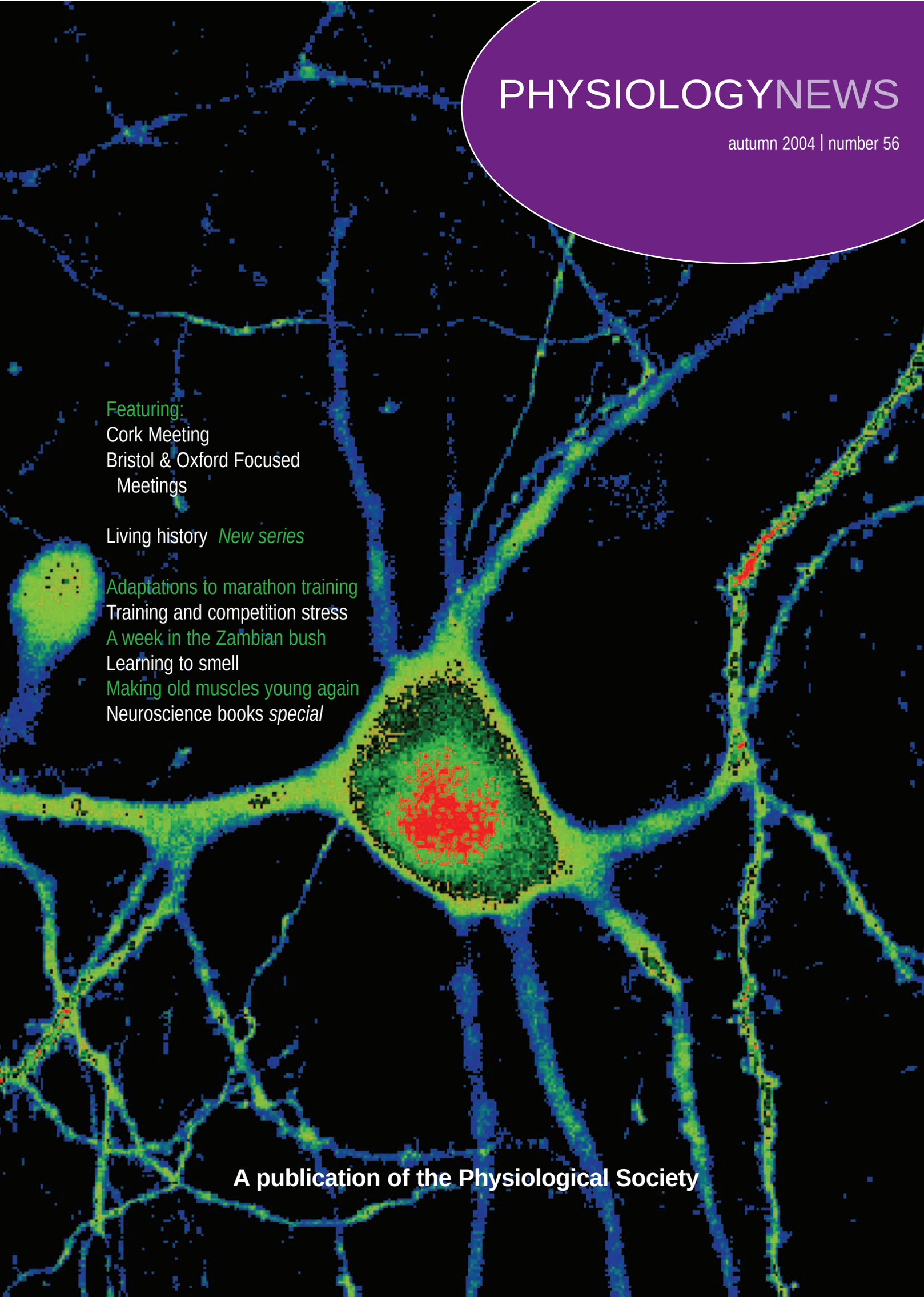

PHYSIOLOGYNEWS

autumn 2004 | number 56

Featuring:
Cork Meeting
Bristol & Oxford Focused Meetings

Living history *New series*

Adaptations to marathon training
Training and competition stress
A week in the Zambian bush
Learning to smell
Making old muscles young again
Neuroscience books *special*

A publication of the Physiological Society

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

Published quarterly by the Physiological Society

Contributions and Queries

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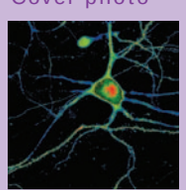

A hippocampal pyramidal neurone expressing green fluorescent protein targeted using an adenovirus with a neurone-specific promoter in organotypic slice culture. Fluorescent neurones remain viable for weeks *in vitro* and allow superb conditions for live cell confocal imaging and other types of experiments

PHYSIOLOGYNEWS

Contents

PHYSIOLOGYNEWS

Action points

Grants

Grant schemes have changed. For full information on Members' and Affiliates' Grants, Pfizer *in vivo* Physiology Grants, Intercalated BSc Bursaries, Network Interaction Grants, Non-Society Symposia Grants, Postgraduate Support Fund information and the Vacation Studentship Scheme please visit:
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Applications for Full and Affiliate Membership are received throughout the year and have no deadlines. A decision is normally made within 8-10 weeks of the Administration Office receiving the application. For full details please visit:

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

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

Physiology News

Deadlines

Letters and articles and all other contributions for inclusion in the Winter 2004 issue, No. 57, should reach the Publications Office (lrimmer@physoc.org) by 20 September, 2004. Short news items are encouraged and can usually be included as late copy if space permits.

Suggestions for articles

Suggestions for future articles are welcome. Please contact either the 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.

In this issue

Welcome to the Autumn 2004 *Physiology News*.

One of the most gratifying things for an editor is to see new features up and running. This issue we introduce what I hope will be a recurring feature, *Living History*. History – and physiology has a rich one – is perhaps best heard through the voices of those who were there. Scientists prize their moments of discovery – times when you saw something new, or first realised something was going to work – and Geoff Burnstock kicks off the *Living History* series on p. 7 by recalling just such a moment. This issue also sees our second diary (p. 13), where Tris Pocock tells us how a physiologist ended up teaching secondary school science in Zambia – a marked contrast to Alan North's working week that we heard about in the last issue.

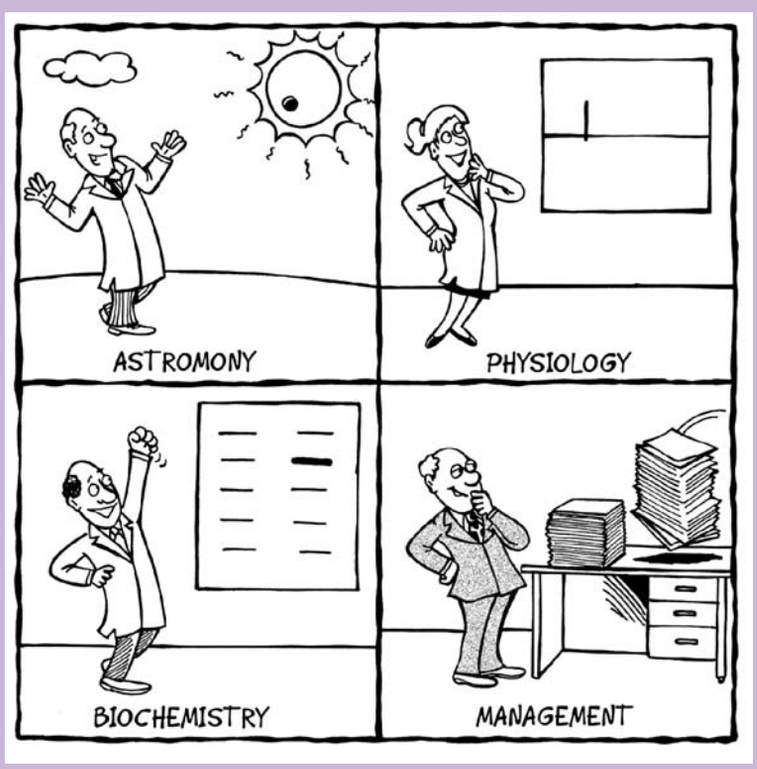
It is fun to try and spot scientific themes in the magazine, and I think I spy at least two this time. One is the continuing Olympic-year strand on muscle and exercise physiology, where we learn about exercising in the heat (p. 9), molecular adaptations to endurance exercise (p. 11), and leaky mitochondria (p. 27). Muscle physiology is not just for the young and fit, of course – in age we want to keep our muscle tone, as James Ryall and friends discuss on p. 33.

A second theme is inner – or rather intercellular - space. In this age of cell and molecular physiology, physiologists can be accused of neglecting the spaces between cells. However, we do so at our peril. The structures and macromolecules in these spaces give us our shape (p. 22), influence the composition of the fluid within the spaces (p. 29), and help determine how solute uptake occurs (p. 16). Finally, mis-programming the structures filling these spaces in early life may lead to adult disease (p. 25).

Plenty of meat and fibre there then! And we have not neglected the body's most (self?) important organ, the brain, since on p. 48 we review five recent books in the field of neuroscience. All in all, we hope, something for everyone.

Austin Elliott

A moment of excitement ...



Talking to the public - whose responsibility?

Not mine! I hear you cry. Or: 'Isn't that what we pay you for?' as a grumpy Council member asked me recently. Well, perhaps, but there are an awful lot of 'the public', and there is only one of me, or my successor.

I should explain. As I am leaving the Society to take up a new post with BBSRC (if you're interested, find out what on p 45), Austin Elliott asked if I would like to write this piece. 'Say what you think needs saying after five years as our External Affairs person' was the commission, which explains why you are hearing about Public Communication two editorials running.

In case you didn't know, the Society's strategic plan singles out 'educating the public' as a particular aim of ours. And in the near future we may get asked what we have done about it. The Charity Commission is busy casting its beady eye over learned societies (ours included), asking if these organisations really should be charities. A quick aside: historically charities have been able to say they 'served the public' (one of their key duties) by serving their members, since their members are part of the public – see e.g. private schools. However, those days are clearly numbered. When this happens, fulfilling stated objectives like 'educating the public' will be critical to holding on to our charitable status, which, if nothing else, saves us a lot of money. I also feel quite strongly that we – you – have a duty to share your knowledge. People really do want to know who you scientists are and what you do. The intricacies of some minor ion channel might be beyond your average layperson, but why you started your research, what the aim is, and how you spend your time will prove surprisingly interesting to Joe Public.

If you read the last editorial you will be aware that the Society already engages with the public to some extent. Frankly, though, this is a drop in the ocean – in my time the amount of the budget spent on these activities has risen from 0 to a whopping 0.1%, although of course a percentage of my time/salary should also be factored in.

So what do I think we should be doing? The Society's best resource is you, our Members. Much as there exists a ballooning'science communication' community, the best people to communicate about science are active scientists. In my experience 'science communicators' can be anything from retired professors who fancy getting on their soapbox, to some kid who has read and possibly failed to understand the latest *New Scientist*. Both can be good or hopeless, but until that community thinks up some way of regulating itself – and even if it does – then it is you who should be out there.

There are two obvious main problems. First, you all have a day job to do. Secondly, talking to a lay audience can be a bit daunting. There have been various suggestions about ways in which public engagement activities can be 'rewarded' - anything from it being a box to tick on a PhD report, to taken as value in the RAE exercise, to universities forcing paid staff out for a couple of days a year. Some people are better at communicating than others, so I personally don't think the last option would be the right way forward. I also fear that the RAE is complicated enough already without trying to add in some kind of measurement of success in public engagement activities. Personally, I would favour making public engagement work part of PhD training as, whichever method one chooses, it should lead to good transferable skills. Also, I think universities should view these activities alongside all the others academics do – so, for instance, if you were the person responsible in your department/group for working with the public, you should be relieved of some admin or similar. However, these are just my views. A consensus among the academic community is needed. As your learned society, we should be actively canvassing your views and then lobbying for whichever is your preferred solution.

Let's suppose a strategy is agreed and universities/funding agencies/the government do whatever is necessary to encourage you to leave your laboratories. What can the Society do to help you? Many of these activities will be local, and depend on local networks (talking to local media, participation with café scientifiques, visiting schools/local clubs etc). Rather than try to reinvent the wheel and set up complicated national networks, the Society should help to raise awareness of local schemes, share success stories, and flexibly provide small amounts of money for Members to use to help you take part. Perhaps funds for some admin help to free up your time to organise/ participate in an event, to contribute to a projector for giving school talks, or help with course fees if you want training in some form of communication skills.

