes in the contractility of the muscle that provide the primary trigger for the enlargement of the heart in FHC (Roberts & Sigwart, 2001).

Do myosin mutations help or hinder?

Given that changes in contractility are the primary trigger for hypertrophy in FHC, the pivotal question is whether there is a *loss* or *gain* of function of myosin. It was initially assumed that it was a depression of contractility (loss of myosin function) caused by FHC mutations that resulted in a compensatory hypertrophy (i.e. the heart gets bigger to compensate for the decline in force/power). In support of this, many of the earlier studies in this field observed a slowing of the biochemical and mechanical properties of mutant myosin. More recently, however, the

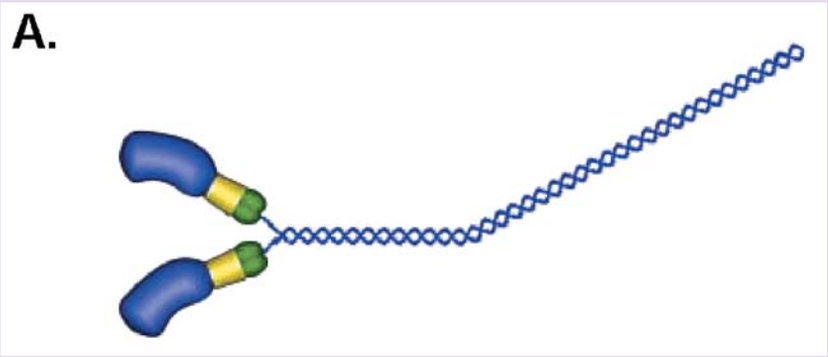
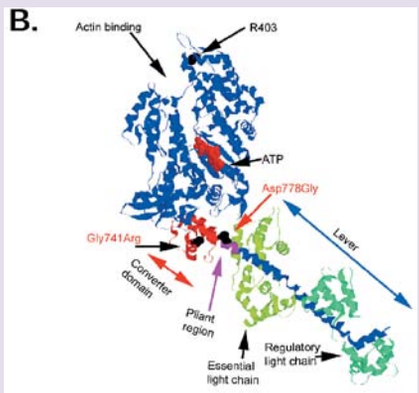


Figure 1. The structure of myosin

A (above). A cartoon of myosin showing 2 myosin heavy chains (blue) associating to produce a long coiled-coil tail attached to globular heads via a neck region. The essential light chain (yellow) and regulatory light chain (green) bind to the neck of the myosin heavy chain. B (below). A ribbon structure of the globular head, or motor domain of scallop myosin (also called subfragment 1) in the 'pre-power stroke' conformation. The globular head consists of a heavy chain (in blue) and two light chains (in yellow and green). The ATP molecule is shown in orange. The converter domain (purple) and pliant region (red) signal changes in the ATP binding pocket to the lever arm (in blue), which connects the pliant region to the thick filament. The lever arm bends around a pivot point (not shown) just before the converter domain. The residue R403 (black) forms part of the actin binding site, and is commonly mutated in FHC. The positions of the two mutations Gly741Arg (in the converter domain) and Asp778Gly (at the start of the pliant region) are shown (black).



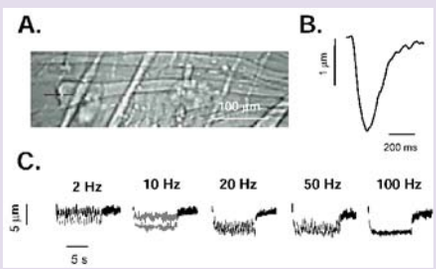


Figure 2. Measuring contractile properties of myotubes expressing mutant myosin

A. The dark/light boundaries at the end of cultured myotubes were tracked using a video-edge detection system.

B. When field stimulation was applied (1 Hz) the myotubes contracted as shown in the representative trace.

C. The relationship between shortening and frequency of stimulation was measured to determine the frequency at which individual contractions fuse, or tetanise. This depends on the duration of the contraction which in turn gives an index of the kinetics of shortening and relaxation of the myotube. In B and C a downward deflection represents shortening.

notion has been raised that it may actually be hypercontractility (gain of myosin function) that causes hypertrophy as a result of the increased energy demand of the myocardium (Moss & Periera, 2000).

Take for example the Arg403Gln mutation, located in the actin binding site of the myosin head (Fig. 1B). This is the most malignant and widely studied FHC mutation in myosin. Early studies from the 1990s suggested that it is a decline in the functional properties of R403Q myosin that triggers FHC in patients with this mutation, whereas more recent work has revealed a gain in function of R403Q myosin (see Lowey, 2002 for a review). The reasons for these seemingly contradictory data may lie in the different ways that myosin function is assessed.

Ways of studying myosin function

Myosin function can be assessed *in vitro*, or *in situ* in myocytes, muscle or the intact heart. Many *in vitro* studies use the actin sliding filament assay, which tracks the movement of fluorescently-labelled actin filaments generated by myosin molecules immunoadsorbed onto a fixed surface. The pulling action of a single myosin molecule attached to actin can be investigated using the optical trap. Actin-activated ATPase activity can also be measured. For *in vitro* techniques such as these, myosin may be extracted from the muscles of patients with FHC, or from transgenic animals. More recently, mutant myosin has been produced using baculovirus/insect expression systems. There are a number of problems with these techniques. The quality and quantity of myosin preparations can have a profound effect on myosin function. In addition, many studies do not use β-myosin heavy chain (Lowey, 2002). Most transgenic work is performed with mice, which express α- rather than β-myosin. It is also much more difficult to produce striated muscle myosins using expression systems, so smooth or non-muscle myosins are used, which can differ markedly from the β-myosin heavy chain affected in FHC.

Myosin can be studied *in situ* by measuring parameters of contractility in myocytes, or diastolic and systolic function of the intact heart from transgenic mice. As β-myosin heavy chain is also expressed in slow twitch skeletal muscle, the contractile properties (maximum velocity of shortening, isometric force generation, power output) of skeletal muscle fibres from patients with FHC can also be used to provide an index of myosin function. One problem associated with these techniques is that there may be concomitant disorganisation of the sarcomere, or compensatory changes in other proteins besides myosin, which can complicate the interpretation of results.

A novel and direct way to look at mutant myosin function

We have recently used a novel and direct approach to examine the functional impact of mutations in myosin heavy chain. This technique has many advantages over those described above. We used myotubes, which are developing muscle fibres, formed from the fusion of mouse myoblasts (muscle precursor cells).

Myotubes do not express β-cardiac myosin, but rather a fast embryonic myosin. We grew myotubes in culture and transfected some of these with wild-type β-cardiac myosin and others with mutant β-cardiac myosin. A week after differentiation, β-cardiac myosin (wild-type or mutant) is expressed at ≈25% of the total level of myosin. At this stage we looked at the effect of mutant myosin on the kinetics of contraction of myotubes stimulated at 1 Hz, and on the relationship between the frequency of stimulation and myotube shortening (see Fig. 2 for more details).

We believe that our method of assessing mutant myosin function circumvents many of the problems associated with other techniques. We are not relying on the quality of an *in vitro* preparation of myosin, and we are using β-cardiac myosin which is the isoform of myosin found in the human heart. In addition, from earlier work we know that the mutant myosin we are using incorporates normally into the sarcomere and does not cause sarcomeric disarray (Miller *et al.* 2000). It also seems unlikely that there is time for compensatory changes in proteins other than myosin to occur in these myotubes.

How do converter domain and pliant region mutations affect contractility?

To date, the hypocontractility-hypercontractility debate has focused on the FHC mutation R403Q located in the motor domain. However, there are mutations in other regions of the myosin molecule that also cause FHC. We elected to look at the impact of 2 mutations: Gly741Arg which is in the converter domain, and Asp778Gly which is at the start of the pliant region, at the junction between the converter domain and the lever arm (Fig. 1B). These mutations have been found in patients with FHC (Harada *et al.* 1993; Arai *et al.* 1995).

When we looked at the differences between myotubes expressing the wild-type β-cardiac myosin and the mutant β-cardiac myosin, it was interesting to note that, although both mutations are closely located in the myosin molecule, they have very different consequences for contraction. These are summarised in the Table. We found that the D778G mutation dramatically increased contraction kinetics and tetanic fusion frequency, compared with that seen in myotubes expressing wild-type β myosin. However, the G741R mutation did not increase the rate of contraction or relaxation at 1 Hz stimulation. There was an increase in fusion frequency with this mutation, but it was less than that seen with the D778G mutation.

The increase in the kinetics of contraction and relaxation that we saw in myotubes expressing D778G myosin is consistent with a gain of function for myosin with this mutation. In fact the contractile parameters that we measured in myotubes expressing D778G myosin were the same as those recorded from untransfected myotubes, suggesting that the speed of D778G myosin is similar to that of the fast endogenous myosin of the myotube.

The only effect we saw in myotubes expressing G741R myosin was an increase in the tetanic fusion frequency; contractile kinetics at 1 Hz stimulation were unaffected. This shows that determination of fusion frequency is a more sensitive way to detect subtle changes in kinetics than the measurement of the rate of activation and relaxation at one frequency of stimulation. Our data suggest that the G741R mutation also causes a gain in function of myosin, but to a much smaller degree than that seen with D778G myosin.

How do our data compare with what little is known about these 2 mutations? Our results for D778G are consistent with a recent study of the equivalent mutation in expressed smooth muscle myosin which reported enhanced myosin activity (Yamashita *et al.* 2000). For G741R, the only report is of reduced V_{max} and isometric force generation in slow skeletal muscle from FHC patients with this mutation, suggesting a loss of myosin function (Lankford *et al.* 1995).

To summarise

Using this novel and direct approach, we are confident of our conclusion that it is an increase in energy demand associated with hypercontractility that is the trigger for hypertrophy in FHC associated with both G741R and D778G mutations in myosin. We would predict that the phenotype of patients with mutation D778G is likely to be more severe that that of patients with

Table. Mutations in the converter domain (G741R) and pliant region (D778G) of myosin, have very different effects on myotube contraction

	Time to peak of contraction	Time for half relaxation	Maximum velocity of shortening	Fusion frequency (Hz)
WT β-myosin	—	—	—	38 Hz
D778G β-myosin	–24 %	–24 %	+31 %	>200 Hz
G741R β-myosin	+1 %	+4 %	0 %	164 Hz

Expression of D778G myosin markedly increased the speed of contraction and relaxation and V_{max} during myotube stimulation at 1 Hz, and also increased the tetanic fusion frequency compared with wild-type (WT) β-cardiac myosin. G741R myosin did not increase the rate of contraction or relaxation during stimulation at 1 Hz, but it did increase fusion frequency compared with wild-type β-cardiac myosin. % values represent the difference from myotubes containing wild-type β-cardiac myosin.

mutation G741R. Interestingly, mutations in other parts of the myosin molecule, and indeed in other sarcomeric proteins, are also thought to be 'energy cost' diseases. Understanding the trigger for hypertrophy in FHC is one step towards finding a treatment for this disease.

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Acknowledgements
The experiments detailed in this report were done in collaboration with Gaynor Miller, Joanne Maycock and Ed White from the School of Biomedical Sciences at Leeds and have been published in the *Journal of Physiology* (Miller *et al.* (2003) **548**, 167-174). We'd like to thank Tim Lee for help with Figure 1, and Peter Knight for valuable discussion of myosin structure. Our work is funded by the British Heart Foundation.

References

Arai S, Matsuoka R, Hirayama K, Tamura M, Aozama T, Kimura M, Imamura S, Furutani Y, Joh-O K, Kawana M, Takao A, Hosoda S & Momma K (1995). Missense mutation of the beta-cardiac myosin heavy chain gene in hypertrophic cardiomyopathy. *Am J Med Genet* **58**, 267-276.

Harada H, Kimura A, Nishi H, Sasazuki T & Toshima H (1993). A missense mutation of cardiac beta-myosin heavy chain gene linked to familial hypertrophic cardiomyopathy in affected Japanese families. *Biochem Biophys Res Comm* **194**, 791-798.

Houdusse A & Sweeney HL (2001). Myosin motors: missing structures and hidden springs. *Curr Opin Struct Biol* 11:182-194.

Lankford EB, Epstein ND, Fananapazir L & Sweeney HL (1995). Abnormal contractile properties of muscle fibres expressing beta-myosin heavy chain gene mutations in patients with hypertrophic cardiomyopathy. *J Clin Invest* **95**,1409-1414.

Lowey S (2002). Functional consequences of mutations in the myosin heavy chain at sites implicated in familial hypertrophic cardiomyopathy. *Trends Cardiovasc Med* **12**, 348-354.

Miller G, Colegrave M & Peckham M (2000). N232S, G741R and D778G β-cardiac myosin mutants, implicated in familial hypertrophic cardiomyopathy, do not disrupt myofibrillar organisation in cultured myotubes. *FEBS Lett* **486**, 325-327.

Moss RL & Periera JS (2000). Enhanced myosin function due to a point mutation causing a familial hypertrophic cardiomyopathy. *Circ Res* **86**, 720-722.

Roberts R & Sigwart U (2001). New concepts in hypertrophic cardiomyopathies, Part 1. *Circ* **104**, 2113-2116.

Yamashita H, Tyska MJ, Warshaw DM, Lowey S & Trybus KM (2000). Functional consequences of mutations in the smooth muscle myosin heavy chain at sits implicated in familial hypertrophic cardiomyopathy. *J Biol Chem* **275**, 28045-28052.

How does your gut grow?

The presence of food in the gut is a powerful driver of cell (and crypt) reproduction

Robert Goodlad

In whatever tissue system you are interested, the gut is likely to either be the largest or the second largest. It is the main endocrine organ and immune organ and is also our second brain. The hindgut is a serious bacterial culture system and it is a strange thought that there are more bacterial cells in the colon than human cells in the entire body.

My main view of the gut is as a cell renewal system and one can argue that it is the only tissue in which to study proliferation and growth control mechanisms. This is due to its accessibility and its well organised anatomy with the proliferative and functional zones being neatly separated. The lining of the intestine divides rapidly, giving it great powers of renewal and of adaptation.

This adaptability means we can easily perturb the system and soon see it respond. One can thus profoundly increase intestinal mucosal cell division by overfeeding or cause proliferative rates to plummet by starvation. Starvation obviously reduces the content of the gut lumen and similar falls in proliferation can be obtained by feeding animals intravenously, as the animal is fed but the gut is starved. A less elaborate model is to feed animals on low residue (elemental) diets. With these diets the work of digestion is all done in the front of the gut so that the distal small intestine and colon atrophy.