Nationally, there are already several organisations with which we could work more closely. The Science Media Centre does an excellent job of proactively badgering journalists to talk to researchers to get their slant on particular stories. I have already suggested we support this organisation, but so far without success. Likewise, the British Association for the Advancement of Science has networks of members and holds events regularly. The Royal Society seems to be doing ever more in this field and, following criticism from the government, the BBSRC has stepped up activities. Helping these organisations with their efforts, both financially and by involving our Members, has to be better than striking out on our own. Although funding a Prize or two, as Nancy Rothwell suggests on p 43, would also be one way for the Society to put its money where its mouth is.

None of this is revolutionary. I am merely suggesting the Society does what it says it will in its (now rather elderly) strategic plan, by formulating a policy through which Members can engage, linking up with other organisations and using a small amount of its budget. However, I have been banging this drum for the last five years and I haven't got very far. Maybe it's time to buy a bigger soap box.

Maggie Leggett

Welcome to University College Cork

Edward Johns details changes in Cork since the Society last met there in 1995

Above: The Boole Library and Lecture Theatre complex, venue for the Society Meeting
Below: Patrick Harrison and his molecular physiology research group (top) and the University quad (bottom)

Queen's College Cork, along with its sister colleges in Galway and Belfast, was brought into existence by the 1845 Act of Parliament under the direction of the Prime Minister, Sir Robert Peel. The building of the College complex in Cork began in 1847. It was completed in 1849 and, having been seen by Queen Victoria in August, was officially opened on 7 November of that year. The educational processes of the College were based on that of the University of London rather than the tutorial system of the Oxbridge universities. Such were the political sensitivities of the time that initially the position of 'Professor' was a Crown Appointment made from London. Medicine was a foundation Faculty with a first year enrolment of 12 students. The demand for teaching in medicine was so great that in the 1860s funds were released for the construction of the Windle Building which to this day houses the Departments of Physiology and Anatomy. It is therefore a singular coincidence that this meeting of the Physiological Society in University College Cork coincides with the 150th anniversary of the graduation of the first cohort of medical students who received their physiology education in Cork.

The provision of teaching and research in physiology today is very different from the early years of University College Cork. The Department of Physiology remains an independent budget unit within the Faculty of Medicine and Health and is responsible for the delivery of physiology teaching to its constituent Schools – Medicine (250 students), Dentistry (80), Nursing and Midwifery (400), Pharmacy (50) and Clinical Therapies (50). Courses in physiology are also delivered to undergraduates in the Faculty of Science (160 students) who provide our final year Honours BSc Physiology class of 10-15 students. It is from these students that a number progress to read for a PhD. In all, just over 1,000 undergraduates each year are exposed to the 'Physiology experience in Cork!'. It is Anne Harris who co-coordinates the teaching and learning approaches which are an essential element of the curriculum.

The Society last met in Cork in 1995 and since that time the Department has changed substantially. John Hall retired, with the new Head arriving in January 2002 (narrowly escaping the demise of the Department of Physiology in Birmingham), while Brian Harvey has moved on to Dublin. There have been three new academic appointments recently to cope with the increased teaching required as a consequence of the opening of the new Schools, bringing the Department complement to eight permanent full time academic staff. Patrick Harrison arrived from the University of Glasgow and he is reinforcing the teaching of molecular physiology in the different programmes. Vincent Healy is a graduate of UCC and, having returned from a fellowship in UCD, is contributing to the cellular physiology courses. Therese Ruane-O'Hora is undertaking teaching in the nursing programmes and is ensuring that there is emphasis on integrative physiology. Along with the increase in personnel, there has been an expansion in quality research space for Physiology on the first floor of the Biosciences Institute – a brand new building which came on stream in October 2003. There are a number of research themes being developed within the Department at present. Patrick Harrison, with Tara Kelly (Research Fellow), Dave O'Sullivan (PhD student) and Rowan Flynn (PhD student), together with collaborators in the Departments of Anatomy and Microbiology, are developing virus vectors for gene delivery. At the molecular level projects are underway to investigate the link between mutant forms of CFTR and mechanisms of infection in cystic

fibrosis, and a separate study to identify the aquaporin and NHE isoforms expressed in *Rana temporaria* bladder (in collaboration with Liz Gebruers). Patrick Harrison also organizes the Physiological Society/Wellcome Trust Molecular Techniques Workshop hosted in the Department since 2000. Each year, the 10-day workshop provides training for 16 physiologists in molecular biological techniques such as sub-cloning, siRNA technology, RT-PCR, site directed mutagenesis and transfection techniques. The next workshop will be held in UCC in Spring/Summer 2005.

Vincent Healy leads a group investigating the mechanisms mediating noradrenaline-induced uptake of sodium in the renal proximal tubule. It would seem that there is intracellular trafficking of the NHE3 from endosomal stores into the apical membrane which requires phosphorylation of intermediates to occur. The impact of the noradrenaline on both these processes is a relatively unexplored area, and Vincent and Claire Thompson (PhD student), are applying not only cell culture techniques but whole animal studies to elucidate the mechanisms involved.

Edward Johns' team is concerned with investigating the mechanisms whereby the sympathetic nerves to the kidney determine both vascular and tubular function and how their regulatory mechanisms may be deficient in pathophysiological states. Together with Chunlung Huang (Research Fellow) renal sympathetic activity is being measured in conscious rats to see how baroreflexcontrol is altered by different dietary sodium intake.

In acute studies in the rat, Rob Gaffney (PhD student) is investigating how leptin within the central nervous system can determine the sensitivity of baroreflex control of renal nerve activity, both normally and in rat models of obesity. Ahmad Ahmeda (PhD student) is assessing the importance of reactive oxygen species in determining renal blood flow distribution and how this may be altered in rat models of hypertension.

Above: Gerry O'Regan with Fionnuala Ni Chiardha (top); Anne Harris (centre); Mike Snow and Roisin Kelly (bottom).
Below: Edward Johns (red jumper) with his team of Chunlong Huang, Ahmad Ahmeda and Rob Gaffney (top); Vincent Healy and Claire Thompson (centre); the new Biosciences Institute which came on stream in October, 2003 (bottom)

The large mammal physiology group is led by Mike Snow and his collaborators are Therese Ruane-O'Hora and Roisín Kelly (PhD student) who are undertaking acute studies in the pig. Together they have two areas of interest; the first concerns understanding the relationship between shear stress and the generation of nitric oxide in large conducting arteries *in vivo*. The second area is the contribution of local factors, for example VIP and NO, in modulating the impact of the vagus on the control of the heart.

Gerry O'Regan and Fionnuala Ni Chiardha together form a team investigating respiratory control in humans. They have been assessing how CO_2 together with tidal volume may determine the degree of respiratory sinus arrhythmias. In this work they are aided by a number of final year BSc students, who effectively recruit other undergraduate subjects.

The Society Meeting will take place within the Boole lecture theatre complex, and administrative and technical staff will be on hand to ensure that audio visual, orientation and any other local issues are dealt with efficiently. There are four symposia organized for the Members to enjoy, 'Cardiovascular-renal interactions' which aims to provide an up-to-date view of central and peripheral regulatory mechanisms; 'Adenosine, purine and nitric oxide in the regulation of kidney function', which is part supported by Pfizer; 'Cellular and integrative aspects of water balance', which is of particular relevance in relation to this years' award of the Nobel Prize in Medicine; and, finally, there is the Molecular Techniques Teaching Symposium; a chance to brush up on the basics and get updated on the latest topics.

We at the Department of Physiology are looking forward to having the Society visit us in Cork in September.

Edward J Johns
Department of Physiology, University College Cork

For further information and registration details visit: http://www.physoc.org/

Bristol Meeting

Viral gene transfer in neuroscience: new tricks of the trade

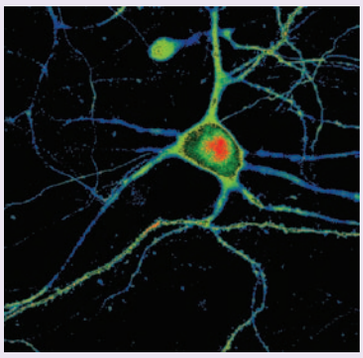
A Physiological Society Focused Meeting will take place at the University of Bristol, Bristol, UK on 4-5 September, 2004

During the last decade, viral vectors have become reliable tools for gene manipulation in the brain. This Meeting will focus on viral gene transfer as one of the powerful techniques in a rapidly growing field of *physiological genomics*. In the context of neuroscience, viral gene delivery helps to determine the roles of individual genes and their families under normal and pathological conditions.

Several types of vectors are currently being used and all offer distinct advantages for neuroscience. The experts speaking at this Meeting will cover such families as alphavirus, adenovirus, adeno-associated, lentivirus and baculovirus-derived vectors, and demonstrate their use to address various physiological and biomedical problems. Special attention will be paid to targeting transgenes to the diverse cellular populations present in the brain and to the future of viral vectors in both basic science and gene therapy.

In addition to the talks there will be an open poster session where participants are invited to demonstrate any piece of recent work using viral gene transfer.

Finally, on the second day of the Meeting, interested participants will have a chance to visit several



laboratories in the University of Bristol where viral gene transfer is carried out (site visit places are strictly limited).

This meeting will provide a great opportunity to learn, meet the experts, and discuss and establish new collaborations between all those interested in viral gene transfer and neuroscience.

Sergey Kasparov
Julian F R Paton
University of Bristol

Oxford Meeting

The city of dreaming spirals

A Physiological Society Focused Meeting at the University of Oxford, Oxford, UK on 1-3 October, 2004.

Dreaming spirals? No, the Special Meeting of the Physiological Society, devoted to *Biocomputation and Modelling in Physiology*, will not focus entirely on modelling cardiac re-entry in the shape of spirals. It will address a whole range of data-based simulation of biological behaviour, from molecule to man.

There will be 18 invited lectures on topics as diverse as molecular dynamics, developmental biology, cancer, the respiratory system and – well, of course – the heart!

The meeting is timed to coincide with the retirement of Denis Noble from his Burdon Sanderson Chair in Cardiovascular Electrophysiology. Even though we do not expect to see much – if any – reduction in his scientific activities (many predict that he will find more time in future to conduct his research than at any point during the last few decades), the 'career change' of a pioneer of our field is certainly worthy of special celebration.

Fittingly, the workshop will be launched by the Physiological Society's Hodgkin-Huxley-Katz Prize Lecture titled *From the Hodgkin-Huxley Axon to the Virtual Heart*, which Denis will deliver on Friday, 1 October at the Saïd Business School. This will be a public and highly publicised event, which coincides with the first day in office of Oxford's new Vice Chancellor, John Hood, who will open proceedings.

The following 2 days will be devoted to lectures by international leaders in the field of biomathematical modelling, covering a wide range of topics from sub-cellular to whole organ and systems modelling.

In addition, there will be a dedicated poster session (all posters will be on display throughout the event).

All abstracts will, as usual, be published on-line by the Physiological Society, while lectures will also be featured in a 2005 focused issue of the *Proceedings of the Royal Society*.

The focus of this workshop is to bring together specialists who are actively conducting biomathematical modelling in physiology. The lecture format (20 min talk + 10 min discussion), lunch arrangements (in the poster area), and social activities are tailored to provide ample time for discussion and informal interaction. Fitting with the concept of a 'workshop', the meeting will be limited to 160 participants, so early registration is encouraged.

In anticipation of a focused and productive Special Meeting, we look forward to welcoming you to the City of Dreaming Spires.

Alan Garny
David Gavaghan
Peter Kohl
Philip Maini
Departments of Physiology, Mathematics and Computing, University of Oxford

For up-to-date information and registration details for the Bristol and Oxford Meetings please visit the Physiological Society website: http://www.physoc.org/

A moment of excitement

To launch our *Living History* series, Geoffrey Burnstock recalls the discovery of non-adrenergic, non-cholinergic autonomic neurotransmission

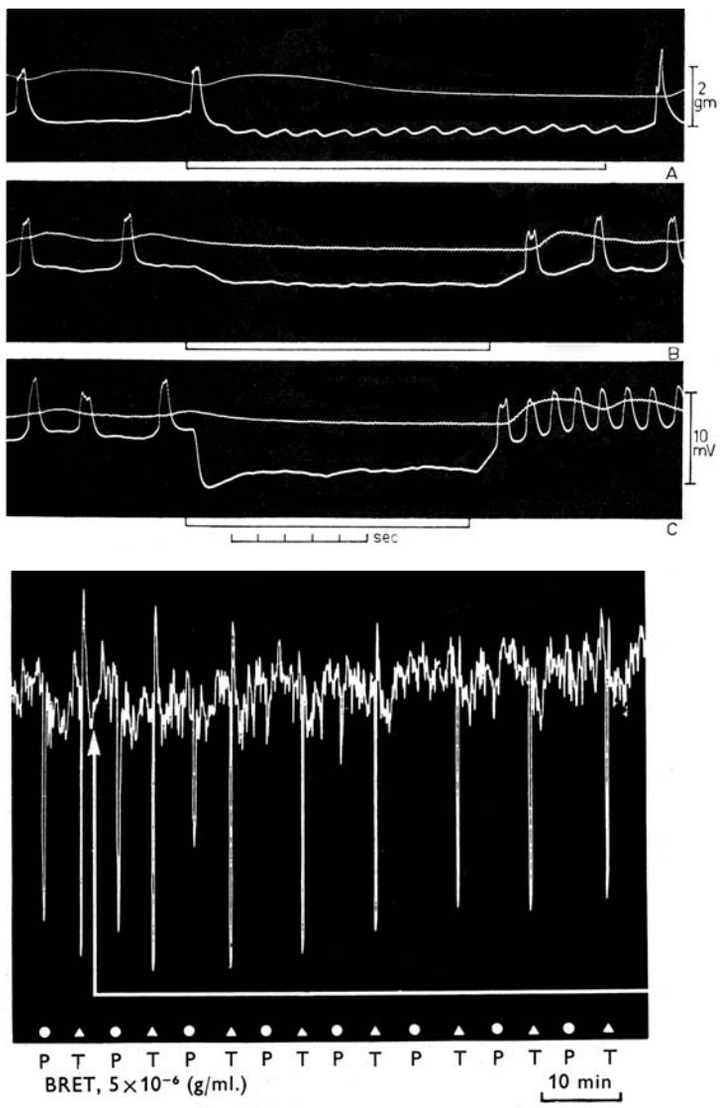
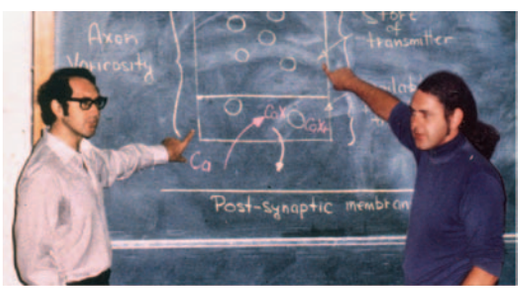


Figure 1 (top). Sucrose gap records from smooth muscle of guinea pig taenia coli showing inhibitory potentials in response to stimulation of intrinsic nerves. Frequencies of stimulation: (A) 1/sec; (B) 10/sec; (c) 20/sec. Upper trace, tension; lower trace, membrane potential. Note the phase of excitation (B and C) which follows cessation of stimulation (from Burnstock, Campbell, Bennett & Holman (1963). *Nature* 200, 581-582)

Figure 2 (above). Effects of adrenergic neurone-blocking drugs on responses to stimulation of the taenia or of the perivascular nerves after atropine. Perivascular nerve taenia preparation. Bretylium (BRET, $5x10^{-6}$ g/ml.) abolished mechanical responses to stimulation of the perivascular nerves at 30 pulses/sec (P, at dots), but only reduced responses to stimulation of the taenia with 10 pulses/sec (T, at triangles). Time marker 10 min (from Burnstock, Campbell & Rand (1966). *J Physiol* 182, 504-526)



Max Bennett (left) with Graeme Campbell, about 1983

I remember vividly the day that we discovered non-adrenergic, non-cholinergic autonomic neurotransmission.

Together with Ralph Straub at the National Institute of Medical Research, the sucrose gap technique for recording continuous, correlated changes in electrical mechanical activity of smooth muscle was developed (Burnstock & Straub, 1958). I moved to Edith Bülbring's lab in the Department of Pharmacology, Oxford, and completed studies of classical adrenergic and cholinergic responses of the guinea-pig taenia coli (Burnstock, 1958a,b).

From Oxford, I moved to Melbourne, Australia where, with the help of an NIH grant held jointly with Mollie Holman, whom I had met in Oxford and whose work I had admired there, I set up the sucrose gap apparatus in my laboratory.

Graeme Campbell, a postgraduate research assistant and Max Bennett, at that time a part-time electronics technician who was completing a degree in Electrical Engineering, were working with me, and one day in 1962 we decided to look at the direct responses of the smooth muscle of the taenia coli after blocking the responses of the two classical neurotransmitters, acetylcholine and noradrenaline. Graeme and Max came into my office to show me the remarkable responses to stimulation, which were rapid hyperpolarisations and associated relaxations in response to single electrical pulses. This was a very exciting moment – we all felt instinctively that this unexpected result was going to be important.

Later we, and others, showed that tetrodotoxin, which had just been discovered in Japan and which blocked nerve conduction but not muscle responses, abolished these hyperpolarisations and we realised that we were looking at inhibitory junction

potentials (IJPs) in response to a non-adrenergic, non-cholinergic (NANC) inhibitory neurotransmitter (Burnstock *et al.* 1963, 1964). This was followed by a detailed study of the mechanical responses of the taenia coli to stimulation of intramural and sympathetic nerves while I was on sabbatical leave at the School of Pharmacy in London, working together with my friend Mike Rand (Burnstock *et al.* 1966). Nearly a decade later, after much work, we published a paper that suggested that the NANC transmitter in the taenia coli was ATP (Burnstock *et al.* 1970).

Geoffrey Burnstock

Director, Autonomic Neuroscience Institute, Royal Free & University College Medical School and Professor of Anatomy in the Department of Anatomy & Developmental Biology at University College London

References

Burnstock G & Straub RW (1958). A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes. *J Physiol* 140, 156-167.

Burnstock G (1958a). The effects of acetylcholine on membrane potential, spike frequency, conduction velocity and excitability in the taenia coli of the guinea-pig. *J Physiol* 143, 165-182.

Burnstock G (1958b). The action of adrenaline on excitability and membrane potential in the taenia coli of the guinea-pig and the effect of DNP on this action and on the action of acetylcholine. *J Physiol* 143, 183-194.

Burnstock G, Campbell G, Bennett M & Holman ME (1963). Inhibition of the smooth muscle of the taenia coli. *Nature* 200, 581-582.

Burnstock G, Campbell G, Bennett M & Holman ME (1964). Innervation of the guinea-pig taenia coli: are there intrinsic inhibitory nerves which are distinct from sympathetic nerves? *Int J Neuropharmacol* 3, 163-166.

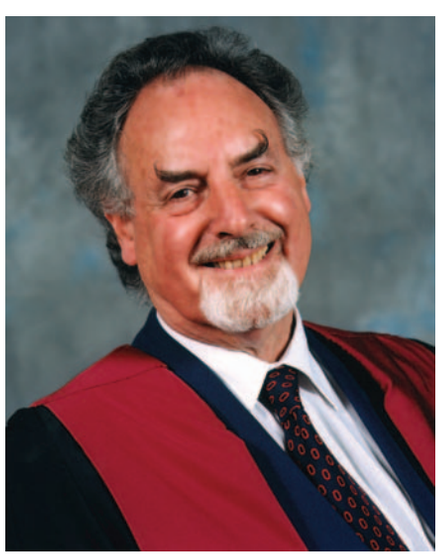
Burnstock G, Campbell G & Rand MJ (1966). The inhibitory innervation of the taenia of the guinea-pig caecum. J Physiol 182, 504-526.

Burnstock G, Campbell G, Satchell D & Smythe A (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br J Pharmacol* 40, 668-688.

Above
Top: Mollie Holman and Geoff Burnstock, 1960
Above: Edith Bülbring and Geoff Burnstock, about 1985

Right: Geoff Burnstock in 2003

Below:
Left: Geoff Burnstock with Mike Rand, about 1992



Biographical notes about all those featured in the article appear on the next page

Biographical notes

Ralph Straub *was a visiting scientist in Feldberg's Department at the National Institute of Medical Research in the late 1950s. He returned to Switzerland in the 1960s to become Professor of Pharmacology at the Centre Médical Universitaire, Geneva. He died in April 1988 when only 60 years old.*

Edith Bülbring *was born in 1903 in Bonn. She joined the Department of Pharmacology in Oxford in 1937 and between 1950 and 1990, when she died, established her laboratory as the leading international laboratory in smooth muscle pharmacology and physiology; she had a major influence on many working in this field today.*

Mollie Holman *was born in 1930 in Australia. She joined Edith Bülbring's group in Oxford to work on the electrophysiology of smooth muscle, completing a DPhil in 1957, and then returned to Melbourne and was eventually appointed as Professor of Physiology at Monash University. She retired in 1995.*

Graeme Campbell *was born in Australia and completed a PhD in Zoology supervised by Geoff Burnstock in 1965. In 1976 he took over the Chair of Zoology in Melbourne vacated by Geoff Burnstock on his move to UCL Anatomy, but is now retired.*

Max Bennett *was born in 1939, completed a degree in Engineering in 1963 and switched to Zoology, completing a PhD in 1967 under the supervision of Geoff Burnstock. He was appointed as a Lecturer in Physiology at Sydney University in 1969 and rose to Professor of Physiology in 1983. He is now one of the most active and distinguished neuroscientists in Australia.*

Mike Rand *was born in 1927 in England, but moved with his mother to Australia in 1941. He completed his PhD in Pharmacology in Sydney University and then accepted a postdoctoral position in Oxford Pharmacology with J H Burn in 1957. After a period of 5 years at the School of Pharmacy in London he accepted the Chair of Pharmacology in Melbourne University in 1965 and after 'retirement' took up an appointment as Adjunct Professor at RMIT University, also in Melbourne. He was a marvellous scientist and had a major international influence; sadly, he died in 2002.*

Training and competition stress: effects on immune function and health

Mike Gleeson continues our series of articles on exercise physiology in the run up to the Olympics by considering the health implications of hard training by endurance athletes

Mike Gleeson

Athletes dread the thought of catching a cold or the 'flu. Infections can interfere with training, impair performance and even prevent an athlete from competing. Unfortunately, athletes engaged in heavy training programmes, particularly those involved in endurance events, appear to be more susceptible than normal to infection. For example, several epidemiological studies in the 1990s indicated that sore throats and flu-like symptoms are more common in endurance athletes than in the general population. The immune system protects the body against infection but the functioning of the immune system is affected by stress and there is some evidence that the increased susceptibility to infection in athletes actually arises from a depression of immune function. Although impairment of immune function sometimes leads to the reactivation of a latent virus, the development of a new infection generally requires exposure to a pathogen, and there are many training and competitive situations in which the athlete's exposure to pathogens is increased.

Heavy prolonged exertion is associated with increased levels of stress hormones (e.g. adrenaline and cortisol) and cytokines (e.g. interleukins 6 and 10) which inhibit some aspects of immune function. Several changes during early recovery from exercise would appear to weaken the potential immune response to pathogens and although the clinical significance of these changes has not been established, it has been suggested that the post-exercise period may provide an 'open window' for infection representing the most vulnerable time for athletes in terms of their susceptibility to infection. The impairment of immune function following a prolonged bout of exercise is associated with decreased expression of the Toll-like receptors on monocytes that detect pathogens, decreased killing capacity of neutrophils and natural killer cells, decreased cytokine production by type 1 T-helper cells together with the disappearance of these lymphocytes from the circulation, decreased lymphocyte proliferative responses to antigens and increased apoptosis under the influence of cortisol. The secretion of cortisol during exercise is stimulated by muscle-derived interleukin-6 (IL-6). Studies from Bente Pedersen's group in Copenhagen indicate that the release of IL-6 from contracting muscle can be attenuated by carbohydrate ingestion during exercise and by long-term antioxidant supplementation. This group has argued that this, however, may be a two-edged sword, as IL-6 has several metabolic effects and shared mechanisms exist regarding immune impairment and training adaptation. The concern for athletes is that although these nutritional interventions may reduce their risk of infection, another effect may be to limit their hard-earned adaptation to training.

Figure 1. Monitoring of saliva immunoglobulin A in swimmers can provide an idea of mucosal immunity

Both heavy exercise and nutrition exert separate influences on immune function; these influences appear to be greater when exercise stress and poor nutrition are present together. Exercise training increases the body's requirement for most nutrients and, in many cases, these increased needs are met by increased food consumption, though for some athletes this may conflict with their weight restriction goals. Athletes can help themselves by eating a well balanced diet that includes adequate energy, carbohydrate, protein and micronutrients. Ensuring sufficient carbohydrate intake to restore glycogen stores on a daily basis will minimise the stress hormone response to training and delay the onset of symptoms associated with overreaching.