We have used the low residue diet in our recent paper in *Experimental Physiology* (Sasaki et al. 2003) to study the actions and interactions of two powerful growth factors, namely epidermal growth factor and keratinocyte growth factor. We used the crypt microdissection method (see Fig 1). Microdissection sounds very complicated, but is actually quite straightforward and is far quicker than scoring histological sections. One 'downside' of its productivity is that we are often tempted to quantify several sites of the gut! The method also avoids many of the problems associated with scoring sections and with simple proliferative or labelling indices, which we have shown on several occasions to miss significant events if both halves of these fractions change at the same time. For example we have shown that in intravenously fed rats the gut weight is dramatically reduced, as is crypt cell production; however, labelling indices showed no difference. This was a result of both the number of

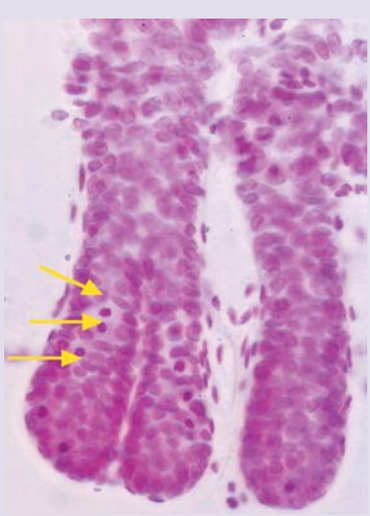

Figure 1. Microdissected mouse small intestinal crypts showing a normal crypt and a crypt in fission. Bulk stained tissue is stained with the Feulgen reaction and then gently teased apart under a dissecting microscope. A cover slip is then applied and gentle pressure causes the crypts to separate. Arrows show the condensed chromatin of vincristine-arrested metaphases, which are scored as a measure of crypt cell production.

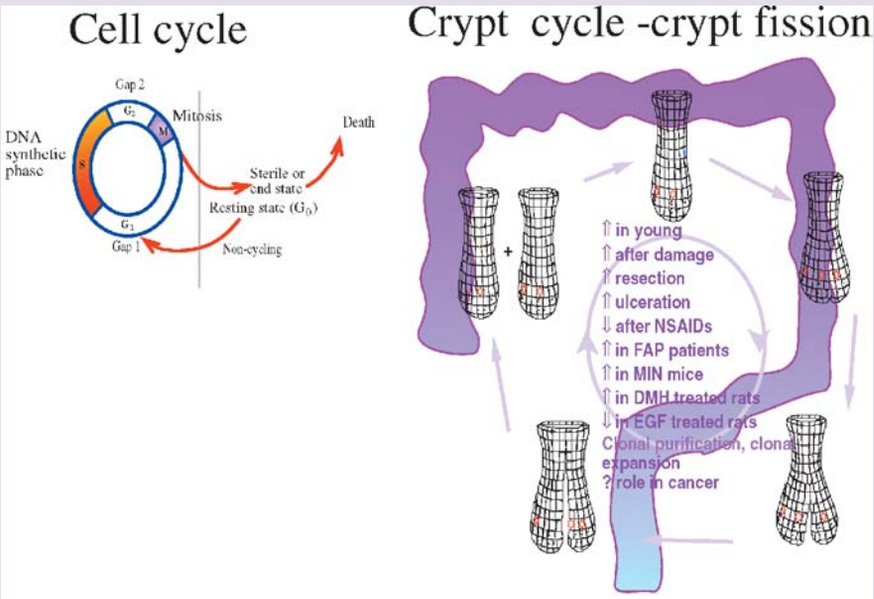

Figure 2. The crypt cycle. Small invaginations are seen at the base of the crypt, which then expand until the crypt 'unzips' to create two new crypts. While providing a valuable means of repair and regeneration, excessive fission is implicated in the clonal expansion of mutated crypts.

cells per crypt and the number of labelled cells decreasing together (Goodlad et al. 1992). If the labelling data was expressed as labelling per crypt the large proliferative changes become apparent, but it is far simpler to score mitotic figures or arrested metaphases directly in the 'microdisected' crypts. A further advantage of the method is that it can be used to quantify crypt fission (Fig. 2). Crypt fission is another way of altering intestinal mass, and although it is mainly seen in the young animal it persists throughout life and can play a role in adaptation following injury or trauma. It is also much increased in rodent and in human cancers (Wong et al. 2002).

When mice are fed an elemental diet there is a large fall in proliferation and in crypt fission, especially in the distal small intestine and colon (Sasaki et al. 2002). This shows the importance of luminal contents for stimulating the gut. Such effects of 'luminal nutrition' may occur directly or more likely they are a consequence of 'intestinal workload' and several gut hormones and growth factors are likely to be involved. Diet induced atrophy can be very useful from an experimental point of view, as the effects of various trophic agents are more readily seen. In the *Experimental Physiology* paper we have used the model to see what happens when EGF and KGF are given alone or in combination. Both growth factors increased proliferation and reduced fission, but when given together the effects (on some sites) were interactive. The ability to alter cell renewal in the gut may be very important, as prevention of atrophy could be useful in several clinical conditions. Lowered proliferation in patients fed elemental diets can reduce mucosal integrity and some diets are already supplemented with 'naturally occurring' growth factors. Patients with short bowel syndrome, ulcers or inflammatory bowel disease, babies with damaged guts and those at risk of mucusitis due to chemotherapy or radiotherapy may all benefit from such therapy; however, cell proliferation is also a two edged sword, as excessive proliferation or fission may increase the growth of cancerous cells.

Of course, such experiments may be occurring every time we eat. We have used similar elemental diets or low fibre semisynthetic diets to look at the effects of starving or feeding the colon by altering the content of dietary fibre and similar colonic substrates. Low fibre diets reduce cell proliferation in the colon and dietary fibre or similar fermentable substrates dramatically increase cell proliferation, but only when the bacteria are present. Thus it is the breakdown of fibre by the bacteria to produce short chain fatty acids that stimulates cell division in the colon. Paradoxically these acids, especially butyric acid, seem to have the opposite effects on cells *in vitro*. Thus whether all 'fibres' are necessarily a good thing is still a matter of intense debate especially in the light of recent epidemiological findings. Diets that are naturally high in fibre are beneficial for many reasons, but recent attempts to broaden the definition of fibre and increase fibre intake through the use of these fermentable substrates could well be harmful (Goodlad 2001; Goodlad & Englyst, 2001).

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References

Goodlad RA (2001). Dietary fibre and the risk of colorectal cancer. *Gut* **48**, 587-589.

Goodlad RA & Englyst HN (2001). Redefining dietary fibre: potentially a recipe for disaster. *Lancet* **358**, 1833-1834.

Goodlad RA, Lee CY & Wright NA (1992). Cell proliferation in the small intestine and colon of intravenously fed rats: effects of urogastrone-epidermal growth factor. *Cell Prolif* **25**, 393-404.

Sasaki M, FitzGerald AJ, Grant G, Wright NA & Goodlad RA (2002). Lectins can reverse the distal intestinal atrophy associated with elemental diets in mice. *Alimentary Pharmacology and Therapeutics* **16**, 633-643.

Sasaki M, FitzGerald AJ, Mandir N, Berlanga-Acosta J & Goodlad RA (2003). Keratinocyte growth factor and epidermal growth factor can reverse the intestinal atrophy associated with elemental diets in mice. *Experimental Physiology* **88**, 261 –267.

Wong WM, Mandir N, Goodlad RA, Wong BC, Garcia SB, Lam SK & Wright NA (2002). Histogenesis of human colorectal adenomas and hyperplastic polyps: the role of cell proliferation and crypt fission. *Gut* **50**, 212-217.

Brain food: the cerebral metabolic response to exercise

Following intense activation of the brain its carbohydrate uptake is enhanced, suggesting to Mads Dalsgaard that central fatigue relates to depletion of cerebral stores of glycogen

Mads Dalsgaard

In order for skeletal muscles to contract a complex chain of events has to be established within the central nervous system (CNS), including generating the idea and probably subconscious planning of learned movement involving adjustment of its force and direction, before the motoneurons are addressed. Only when performing a task that has not been practiced, or when a contraction is intense or repeated to fatigue, it is conscious or directed by a 'will'. In more complex tasks such as walking or running an even higher level of integration is required to maintain balance and to excite the cardiovascular and respiratory systems. Also, psychological factors come into play, such as verbal encouragement or critical feedback provided by the coach. Paradoxically, such external stimuli may in themselves induce fatigue as seemingly the brain has a limit to how much information it can process without compromising its ability to recruit the motor system. The influence of external stimuli on the brain's contribution to performance was already established in 1904 as Mosso coined the phrase 'mental fatigue' when a colleague of his could not accomplish the usual work with a finger ergograph after giving a lecture. Similar apparent central fatigue is established when subjects, after work performed until exhaustion with the eyes closed, become able to continue the work by opening their eyes. Equally, muscle strength and endurance may be enhanced if some 'diverting activity', i.e. calculations or activity of a different kind, is performed at the same time.

Cerebral blood flow and metabolism in exercise

With such complex integration of motor control, it would seem that brain metabolism, and in turn its blood flow, would be enhanced with physical activity. Yet, the pioneering observation from the time of Mosso's experiments by Atwater and Benedict which showed an increase in whole body metabolism during thinking has not been confirmed and may be ascribed to muscle tension when concentrating on a given task. With the introduction of the Kety-Schmidt technique global cerebral blood flow (gCBF) and metabolic rate of oxygen ($CMRO_2$) were found to remain remarkably stable in all conditions. Reductions in these variables are noted during deep sleep and hypoglycaemia, while only exercise in the heat increases $CMRO_2$. However, as demonstrated by Lassen and coworkers, such stability of gCBF and $gCMRO_2$ during mental activity reflects marked changes in the regional values while during exercise, these increases take place in areas of the brain that are not only involved in motor control but also in integration of sensory input, and control of ventilation and cardiovascular variables. It may be that activity in one or several parts of the brain is compensated by down-regulation of blood flow and metabolism in other parts of the brain. This notion would explain why it is difficult to concentrate on more than one thing at a time with respect to both intellectual performance and complex patterns of movement.

The cerebral metabolic ratio

In contrast to skeletal muscle where activity results in a disproportional increase in metabolism compared to blood flow supply (as inferred by a decrease in muscle oxygenation), CNS responds to activation with a larger increase in regional blood flow than the concomitant increase in $CMRO_2$, as detected both by near infrared spectroscopy, positron emission tomography and functional magnetic resonance. However, activated brain tissue appears to take up glucose out of proportion to its uptake of oxygen both on a local and a global level. Mental activity and visual stimulation decrease the global cerebral metabolic ratio (gCMR; O_2 uptake/glucose uptake) from the resting value of close to 6 to about 5.4 (Madsen *et al.* 1995).

With respect to exercise, a submaximal effort does not influence gCMR, while during maximal

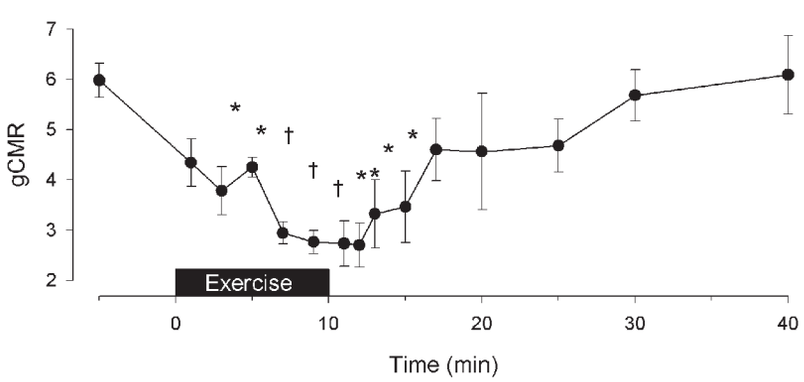

Figure 1. gCMR in response to exhaustive whole body exercise.
gCMR, cerebral uptake of O_2/(glucose ± lactate). Values are mean ± S.E. for 9 subjects. Different from to rest: * $P < 0.05$; † $P < 0.01$.

exercise it decreases to a low of ~ 3.7 (Dalsgaard *et al.* 2002) or to ~ 3 during whole body exercise, when lactate is also taken into consideration (Fig. 1). During prolonged submaximal exercise gCMR stays stable for as long as the workload seems relatively easy, but when the subject struggles to continue the work after perhaps 1–2 hours, gCMR decreases (Nybo *et al.* 2003). During intense exercise that requires the subject's instant attention, or with unfamiliar exercise such as arm-cranking, gCMR decreases from the onset of exercise. Moreover, it is a consistent finding that gCMR remains low for some five minutes after exercise.

We evaluated the influence of central command on gCMR during exercise with partial neuromuscular blockade (Dalsgaard *et al.* 2002). During pharmacologically-induced neuromuscular blockade, exercise at a given workload requires extra effort in order to recruit more muscle and compensate for the fraction of motor units that do not contribute to the contraction. In that situation, gCMR is reduced to ~ 5, or slightly less, compared to control maximal exercise, although the effort in both conditions is maximal. The moderate reduction of gCMR during exercise with neuromuscular blockade may be explained by an influence by sensory input from the muscles. The impact of such sensory stimuli on brain metabolism is addressed when thigh cuffs impede blood flow to the legs and cause severe pain (Dalsgaard *et al.* 2003). During exercise with muscle ischaemia, as with post-exercise muscle ischaemia, gCMR decreases to about 4.0–4.5. Thus, the very low gCMR developed during maximal exercise may reflect the combined effect of mental activity associated with central command and sensory input from the muscles on cerebral metabolism.

Energy sources for the brain

While it is evident that the gCMR decreases in response to a mental effort, including intense exercise, it is less clear why this happens. For the brain at rest oxygen and glucose are taken up in a ratio close to ~ 6, which implies that glucose undergoes complete oxidation and therefore is the primary substrate for the brain. A slightly lower gCMR of about ~ 5.7 is often noted at rest, consistent with the idea that part of the energy production results from glycolysis, which is also signified by a discrete lactate efflux from the brain. However, substrates other than glucose may serve as an energy source for the brain. Lactate becomes of value when the blood concentration increases as observed during cardiopulmonary resuscitation, insulin dependent diabetes mellitus, hypoglycaemia, and in particular when the brain is activated during exercise where lactate uptake may exceed that of glucose. Since the lactate taken up by the brain during and after exercise is not released back into the blood it is presumably metabolised. Alternatively, lactate taken up by the brain could accumulate in a distribution volume, but even after a marked cerebral uptake of lactate, it does not build up in the cerebrospinal fluid or within the brain as determined by magnetic resonance spectroscopy. Also, on a local level, cultures of brain cells, and especially neurons, metabolise lactate and it is preferred to glucose when the brain is recovering from hypoxia. In humans infusion of lactate prevents hypoglycaemic symptoms and reduces glucose metabolism.