Hard exercise is associated with an increased level of free radicals because of the high rate of oxygen consumption. The superoxide radical is formed during the passage of electrons through the mitochondrial respiratory chain. Free radicals are also formed in the blood and tissues by activated phagocytic white blood cells. Finally, the reperfusion of tissues such as the gut after exercise may also give rise to increased free radical production through the actions of endothelial xanthine oxidase. Some immune cell functions can be impaired by an excess of free radicals and so this is another possible mechanism of exercise-induced immune function depression. In Athens, the high level of air pollutants (particularly ozone) is likely to exacerbate oxidative stress and may also impair lung function. This, of course, affects athletes more than sedentary people because of the high level of pollutant exposure with high rates of ventilation during exercise (as high as 200 litres per minute in very fit individuals). Obviously, the longer the event, the greater the exposure to ozone, and asthmatic athletes such as Paula Radcliffe will be more susceptible to airborne pollutants. To protect against the harmful effects of oxidative stress, the body contains numerous anti-oxidant compounds (e.g. glutathione) and enzymes (e.g. superoxide dismutase), along with the ability to absorb several dietary anti-

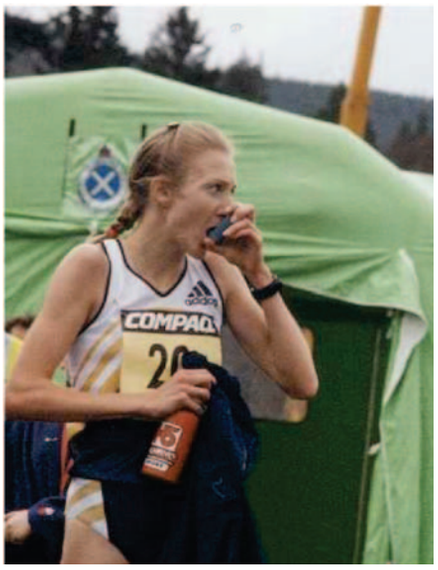


Figure 2. High levels of air pollution may impair lung function in athletes

oxidant compounds, such as vitamins C and E, that are involved in the quenching of free radicals. Supplementation with vitamins C and E appears to alleviate the effects of ozone on exercise performance and, although there is some controversy regarding the effects of anti-oxidant vitamin supplements on immune function and resistance to infections, a handful of studies have reported fewer infectious episodes in athletes supplemented with vitamin C prior to long distance foot races.

The other big environmental problem in Athens is, of course, the heat. Exercising in these hot environmental temperatures is associated with an increased stress hormone response and greater perception of effort. The general consensus is that exhaustive physical activity and severe environmental stress generally have at least an additive effect on stress responses and immunodepression. Limiting initial exposure when training or competing in adverse environmental conditions, and acclimating or acclimatising where appropriate, will reduce the effects of environmental extremes on the stress hormone response to exercise. This should be beneficial for the maintenance of immunocompetence.

In addition to physical stress, there is the added psychological stress of competition, team and commercial pressures, international travel, selection pressures, funding pressures and other major life events. Chronic psychological stress is also known to depress immune function. The aim of the coach, working with a sport psychologist, should be to anticipate these additional stressors and, through appropriate evaluation and planning, eliminate or minimise as far as possible their impact upon the athlete. Sleep disturbance is not uncommon in athletes who are training hard, and it is important to realise that chronic lack of sleep is itself associated with impaired immunity. Athletes should be encouraged to get adequate sleep and 6 hours sleep per night is probably the minimum required by most. Other behavioural lifestyle changes, such as good hygiene practice, may limit transmission of contagious illnesses by reducing exposure to common sources of infection including airborne pathogens and physical contact with infected individuals. Medical support, including regular check ups, appropriate immunisation and prophylactics, may be particularly important for athletes who are at high risk of succumbing to recurrent infection.

Finally, do not take this as a message that exercise is bad for you! Of course, it is well established that regular moderate exercise is good for health and is associated with reduced incidence of both cardiovascular and metabolic diseases. Even immune function may benefit from regular exercise, provided that you don't over do it. A recent study found that the incidence of respiratory infections was 30% lower in people who exercised moderately for 1-2 hours per day compared with those with a coach potato lifestyle! To have any chance of winning an Olympic medal, though, athletes have to train much longer and harder than this.

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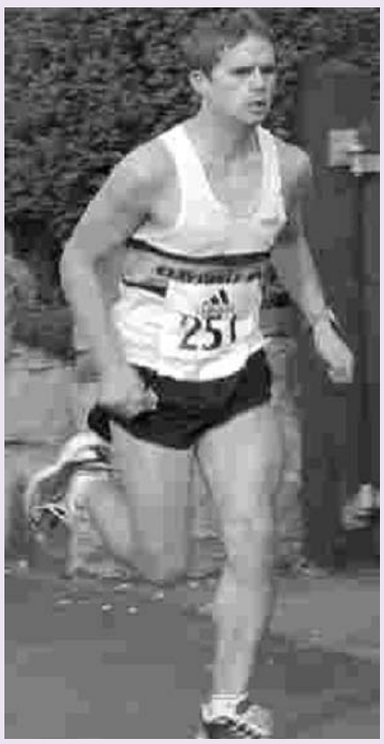
The final article in this series, to be published in issue 57 of* Physiology News*, will look at doping issues.

Inside the 'black box'

Henning Wackerhage and Philip Atherton explain the molecular adaptations to marathon training

'We are now well on the way to characterising the mechanisms that make athlete's hearts grow, their muscle capillaries sprout and their bones and their cartilage more resistant to mechanical impact' – Henning Wackerhage (above) and Philip Atherton (below)



The marathon run is a benchmark endurance test. It was born during the first modern Olympic Games in Athens in 1896, and subsequently stretched in steps to today's 42.195 km or 26.2 miles. The jogging and running boom of the 70s and 80s, and improvements in endurance training, have changed it from something that was seen as a dangerous, over-exhaustive, males-only activity to a serious test that anyone can do – including the young and old, heart patients and disabled people. Marathon running was also an important stage for female emancipation in sport, starting with unofficial attempts in the 60s up to the present day record of 2:15 h held by Paula Radcliffe.

The high fitness of marathon runners and other endurance athletes, and phenomena such as 'hitting the wall', have stimulated a generation of exercise physiologists to study marathon running and endurance training. They focused on the determinants of marathon running performance and on the adaptations that are stimulated by endurance training. An early, major finding was that endurance training promoted 'healthy' cardiac growth, resulting in the 'athlete's heart'. The endurance athlete's larger heart can increase its pumping of blood to a maximum of 40 litres per minute (compared to about 20 litres in untrained subjects). The higher capacity for blood transport also means a higher capacity for oxygen and nutrient transport round the body.

Skeletal muscles adapt as well: endurance training promotes a limited fast-to-slow exchange of motor proteins. That is one reason why marathon runners are poor sprinters. Marathon runners, however, have a very high capacity for producing ATP by oxidative phosphorylation because mitochondrial biogenesis is stimulated by endurance exercise. The fuel supply for oxidative phosphorylation also changes, which enables the runner to better sustain the fuel supply during long duration exercise. As a rule of thumb, the concentrations of enzymes that use glycogen decrease, whereas the concentrations of enzymes that synthesize glycogen and utilize fat increase. As a result, marathon runners save their relatively small glycogen store and rely more on the plentiful fat reserves.

Classical exercise physiologists used a 'black box' approach to study the adaptation to endurance training. Their model was:

endurance training → black box → adaptation.

Molecular exercise physiologists now seek to open the black box and to identify the mechanisms that regulate the well-described adaptive responses to endurance training. Their aim is to identify the chain of events, starting with an exercise-related signal such as calcium or muscle tension, followed by the activation of a signal transduction pathway and its effect on gene regulation and ending with a known adaptation to exercise.

The major breakthrough in this new field was made by Eva Chin and co-workers in 1998. Chin *et al*. found that the immunosuppressant drug cyclosporin A increased the percentage of fast muscle fibres in rodents. Cyclosporin A is known to inhibit the calcium-activated calcineurin pathway.

This and other findings suggested that activated calcineurin stimulated the production of proteins such as myoglobin and slow troponin that are known to be upregulated by endurance training. The findings could be summarised as a mechanistic model (Fig. 1):

endurance training → calcium concentration increase → calcineurin activation → increased expression of proteins known to increase in response to endurance training.

Soon after, it became clear that the calcineurin pathway was only part of the story. Murgia *et al*. (2000) showed that the activated ERK1/2 pathway also increased the percentage of slow muscle fibres (Fig. 1).

Other studies showed that the ERK1/2 pathway is activated by endurance exercise and thus both pathways are

likely to co-operatively regulate the adaptive response to endurance exercise.

Much progress has also been made in identifying the mechanisms that regulate the exercise-induced increase in the division of mitochondria, termed mitochondrial biogenesis. Mitochondria are the sites of oxidative phosphorylation. The majority of mitochondrial proteins are encoded in nuclear DNA, but mitochondria have their own 16,600 base pair-long DNA which encodes some of the proteins. This is an evolutionary 'leftover' and was the target of the first major human DNA sequencing project. Because of the existence of mitochondrial DNA, the regulation of mitochondrial biogenesis must involve the activation of the expression of genes that are encoded in nuclear and mitochondrial DNA.

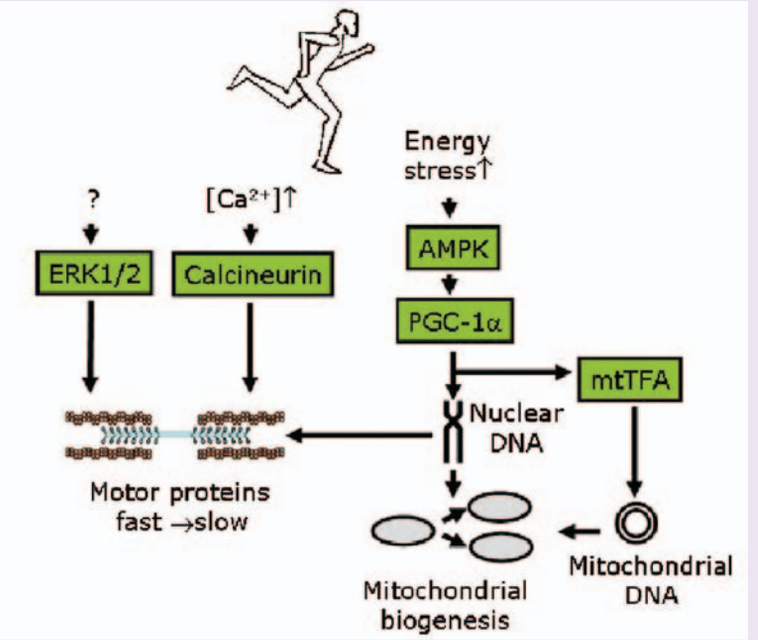


Figure 1. Major signalling events that mediate the adaptation to endurance training

Scarpulla (2002) has identified so-called nuclear respiratory factors that were important for the expression of mitochondrial genes encoded in the nuclear DNA. Tiranti *et al*. (1995) then identified the mitochondrial transcription factor A (mtTFA or TFAM), which is encoded by nuclear DNA but switches on the expression of genes encoded in mitochondrial DNA.

A breakthrough was the discovery of the transcriptional co-factor PGC-1. Puigserver & Spiegelman (2003) found that the overexpression of PGC-1 stimulated mitochondrial biogenesis. Interestingly, mice that overexpress PGC-1 not only have a very high mitochondrial content but also express large amounts of other slow fibre proteins such as myoglobin (their muscles appear red) and slow troponin.

Recently Terada *et al*. (2002) have shown that endurance exercise and the activation of the AMP kinase increased PGC-1 expression. AMP kinase is activated by increases in the concentration of AMP which is associated with the 'energy stress' during exercise and with low glycogen, which results from endurance training.

To summarise, the major regulating events during mitochondrial biogenesis might be formalised as (Fig. 1):

endurance exercise → 'energy stress' → AMP kinase activation → upregulation of PGC-1 → expression of a) nuclear DNA-encoded mitochondrial genes (including mtTFA) via PGC-1 and b) mRNA and mitochondrial DNA-encoded mitochondrial genes via mtTFA → synthesis of all mitochondrial proteins and mitochondrion assembly (more mitochondria) → higher capacity for ATP synthesis by oxidative phosphorylation.

The above findings show that molecular exercise physiology has entered its golden era fuelled by technological advances such as antibodies against phosphoproteins and microarray technology. We are now well on the way to characterising the mechanisms that make athlete's hearts grow, their muscle capillaries sprout and their bones and their cartilage more resistant to mechanical impact. In addition, genotype chips are available that allow researchers to link an individual's genotype to performance-related characteristics.

The future will show whether this increase in knowledge can be exploited for guiding marathon runners at all levels towards better performances.

Acknowledgements

We should like to thank the Wellcome Trust, the University of Dundee and the University of Central Lancashire for supporting our research.