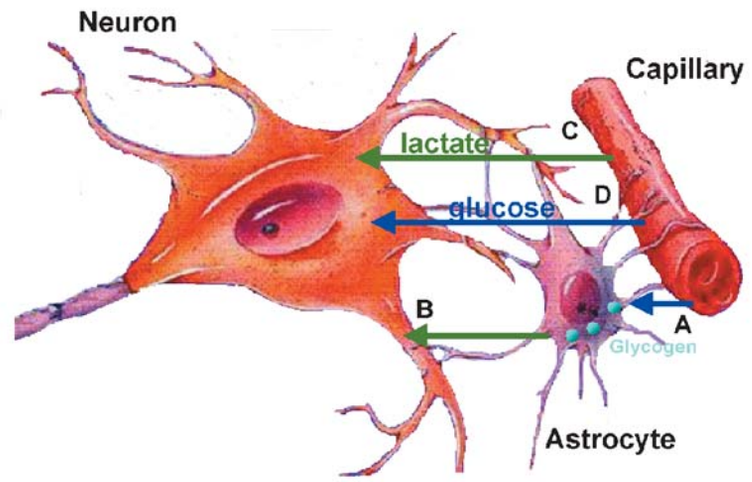

Figure 2. Schematic representation of a neuron, an astrocyte and a capillary, including their histological arrangement and the trafficking of substrates.
A, The 'lactate shuttle' implies that part of the glucose taken up by astrocytes maintains a glycogen level, and B, neuronal activity induces break down of glycogen to provide instant energy for the neuron as lactate is released. C, During exercise, lactate taken up from the blood may be provided directly to the neuron. D, The neuron requires glucose for de novo synthesis of neurotransmitters such as as glutamate.

The brain possesses the capacity to oxidise fatty acids and with their large carbon skeleton even a small cerebral uptake of fatty acids could challenge gCMR. However, with exercise the uptake of free fatty acids by the brain is negligible (Dalsgaard *et al.* 2002). Moreover, glycerol, glutamate, glutamine and alanine uptake from the blood is not of functional importance for whole brain metabolism during exercise. In addition, even though ketone bodies can serve as an energy source for the brain, particularly during a fast and in insulin dependent diabetes mellitus patients, this is not the case during exercise where plasma concentration remains low. Notwithstanding that the brain can utilise a variety of substrates if their availability increases, or if the supply of glucose is low, gCMR would remain stable if neuronal activity was fuelled by a balanced oxidative metabolism.

Glycogen and lactate metabolism

The uptake of carbohydrate in excess of oxygen, as implied by a reduced gCMR, provokes speculation as to

whether the brain metabolises glycogen. Besides lactate taken up from blood, neurons may be provided with lactate released from the breakdown of glycogen in neighbouring astrocytes (Fig. 2) (Brown *et al.* 2003, see also page 18). With cerebral activation in the rat, the cerebral glycogen store is diminished from the resting value of ~ 6–8 µmol glucose equivalents per gram, similar to the level found during tumour surgery in humans. After such depression of brain glycogen, its replenishment may reach a higher value than at rest ('super-compensation') a phenomenon also known to occur in skeletal muscle during recovery from prolonged exercise and in the rat brain after hypoglycaemia. Also lack of sleep affects the cerebral glycogen level supporting the notion that glycogen metabolism and generation of lactate form an integrated part of brain metabolism.

Changes in the level of glycogen within the brain may not be the only reason why gCMR decreases in response to intense activation. Accelerated glucose uptake and metabolism are likely to depend on accumulation of intermediates. Furthermore, glucose serves as precursor for amino acids and, after activation of the brain in the rat, about half of the decrease in gCMR has been ascribed to accumulation of glutamate and other metabolites. Glutamate has been suggested to be involved in central fatigue, but that is not supported by the arterio-venous difference over the brain when gCMR is low. However, it remains a possibility that brain metabolism of amino acids does not become manifest in blood within a time frame that affects gCMR during or immediately after exercise.

gCMR and central fatigue

The implication of a reduced gCMR during neuronal activation remains unknown. Intense cerebral activity causes the energy demand to exceed the production and a reduced glycogen level in the astrocytes could influence neuronal function. Conversely, the reduced gCMR after exhaustive exercise suggests glycogen repletion. If this occurs brain glycogen deposits appear to recover within ~ 5 min, and this time frame corresponds with the observation that following exhaustive exercise athletes are willing to continue the work after only a few minutes of recovery.

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References

Brown AM, Tekkok SB & Ransom BR (2003). Glycogen regulation and functional role in mouse white matter. *J Physiol* **549**, 501-512.

Dalsgaard MK, Ide K, Cai Y, Quistorff & Secher NH (2002). The intent to exercise influences the cerebral O_2/carbohydrate uptake ratio in humans. *J Physiol* **540**, 681-689.

Dalsgaard MK, Nybo L, Cai Y & Secher NH (2003). Cerebral metabolism is influenced by muscle ischaemia during exercise in humans. *Exp Physiol* **88**, 297-302.

Madsen PL, Hasselbalch SG, Hagemann LP, Olsen KS, Bulow J, Holm S, Wildschiodtz G, Paulson OB & Lassen NA (1995). Persistent resetting of the cerebral oxygen/glucose uptake ratio by brain activation: evidence obtained with the Kety-Schmidt technique. *J Cereb Blood Flow Metab* **15**, 485-491.

Nybo L, Nielsen B, Blomstrand E, Moller K & Secher NH (2003). Neurohumoral responses during prolonged exercise in humans. *J Appl Physiol* **95**, *1125-1131*

Managing magnitude and duration of amino acid availability as a strategy to optimize protein gain ?

The effects of the pattern of amino acid availability on protein metabolism suggest that this factor might be used to optimize protein gain in various circumstances

Left to right: Yves Boirie, Martial Dangin and Pierre Gachon

It has long been recognized that optimization of protein retention in various circumstances requires to consider numerous parameters such as nutritional factors. Main dietary parameters that influence protein metabolism include the amount, the composition and the digestibility of dietary proteins as well as the dose and the composition of non-protein sources (fat, carbohydrates). More recently, human studies have provided new insight into the factors modulating protein metabolism and consequently protein retention. The bio-availability of dietary amino acids over time is one of them. It can be modified either by adapting rate of digestion or changing pattern of feeding.

With respect to digestion rate, the two major bovine milk protein fractions, i.e. whey protein and casein, have quite different behaviour (Boirie *et al.* 1997). Whey protein that remains in a liquid form in the stomach is emptied into the duodenum and appears rapidly in the blood after intestinal absorption of its constitutive amino acids. By contrast casein, that makes a clot at low pH in the stomach, is emptied much more slowly and the rate of delivery of its amino acids in the blood is slower, lower and more prolonged. The pattern of amino acid availability, as reflected by plasma aminoacidemia, is similar to the one of the appearance of dietary amino acids. With fast digested proteins, such as whey protein, the elevation of amino acid availability is high but of short duration while with a slow one, such as casein, the increase is lower but more persistent. 'Fast' protein induces a transient stimulation of protein synthesis, a dramatic increase of amino acid oxidation and has

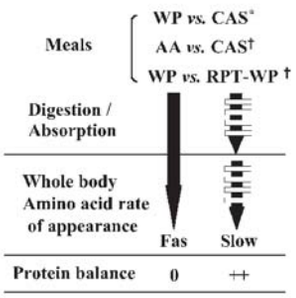

Figure. Schematic summary of the results related to the effects of protein digestion rate on protein metabolism. WP: whey protein, CAS: casein, AA: mixture of free amino acids mimicking casein composition, RPT-WP: repeated small meals made of whey protein given every 20 min (for 240 min). Adapted from Boirie *et al.* (1997) and Dangin *et al.* (2001)

minimal effect on proteolysis. It is quite different with a 'slow' protein: protein synthesis is unaffected, amino acid oxidation increases moderately and proteolysis is inhibited for a long period. Finally, protein balance is lower with a fast digested protein, than with a slow one (Fig.). Taken together, these results suggest that protein digestion rate affects protein gain by modulating amino acid availability (Boirie *et al*. 1997).

This interpretation is fully confirmed by two set of experiments (Dangin *et al*. 2001). Indeed, when casein ('slow' protein) is compared with a mixture of free amino acids mimicking the composition of casein (rapidly digested) or when a single whey protein meal ('fast' protein) is compared with a sequence of small meals made of whey protein and given every 20 minutes (paradigm for a 'slow' protein), the results are consistent with those above mentioned. Indeed, in the two pairs of studies, 'fast' digested meals stimulate protein synthesis and amino acid oxidation and induce a lower protein gain than "slow" digested ones that inhibit proteolysis durably and increase amino acid oxidation to a lower extent (Fig.). Since the meals differ only by their digestion rate, their amino acid content being identical, protein digestion rate independently modulates protein gain (Dangin *et al*. 2001).

The key factors responsible for the effects of protein digestion rate on protein anabolism appear to be mainly amino acid availability. Indeed, it is known that amino acid availability, within a physiological range, affects, as a dose dependent manner, protein synthesis and amino acid oxidation (Giordano *et al.* 1996). For proteolysis, the differential effects of 'slow and fast' proteins may be attributed to the duration of the hyperaminoacidemia, because amino acids are known to inhibit proteolysis, which is more prolonged with 'slow' than with 'fast' proteins. However, all these results have been obtained after ingestion of protein meals without any non-protein energy, factor known to possibly affect protein metabolism.

Modulation of amino acid availability can be obtained by ingesting 'slow or fast' protein, but it can also be modified by changing the pattern of feeding over the day. For example, it is known that total parenteral nutrition provided as bolus or as constant infusion affects plasma aminoacidemia and nitrogen balance according to the physiological state of the subjects. It has also been reported, in young healthy women (Arnal *et al*. 2000), a trend of higher nitrogen balance with a 'spread' feeding pattern, i.e. daily protein intake evenly distributed over four meals, than with a 'pulse' one (80% of the daily protein intake consumed at noon). Since one might consider that the spread feeding pattern induces a lower but more prolonged rise in plasma aminoacidemia than the pulse one, these results are in agreement with those obtained after 'slow and fast' protein ingestion. In contrast, in elderly women, the results are quite different: the pulse diet induces a better nitrogen balance (Arnal *et al*. 1999). This effect appears to be to age-related, since there is a greater efficiency of a 'fast' protein than a 'slow' one in improving protein gain in elderly men, in contrast with the younger group, when consuming mixed meals (Dangin *et al*. 2003). In addition, it is described that stimulation of protein synthesis of old muscle is impaired at low increment in amino acid availability, but that response can be normalized by high levels of amino acids and/or leucine.

Collectively, these studies support the general idea that the magnitude and the duration of change in amino acid availability regulate protein gain after meal ingestion. That factor can be controlled by choosing protein with specific digestion rates, adapting the infusion rate of nutrients or by changing protein feeding pattern. It may thus represent an adjunctive dietary strategy to optimize protein gain in various pathological circumstances. However, since these effects can be modulated by extrinsic factors such as age or metabolic stress or by intrinsic factors such as non protein nutrients in the meals, their implications for human nutrition warrant further examinations.

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References

Arnal MA, Mosoni L, Boirie Y, Houlier ML, Morin L, Verdier E, Ritz P, Antoine JM, Prugnaud J, Beaufrère B & Mirand PP (1999). Protein pulse feeding improves protein retention in elderly women. *Am J Clin Nutr* **69**, 1202-1208.

Arnal MA, Mosoni L, Boirie Y, Houlier ML, Morin L, Verdier E, Ritz P, Antoine JM, Prugnaud J, Beaufrère B & Mirand PP (2000). Protein feeding pattern does not affect protein retention in young women. *J Nutr* **130**, 1700-1704.

Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL & Beaufrère, B. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci USA* **94**, 14930-14935.

Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballèvre O & Beaufrère B (2001). The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am J Physiol Endocrinol Metab* **280**, E340-E348

Dangin M, Guillet C, Garcia-Rodenas C, Gachon P, Bouteloup-Demange C, Reiffers-Magnani K, Fauquant J, Ballèvre O & Beaufrère B (2003). Protein digestion rate affects differently protein gain during aging. *J. Physiol(Lond)* **549**, 635-644.

Giordano M, Castellino P, DeFronzo RA (1996). Differential responsiveness of protein synthesis and degradation to amino acid availability in humans. *Diabetes* **45**, 393-399.

Pulmonary Na^+ transport

The distal lung epithelia continually absorb Na from the lung lumen and this process ensures that water cannot accumulate in the alveolar region where it could endanger life by impairing oxygenation of the blood. In this article Stuart Wilson discusses some of the factors underlying the development and maintenance of this phenotype

Stuart Wilson

During fetal life liquid is secreted into the lumen of the developing lung generating a distending pressure that inflates the lung to a volume comparable to the functional residual capacity of the neonatal lung. This process is important to lung morphogenesis as clinical syndromes that disrupt this pressure cause abnormal lung growth. However, this secreted liquid must be entirely removed from the lung by the time of birth as the retention of even a small volume of liquid will impair oxygenation of the blood. Indeed, the postnatal retention of lung liquid is a dangerous feature of neonatal respiratory distress syndrome (RDS), the commonest cause of death amongst newborn and premature infants in the developed world. It was long assumed that liquid was simply squeezed from the lungs during labour and birth but this myth was dispelled by the observation that blockage of the trachea did not prevent a fall in lung liquid volume. The removal of liquid must therefore be an absorptive process dependent upon the lung tissue itself.

Hormonal control of lung liquid absorption

The physiology of lung liquid absorption was investigated by monitoring the volume of liquid in the lungs of fetal lambs. The first such studies explored the effects of adrenoceptor agonists/antagonists as the level of adrenaline in the fetal circulation is known to reach very high levels during labour. As anticipated, lung liquid volume normally rose as liquid was secreted into the lumen, but then fell during labour and birth as it was subsequently reabsorbed. A central role for adrenaline in this process was established by the fact that β-adrenoceptor agonists evoked liquid absorption in mature fetuses whilst antagonists reduced the liquid absorption seen during labour. However, in relatively immature fetuses, *β*-adrenoceptor antagonists merely slowed the rate of fluid accumulation and so this absorptive response must develop over the last stages of gestation. Moreover, the maturation of this response was blocked by fetal thyroidectomy and, although this effect was reversed by exogenous thyroid hormone (T_3), T_3 did not cause precocious development of absorptive capacity in immature fetuses unless given with a glucocorticoid. The circulating levels of T_3 and glucocorticoids both rise during the perinatal period and so these hormones appear to prime the lung by evoking the development of an absorptive capacity that is then unmasked when the lungs are exposed to high levels of adrenaline during labour.

Pulmonary Na^+ transport

Subsequent studies showed that the absorption of fluid from the lung lumen is driven by the active absorption of Na^+. At that time, it was known that transepithelial Na^+ transport is normally limited by the rate at which Na^+ can cross the apical membrane but the channels permitting this Na^+ entry were unknown until the early 1990s when Canessa *et al.* (1993) identified three genes encoding the component subunits of the epithelial Na^+ channel (α-, β- and γ-ENaC). Subsequent studies showed that genetic deletion of α-ENaC caused death from severe RDS by blocking the absorption of liquid from the perinatal lung (Hummler *et al*. 1996). Perinatal lung liquid absorption is thus dependent upon pulmonary Na^+ transport and this process has now been extensively studied using isolated rat fetal distal lung epithelial (FDLE) cells, which form Na^+ absorbing epithelia when cultured on permeable supports. *b*-adrenoceptor agonists stimulate Na^+ transport in these cells by increasing apical Na^+ conductance (G_{Na^+}) and this control over G_{Na^+} is dependent upon T_3 / glucocorticoids, a finding that explains the importance of these hormones to lung liquid clearance (see above). These observations thus predict that, as well as inhibiting the secretion/synthesis of surfactant, a lack of T_3 would impair lung liquid clearance and this may explain why RDS is abnormally prevalent amongst hypothyroid infants. Moreover, premature delivery by Caesarean Section appears to attenuate the perinatal surge in circulating T_3 (Baines *et al*. 2000) and the role of this hormone in the control of G_{Na^+} may explain why RDS is more prevalent in babies delivered in this way.

Role of atmospheric O_2

Whilst adrenaline is crucial to the initiation of pulmonary Na^+ absorption, other factors must contribute to the maintenance of this phenotype as Na^+ absorption normally continues throughout adult life despite a rapid, postnatal fall in circulating adrenaline levels. It is interesting, in this context, that fetal development takes place under

hypoxic conditions implying that the distal lung epithelia experience a rise in P_{O_2} as the newborn infant takes its first breaths. The first evidence that this might play a role in the functional maturation of the lung came from studies of fetal rat lung explants, which showed that P_{O_2} could influence pulmonary fluid balance. Subsequent studies of FDLE cells showed clearly that increased P_{O_2} stimulates Na^+ transport (Pitkänen *et al*. 1996) and these data raised the possibility that the rise in alveolar P_{O_2} seen at birth may provide a drive for maintained Na^+ transport despite falling adrenaline levels. Interestingly, this effect of O_2 was reversible implying that, even in adult life, a fall in alveolar P_{O_2} would reduce the drive for Na^+ transport and thus allow liquid to remain in the lungs. Such alveolar flooding often occurs in pulmonary oedema, a condition with many underlying causes including heart failure, septic or haemorrhagic shock and ascent to high altitude and, irrespective of its underlying cause, the resolution of this condition depends upon pulmonary Na^+ absorption (Ware & Matthay, 2001). Whilst the alveolar flooding in pulmonary oedema is usually attributed to disturbed pulmonary haemodynamics, there is evidence that hypoxic inhibition of Na^+ absorption contributes to the liquid accumulation seen in at least some forms of oedema (Scherrer *et al*. 1999).

Atmospheric O_2 may thus play an important role in pulmonary physiology by stabilising the lungs' Na^+ absorbing phenotype. Despite this potential importance, however, the way in which O_2 can control Na^+ transport is not well understood. However, raising P_{O_2} increases the abundance of α-, β- and γ-ENaC mRNA, suggesting that the response may involve increased ENaC gene expression. Moreover, the promoter region of the α-ENaC gene contains a binding site for NF-κB (Otulakowski *et al*. 1999), a transcription factor activated by physiologically relevant increases in P_{O_2} and so it was suggested that O_2 may stimulate Na^+ transport by evoking NF-κB-dependent ENaC expression (Pitkanen *et al*. 1996). Although subsequent work showed that increases in P_{O_2} could activate the α-ENaC promoter (Baines *et al*. 2001), this response was weak and seen only after P_{O_2} was raised for 24–48 h. However, the stimulation of Na^+ transport was fully developed by 24 h and cannot, therefore, be a consequence of increased gene expression. However, physiologically relevant increases in P_{O_2} increase the capacity of the basolateral Na^+ pump and this response precedes the increased Na^+ transport by 3–4 h. This event may well be an important early step in the functional response to increased P_{O_2}, whereas increased ENaC expression could be a consequence, rather than the cause, of the increased Na^+ transport. The mechanisms that allow the Na^+ pump to respond to an increased P_{O_2} are unknown and neither is it clear how this effect is transduced into increased transepithelial Na^+ transport.

Conclusions

Pulmonary Na^+ transport is important to the integrated functioning of the respiratory tract and the initiation and maintenance of this phenotype is dependent upon the interaction between physiological (circulating hormones) and environmental factors (atmospheric O_2). Improved understanding of the way in which these factors control Na^+ transport may reveal the pathophysiological basis of RDS and certain forms of pulmonary oedema.

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References

Baines DL, Folkesson HG, Norlin A, Bingle CD, Yuan HT & Olver RE (2000). The influence of mode of delivery, hormonal status and postnatal O_2 environment on epithelial sodium channel (ENaC) expression in guinea pig lung. *J Physiol* **522**, 147-157.

Baines DL, Ramminger SJ, Collett A, Haddad JJE, Best OG, Land SC, Olver RE, & Wilson SM (2001). Oxygen-evoked Na^+ transport in rat fetal distal lung epithelial cells. *J Physiol* **532**, 105-113

Canessa CM, Horisberger JD & Rossier BC (1993). Epithelial sodium channel related to proteins involved in neurodegeneration. *Nature* **361**, 467-470.

Hummler E, Baker P, Gatzy J, Berrmann F, Verdumo C, Schmidt A, Boucher R & Rossier RC (1996). Early death due to defective neonatal lung liquid clearance in α-ENaC-deficient mice. *Nature Genetics* **12**, 325-328.

Otulakowski G, Raffii B, Bremner HR & O'Brodovich H (1999). Structure and hormone responsiveness of the gene encoding the α-subunit of the rat amiloride-sensitive epithelial sodium channel. *Am J Respir Cell Mol Biol* **20**, 1028-1040

Pitkänen OM, Tanswell AK, Downey G & O'Brodovich H (1996). Increased PO_2 alters the bioelectric properties of the fetal distal lung epithelium. *Am J PHysiol* **270**, L1060-L1066.

Scherrer U, Sartori C, Lepori M, Allemann Y, Duplain H, Trueb L & Nicod P (1999). High-altitude pulmonary edema: from exaggerated pulmonary hypertension to a defect in transepithelial sodium transport. *Adv Exp Med Biol* **474**, 93-107.

Ware LB & Matthay MA (2001). Alveolar epithelial fluid clearance is impaired in the majority of patients with acute lung injury and acute respiratory distress syndrome. *Am J Resp Crit Care Med* **163**, 1376-1383.

Staff changes in the Cambridge Publications Office

With the change of publisher for the Society journals from January 2004 there will unfortunately be several members of staff leaving at the end of November. One staff member who is known to many Members is Diana Greenslade Jones, who has been producing the Proceedings issues of *The Journal of Physiology* for over 12 years. She attended the scientific meetings regularly until 1999, when she took her first maternity leave. She returned to work on a part-time basis so has not been such a regular visitor at meetings since then. She is currently on her second period of maternity leave, the end of which will coincide almost exactly with her finishing with the Society. Diana will become a full-time mum for a couple of years.

We will also sadly be losing five other members of staff who have contributed in many ways to life in the office – Emma Kelly, Claire Varley, Lydia Grove, Charlene Gibbons and May Block.

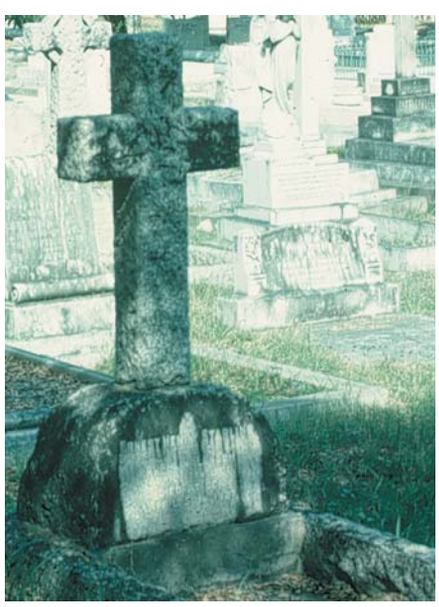
Emma first joined the Society in 1997, following a postdoc in molecular biology. After the birth of her twins on Christmas Day, 2001 she worked 2 days a week. Claire was a formulation development scientist at Cantab Pharmaceuticals and has been with us for just over 2 years. Our two Publications Assistants, Lydia (a science graduate) and Charlene (a design graduate) have been with us for 3 and 2 years respectively. May retired earlier this year after working on the journals since 1992. All five will be greatly missed.

We wish them all the very best for the future and would like to thank them, on behalf of the Society and all the staff, for all their help in maintaining the high quality of the journals.

Ernest Starling (1866–1927)

Tim Biscoe recalls his visit to the Jamaican grave of an outstanding physiologist

Tim Biscoe



Ernest Starling (above) and his grave in the English churchyard, Kingston, Jamaica (below)

In *Physiology News* number 51, David Miller wrote about the grave of Sydney Ringer. His piece caused Ann Silver to remind me that I had once visited the grave of Starling. In 1976 I went to Kingston, Jamaica to examine in physiology at the Medical School. Professor Jones took me to Starling's grave in the English churchyard. The picture shows the gravestone at that time.

The life of Starling and his colleague William Bayliss is described with charm and elegance by Sir Charles Lovatt Evans in the First Bayliss-Starling memorial lecture entitled *Reminiscences of Bayliss and Starling*. The lecture was given at University College London on 22 March, 1963 and was published by the Physiological Society in 1964 as a separate pamphlet bound, as was the custom for *The Journal* in those days, in pink paper.

Lovatt Evans should be read for his wit and for conjuring a lost time. The connection to Ringer is close since they all three worked at University College and, according to Evans, Starling and Bayliss were both elected members of the Physiological Society at the Annual Meeting at UCL on the 15 February,1890 with Sydney Ringer in the chair. Furthermore, Bayliss was married to Starling's sister. Bayliss was 'gentle, retiring and kindly', whilst Starling was brisk, ambitious, a bit quixotic, serious, generous, highly strung'.

Their research separately and together was outstanding. Starling formulated the hypothesis about the balance of forces across capillaries, together they showed that the excitation wave in the heart travels from base to apex, and much else. They studied the innervation and movements of the intestines. Their most revolutionary work was the experiment one afternoon that showed the pancreatic secretion to be controlled by an extract of the jejunal mucosa. This discovery, of secretin, led to the general name hormone and the development of a new understanding of the way in which living things function. It led to a revolution in biology, showing that substances may be released at one site and act at another to change function. The work is beautifully described by Bayliss and Starling (1902) and it is a model paper. Anyone interested might also wish to read the comprehensive account by Gregory (1977).

There was also Starling's law of the heart and The Principles of Human Physiology and, of course, Bayliss on the Principles of General Physiology which is maybe even more of a treasure in the first or second edition.

Evans says on '...11th April he started off on the ill-fated journey to Jamaica, and by the irony of fate died of heart failure as the ship approached Kingston, on 2 May. He was buried at St. Andrew's Parish Church, Halfway Tree, near Kingston the next day'.

Tim Biscoe
Emeritus Professor of Physiology
University College London

References

Bayliss WM, Starling EH (1902). The mechanism of pancreatic secretion. *J Physiol* **28**, 325-353.

Gregory RA (1977). The gastrointestinal hormones: an historical review. In *The Pursuit of Nature*, pp **105-132**. Cambridge University Press, Cambridge, UK.

Experimental Physiology

Rod Dimaline selects highlights from a recent meeting of the EP Editorial Board

The EP Editorial Board. Back row (left to right) David Paterson, Rod Dimaline, Mike Gray.
Middle row (l to r) Lynn Jeppesen, Emma Ward, Mohan Raizada, John Coote, Peter Ellaway.
Front row (l to r) Christian Bauer, K.W. Ranatunga, Bridget Lumb, Mary Forsling

The Editorial Board of *Experimental Physiology* (EP) met in Dublin on 10 July, with the city in the grip of an unexpected heat wave. The meeting followed on immediately from the Dublin Scientific Meeting of the Society, and was held in the intimate surroundings of the seminar room of the Physiology Department. In addition to the Editors and Society Officers who normally attend these meetings, the Chairman, John Coote, welcomed the Chairman of the Executive Committee, Deputy Chair of *The Journal of Physiology*, and representatives from the Blackwell publishing group. He also extended a cordial welcome to Mohan Raizada, who had travelled from Florida to attend his first meeting.

There are to be some important changes to the constitution of the Editorial Board. A panel of distinguished senior scientists has agreed to become Consultant Editors, to provide advice on strategic issues concerning the focus and further development of EP. At the same time, the post of Associate Editor is to be disestablished. A number of former Associate Editors will retire by rotation, while the others will become full Editors.

One of the first items to be dealt with by the Board was the perennial – some might say thorny – issue of the relationship between the Society's journals. It is probably true to say there is a fairly wide perception that the Society should not be publishing two journals with very similar scope. In this context, the clear steer towards a distinctive role for EP in having a major emphasis on integrative and translational physiology, which has been a feature of John Coote's Chairmanship, is an important development. A more detailed appraisal of this philosophy was presented in John's article in the Spring 2002 *Physiology News* (Number 46, p.21). The issue has recently been thrown rather sharply into focus with the impending appointment of new publishers, and there is now a genuine feeling emerging that a clearer and more comfortable relationship between the two journals is within our grasp. The Board expressed a view that the situation might be still further improved through the active participation of the Society's Publications Committee.

The Chairman initiated discussion on a range of other important issues that included impact factors, citation rates, focussed issues and reviews. The Board was greatly encouraged to note that the impact factor of EP had increased steadily year on year, over the last three years. This was combined with an almost 20 point improvement in the ranking of the journal. It is only fair to say that this welcome trend directly reflects deliberate editorial policies and initiatives over the last few years, and the strong leadership of the Chairman. A wide-ranging discussion followed on how to maintain and improve the position of EP. One clearly identified target was the recruitment of more contributions from the USA, a scientific community that is substantially underrepresented in the journal, and generally raising its profile in that region. The importance to the journal of full reviews, and shorter invited articles such as 'hot topics' mini-reviews, is well recognised, and it was decided that for the future a member of the Board would act as Reviews Editor.