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References

Chin ER, Olson EN, Richardson JA, Yang Q, Humphries C, Shelton JM, Wu H, Zhu W, Bassel-Duby R & Williams RS (1998). A calcineurin-dependent transcriptional pathway controls skeletal muscle fiber type. *Genes Dev* 12, 2499-2509.

Murgia M, Serrano AL, Calabria E, Pallafacchina, G, Lomo T & Schiaffino S (2000). Ras is involved in nerve-activity-dependent regulation of muscle genes. *Nat Cell Biol* 2, 142-147.

Puigserver P & Spiegelman BM (2003). Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 24, 78-90.

Scarpulla RC (2002). Nuclear activators and coactivators in mammalian mitochondrial biogenesis. *Biochim Biophys Acta* 1576, 1-14.

Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T & Tabata I (2002). Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. *Biochem Biophys Res Commun* 296, 350-354.

Tiranti V, Rossi E, Ruiz-Carrillo A, Rossi G, Rocchi M, DiDonato S, Zuffardi O & Zeviani M (1995). Chromosomal localization of mitochondrial transcription factor A (TCF6), single-stranded DNA-binding protein (SSBP), and endonuclease G (ENDOG), three human housekeeping genes involved in mitochondrial biogenesis. *Genomics* 25, 559-564.

A week in the Zambian Bush

In May 2002, Physiological Society Member Tristan Pocock left the Physiology Department at the University of Bristol, where he was a senior post-doc researching the microcirculation, to spend 2 years as a VSO[1] volunteer teaching high school biology in the Zambian bush

A typical week begins with the Monday morning staff briefing. I usually emerge from under my mosquito net at 6.15am, having dozed since being awoken by chickens crowing at 4.00 (everyone in rural Zambia seems to own at least one chicken and they are usually let out well before dawn). The water is turned on at 5.30 in the morning for 30 minutes – if I want a wash, I have to jump in the cold bath for a few minutes and then refill it for washing clothes, plus filling bottles for drinking. Getting up this early was not part of my routine as a PhD student or postdoc! However, it is much easier to get up at such an unearthly hour with the sun streaming through the curtains 360 days a year.

Tristan Pocock with pupils from the Mambwe High School, where every day offers something new

Being better at getting up than my VSO housemate, Jenny, I make breakfast for us both (imported porridge oats), unless I am 'Teacher on Duty', in which case I am obliged to go to the dining hall to taste the ground maize porridge which the pupils get for breakfast. I leave the house for the 6.45 staff briefing, invariably arriving 2 minutes late. Other teachers arrive over the course of the next half an hour. With the staff suitably up-to-speed (or more likely confused) as to what lies in store for the week, we proceed to assembly. The best thing about assembly is listening to the very talented choir and joining in with the National Anthem (provided it's sung in English, rather than the local language, Chinyanja). The worst thing is that it delays the start of teaching (often to the extent that the first lesson is lost entirely!). On other days of the week lessons start at 7.00 and go through until 12.40.

Before lessons, I take my class register. With 56 pupils in my class this often takes up all 10 minutes of registration. Lessons are generally 80 minutes in length, but seem to get longer and longer as the morning progresses and the temperature rises (in the hot season, it is 30ºC at 7 am and often over 40ºC by 12.40 pm!) Many pupils get up in the middle of lessons and lean against the wall. It took me a while to realise that this is to stop them from falling asleep!

I am the only biology teacher, but also teach chemistry sometimes. It was a bit of a shock having to dig deep into the memory banks for my long-neglected GCSE knowledge of atomic structure! Most of the higher education level biology I learnt is pretty irrelevant here. At the beginning I occasionally had to stop myself getting carried away and ranting on about growth factors when teaching blood vessel structure and function. In fact, it is the sometimes-derided 'transferable skills' from my time in higher education and research that are the most useful here – the ability to judge the appropriate level of explanation for the audience/class, to decide how detailed to make a diagram, and so on.

So how did I end up here? After nearly 10 years in research, I had the growing feeling that it was not a career for me in the long term, although I had no clear idea what to do instead. Having glimpsed different cultures around the world as a tourist, including South Africa, I knew I wanted to learn more. When I contacted VSO, they decided I was made to be a biology teacher! I do feel privileged to have received such a good education in the UK (honestly!), and VSO offered me a chance to use my knowledge to benefit others, as well as to live in a different culture for a couple of years. Having made the decision, I finished my contract, and then went, within the space of about a week, from the lab in Bristol to the bush.

I first arrived at the school after an arduous journey from the Zambian capital, Lusaka, where I had spent a few days acclimatising and learning about Zambian culture. From Lusaka I boarded the 'chicken bus' (so-called because of the ever-present chickens, and with hundreds of people crammed into every available space) for the long journey to Chipata, my 'local' town. After that, I endured a further 3 hour journey to the school along a heavily

1 Voluntary Services Overseas (VSO) is an international development charity, started in 1958 and currently has over 2,000 volunteers working in 'developing countries' worldwide.

Classroom lessons generally last for 80 minutes, but seem to get longer as the morning progresses and the temperature rises to over 40°C. Pupils often get up in the middle of lessons and lean against the wall - to stop them from falling asleep!

pot-holed dirt track, perched precariously on the back of a truck with all my worldly possessions and several nonchalant locals (with their luggage, bags of maize and, inevitably, more chickens).

My school, Mambwe High School, is a government-run mixed boarding school with almost 400 pupils, aged between 14 and 25. The pupils study in classes ranging in size from 22 to 60 (!) and sit exams similar to British GCSEs. The teachers (all Zambian except for Jenny and I) live on the school compound. The nearest post office, bank and supermarket are 87 km away in Chipata. Fortunately, the local BOMA (administrative centre), a mere 7 km down the road, has a few shops (which stock a wide range of biscuits and soap), a market (which sometimes has fresh vegetables) and even a bar (which, until electricity arrived in the District last year, served only warm beer!) There is a mission hospital 3 km away, which has one doctor (when he's not called away to town) and several nurses, an X-ray machine and an operating theatre. My only experience of treatment there was when I got plastered after breaking my arm playing football.

I settled into teaching quickly – in contrast to my experiences of British schools (and universities!), the pupils are extremely respectful of teachers and were very willing to accept me. Unlike in the UK, secondary school teaching is a privilege many children here cannot afford. My experience as a physiology tutor at Bristol, and of presenting at national and international scientific meetings, helped me to come to terms with teaching - just as well, since my only previous experience at this level came from a week of shadowing teachers in a Gloucestershire secondary school. Learning the names of over 300 pupils was infinitely more difficult. All teaching is conducted in English, but it is not the first language (there are around 70 tribal languages in Zambia). Although pupils must pass an English exam at a fairly high level, for many their lack of English understanding is a major stumbling block to learning. Another hindrance is pupils being dragged out of lessons to draw water, collect firewood, mend the diesel generator (which powers the water pump and supplies lighting in the evening) or for punishment. Punishment usually involves an extended stint cutting the long grass on the school compound. This is useful because the long grass is where the (malarial) mosquitoes breed. Cutting the grass is also an important part of impressing visiting dignitaries from the Education Ministry! Just as back in the UK, whistle-stop tours by the Great and the Good mean a frenzy to get things looking right, since visiting Big-wigs are rarely around long enough to do more than look.

Back to the teaching. Due to the large class sizes, science practicals are a logistical nightmare. However, I decided to take the plunge and teach groups of 20-25 in the afternoons for practicals. The lab is surprisingly well-stocked for an African school – plenty of glassware and a cupboard full of chemicals, although most of them inappropriate for the level of teaching. Many of them are the kind of solvents you still find in UK university biology labs, but which were long ago banned from British schools for being too dangerous, like xylene and phenol. There are no COSHH regulations to worry about, though, and most of the pupils would be happy to dissect whatever wildlife they can get their

Classes of up to 60 pupils make science practicals a logistic nightmare, but the lab is surprisingly well stocked for an African school

hands on! Lack of running water and electricity is a hindrance, but not an insurmountable one – examples of practicals we do include testing the local produce for fats, proteins or carbohydrates, or comparing the features of local flora.

Morning tea break is a chance to re-energize with a cup of Zambian tea (more like a concentrated sugar solution with a hint of tea) and a scone. This probably saved me on a number of occasions from collapsing in the heat of the late morning! At 12.40, I return home for further vital rehydration and lunch (bread buns bought from a local village and sometimes tomato, followed by local bananas, all washed down with warm squash). Over lunch I play Scrabble with Jenny. This rapidly became an addiction, and we recorded over 150 games in one term!

Apart from the practicals, afternoons are spent marking, or on extracurricular activities, such as coaching the girls' football team or meeting with the Anti-AIDS Club. AIDS is probably the major health issue facing the population here. The proportion of Zambians infected with HIV is around 25%, and about 1 million Zambian children have lost at least one parent to AIDS. Just on the laws of statistics, a significant proportion of my pupils must be HIV-positive. It is therefore very important that this generation of Zambian youth are active in instigating the behaviour changes necessary to reduce the infection rate. Fortunately, Mambwe High School has an enthusiastic Anti-AIDS Club. My involvement, as club 'Patron', is to facilitate discussion and to organise visits to other schools and villages. The pupils perform drama and poetry, sing and dance to raise awareness and educate other people about HIV/AIDS. The other Club I supervise is the Wildlife Conservation Club. The school is in a 'Game Management Area', so it is important for the pupils to understand that, while poaching is a way of life for many people here, conservation of rare species is imperative for maintaining the National Park (South Luangwa) as a popular tourist destination. This then provides income and employment for many local communities. The pupils know far more about the local wildlife than I do, and many are keen to work as guides when they finish school.

Typical early evening activities include badminton or tennis, on a specially constructed court in front of the house, followed by a cold bath (shared – but one at a time!) to coincide with the water being turned on, again for just 30 minutes. Dinner is a combination of tomatoes, onions (plus peppers if we can get them), and pasta or rice from the supermarket in Chipata. Dinner is followed by at least one game of Scrabble (by candlelight if the generator is not working), often interrupted by having to remove a gigantic insect or turf out noisy frogs. These latter might be *Xenopus*, although I couldn't swear to it. I occasionally find myself wondering whether they have interesting muscle fibres, oocytes, or microcirculatory vessels, or whether I could ship them to the UK for experimental purposes.

If I'm feeling brave, I might venture up to school in the dark (no outdoor light except moon and stars here) to supervise evening 'prep', or do some marking before the power is switched off at 9.30 pm. After that, the compound is silent, except for the (often ear-piercing) sounds of cicadas and the occasional distant howl of a hyena or wild dog.

Weekends are usually spent at the school, preparing lessons, taking the Anti-AIDS Club on visits, watching the school football team play or relaxing. Perhaps the most unusual after-school activity so far was getting roped in to judge a pupils' modelling contest! A downside of being on-site is that the pupils have no qualms about knocking on the door at 6 am on a Sunday morning to request a book. Lie-ins are a rare luxury given the intense heat during most of the year. Sometimes on Saturday nights I join the 'drinkers' on the 7 km excursion to the 'local' bar. If transport can't be commandeered then we have to walk back in darkness, running the risk of meeting the odd hunting hyena *en route* – probably an insignificant risk, though, compared with walking back from a British pub after closing time! All in all, a far cry from my life in Bristol, where I had a choice of six pubs within 10 minutes walk from home. Weekends away start with hitching to Chipata on the back of a truck – a journey which can take anything from 1 to 6 hours, depending on the state of the pot-holed road, the recklessness of the driver and the degree of overloading.

Leaving research, after completing a Pharmacology degree, a PhD and then 4 years as a post-doc, to teach secondary school biology in Zambia was perhaps not the most obvious career move, but I have no regrets. Instead of being cooped up in a dark lab looking down a microscope at capillaries fluorescing (or not, on a bad day), I get to stand up in an open classroom in front of a large crowd of teenagers explaining how blood moves around the body. Like other teachers, I have more responsibilities concerned with the well-being of other people and hope that I have made a lasting impact on some of those I have met. If only one of my pupils decides to use condoms as a result of my attempts to explain how the HIV virus is transmitted, then several people might be spared infection with HIV. Aside from teaching, I'm sure I have learned as much from the people I have met in Zambia as they have from me. Every day has offered something new, and I have had some amazing adventures.

My experiences in Zambia have certainly changed my outlook on life. Teaching has been a refreshing change from the confines of a lab, and I am keen to continue teaching in some capacity when I return to the UK. Perhaps as a teaching fellow or demonstrator in a university department, or even as a secondary school teacher. Anyway, it has been great fun (if exhausting), and I would heartily recommend VSO to any young physiologist contemplating a career change from research to teaching.