Simon Rallison and Amanda McLean-Inglis from Blackwell Publishing gave us an interesting and informative presentation on their company's publishing operation, and specifically how it related to *Experimental Physiology*. A number of areas were identified where the publishers and the Editorial Board could work together to improve the position of the journal; one immediate benefit will be the great increase in sales and readership of EP through Blackwell's Consortial Licensing arrangements. With the imminent change of publisher, the Board was also keen to take the opportunity that this afforded, to update the cover of EP. The

Chairman had produced some innovative Da Vinciesque sketches (which will doubtless become valuable artefacts in their own right) and Blackwell had incorporated these into an impressive sample cover. The plain red cover of EP was to be replaced by a montage with elements designed to reflect the scope of the journal. The practice of featuring on the cover a figure from a paper published in that issue, was to be retained; but in the future, multi-colour reproduction of the figure would be possible. The board was highly supportive of the material presented, and a number of valuable, constructive suggestions were made for further improvements.

A further issue discussed was that of blind versus open review - no dissent was expressed from the current practice that reviewers retain their anonymity. It will, however, be interesting to see if this view is endorsed by the wider canvassing of opinion amongst expert reviewers that was recently undertaken by *The Journal of Physiology* (see article below). One decision that needs to be taken, as a result of rapid electronic publication of papers shortly after they are accepted, is precisely which version of the accepted manuscript should appear on line. The choice falls broadly between uncorrected and corrected proofs, and there are cogent arguments in favour of both alternatives. Nevertheless, the Editorial Board was strongly in favour of publishing corrected proofs, which is apparently the preferred option with the majority of Blackwell science journals.

A number of other matters for discussion or information were briskly covered as we approached the end of the time allocated for the meeting. The Chairman thanked retiring Editors for their hard work on behalf of the journal, and closed what had been a full and productive Board meeting. However, we had not quite finished yet. The Deputy Chair of The Journal of Physiology suddenly produced a formidable looking item of high-tech digital photographic equipment and requested that we assemble on the front steps of the Department of Physiology for a group photograph. Previous occupants of the steps, who had been enjoying the late afternoon sunshine, were politely requested to move on and the deed was swiftly done. Readers can judge for themselves the degree of digital enhancement that was subsequently required.

Rod Dimaline
Department of Physiology
University of Liverpool

The Journal of Physiology

Managing Editor Jill Berriman details recent decisions by the Editorial Board

There has been quite a bit of discussion over the last few months on the subject of anonymous reviewing. As reported in the summer 2003 issue of *Physiology News*, *The Journal of Physiology* decided at its Editorial Board in Tenerife in February to seek the opinion of all of the reviewers that they have used over the last 2 years. An email was sent to about 3,000 people asking them if they would prefer *The Journal* to maintain its present policy of having an anonymous system. We received replies from about half of those canvassed and the overwhelming opinion (82%) was that we should continue with the present policy. However, many respondents did include comments, which were very interesting to read. Many people are of the opinion that, although the present system is not ideal, it does work and is probably better than any alternatives. Quite a few felt that reviewers should be able to add their names to the review if they choose to, which *The Journal* does not allow presently and several also thought that the whole system should be anonymous, i.e. authors names should be withheld from reviewers. Many also expressed the opinion that Editors should be named.

After further discussion by the Editorial Board, it was agreed that *The Journal* would retain its present policy.

Submissions to *The Journal* have continued to rise, with an increase during 2003 to date of almost 10% over 2002. The average time from submission to acceptance is 10.8 weeks for regular manuscripts and 6.1 weeks for Rapid Reports. All papers are now published online upon acceptance, so delays to publication are now less than 1 week. The latest impact factor for *The Journal* is 4.65, an increase of almost 4% over the 2001 figure. The Board are working hard to increase this further.

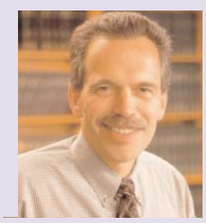
From January 2004 *The Journal of Physiology* and *Experimental Physiology* will be published by Blackwell Publishing. The Society will continue to employ three copy editors to edit *The Journal of Physiology* (*Experimental Physiology* will be copy edited by Blackwell). The main web site for both journals will be on HighWire Press, although both will also appear on the Blackwell Synergy web site. Both journals will now be under an 'umbrella' site on the HighWire system which means that Members will be able to access both journals from the Society web site by entering the Members only web page. They will also be able to access both journals when entering passwords for either, without the bother of entering the password a second time.

The move from Cambridge University Press will mean that the Publications Office has moved to alternative offices from September 2003. All the contact details will









From the top: New Editors Gordon Drummond, Dwain Eckberg, Masamitsu Iino Michael Kjaer and Richard Fitzpatrick, Deputy Chair Prem Kumar and International Deputy Chair Richard Moss

change and full information is given below.

The Journal's highly successful symposia continued with the 8th symposium in March in Fukuoka, Japan on 'Ion channels: their structure, function and control'. Plans for two symposia in 2004 and 2005 are in hand.

June saw the retirement of Michael Rennie (who had also served as a Senior Editor, Designated Senior Editor and Statistics Editor), Charles Bourque, Thelma Lovick and Peter Jonas. Their contributions to the continuing development of *The Journal* have been invaluable.

The Editorial Board has been joined this year by Gordon Drummond, Dwain Eckberg, Richard Fitzpatrick, Masamitsu Iino and Michael Kjaer. The role of Chairman has been renamed Chair, and new roles of Deputy Chair and International Deputy Chair have been filled by Prem Kumar and Richard Moss, respectively. Distributing Editors have been renamed Senior Editors, with Corne Kros elected by them as Designated Senior Editor. The Senior Editors are currently George Augustine, William Large, Steven Mifflin and Jens Bo Nielsen. Geraldine Clough has taken on responsibility for Topical Reviews. The post of Press Secretary has been discontinued, with the responsibilities of that role reverting to me, the Managing Editor.

Jill Berriman

The Physiological Society

PUBLICATIONS OFFICE

Change of address from 19 September, 2003

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Experimental Physiology
Physiology News

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Fax: **+44 (0)1223 246858**

Experimental Physiology **is now using Bench>Press online manuscript processing system. All future submissions should be made to:**

http://submit.expphysiol.org/

Receptors and cell signalling on oxidative stress

Giovanni Mann and Imre Kalló report on the Physiological Society's Spring Workshop at the Hungarian Academy of Sciences in Budapest, Hungary

Giovanni Mann (above) and Imre Kalló (below)

The Hungarian Academy of Sciences (top), Ron Jacob and his students (middle) and David Brown with Ian Scott, Richard Siow and Jose Viña (bottom)

The 4th International Workshop for young physiologists was held in Budapest from 3–5 April 2003, organized by Giovanni Mann, Catherine Rice-Evans and Imre Kalló. The venue for the workshop was the magnificent building of the Hungarian Academy of Sciences that provided an inspiring environment for the invited speakers and the young scientists gathered from 14 different countries (registrants in brackets): Russia (13), Hungary (12), United Kingdom (4), Bulgaria (3), Romania (3), Ukraine (3), Croatia (2), Portugal (2), and one each from Azerbaijan, Belarus, Czech Republic, Denmark, Poland and Spain.

After a brief introduction by Giovanni Mann, Ian Scott and David Brown gave a detailed overview of the international grants programme and funding schemes available from the Wellcome Trust and the Physiological Society, respectively. The workshop continued with a two and a half day scientific programme, during which a series of lectures were presented by the invited speakers on oxidative stress-induced changes in receptors and cell signalling in mammalian cells. The invited speakers were Richard Siow, Ron Jacob and Giovanni Mann from King's College London, Giuseppe Poli (University of Torino), Jose Viña (Universidad de Valencia), Maurizio Taglialatela (University of Naples Federico II), Malcolm Jackson (University of Liverpool), Vera Ádám-Vizi (Semmelweis Medical University) and Imre Kalló (Institute of Experimental Medicine of the Hungarian Academy of Sciences).

The review lectures stimulated lively discussion which continued during the lunchtime and afternoon poster sessions and designated workshop discussion groups. Invited speakers chaired these informal workshop discussion groups, which were based on their lectures. Young scientists had the opportunity to discuss their research programme with at least two invited speakers and in their exit questionnaire confirmed that they had appreciated these sessions.

Each of the registered participants presented a poster, which they discussed with the invited speakers and other poster presenters. A small poster evaluation committee then selected four posters for International Workshop Poster Prizes. David Brown, on behalf of the Physiological Society, officially presented Poster Prize certificates and the promise to send cheques to Vassily I Kazey (Institute of Neurology of the Russian Academy of Medical Sciences), Erzsébet Szatmári (Babes-Bolyai University and the Biological Research Centre), Eva Machová (Institute of Physiology CAS, Prague, Czech Republic) and Paula Isabel Moreira (Centre for Neuroscience of Coimbra, Department of Zoology, Faculty of Medicine, University of Coimbra, Portugal). The poster awards were a highlight of the closing ceremony of the workshop.

Besides science, all participants had a chance for sightseeing during bus transport to and from their residences in Buda. Furthermore, on the second day, participants and lecturers were treated to a bus trip to the Pest side of Budapest to attend an organ recital in the Jaki Chapel, which was built on the occasion of a world exhibition. In the evening we were taken to Budafok, the wine-growing part of the capital, where during the banquet activities other than scientific were pursued, e.g. whip cracking, dancing with locals, balancing wine bottles on one's head, cooking and above all tasting significant amounts of local wine. As we left the banquet, some of the more relaxed participants stopped to purchase wine bottles with their

photos on the label!

The closing event of the workshop was a boat-trip on the Danube, which has its source in the West and a wide mouth in the East. The Workshop in Budapest upheld the established tradition of previous workshops and provided young scientists from Eastern Europe and the former Soviet Union a unique opportunity to discuss their research with colleagues from Europe. The most rewarding experience for the organizers was that students and staff from all over Europe met and interacted so effectively. Several of the younger participants have already made contacts to develop collaboration with leading laboratories in their field.

Finally, the success of this Workshop was largely due to Imre's excellent organizational skills and the help he received from Franciska Morlin and Zita Horváth, the local conference company!

The full programme of the Workshop, including the abstracts of the lectures and posters, is available at:
http://www.physoc.org/international

Additional photographs of the Workshop are available from:
http://www.kcl.ac.uk/depsta/biomedical/hungary

Giovanni E. Mann
Catherine Rice-Evans
David Brown
Imre Kalló

Neuroscience Workshop in Romania

Bucharest was the venue for the latest Physiological Society Workshop from 23–25 September. The theme – *Ischemial Hypoxia in the Brain: Experimental Methods and Basic Mechanism* – attracted over 40 students, mainly from Romania and other Eastern European countries but with a sprinkling from the UK, with some students making heroic journeys to be there.

Speakers and workshop participants were welcomed on the evening of 23 September by Leon Zagrean and David Brown and treated to a buffet and congenial get together at the 'Carol Davilla' University of Medicine and Pharmacy. The University, which was founded in the mid 18th century, sports a magnificent entrance hall, off which lies an ornate, semi-circular tiered lecture theatre embellished with a carved ballustrade, splendid ceiling mouldings highlighted in gold and a huge chandelier.

A team of seven speakers from the UK and three from Romania filled the mornings with lectures devoted to considerations of some of the experimental methods used to study the effects of ischemia/hypoxia on brain function. Speakers highlighted their successes with the techniques used in their own labs, but were careful to point out limitations and potential pitfalls as well. Emil Toescu's summing up of his presentation on methods for investigating mitochondrial function using fluorescent dyes was particularly succinct – 'measuring free radicals is a bitch' – this may have lost something in translation! Other topics on offer included the use of brain slices to investigate activity – related dilatation (Thelma Lovick), methods of inducing experimental cerebral ischaemia in rodents and assessment of subsequent brain damage (Mhairi Macrae), stereological measurement (Mircea Oprica) and calcium imaging (Alex Verkhratsky). On day 2, Paul Kemp discussed cloning and expression of K^+ channels and how to test their responses to acute and chronic hypoxia. This was followed by talks on the use of organotypic brain slices to model ischaemia-induced neuronal changes (Ashley Pringle), the use of adenosine microsensors to measure purine release during ischaemia (Bruno Frenguelli) and how changes in the EEG might be used as an index of recovery from ischaemic insults (Mihai Moldovan). The neurosurgeon Vlad Ciurea offered us tips for 'perfect surgery' and numerous examples of his expertise in treating vascular and tumoural lesions in patients.

After lunch there was an early afternoon poster session followed by lab work until the evening dinner. Students chose from a wide range of practical demonstrations that included methods for inducing ischaemia *in vivo* and *in vitro*, assessment of neuronal damage, patch clamp techniques, calcium measuring, immunohistochemistry, immunoblotting and more. These students did not rest!

What do students get out of a workshop like this? We must await the analysis of the exit questionnaires to get the full picture. However, a straw poll I conducted during the tea breaks and over dinner revealed many things. They really liked the practicals, especially seeing new techniques, and felt that they will now have a better appreciation of related literature. They found the lectures hard work but informative and they particularly appreciated speakers who went slowly. There are other benefits that are less tangible. They met other students with similar interests – a great morale booster. They listened to experienced speakers who mounted slick presentations and kept to time. They discovered that senior scientists need not be distant figures and were surprised by the friendliness and informality of the UK speakers when 'off duty'! And importantly, they experienced a well organised meeting. Ana-Maria Zagrean (Bucharest) must take most of the credit for this. She did a splendid job, thinking of everything and making it all happen.

Thelma Lovick

Learning with electrodes

Laura Blackburn reports on the 20th Plymouth Microelectrode Workshop

The Citadel, headquarters of the Marine Biological Association in Plymouth and venue for the Microelectrode Workshop

Any electrophysiologist will tell you that their subject can be something of a black art at the best of times, requiring infinite amounts of patience and even a certain degree of luck!

This year saw the 20th Plymouth Microelectrode Workshop (3–17 September, 2003) at the Marine Biological Association (MBA) – a course that is designed to teach and introduce students to techniques, and some of the tricks of the trade that are invaluable to their research. Twenty students from departments ranging in diversity from pharmacology to plant sciences spent two rewarding weeks getting hands on experience of patch clamp, voltage clamp, ion sensitive microelectrodes, fluorescent indicators and dye injection techniques.

The first day hosted the electronics workshop, where we made our own operational amplifiers – something that most of us had never done before. Lectures on the techniques that we could sample followed on day two, with experiments starting in earnest on day three. Experiments were carried out in three blocks of three days, during which time researchers from both Europe and the United States guided us through all the techniques available.

It was possible to try a patch clamp single channel recording, whole cell recording and recording in brain slices; or use voltage clamping to look at membrane ion conductances in oocytes and snail neurons. Imaging and labelling techniques complemented the experimental work and produced some wonderful images. There was a high degree of success with the experiments, even for those with little or no experience of particular techniques.