Tristan Pocock
Since this piece was written, Tris Pocock has returned to the UK and is now a Teaching Fellow in Pharmacology and Physiology at Manchester University

Role of villus microcirculation in intestinal absorption: coupling of epithelial with endothelial transport

Capillaries in jejunal villi can absorb glucose at rates several hundred times greater per gram of tissue than in brain or contracting skeletal muscles. These high rates are made possible by delivery of glucose in high concentration from epithelium to capillaries via paracellular diffusion and solvent drag combined with increases of villus capillary blood flow and surface area

John Pappenheimer (top)and Charles Michel

Investigations of nutrient absorption from the small intestine have focussed almost entirely on transport into and out of the epithelial cells. However, the transport pathways extend beyond the epithelial absorptive cells to villus capillaries so that, in addition to the apical and lateral epithelial cell membranes, transport continues through the epithelial basement membrane and the walls of the villus capillaries into flowing capillary blood. Previous investigators have assumed that the basement membrane and capillary walls offer little resistance to absorbed solutes, and that the concentration differences across them are negligible. Our recent analysis of glucose absorption shows that this assumption is far from correct (Pappenheimer & Michel, 2003). The capillaries present a barrier comparable with that of the epithelium, and increases in villus capillary blood flow and surface area during absorption are an important part of the absorptive mechanism.

A schematic diagram of the transport pathways is shown in Fig. 1. Glucose enters the epithelial cells with Na^+ on the SGLT-1 transporter and leaves by facilitated diffusion through the lateral membranes on Glut-2. It is seldom appreciated that the nucleus, mitochondria, and other membranous organelles occlude most of the cross sectional area of the epithelial cells (Buschmann & Manke, 1981). Consequently, diffusion into the basal regions of the cell is hindered and the preferential pathway lies through the lateral membranes immediately below the tight junctions. Subsequent transport to the abluminal surfaces of the capillaries is by convection and diffusion through the post-junctional intercellular channels. Thus paracellular transport is the connecting link between epithelium and capillaries, and there is strong anatomical and electro-physiological evidence that the channels are widened during Na^+-coupled transport of sugars and amino acids (Madara & Pappenheimer, 1987; Pappenheimer & Volpp, 1992).

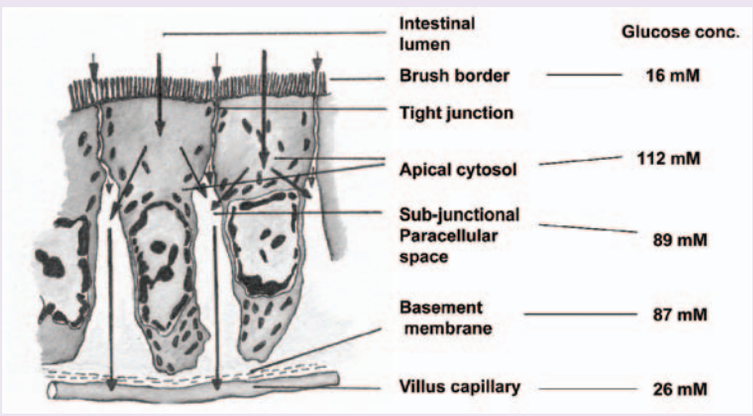

Figure 1. Diagrammatic representation of pathways for transport of glucose from lumen of small intestine to villus capillary blood. The pathways are shown as thick arrows passing through apical regions of the epithelial cells into the paracellular spaces and into the capillary. To the right of the diagram are listed the estimated glucose concentrations at various points along the pathway during glucose absorption at a moderate rate (40 µ-moles h^{-1} cm^{-2}) under normal conditions in unanaesthetised rats. Note that the fall in concentration across the capillary walls is more than twice that needed for transport between the apical cytosol of the epithelial cells and the abluminal surface of the capillaries.

We have estimated glucose concentrations along the pathway as a function of glucose absorption rates in unanaesthetised rats and in normal human subjects. Only small concentration gradients are necessary to account for diffusion-convection in intercellular channels. However, large trans-endothelial concentration gradients are required to account for observed absorption rates of glucose into villus capillary blood. Figure 2 shows our estimates of glucose concentrations at the epithelial basement membrane immediately outside the capillaries as a function of glucose load and villus blood flow. With normal hyperaemic responses to glucose absorption the concentrations in abluminal fluid required for diffusion into capillary blood are in the range 60 -100 mM; without the normal hyperaemic response, concentrations exceeding 400 mM would be required to sustain observed absorption rates.

In addition to the large concentrations required for transport across villus capillaries, there may be significant gradients across the lateral membranes of epithelial absorptive cells. Calculation of these gradients, using available kinetic parameters of Glut-2 (Kellett, 2001), has led to estimates of

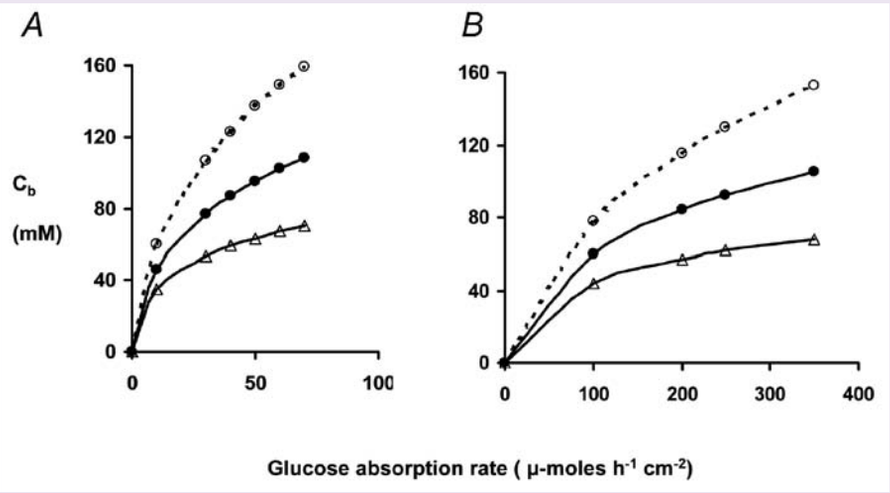

Figure 2. Effects of blood flow on glucose concentrations immediately outside villus capillaries at different rates of glucose absorption. C_b is mean glucose concentration at the abluminal surface of the capillaries under conditions where the increase in blood flow is normal (middle curves •), twice as great as normal (lower curves Δ) and only half as great as normal (upper curves, o). Glucose absorption rates are expressed in µ-moles h^{-1} cm^{-2} of smooth luminal surface of small intestine. Panel *A* shows data for unanaesthetised rats and panel *B* shows data for conscious human subjects.

intracellular glucose concentrations as shown in Fig. 3. From this it is easy to see how failure of blood flow to increase during absorption could lead to cellular glucose concentrations that compromise the efficiency of SLGT-1 upon which all glucose absorption depends.

Conclusions

The jejunal epithelium of normal animals can present the villus capillaries with absorptive loads that are several hundred times greater (per gram tissue) than found in capillaries elsewhere, including contracting skeletal muscle and brain. Far from being negligible, the villus capillary walls account for 50% or more of the resistance to glucose transport between the apical cytosol of the epithelial cells and the capillary blood during normal rates of absorption. Increases in villus capillary blood flow and permeability-surface area product are thus essential for sustaining high rates of glucose absorption. Na^+-coupled concentrative transport of sugars and amino acids must trigger these increases in blood flow and permeability-surface area product; otherwise, concentrations in the sub-junctional fluids and within the epithelial cells would rise to levels that would compromise carrier mediated transport. Signals that trigger the microcirculatory responses remain to be discovered.

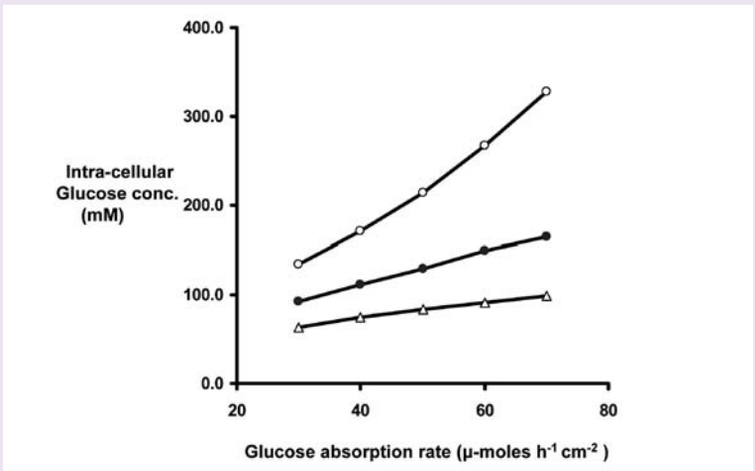

Figure 3. Effects of blood flow on estimated glucose concentrations in the apical cytosol of rat epithelial cells at different rates of glucose absorption. The three curves show the relations when the increase in blood flow with absorption rate is normal (•), twice as great as normal (Δ) and half as great as normal (o).

The low rates of absorption found in anaesthetized animals (Ugolev, 1987) may be attributed to inhibition of the normal microcirculatory responses associated with Na^+-coupled transport by the epithelium. In anaesthetised animals with low villus blood flows, glucose concentrations outside the capillaries may exceed 150 mM (Fig. 2) and with increased gradients across the lateral membranes (Fig. 3) the intracellular concentrations will rise to 250-300 mM. Total osmolarity, including the Na^+ brought in with glucose, would then exceed 600 m-Osmolar, a value comparable to that found experimentally by Hällback *et al.* (1991).

August Krogh (1922) appears to have been the first to recognize the special problems posed by nutrient transport within the villi after absorption by the epithelium. The hypothesis outlined briefly in this essay provides a solution to these problems in terms of villus capillary permeability, surface area and blood flow on the one hand, coupled with properties of the epithelium on the other.

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References

Buschman RJ & Manke DJ (1981). Morphometric analysis of the membranes and organelles of the small intestinal enterocytes. I Fasted hamster. *J Ultrastruct Res* 76, 1-14.

Hallbäck DA, Jodal M, Mannischeff M, & Lundgren O (1991). Tissue osmolality in intestinal villi of four mammals *in vivo* and in *vitro*. *Acta Physiol Scand* 143, 271-277.

Kellett JL (2001). The facilitated component of intestinal glucose absorption. *J Physiol* 531, 585-595.

Krogh A (1922). *The Anatomy and Physiology of Capillaries.* pp 244-249. Yale University Press, New Haven.

Madara JL & Pappenheimer JR (1987). The structural basis for physiological regulation of paracellular pathways in intestinal epithelium. *J Memb Biol* 100, 149-164.

Pappenheimer JR & Michel CC (2003). Role of the villus microcirculation in intestinal absorption of glucose: coupling of epithelial with endothelial transport. *J Physiol* 553, 561-574.

Pappenheimer JR & Volpp K (1992). Transmucosal impedance of small intestine: correlation with transport of sugars and amino acids. *Am J Physiol* 263, C480-C493.

Ugolev AM (1987). Membrane transport and hydrolytic enzymes under physiological vs acute experimental conditions. *News in Physiol Sci* 2, 186-190.

Learning to smell

The human olfactory system can 'learn' to smell androstenone. Why this should occur is uncertain and, because androstenone can have different smells to different people, it challenges the relationship between odour and receptors. There is perhaps more to this smelling business than meets the nose, says Tim Jacob

Tim Jacob

There is a long-standing debate about how much, if any, of the olfactory system is hard-wired and therefore whether the discrimination of certain smells is innate or, alternatively, whether we have to learn to smell. This article concerns the phenomenon of induced sensitivity to the steroid androstenone which may give some insights into the process of acquisition of smell discrimination.

Androstenone is a member of the 16-androstene family of steroids, one source of which is the testes in pigs. The steroids gain access to the systemic circulation via the spermatic vein. In the salivary gland the steroids are stored and concentrated to be released into the saliva at moments of aggression or sexual activity. The response of the sow is to take up the mating position ('lordosis', Fig.1). Such a rapid response is typical of a releaser (or signalling) pheromone effect.

Cooks have long known that an unpleasant odour emanates from the meat of mature boar when it is cooked. This is the so-called 'boar taint' and one of the compounds responsible is 5α-androstenone.

Androstenone also occurs in human saliva, urine and sweat. It is found in much higher concentrations in men than women (Gower & Ruparelia, 1993).

A proportion of people (~30%) cannot smell androstenone and those who can fall into two groups: a) a very sensitive group, who can detect less than 10 parts per trillion and who find the odour extremely unpleasant (urinous), and b) a group who are not only less sensitive but perceive the odour in different ways such as 'sweet', 'musky', 'perfume-like'. The distribution of thresholds for androstenone, unlike most other odorants, is not normally distributed, but heavily skewed toward the high threshold end.