The large experimental laboratory at the MBA allowed us to get involved with as many things as possible, and also to pop outside for a quick cup of tea and admire the stunning views over the sea. The experiments were complemented with lectures on both new and established experimental and analytical techniques, as well as demonstrations of microscopy and of the latest developments in equipment and software. The busy schedule was accompanied by fabulous lunches, courtesy of the canteen staff at the MBA, and by visits to local attractions, most notably the spectacular Plymouth Marine Aquarium, and the local pubs!

The course co-organisers, David Ogden and Colin Brownlee, have been involved for 20 years and have seen the workshop teach over 320 students. 'The course is popular, and we are very keen to attract applications from people studying a wide range of disciplines, especially those from Zoology and Plant Sciences departments. There is competition for places, but we would encourage people to apply even if they are not successful the first time, and there are bursaries available from our sponsors to those who might need help with the course fees,' say Ogden and Brownlee.

The extensive training was very useful, and answered many of the difficult questions that can confront anyone studying electrophysiology, as well as giving students the opportunity to learn from and question many of the researchers in the field.

Next year's course will run from 8–22 September and further information is available from the MBA website http://www.mba.ac.uk and from the NIMR http://www.nimr.mrc.ac.uk.

Laura Blackburn
Department of Zoology
University of Cambridge

Virtual abstracts

Dear Editor,
Somewhat belatedly, we have risen from our torpor to comment on the decision at the Society's 2002 Annual General Meeting to abandon publication in *The Journal of Physiology* of abstracts from the Society's meetings in favour of virtual 'publication' on a website. We would like to make the following points about the decision and, in the context of the present debate on the future of Society meetings, how it might have a bearing on future policy:

The decision

- It seems to us regrettable that the decision was made on the basis of a handful of votes at the Leeds AGM rather than by the membership as a whole.

- It is also regrettable that no account was taken of the views of young members who find publication of abstracts a source of pride and encouragement.

- We deprecate the dismissive attitude to abstracts by some members (at the AGM) on the basis that any decent work will be published in full. In reality, some findings do not warrant 'full' publication (e.g. minor points that require no further development).

- The disappearance of printed Proceedings from library shelves will be a sad loss, and it seems rash to 'go electronic' when this form of publication is relatively new, untested and in a constant state of flux.

Future policy

- If the decision is irreversible, and abstracts will no longer be worth the paper they're not written on, surely this adds further weight to an already powerful argument to discontinue the stress-inducing process of voting at the end of each communication/poster. It is patently clear that there is no uniformity in the scrutiny of communications, so that at the same meeting an appalling piece of insignificant drivel might get through without comment whereas a well-performed but provocative piece of work might come under fire from interested parties.

- In fact, we believe that there is already tacit agreement that the voting system is a nonsense: how many abstracts have been voted out in the last two years? In which case, why continue with it?

- The only excuse for continuing with the voting system was that it produced abstracts that were ostensibly 'refereed' and were therefore held in high esteem by the outside world. If abstracts are no longer to be published, this excuse disappears.

- Absence of the threat of being voted out might well result in improved quality of discussion, with participants not holding back from saying something that might otherwise result in hostile voting.

In conclusion, whatever agreement is reached on the revised format of Society meetings, we urge that voting on abstracts be abolished!

Dave Shirley
Department of Physiology
Royal Free & University College Medical School, London
Matt Bailey
Department of Cellular & Molecular Physiology
Yale University School of Medicine, New Haven, CT, USA

The outback experience

Dear Editor,
I don't recollect whether it was Stephen Kuffler, Bernard Katz or John Eccles who told me the following story – which I gathered took place right at the beginning of their time together in a little temporary laboratory off one of Sydney's main streets.

One day there was a knock on the door. Eccles went to answer and came back to say it was a woman who had heard of their experiments and had a question: '*Why do some people have nerves and others not*?' But perhaps it was the Kanematsu Institute, and perhaps it was Kuffler who went to the door... or Katz...

Now that they are all gone, can Liam Burke, Jonathan Ashmore – or anyone else – enlighten?

Andrew Packard
Stazione Zoologica 'Anton Dohm'
Naples, Italy

Organising congresses

Dear Editor,
I read with interest Mark Cain's articles on arranging scientific meetings. On a slightly more serious and more boring note, we offer the following collection of salutory lessons learned after arranging many meetings over the years, which he might be interested in:
http://oslovet.veths.no/congresshelp

Adrian Smith
Norwegian School of Veterinary Science
Oslo, Norway

CRAC Special Interest Group

Dear Editor,
As you are probably all aware, the two Special Interest Groups (SIGs) on Autonomic Function and Cardiorespiratory Control have now merged to form a new group called Cardiovascular, Respiratory and Autonomic Control or CRAC (this acronym was inspired by the Dublin meeting). We pondered long and hard over the name which needs to incorporate all areas of research in this group and we would be quite willing to change the name if anyone has a more inspired title. We really would like to keep this new group as lively as possible, with high profile speakers and at least two designated sessions a year.

At the Dublin meeting the separate Autonomic Function and Cardiovascular/Respiratory Control SIG sessions were amongst the largest in the meeting, with 13 communications and seven posters. We are hoping that in merging the two groups we can have even bigger and livelier sessions at future meetings.

Our next designated session will be at the Cambridge Meeting in December. We hope to have one, or maybe two, excellent speakers at this meeting and would really encourage people to submit abstracts. The more abstracts at the meeting the greater the likelihood of people in the field attending the meeting, which hopefully will mean a high level of interaction and discussion.

If you have any ideas of speakers that you would like in the future or feel that the SIG needs a symposium in any particular area, we would love to hear from you and will try and accommodate your wishes!

Susan A Deuchars
Jim Deuchars
School of Biomedical Sciences
University of Leeds

Geoffrey Walsh

Dear Editor,
In his excellent obituary of Geoffrey Walsh, Martin Lakie mentions Geoffrey's fondness for the headline 'Heads roll on the 09.45', used by *The Times* for an article on his study of the oscillations of the head in railway carriages. Geoffrey also enjoyed his experience of talking on Scottish TV about this project. Since his slot came just before the advertisements, he was warned not to use any complicated words that might make viewers switch off. He was happy to replace 'oscillate' with the good Scots word 'shoogle'.

Ann Silver

Physiologists in the news ...

Colin Blakemore's appointment as Chief Executive of the MRC has been widely reported, and welcomed, in the national press, with many stories highlighting his skills as a science communicator. Colin's key role in defending animal experimentation was also discussed in several features, including one in the *British Medical Journal*.

Blaine speaking

With the occasional exception, physiology and physiologists do not exactly feature regularly in the national press, especially on the news pages. However, in September several Society members were in demand, offering opinions on the purported '44 days in a perspex box' stunt in London by magician/illusionist/entertainer David Blaine. As this article went to press (3 October) the stunt had reached Day 27.

Both Jeremy Ward (King's College), and Mike Rennie (recently relocated from Dundee to Nottingham)

pointed out that 44 days without food was well within the bounds of the possible. Mike Rennie stated: 'There is no reason why Blaine cannot last for 44 days without any long-term damage. The only risk is infection or low temperature but a fit man in his 30s should come through it.'

Other potential physiological problems noted in articles discussing the stunt were muscle wasting, electrolyte disturbances, acidosis, and kidney and liver dysfunction. On Day 13 (19 September) the Channel 4 website devoted to Blaine's stunt (URL below) had as its headline news that 'Chronic salt deprivation is emerging as the biggest physical problem that could be faced by David Blaine'. Jeremy Ward had earlier pointed out in *The Independent* that adding electrolytes to Blaine's water supply would be an easy way to give the stunt a helping hand, but Blaine has insisted that he will be taking only pure water.

Several commentators, including the scientists, cited the evidence from hunger strikes that even people who were not in peak condition had gone 60 days or more without food. Finally, many pundits, including Jeremy Ward, wondered aloud what the point of the whole stunt was meant to be. Five million pounds is the widely quoted figure.

Given the lack of independent monitoring, and that Blaine is an illusionist by profession, there must be considerable doubt whether the stunt is/was exactly what it seems. However, Blaine has clearly prepared to undergo substantial weight loss. One rather under-reported fact was that Blaine had gained 20 kilos or more in weight pre-stunt, to judge from TV pictures. The Channel 4 website (http://www.channel4.com/entertainment/tv/microsites/D/david_blaine/index.html) indicated Blaine had taken 'nutritional advice' from doctor, author and adventurer (and Society member) Mike Stroud (Southampton University), who is probably best known for his 1993 unsupported polar expedition with Ran Fiennes.

Alan North – new President

Alan North, new Society President

At its last meeting, Council elected Alan North, FRS as the new President of the Society. He took over the job from Colin Blakemore after the AGM in September.

For those members who don't know him (very few, I imagine!), a brief biography. Alan is a native of Halifax, Yorkshire. He graduated from Aberdeen with a BSc and MB, ChB, and subsequently gained a PhD in Pharmacology with Hans Kosterlitz in 1973. He was elected FRS in 1995 and FRCP in 2000, and also has a DSc from Aberdeen.

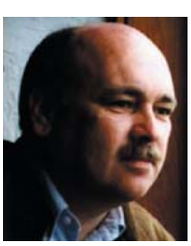
Alan stayed at Aberdeen until 1975, then migrated to the Department of Pharmacology at Loyola University, Chicago (then the home of A G Karczmar and Syogoro Nishi) and (in 1981) from there to MIT where he became Professor of Neuropharmacology. From 1987 to 1993 he was Senior Scientist at the Vollum Institute in Portland, Oregon. He then came back to Europe, to the Glaxo Institute for Molecular Biology in Geneva, Switzerland. The name changed in 1996 (to Glaxo Wellcome Research and Development), but Alan stayed until 1998, when he came back to the UK and took up his current position as Professor of Molecular Physiology at the University of Sheffield. There he has done a great job helping to mastermind Sheffield's resurrection, his Department gaining a well-deserved 5* rating in the last RAE exercise.

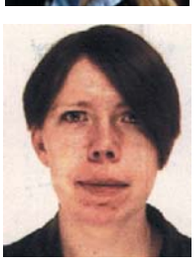
Alan was elected a member of the Physiological Society in 1976, and has been a regular participant in Society meetings even during his long years abroad. He was a member of the Society's Committee from 1996 to 1999 (during which period he also served on the then-Foreign Secretary's Advisory Committee). He gave one of our Annual Review lectures in 1999 and has served *two* terms on the Editorial Board of *The Journal of Physiology*, including two years as a Distributing Editor.

He is very much an international scientist: apart from his long-term appointments in the States and Switzerland, he has spent time in labs in Kiev, Frankfurt, Canberra, Melbourne and Adelaide; his lab has hosted post-docs from at least 13 different countries; and he has given lectures all over the world. His work on signalling from muscarinic, catecholamine and opioid receptors has been at the forefront of physiological research for three decades. His experimental approaches have ranged from intact animals to the molecular structure of ion channels, and his current work deals with ATP-gated channels. Alan is a Council member of the MRC, past Council member of the Royal Society and a member of the last HEFCE RAE panel for Units of Assessment 5–8. Hence, he brings to the job a breadth of physiological interests, a wealth of international experience and connections, backed by the respect of the world's scientific community for his research.

Dafydd Walters

New appointments





Giovanni Mann (top) and Helen Close

We are pleased to welcome Giovanni Mann, Professor of Vascular Physiology and Head of Graduate Studies at King's College London, as the New Deputy Chairman of the Executive Committee. Professor Mann served on Committee for four years and chaired the Higher Education Sub-Committee.

Congratulations also to the other newly elected Council members - Doug Corfield (University of Keele, Convenor for the Respiratory Special Interest Group), Chris Peers (University of Leeds), Sergey Smirnov (University of Bath) and Keith Thornbury (Queen's University, Belfast). The Affiliate Member elected as observer to Council is Catherine Bleasdale (University of Bristol).

Thanks to those who now stand down - Rob Clarke (Deputy Chairman), Stephen Fitzjohn, Thelma Lovick, Godfrey Smith and Bill Winlow.

Helen Close joined the Society in August. Helen completed her Masters in International Relations in 2001 and joins us from De Montfort University, where she was working in the Health and Safety Department. She will be working from the Society's Administration Office in London and, in addition to her international responsibilities, she will be involved in meetings' administration and external affairs.

The Society is most grateful to Tina Bashford, who has supported David Brown as International Secretary, and will now resume her full time position as Academic Administrator in the Department of Pharmacology in UCL.

2003 Molecular Techniques Workshop

What's the difference between a 5'-overhang and a 5'-splice site – and why are they important? These are just two of the questions raised, and answered, at this Easter's Molecular Techniques Workshop held in the Department of Physiology, University College, Cork (UCC). The 10-day residential course, funded by the Physiological Society and the Wellcome Trust, saw the transformation of 15 physiologists into molecular physiologists.

Since all the participants had little or no previous experience in molecular techniques this adaptation to molecular physiology was particularly challenging. Each day comprised a mixture of theory, practice and application of techniques. The course is loosely based on a real research project involving the isolation of RNA from tissue and detecting gene expression by northern blot and RT-PCR. The PCR product was cloned, sub-cloned and mutated creating a GFP-fusion protein. Each participant had the opportunity to transfect cells and compare different transfection reagents. Finally, the consequences of the mutations and the effect of creating a GFP-fusion protein were compared to the wild type receptor.

The course thrives on the generosity of a number of speakers from the UK and UCC who gave freely of their time. Special mention goes to Rod Dimaline from the University of Liverpool who spent four days running the first half of the course in aspects of RNA handling, isolation and amplification. Additional acknowledgement goes to the 10 speakers, who helped deliver a packed lecture programme on a diverse range of topics including control of gene expression, transgenesis, real-time PCR, synthesis and screening libraries, virus vectors, SNP analysis, bioinformatics and applied molecular physiology.

Funding has been generously provided by the Physiological Society and the Wellcome Trust to run the course for a further three years at UCC. With over 100 physiologists benefitting from this course since 1996, these future workshops should increase that figure to over 150. The next workshop will be held in UCC at Easter 2004. Further details on how to apply for a place on the course will be provided in *Physiology News* and on the UCC Department of Physiology's web site later in the year (http://www.ucc.ie/ucc/depts/physio).

Patrick Harrison
Department of Physiology
University College, Cork

Membership subscriptions

The subscription rates detailed in the table below were confirmed at the Annual General Meeting held in Manchester on 11 September, 2003 and refer to subscription rates from 1 January, 2004.

Ordinary Members, junior Members and retired Members receive a £5.00 discount for paying by direct debit.