Androstenone is thought to be formed from non-odorous precursors (perhaps testosterone and progesterone) by the action of bacteria that inhabit the axillae (armpits). Since the type of microflora present will depend on the immune sensitivity of the individual this is one of the proposed mechanisms for how immunotype is related to an individual's unique smell or 'odortype'.

In the 1980s, Charles Wysocki, a smell researcher anosmic to androstenone, noticed in the course of an experiment involving the steroid what appeared to be induced sensitivity. After months of intermittent exposure he found that he could detect a distinct odour (Wysocki *et al.* 1989). He went on to study this phenomenon and found that subjects initially insensitive to androstenone could be induced to smell it by repetitive exposure. Subsequent studies have shown that even those who can smell it can lower their threshold by this treatment.

This phenomenon of sensitization to an odour is actually quite rare. Mostly repetitive exposure to odours leads to a decrease in responsiveness as the olfactory system adapts and/or habituates. Adaptation is a peripheral process and habituation is a central process, both involving an attenuation of the reponse to the repeated stimulus.

To date this sensitization phenomenon has only been shown to occur with androstenone in humans, and it occurs in both sexes. Women of reproductive age can become sensitized to benzaldehyde and citralva, something that does not happen to men or pre-pubertal or post-menopausal women. Another odorant commonly used in smell research is amyl acetate and it does not induce the same sensitivity increase (Dalton *et al.* 2002).

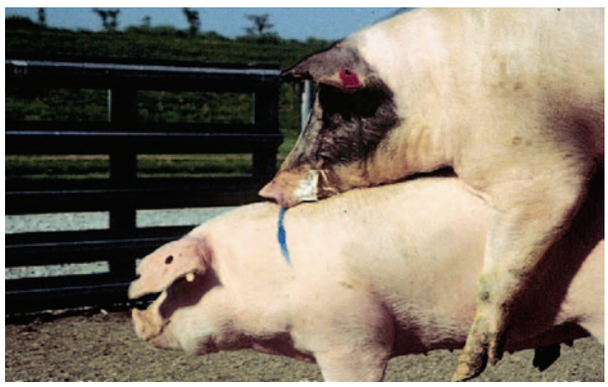
Initially, it was thought that the proportion of people that couldn't smell androstenone had a 'specific' anosmia and were lacking the gene that coded for the receptor that bound androstenone. Examples of supposed specific anosmias have been reported; one person in 10 cannot smell the poisonous gas hydrogen cyanide and about one in 1,000 people are immune to the smell of skunk (butyl mercaptan). But the idea of specific anosmias has led people to underestimate the complexity of odour detection and discrimination. It is now known that there is not one receptor type for a given smell but that an odorous compound will activate a range of different receptors. These receptors and their respective glomeruli to which they project in the olfactory bulb are molecular 'feature' detectors. An odour is recognised because of the unique pattern of activation of these feature detectors – the odour code.

Recent work on the threshold for androstenone has shown that far fewer people than previously thought are really anosmic to it. When signal detection studies are performed, only between 2-6% of people seem to be unable to detect androstenone (Bremner *et al.* 2003). Detection thresholds are more similar among identical twins as compared to dizygotic twins; hence insensitivity to androstenone appears to have a genetic basis. One might expect it therefore to be stable over time. Early explanations put forward to explain the androstenone sensitization process invoked clonal expansion of the olfactory receptor

cells containing the receptors for androstenone. An implication of this is that there must be a few cells that respond from which the expansion originates. This is difficult to reconcile with the notion of an odour code that requires the activation of many different receptors per odour. For example, one crucial receptor for the discrimination of androstenone might be missing, leading one to miscode it. But how, if that receptor is missing, is sensitivity induced? The only explanation that fits the facts is that there is a set of androstenone receptors, but they are expressed at such a low level that their activation does not result in perception. This argues against the existence of specific anosmias, at least for androstenone.

The search for specific anosmias was begun by John Amoore in an attempt to prove his 'molecular shape theory' of olfactory transduction. He believed that there were primary odours, much like the visual system, each with its own particularly shaped receptor. Odours fitted into one or more sites with varying affinities and the combination of binding gave rise to the multiplicity of smells that we can detect. Amoore thought that the known types of specific anosmias would represent the start of a list of primary odours (Amoore, 1967). But his definition of specific anosmia was arbitrary, a detection threshold greater than two standard deviations from the population mean. The 'specific anosmics' detected the odour, but at much higher concentrations.

How this phenomenon of induced sensitivity occurs is unclear. Yee and Wysocki (2001) provided evidence of the involvement of the olfactory epithelium by repetitively exposing mice with olfactory nerve transection to androstenone and demonstrating increased sensitivity (compared to pre-surgery levels) upon regrowth of the olfactory nerves. However, Mainland *et al.* in a recent study (2002) on humans in which they exposed only one nostril to repetitive stimulation and showed acquisition of sensitivity by the other, unexposed nostril, suggested that this learning occurred in central brain regions of the olfactory system.



Reproduced with permission from Wayne Singleton, Purdue University

Figure 1. The pig pheromone androstenone is released into the boar's saliva at moments of sexual activity and causes the sow to adopt the mating position.

We set out to monitor the response of the olfactory system during the acquisition of increased androstenone sensitivity by measuring the evoked potentials of the olfactory epithelium (EOGs), simultaneously with the event-related potentials (OERPs) recorded on the scalp using EEG electrodes. We correlated the results with the detection thresholds for androstenone. The EOG represents solely peripheral events, whereas the OERP reflects the activity of both peripheral and central elements of the olfactory system. It is thus possible to dissect out the location of any induced changes. We found that the androstenone-evoked EOG increased with increasing sensitivity and the OERP followed the EOG suggesting that this is a peripheral change occurring at the level of the olfactory receptors (*Wang et al.* 2004).

The fact that androstenone sensitivity can be induced or enhanced by exposure tends to militate against the current wisdom of the involvement of multiple receptors which 'analyse' the molecular features of androstenone (i.e. the odour code for androstenone), for one would have to postulate a concurrent increase in a number of different receptors or receptor cells, and rather suggests that a single receptor type or receptor cell is involved. Two plausible alternatives, those of rapid adaptation or central inhibition, were ruled out by our finding that if there is no sensitivity then there is no EOG.

Many questions remain: is there just one receptor for androstenone? Are new androstenone-sensitive olfactory receptor cells formed by clonal expansion of a small number of pre-existing receptor cells, or do existing cells express more androstenone receptors? Is just one population of cells stimulated to divide or make more receptors, or are all cells that detect androstenone's molecular features stimulated to divide? Does this phenomenon extend to other steroids or, indeed, other compounds? What exactly is the significance of exposure-dependent smell learning? Once learned, is it reversible?

Further study of this phenomenon may answer some of these questions but, for the moment, androstenone-sensitization gives an indication of the kind of processes that might occur in babies as they learn to smell their new world and, most importantly, learn the smell of their own mothers – something they are able to do within 3 days of birth. A timescale that, coincidentally, is about the same as that taken for androstenone sensitization.

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References

Amoore JE (1967). Specific anosmia: a clue to the olfactory code. *Nature* 214, 1095-1098.

Bremner EA, Mainland JD, KhanRM &Sobel N (2003). The prevalence of androstenone anosmia. *Chem Senses* 28, 423-432.

Dalton P, Doolittle N & Breslin PAS (2002). Gender-specific induction of enhanced sensitivity to odors. *Nature Neuroscience* 5, 199-202.

Gower DB & Ruparelia BA (1993). Olfaction in humans with special reference to odorous 16-androstenes: their occurrence, perception, possible social, psychological and sexual impact. *J Endocrinol* 137, 167-187.

Mainland JD, Bremner EA, Young N, Johnson BN, Khan RM, Bensafi M & Sobel N (2002). One nostril knows what the other learns. *Nature* 419, 802.

Wang L, Chen L & Jacob, TJC (2004). Evidence for peripheral plasticity in human odour response. *J Physiol* 554, 236-244.

Wysocki CJ, Dorries KM & Beauchamp GK (1989). Ability to perceive androstenone can be acquired by ostensibly anosmic people. *Proc Natl Acad Sci USA* 86, 7976-7978.

Yee KK & Wysocki CJ (2001). Odorant exposure increases olfactory sensitivity: olfactory epithelium is implicated. *Physiol Behav* 72, 705-711.

Conditions under which systemic lactate may act as a metabolic substrate for the brain

Ask any student what substrate the brain uses as a fuel and the answer will invariably be glucose. So ingrained is the concept of glucose as the brain's only energy substrate that it is the central dogma of brain energy metabolism and can be summarised as follows: the brain contains insignificant energy reserves, and is thus entirely dependent on the blood to deliver a constant, uninterrupted supply of glucose in excess of the brain's demand.

The reasons for this universally accepted assumption of glucose as the sole substrate for the brain include:

- complex homeostatic mechanisms have evolved that ensure a stable concentration of glucose in the blood ($[glucose]_{blood}$) of ~ 6 mM, thus the delivery of glucose to the brain is constant;
- non-glucose substrates in the blood are converted to glucose by the liver and kidneys implying glucose is the preferred substrate;
- the brain contains neurones which respond to decreases in $[glucose]_{blood}$ and promote compensatory mechanisms designed to elevate $[glucose]_{blood}$;
- the a-v difference in $[glucose]_{blood}$ is always positive, implying that the brain takes up glucose;
- NMR studies have shown that labelled glucose in the blood ends up as labelled glycolytic and oxidative intermediaries in the brain;
- insulin overdose which causes systemic hypoglycaemia and results in decreased delivery of glucose to the brain, results in deficits in brain function.

These factors appear to present a cast-iron case for glucose as the sole energy substrate used by the brain. However, there are certain circumstances where glucose is not the sole substrate used by the brain. In neonatal mammals the fatty acids derived from mothers' milk provide about half of the energy substrate metabolised by the brain, and adult brain can survive on ketone bodies derived from fatty acids or

Malcolm Prior (left) with Angus Brown

pyruvate oxidation. It should be stated quite clearly at this point that *in vitro* brain tissue preparations can survive for extended periods of time on a host of non-glucose substrates (including the sugars mannose and fructose, the monocarboxylates lactate and pyruvate, and the ketone bodies acetoacetate and β-hydroxybutyrate), but in these preparations the blood brain barrier (BBB) is circumvented and substrates in the perfusate have unlimited access to tissue. The topic of this review is the viability of lactate in the systemic circulation supporting brain function.

Lactate infusion improves cognitive function during systemic hypoglycaemia

An intriguing possibility that has recently been proposed is that under certain pathological conditions systemic lactate can support brain function in adults. This concept flies in the face of current convention, as it is widely assumed that in adults lactate has limited access to the brain due to the selective permeability of the BBB. Using human volunteers, Stephanie Amiel's group studied the ability of exogenously applied lactate delivered to the systemic circulation to support brain function when $[glucose]_{blood}$ had been rendered hypoglycaemic by the infusion of insulin (Maran *et al.* 1994). In these carefully controlled experiments when $[glucose]_{blood}$ was driven to hypoglycaemic levels (~2.5 mM: < 4 mM glucose is considered hypoglycaemic) infusion of lactate, leading to an eventual $[lactate]_{blood}$ concentration of 3.5 mM, resulted in an improvement of cognitive function when compared to volunteers who were deprived lactate infusion, implying that lactate can freely pass into the brain and be oxidatively metabolised. In addition, the counter-regulatory responses to systemic hypoglycaemia were substantially diminished. Under normal physiological conditions the a-v difference of lactate is negative, i.e. the brain produces lactate which then passes into the venous circulation. At physiological pH (pH 7.4) lactate is 99% ionised, and is co-transported with H^+ ions by monocarboxylate transporters. The transport is freely reversible with equilibrium reached when:

$$\frac{[lactate]_i}{[lactate]_o} = \frac{[H^+]_o}{[H^+]_i} \quad \text{i.e.} \quad \frac{[lactate]_{brain}}{[lactate]_{blood}} = \frac{[H^+]_{blood}}{[H^+]_{brain}}$$

Under physiological conditions blood pH is 7.4 ($[H^+]$ = 40 nM) and brain pH is 7.2 ($[H^+]$ = 63 nM), the $[lactate]_{brain}$ is ~2 mM and the $[lactate]_{blood}$ is ~1 mM (Abi-Saab *et al.* 2002). Inserting these values into the equation demonstrates that the system is not in equilibrium, resulting in net efflux of lactate out of the brain. Assuming that all the parameters except the $[lactate]_{blood}$ remain constant, $[lactate]_{blood}$ must increase above 3.2 mM in order for net lactate transport into the brain to occur, i.e. $[lactate]_{blood}$ must reach a critical threshold determined by the pH gradient as well as the lactate gradient before net lactate influx into the brain occurs. This is illustrated in Fig. 1, where the $[lactate]_{blood}$ required to reverse transport of lactate into the brain is plotted against brain pH, with blood pH constant at 7.4, and $[lactate]_{brain}$ constant at 2 mM. It can be seen that as the brain becomes more alkaline, the $[lactate]_{blood}$ required to promote net reverse transport decreases. It has been shown by several groups that hypoglycaemia results in an alkalisation of the brain, such that brain pH ~ blood pH (Fig. 2), implying that all that is required for net lactate transport into the brain is for $[lactate]_{blood}$ to exceed $[lactate]_{brain}$. Under hypoglycaemic conditions it is

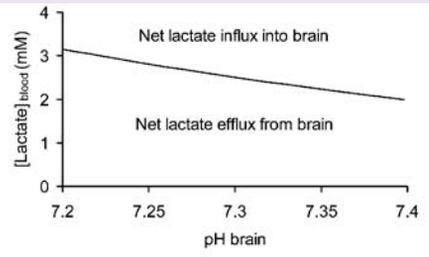

Figure 1. The relationship between brain pH and $[Lactate]_{blood}$. At physiological brain pH (7.2) it is calculated that $[Lactate]_{blood}$ must rise to 3.2 mM in order for net transport of lactate into the brain to occur. However as the brain becomes more alkaline, this concentration decreases such that at brain pH = 7.4 (~ blood pH), $[Lactate]_{blood}$ must only exceed $[Lactate]_{brain}$ in order for net lactate transport into the brain to occur.

likely that the $[lactate]_{brain}$ stays constant or decreases, further lowering the $[lactate]_{blood}$ required to drive net lactate transport into the brain. Thus the response of the brain to hypoglycaemia lowers the $[lactate]_{blood}$ threshold at which net movement of lactate into the brain occurs.