Members and Affiliates will note that while there has been a slight increase in subscriptions for full Members in line with inflation, I was pleased to be able to recommend to the AGM that the rates for Affiliates remained the same as last year. In addition, Council recognizes that whilst PhD students living in this country struggle financially those living in some other parts really cannot afford even these moderate subscription rates. We therefore intend to introduce a reduction to the subscription rates for Affiliates, for web-only access to information. Affiliates would have all the same benefits as those paying the normal rates except they will not receive hard copies of the Magazine, meetings programmes or Affiliate handbook. They will have a membership number and be able to access information via the website. The reduction for this form of membership will be to £15, and will be constant no matter where the person lives.

Jeremy Ward
Treasurer

New membership rates for 2004

Category	Cost
Full Members	£70.00
Full Members living in UK or RoI with subscription to *The Journal of Physiology* hard copy	£180
Full Members living outside UK or RoI with subscription to *The Journal of Physiology* hard copy	£255
Full Members with subscription to *Experimental Physiology* hard copy (regardless of member's location)	Additional £64
Junior/Retired Members	£50.00
Junior/Retired Members living in UK or RoI with subscription to *The Journal of Physiology* hard copy	£170
Junior/Retired Members living outside UK or RoI with subscription to *The Journal of Physiology* hard copy	£245
Affiliate Members living in UK or RoI	£20
Affiliate Members living in rest of EU	£40
Affiliate Members living outside the EU	£45

What is the Novartis Foundation?

'This place itself is not a laboratory for mixing compounds, but a laboratory for mixing scientists,' declared Lord Beveridge at the opening ceremony in 1949, neatly distilling the Foundation's aims into one sentence. Today, the same original spirit prevails. The Foundation – a scientific and educational charity - continues to provide a unique forum where scientists can meet and freely exchange information and ideas.

The list of participants in the Foundation's meetings reads like the Who's Who of biomedical science. From Paul Nurse and Tim Hunt, recent Nobel laureates in medicine, to James Watson, Francis Crick, and Rosalind Franklin back in 1956, the Foundation has attracted hundreds of scientific luminaries during the 54 years of its existence.

The Foundation, which is based in London, receives approximately 55 per cent of its annual income from

Novartis, the Swiss pharmaceutical company, yet it has no ties with the company's business. The original view, which the company still supports, is that the exchange of ideas and information ought to remain independent of commercial pressures.

When the Foundation's meeting rooms and 15 bedrooms are not being used for the in-house scientific programme, these are made available at affordable charges to other scientific organizations and to scientists on working visits to London. Income from this 'hospitality' function combined with royalties on publications and bank interest comprises the remaining 45% of the Foundation's annual income.

There are eight symposia a year, focusing mainly on the biological and biomedical disciplines. What makes these symposia so special is that they represent a total departure from most other scientific meetings. For starters, they are invitation only and numbers are deliberately limited to 20 to 30 experts. Why so small? To encourage an open and frank discussion among participants. Another trademark feature is that over the two and a half days of the symposium, as much time is allocated to discussion as to presentations. The result is a fast-paced exchange of ideas between the sharpest minds in that research area. There is a focus on interdisciplinarity, which helps fuel discussions even further.

The science may be discussed behind closed doors, but the proceedings are transcribed and published as books, providing a highly covetable record of the latest thinking in that field. Also, the Foundation organizes one-day discussion meetings run on similar lines to the symposia but without the commitment to publication, and one-day open meetings directly following the symposia to which anyone with an interest can attend. These meetings are advertised by the Physiological Society and members wishing to attend can do so at a reduced rate.

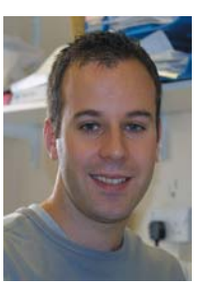
In addition, there is a bursary scheme targeted at young researchers that enables them to attend the symposia. The aim is to enable a person who is starting out in his or her scientific career not only to attend a symposium but also, immediately afterwards, to spend 4 to 12 weeks in the laboratory of one of the symposium participants. Bursaries are advertised on our website (http://www.novartisfound.org.uk) and through our e-mail newsletter (to receive our free newsletter please send a message to: bulletin@novartisfound.org.uk).

Scientists who have attended the Foundation's symposia remain devoted fans. The opportunity to interact is what they prize most highly. 'That is what encourages very busy people, who are invited to hundreds of meetings, to accept our invitation,' says Dr Derek Chadwick, the Foundation's Director. 'They know it will be worth their while.'

For book listings and forthcoming meetings please visit:
http://www.novartisfound.org.uk

Lisa Melton
Novartis Foundation

Pfizer and the Physiological Society



Samuel Fountain, winner of the Pfizer prize at the Leeds Meeting, September 2002. The title of Sam's presentation was Real-time RT-PCR analysis of Kv1 channel gene expression in mouse aorta. The prize was awarded to Sam by Mike Collis at the Dublin Meeting in July 2003.

Pfizer is now the world's largest pharmaceutical company and has discovered many important new drugs for both human and for animal diseases. The Pfizer Global Research and Development Centre in Sandwich, Kent employs about 2,500 scientists and is Pfizer`s major European site. At Sandwich we have discovered and developed some of Pfizer`s most successful drugs such as Amlodipine for hypertension and Sildenafil for male erectile dysfunction. The future success of the Sandwich Research Labs depends to a major extent on the quality of our scientists.

Support for the Physiological Society

At Pfizer we are keen to support academic physiology in the UK. One way in which we do this is by the Pfizer Physiological Society Prizes. These prizes are awarded to young physiologists (within 4 years of registration for a higher degree) and are made on the basis of the quality of their research presented as an oral communication at a designated session of the Society's meetings. From the December 2003 meeting the value of these prizes will be increased to £250 and we encourage young physiologists (with the support of their supervisors) to apply for them. An area of particular importance to the pharmaceutical industry is integrative whole animal physiology. This type of research is key to the understanding of the genome through the use of transgenic organisms and is essential to allow us to investigate the efficacy and safety of novel drugs. Pfizer is involved in a number of initiatives to support training and research in integrative *in vivo* biology including sponsorship of the joint Physiological/Pharmacological Society short training courses for undergraduates. As part of these initiatives we are introducing a new category of Pfizer Prize for Integrative Physiology. This £500 prize will be available to scientists within the first 6 years of their career following registration for a higher degree and will be awarded on the basis of presentation at designated sessions of a Physiological Society meeting. In addition, we are

providing funds to the Physiological Society to support attendance by integrative physiologists presenting at scientific meetings and visits to other institutions to exchange best practice. These funds will be available in 2004. Please see http://www.physoc.org/grants for further details.

The role of physiologists in the pharmaceutical industry

Drug discovery in the 21st century requires a wide range of scientific skills and some very high throughput technologies, to probe both the genome and the proteome, and to screen enormous numbers of compounds. But does the physiologist have a role in this process? At Pfizer we think the answer is definitely 'yes'. Physiology is about biological function, from cells to whole organisms and drug discovery involves identifying chemical compounds that influence biological function in a whole organism, usually man. Physiology underpins pharmacology and medicine and an understanding of physiology is always important when the benefits and the safety of novel drugs are being evaluated.

The translation of the human genome has given biologists a unique opportunity to understand and influence human health and disease. The genome, however, is just the start of this journey. The challenge for biologists now is to understand how genomics relate to function. We have the code but we don't understand much of what it does. Having gone through a decade of reductionist biology where the focus has been on the gene, we now need to use integrative biology to understand the functional significance of the genome in cells, tissues and organisms. Physiology has always been an integrative science concerned with function. The genomic revolution needs physiology and physiologists to assign function, significance and therapeutic utility.

One of the major challenges the pharmaceutical industry faces is to sift through the wealth of information in the genome and to find those genes that encode proteins that have an important biological role of relevance to disease and that are also suitable for high affinity interactions with potential drugs. Relevance to disease in this context does not necessarily mean that the particular protein causes the disease, but that it has an important influence on a process of significance in that disease. For example, there are a number of proteins that are good targets for efficacious anti-hypertensive drugs – components of L-type calcium channels, angiotensin II receptors and beta 1 adrenergic receptors. Although drugs interacting with these mechanisms can result in a lowering of blood pressure, these molecular drug targets are unlikely to be the underlying cause of the disease. Picking the right drug target is one of the keys to success in drug discovery, and a knowledge of integrative biology and physiology is important in order to develop an understanding of the potential impact of an intervention on the whole organism. Whilst powerful bio-informatic tools can access and deliver impressive amounts of information about the putative functions of novel genes, it takes an integrative biologist to see the big picture on the organism level.

As potential new drugs are evaluated it is essential that we understand the way that they interact with human cells and with intact organisms. This is another area where scientists with a good understanding of physiology are needed. Many new drugs fail because they are not efficacious in man. We need to build up a detailed picture of how a new drug class influences function in the intact cell and intact organism to increase the chances that it will show beneficial effects in clinical trials

The evaluation of drug safety also requires an integrative approach, at both the cellular and the organism level. A good example of where physiologists have lead the way in an area of particular importance to drug safety is in our understanding of cellular electrophysiology. The effects of drugs on cardiac repolarisation, as reflected clinically by Q-T interval, have become a high priority for the pharmaceutical industry and regulatory groups as they can be associated with dangerous dysrythmias in man. In our Sandwich labs we have a dedicated ion channel group, led by a physiologist, to ensure that our new drugs do not exhibit these adverse effects.

Pfizer employs physiologists in a variety of different departments. Their initial role is usually in discovery research and many make this their long-term career. We value practical experimental experience and a quantitative approach very highly in new recruits, as we know that drugs are discovered by performing rigorous experiments in the laboratory. Some physiologists who start out in discovery research move to other groups such as the development and clinical departments, where their skills are also of great value. In addition to recruiting physiologists, Pfizer has collaborations with academic physiology departments across the UK to investigate basic mechanisms of relevance to disease and to develop new experimental models.

If you would like to know more about Pfizer, and how we support physiology in the UK please contact the author.

Samuel Fountain

Some recent references to published studies involving Pfizer (Sandwich) scientists

Fairman DA, Cota A, Brearley C & Schmid E (2000). Systemic markers of inflammation in response to treadmill exercise in patients with intermittent claudication. *J Physiol* **523**, 240.

Fairman EJ, Ridden J, Benson N, Gill J & Bungay PJ (2000). Effects of K^+ on human pyruvate dehydrogenase kinase. *J Physiol* **523**, 240.

Wibberley A, Nunn PA, Naylor AM & Ramage AG (2000). Evidence that decreases in urethral smooth muscle tone produce reflex increases in urethral striated muscle activity in the anaesthetized female rat. *J Physiol* **526**, 173.

Webster R, Allan G, Anto AK, Harrison A, Kidd T, Leishman D, Phipps J & Walker D (2001). Pharmacokinetic/pharmacodynamic assessment of the effects of E4031, cisapride, terfenadine and

terodiline on monophasic action potential duration in dog. *Xenobiotica* **31**, 633-650.

England S, Heblich F, James IF, Robbins J & Docherty RJ (2001). Bradykinin evokes a Ca^{2+}-activated chloride current in non-neuronal cells isolated from neonatal rat dorsal root ganglia. *J Physiol*, **530**, 395-403.

Harvey RA, Skennerton DE, Newgreen D & Fry CH (2002). The contractile potency of adenosine triphosphate and ecto-adenosine triphosphatase activity in guinea pig detrusor and detrusor from patients with a stable, unstable or obstructed bladder. *J Urol* **168**, 1235-1239.

Falconer M, Smith F, Surah-Narwal S, Congrave G, Liu Z, Hayter P, Ciaramella G., Keighley W, Haddock, Waldron PG & Sewing A (2002). High-throughput screening for ion channel modulators. *J Biomolecular Screening* **7**, 460-465.

Ajiis A, Bagnall NM, Collis MG & Johns EJ (2003). Effect of endothelin antagonists on the renal haemodynamic and tubular responses to ischaemia-reperfusion injury in anaesthetised rats. Exp Physiol **88**, 483-490.

Coalition for medical progress

The Coalition for Medical Progress (CMP) comprises a wide range of organisations working together to engage in public communication about biomedical research involving animals. There are over 20 member organisations including the Association of Medical Research Charities, the Research Defence Society, the Wellcome Trust and most of the major pharmaceutical companies. The Physiological Society, along with the British Pharmacological Society, joined in its own right recently as well as being a member through the Biosciences Federation.

CMP is not a lobbying organisation. Instead, it aims to focus on the concerns of the public. Mori polls have shown that the level of general acceptance of the need for animal experimentation has improved. However, even in the most recent polls there are some worrying statistics such as 83% of the public thinking that illegal experiments take place. Through a targeted campaign with the media, a website and, by using MPs to reach people, CMP aims to answer concerns like these and achieve better understanding and acceptance of research involving animals.

Further information about the work of CMP is available at: http://www.medicalprogress.org/

Maggie Leggett

Emily Ferenczi

On Tuesday, 9 September, at the London Guildhall, one of Cambridge's Part II students, Emily Ferenczi (New Hall) was awarded the 2003 UK Science, Engineering and Technology Award for best Biology student of the year by the Institute of Biology, on the basis of her academic record, extracurricular activities and project entitled 'Membrane potential regulation following osmotic stress in amphibian skeletal muscle'. Emily is the daughter of *The Journal of Physiology* Editorial Board member Michael Ferenczi.

This is the second year running that Cambridge Part II Physiology has won this national award (last year's winner was Claire Martin, Caius).

Aileen Briggs

Gnomic –omics™

We are now living, everyone tells me, in the age of 'Omes and –omics'.

What, some of you may ask, is an -Ome?

It hardly needs to be said that this is an 'Ome', O-M-E, and NOT an 'Ohm', O-H-M, of the type long beloved by physiologists – or at least by electrophysiologists.

Those Ohms were manageable, if mildly tricky for the non-physics-literate. Omes are quite a different proposition.

And: RESISTANCE IS USELESS. OK, lame joke, I admit.

Anyway, for the uninitiated, if there are any left, an Ome is one of those made-up words, more and more common nowadays, produced by taking a sensible word – like 'transcription' or 'protein' or 'physiology' – and tagging –ome on the end. This instantly turns it into something incredibly modern-sounding but whose precise meaning no-one understands. Transcriptome. Proteome. Physiome. And there are more. Many, many more.

Next, you can go another step, and make your Ome into an 'Omic' or 'Omics' . That way you can define a whole sub-science, or discipline, which is completely opaque to outsiders.

Transcriptomics. Proteomics. Metabolomics.

Admittedly, some people may feel they have an idea what those last three are getting at.

But how about these ones? Methylomics. Diagnomics. Even Degradomics.

The last of these sounds like something you could get arrested for, unless you were consenting adults behind closed doors. Believe me, though, all of the above are real. They have been coined, and used[1].

And 'Diagnomics' was evidently so catchy that someone has trademarked it: so I should really have written it: Diagnomics™.

Like many crazy trends, it all started out with good intentions. After all, it makes perfect sense to have a single short word for all of the genetic information of an organism. Hence 'genome'. A useful word because it has a clear meaning and saves unnecessary verbiage, as we can now say 'cell division occurs after replication of the cell's genome'. Or 'every single cell in the body carries the complete human genome.'

But it didn't stop there. Of course.

The problem is that we live, as if it needed repeating, in an age where the appearance, or impression, something gives is at least as important as the reality of what it does, or is.

Since this applies to entire Governments, it is no surprise it also applies to University Departments, or to scientific disciplines

Everyone has to look as if they are 'cutting edge', and are riding whatever new wave is this year's Big Idea.

In a nutshell, what you do has to sound 'Now'. Or you're history. Literally.

And having lots of the right leading-edge terminology is a big part of sounding Now.

So once genomics became the latest buzz-word, it was only a matter of time before other areas of biology followed suit. Because Physiology sounds, well, a bit dull. After all, we've had a *Journal of Physiology* for more than a century. Talk about dusty. And as for metabolism... let's face it, what could sound more Yesterday? Metabolism was what biochemistry departments did 25 years ago, before they all embraced recombinant DNA with the fervour of the born-again. Metabolism? Old hat. But Metabolomics– completely different.

Just the - omic alone tells you that Metabolomics is definitely Very Now.

And so it spreads.

Studying physiology? Yawn. Yesterday.

But 'Mapping physiological genomics' or 'Interrogating the physiome'. Now that's TODAY.

And so, by definition, no area of biological science can compete for headlines, Now-ness and – critically – funding unless it can coin a term as catchy and Now as 'Genomics' for it's bit of science.

Next, these words start proliferating in the titles of departments, and institutes, as universities and other institutions strive to appear ever more Now.

I predict that within the next 5 years many physiologists will be working in departments or schools of 'Physiological Genomics' (best case) or 'Integrative Biomics' or even just 'Integratomics'.

After all, it's another easy one for the managers. Want to make clear that the biological sciences in your institution are leading-edge? No problem. Just change the word 'Biology' by a kind of Global Replace to 'Biomics'. As one UK medical school – no name to save embarrassment – has already done.

But there is a snag for those bioscientists keen to appear truly Ultra-Now:

Because if every bioscience department or institute re-christens itself 'Biomics', how will anyone know which of them are REALLY Now? As opposed to just aspiring?

Basically, how can we stand out from all those other Centres for Integrative Biomics?

As a responsible staff member, I have already written to my Vice Chancellor – I think he's still a Vice Chancellor, although these days he may well have been renamed something more Now, such as a Principal or a President – to suggest that we need to stand out from the herd of newly–arising Centres for Biomics. We need a name that is suitably Now, but also subtly different.

So I have proposed we set up an Interdisciplinary Centre for Integrative...No, not Biomics, like everyone else. Ours will be for Integrative BIOLICS.

The quick-witted among you will have already guessed that the first 'i' in 'Biolics' is silent.

Have a good holiday. And I hope you have a happy Ome to go to.

Mark Cain

Got a good story to tell? Or something you want to get off your chest? Fancy yourself as a humourist? Now is your chance. We are keen to receive contributions for the 'Unbelievable' column. £50 paid for any article published.

[1] See http://www.genomicglossaries.com/content/omes.asp

Fazlul Karim

1936–2003

Fazlul Karim came to Leeds in 1968 as a Commonwealth Scholar into the then Cardiovascular Unit of the Department of Physiology. Before coming to Leeds he had obtained a medical degree from Dhaka Medical College and an MPhil in Renal Physiology at the Jinnah Postgraduate Medical Centre, Karachi. He was thus well prepared for a successful career in experimental physiology. Faz obtained his PhD in Leeds working with Cec Kidd and myself on the control of the cardiovascular system, particularly from atrial receptors and aortic baroreceptors. His work on the efferent sympathetic responses to atrial receptor stimulation has become something of a classic. He elegantly showed that the same stimulus, atrial distension, caused sympathetic activity to increase (heart), decrease (kidney) and not to change (lumbar and splanchnic). Faz and I had several years of fruitful cooperation which led to several papers in *The Journal* on circulatory control, in particular of the venous system, and from a variety of afferent nerves originating in the heart, lungs and major arteries. In the mid '80s, Faz turned his attention to the kidney and, with Roger Summerill and Simon Poucher, examined the reflex control of renal haemodynamics and renal function. The other area in which he made an important contribution, with David Cotterrell and Heather Ballard, was in the role of adenosine in the control of blood flow.

In addition to being one of a now rare breed, a whole animal or systems physiologist, Faz was a thoughtful, caring and often quite amusing person. I recall the IUPS Congress several years ago in India where he took complete charge of his colleagues and even insisted on inspecting the kitchens of any establishment where we planned to eat before we were allowed to enter!

Faz's legacy is not just the scientific papers that he published, important as they are, but also in the knowledge, inspiration and enthusiasm that he injected into the many research students and colleagues with whom he worked. His influence extends to many parts of the world through his many former students and co-workers. In recent years, he devoted much effort to enhancing medical and scientific standards in his native Bangladesh. His influence there will be hard to replace. Faz was a dedicated teacher at all levels. Medical, dental and physiology students all enjoyed and positively responded to his outgoing nature and enthusiasm. He became Senior Lecturer in 1980 and Reader in Cardiovascular Physiology in 1997. He retired from the University, a little early due to poor health, in 2000. He then seemed to recover his health following his retirement and was frequently present at departmental meetings. It was a shock to learn of his death in July this year. Things will not be the same in Leeds. It will certainly be quieter but Faz's lively and incisive contributions to physiological gatherings will sadly be missed.

Roger Hainsworth
University of Leeds

Marthe Vogt

1903–2003
Elected Member 1939
Elected Honorary Member 1974

Marthe Vogt died on Monday, 8 September – her 100th birthday. A full obituary will be published in a future issue of *Physiology News.*

Tony Edwards retires

A.V. Edwards, a Member since 1968 and one-time Secretary and Chairman of the Editorial Board of *The Journal of Physiology,* retired on 30 September from his Readership in the Physiological Laboratory in Cambridge.

An Holistic Guide to Anatomy and Physiology

Tina Parsons
Thomson Publishing, £15.99, 282 pp
ISBN, 1-86152-976-7

Book reviewers receive many books, some of which are good, some bad and some indifferent. When Tina Parsons' book landed on my desk a few weeks ago, I wasn't quite sure what to make of it. It is well written, but the illustrations are not quite what I expected in a book of this sort. They are greatly simplified to suit an audience I had not previously considered. The clue lies in the fact that the book is published as part of a series for HABIA – the Hairdressing and Beauty Industry Authority – which I have to admit to never having heard of before.

The publishers are involved in medical education and are dedicated to providing 'innovative approaches to lifelong learning' and this book is certainly innovative. However, I could not possibly recommend it to medical or science students because of the number of non-scientific concepts introduced at various points. For example, in a section on the axial skeleton there is a whole page on colour, which is a bit of a surprise, until you realise that the 'axial skeleton is the site of the seven primary chakras' (wheels of light that attract energy in seven different colours) and affect our physical well being. For example, the fifth chakra is associated with the colour blue and is located in the cervical region of the spine, which means that you should wear something blue around the neck to ease the stress of public speaking!

Leaving things like this aside, Tina Parsons has produced quite an interesting book, much of which is basic physiology and which will I hope will popularise our subject among the therapists, students and clients at whom it is aimed. I just hope that they can segregate the science from the mythology.

Bill Winlow

Signalers and Receivers Mechanisms and Evolution of Arthropod Communication

Michael D. Greenfield
Oxford University Press, New York, 414 pp
ISBN: 0-19-513452-4

Michael Greenfield is a Professor of Biological Sciences at the University of Kansas and in this excellent book he presents the story of how arthropods use 'pheromones, sound, vibration and light for sexual and social communications'.

Arthropods comprise more than 80% of all catalogued species, but most are minute, making physical signalling, perception and sensitivity more a question of resolving problems of scale, rather than merely miniaturising those sensory components with which vertebrate physiologists are familiar. However, most arthropods use chemical signalling as a major means of communication and this is almost impervious to scaling effects.

The book is very clearly written and the author has made great efforts to ensure that each chapter, following a short introductory chapter, stands on its own with a minimum of cross-referencing. He considers five major areas: signal theory, chemical signalling and olfaction, sound and vibration, visual signalling including bioluminescence, sexual selection and the evolution of signals. Finally, there is a short chapter on the diversification and modification of signals in evolution. There is a comprehensive reference section.

Although the arthropods appear to have separated from the chordates at least 550 million years ago, they have developed communication systems that in many ways parallel those of vertebrates. This makes the book fascinating reading for comparative physiologists and neuroscientists.

Bill Winlow

And finally ...

Readers advertisements

Readers wishing to dispose of surplus equipment, etc are invited to send in short advertisements for inclusion in this column at no charge.

BIG BLUE TANK
We have a new, unused tank for weighing people in water to determine body composition by hydrodensitometry. It is surplus to requirements aand no reasonable (or even unreasonable) offer refused.

Contact John Mellerio, Westminster University at: mellerj@wmin.ac.uk or 020 7911 5000, ext 3564

Images of physiology

A number of you recognised Bridget Lumb and Thelma Lovick (below) as the infamous physiologists featured

in Images of Physiology (*Physiology News*, **52**, 21). Prizes are on their way to:

Anthony Dickenson
(University College, London)

Simon McMullan
(University of Bristol)

FORTHCOMING PHYSIOLOGICAL SOCIETY MEETINGS

For further details please visit the Society's web site (http://www.physoc.org)

2003

Cambridge 17–19 December

2004

* Glasgow: 29–31 March
Cardiff: 6–7 July
Cork: 1–3 September
King's College London: 16–18 December
(Joint Meeting with the Chilean Physiological Society)

* The designated sessions from the Babraham meeting (originally planned for May, 2004) will now be incorporated into the Glasgow programme. Details will be posted on the Society's web site in due course. Symposia are planned in the following subject areas:

- Calcium imaging in smooth and cardiac muscle
- Cell signalling
- Genetic and molecular approaches to investigate spinal cord circuitry
- TRP channels

2005

Bristol 20–22 July

IUPS 2005 – 35th CONGRESS OF THE INTERNATIONAL UNION OF PHYSIOLOGICAL SCIENCES

San Diego, CA, USA
31 March–5 April

IUPS 2005 is being organised by the six member societies of the US National Committee of the IUPS, the American Physiological Society, the Society for Neuroscience, the Microcirculatory Society, the Society of General Physiologists, the Biomedical Engineering Society and the Society for Integrative and Comparative Biology, under the auspices of the US National Academy of Sciences.

http://www.IUPS2005.org

INTERNATIONAL BURSARIES

The Society will offer a limited number of bursaries of up to £250 each for Young Physiologists from countries outside the UK and Ireland to attend domestic Scientific Meetings of the Physiological Society. Applications are open to Members and Affiliates, and also to non-Members of the Society. Candidates will normally be expected to present an Oral Communication or Poster Communication at the Scientific Meeting, and will be encouraged to join the Physiological Society if not already a Member. Applicants should be sponsored by the head of their Department or Institute, or by their supervisor (if a PhD student), or a Member of the Physiological Society (if not a Member). There is no deadline for applications, but they should be made **not less than 10 weeks before the Scientific Meeting** that the applicant wishes to attend. Three hard copies must be posted to the Society in order to be considered. Faxed copies will be considered if a deadline is near, on the understanding that the hard copies will be posted as well.

Applications should be made on forms available from Jamie Gould (Membership and Grants Administrator) at The Physiological Society, PO Box 11319, London WC1V 6YB, UK
Email: jgould@physoc.org
http://www.physoc.org

MOLECULAR TECHNIQUES FOR LIFE SCIENCES

26–30 January, 2004
Manipulating Nucleic Acids

A five day practical workshop to introduce participants to techniques used in molecular biology investigations and to facilitate development of core molecular biology skills
Price: £715

Further details from: Mrs J Pierotti, MTLS Administrator, Biological and Biomedical Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow, G4 0BA, Scotland, UK.

Tel: 0141 331 3209
Fax: 0141 331 3208
Email: biological.biomedical@gcal.ac.uk
Website: http://www.caledonian.ac.uk/mtls

YOUNG PHYSIOLOGISTS SYMPOSIA

University of Bristol
16 April, 2004

In Vivo Cellular Mechanisms of Cardiovascular Disease. More details will be circulated by email and made available on the Society's website. This event is co-sponsored by Pfizer.

Noticeboard

Notices for the Spring 2004 issue of *Physiology News* should reach the Publications Office by 10 January, 2004 (lrimmer@physoc.org).

Please note that whilst members are welcome to advertise relevant events in *Physiology News* and on the Society's website, advertisements via email will be restricted to events sponsored by the Physiological Society.

10 years ago...plus ça change

The tyranny of the scientific paper

Some thoughts on science broadcasting. So, how should I begin...? *"A request was received asking for a contribution to The Physiological Society Magazine. It was considered, and a positive conclusion was reached. The Editor was phoned and advised of this decision."*
In truth, if I were to begin like this, and then continue in the same vein, I doubt that anyone would read far beyond those three sentences. So, an alternative: *"The Editor asked me to contribute to The Physiological Society Magazine. I thought about it, decided I would, and phoned to say so."* Much better. But what point am I trying to make? After all, you may say, nobody would actually write in the way I phrased that first version; and certainly nobody would speak like that ...
No? Well perhaps this will seem more familiar: *"The cells were placed in a test tube. Three mls of the first solution were placed in the test tube. Three mls of the first solution were added, and the tube was shaken briskly. A distinct colour change was then observed".*
But people - scientists - don't speak like that, you may insist. Well, after some 15 years spent listening to them through the microphone, I'm here to assure you that they do. Not when talking about the weather, or last weekend's golf, or the price of tomatoes; just when they're talking about science. This bizarre syntax, this sterile, mind-numbing use of the passive voice is the bane of my life as a broadcaster.

It was Peter Medawar who pointed out that the scientific paper, with its neatly compartmentalised sections on "method", "results", "discussion" and the rest of them, is a fraud. The practice of science, he argued, is altogether less tidy, less systematic than the published paper would suggest. The conventions by which scientists report their work to each other may or may not be sensible; that is not my concern. What upsets me is the tyrannical grasp in which the scientific paper continues to hold so many scientists when they are trying to communicate, not with their peers, but with an audience unaccustomed to the ways of academics. Such stilted expression renders even simple procedures or ideas harder to follow or, worse, incomprehensible...

Geoff Watts
(Presenter of "Medicine Now", BBC Radio 4)

Extracted from *The Physiological Society Magazine*, 1993, No 7, p 26