Exercise-induced hypoglycaemia and elevated plasma lactate

Experiments under more physiological conditions support the hypothesis that systemic lactate can support brain function. During heavy exercise $[glucose]_{blood}$ can fall to levels considered hypoglycaemic (~2.5 mM), without producing the warning signs of systemic hypoglycaemia that a non-exercising individual would display (Felig *et al.* 1982). This is almost certainly correlated with the fact that $[lactate]_{blood}$ can increase to 8 mM during extreme exercise. Under these conditions the a-v difference in lactate reverses as the brain takes up lactate, presumably for oxidative metabolism to fuel brain function (Ide *et al.* 2000). As is the case with insulin-induced hypoglycaemia the response to exercise (i.e. acidification of the blood) lowers the $[lactate]_{blood}$ threshold at which lactate will enter the brain. These experiments are rather confused by the fact that there is still considerable glucose present in the blood, and it thus appears that the brain survives on a combination of glucose and lactate. Can it be a coincidence that the deficit in $[glucose]_{blood}$ during exercise compared to baseline levels (6 mM - 2.5 mM = 3.5 mM) is matched by an equivalent increase in $[lactate]_{blood}$ (8 mM - 1 mM = 7 mM ~ 3.5 mM glucose)?

Of considerable interest is the fact that during exercise-induced hypoglycaemia there is no activation of counter-regulatory mechanisms suggesting that the glucose-sensing neurones in the brain are not activated due to the increased $[lactate]_{blood}$. This has been verified in a separate study in rats subjected to systemic hypoglycaemia (Borg *et al.* 2003). Animals in which lactate was infused into the ventromedial hypothalamus exhibited a reduction of 80 - 85% of the counter-regulatory hormonal response, implying that these glucose-sensitive cells sense glucose through glycolysis, as occurs in pancreatic β cells. However, in this study it was argued that the lactate was generated within the brain parenchyma, rather than transported across the BBB.

What is the fate of glucose and lactate once they have entered the brain? It is widely agreed that astrocytes, which comprise 50% of the brain volume, are mainly glycolytic, taking up glucose and converting it to lactate which is subsequently released into the extracellular space. Neurones, on the other hand, are clearly oxidative and there is accumulating evidence that they can take up and oxidatively metabolise astrocyte-derived lactate. Is it possible that there is cellular compartmentalisation of the substrates available to the brain during exercise, and that the systemic glucose is used by astrocytes, whereas the muscle-derived lactate is used to fuel neurones?

The data presented in this article to support the hypothesis that systemic lactate is transported across the BBB and metabolised by the brain, although convincing, are entirely circumstantial.

The extensive magnetic resonance facilities at the University of Nottingham offer an ideal environment in which to address some of the key points raised in this article, and experiments are currently underway in which NMR spectroscopic detection of metabolites derived from systemically injected labelled lactate will determine conditions under which systemic lactate crosses the BBB and is metabolised.

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References

Abi-Saab WM, Maggs DG, Jones T, Jacob R, Srihari V, Thompson J, Kerr D, Leone P, Krystal JH, Spencer DD, During MJ & Sherwin RS (2002). Striking differences in glucose and lactate levels between brain extracellular fluid and plasma in conscious human subjects: effects of hyperglycemia and hypoglycemia. J Cereb Blood Flow Metab 22, 271-279.

Borg MA, Tamborlane WV, Shulman GI & Sherwin RS (2003). Local lactate perfusion of the ventromedial hypothalamus suppresses hypoglycemic counterregulation. Diabetes 52, 663-666.

Felig P, Cherif A, Minagawa A & Wahren J (1982). Hypoglycemia during prolonged exercise in normal men. N Engl J Med 306, 895-900.

Ide K, Schmalbruch IK, Quistorff B, Horn A & Secher NH (2000). Lactate, glucose and O2 uptake in human brain during recovery from maximal exercise. J Physiol 522, 159-164.

Maran A, Cranston I, Lomas J, Macdonald I & Amiel SA (1994). Protection by lactate of cerebral function during hypoglycaemia. Lancet 343, 16-20.

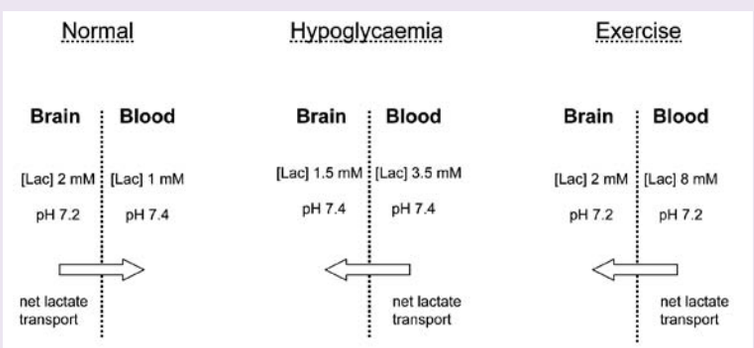

Figure 2. Net lactate transport under normal, hypoglycaemic or exercise conditions. Under normal conditions there is a net efflux of lactate from the brain, implying that the brain generates lactate from glucose and transports it into the venous circulation. Under hypoglycaemic conditions the alkalization of the brain reduces the threshold for net transport of lactate into the brain. It should be noted that this is an experimental paradigm in which exogenous lactate is applied to the systemic circulation. During exercise $[lactate]_{blood}$ rises considerably accompanied by an acidification of blood, facilitating net lactate transport into the brain.

Our shape is elastic, modular and held together by carbohydrate strings

Glycan strings tie collagen fibrils into shape-defining modules with intrinsic elasticity based on a springy sugar (Iduronate) and reversible intermolecular ratchets

John Scott

Bodies are supramolecular organisations, but we are not molecular Lego-men. We respond elastically to internal and external stresses. This enables us to move and to recover from our encounters with the outside world. Much of this elasticity is proposed to reside in carbohydrate strings that tie us together.

Two views of connective tissues

'*Connective tissue is what you get rid of in order to work with cells*' (anonymous physiologist).

'*If by some magic solution one could dissolve all the connective tissues of the body, all that would remain would be a mass of slimy epithelium, quivering muscle and frustrated nerve cells*' (Arcadi (1952) quoting his unnamed teacher).

Complete agreement! But these two anonymous commentators are going in opposite directions. There is a third way. Connective tissue (CT) (skin, bones, tendon, cartilage, etc.) is shape. Shape is one of the most important issues in biology. Without a permanent shape central functions (digestion, circulation, etc.) could not have evolved. *Ergo*, CTs are of supreme importance. How do they do it? Is there a core concept?

Nearly 30 years ago I proposed a global definition, applicable to all CTs (or more precisely their extracellular matrices, ECMs) in all species at all stages in development as follows: 'animal CTs are systems of insoluble fibrils and soluble polymers that evolved to take the stresses of movement and the maintenance of shape' (Scott, 1975). The fibrils are proteins - collagen and elastin. They are ropes, resisting and transmitting *tensile* (pulling) stresses, visible as such from the very thin (~10nm) collagen fibrils in vitreous humour to the huge tendons that support large animals.

The soluble polymers are carbohydrates, anionic glycosaminoglycans (AGAGs, e.g. chondroitan, keratan and dermochondan sulphates). They are attached to proteins, thereby becoming proteoglycans (PGs). They resist *compressive* forces because they tend to swell in aqueous solutions, as all polymers do in good solvents, thus increasing their entropy. The many anionic groups (carboxylates, sulphate esters) they carry repel each other, expanding the polymers they are covalently attached to. Also important is the osmotic pressure of counterions (Na^+, etc.) associated with the GAG

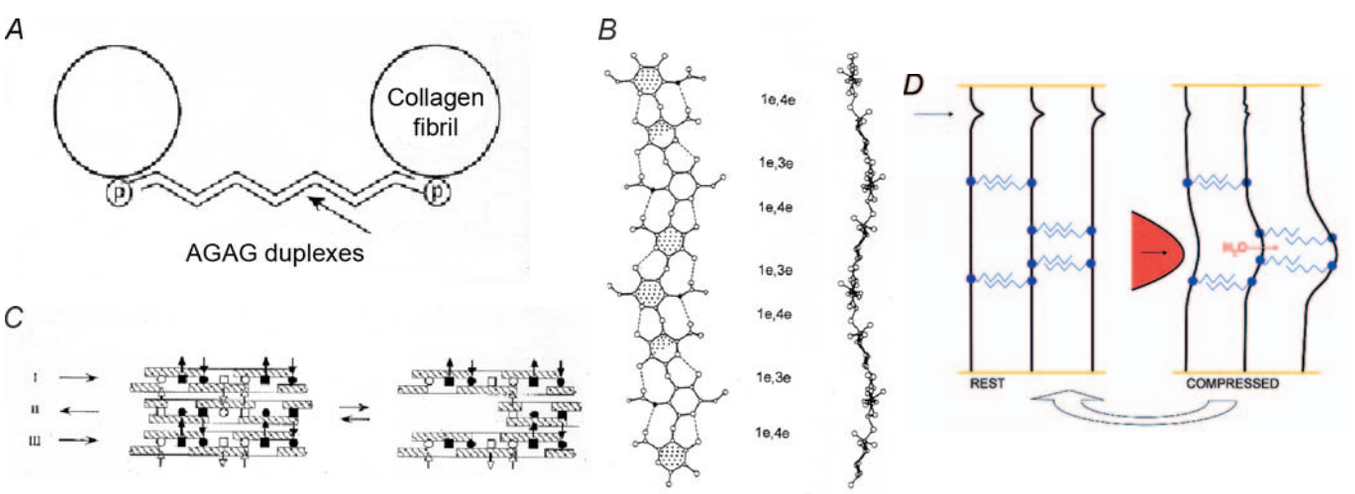

Figure 1. *A* The shape module. Antiparallel AGAG aggregates (shown as duplexes) link collagen fibrils (in section). (p); proteoglycan protein.
B Plan (left) and elevation of twofold helices preferred in solution by shape module AGAGs (chondroitins, keratans) and HA (hyaluronan) in which all glycosidic bonds are equatorial 1-3, 1-4; and hydrophobic patches (stippled) are identically placed, as are the waves and inter-residue H-bonds (dotted lines). Filled circles; N atoms. HA is illustrated.
C Side views of tertiary structures of twofold helical AGAGs (see *B*). Hydrophobic patches (cross-hatched) form hydrophobic bonds and acetamido NH (■□) H-bonds to carboxylate (●○) on the adjacent AGAG. Filled symbols are below, and open symbols above, the plane of the diagram. The waves in *B* complement each other in these antiparallel aggregates. Arrows (left) indicate reducing end (Scott, 2003 for review). Displacement of chain II vis-a-vis chains I and III (see also Fig 1*D*) is reversible, driven by the energy loss in reforming the broken hydrophobic and H-bonds (from Scott, 2003).
D Elastic deformation (reversible slippage) within the shape module AGAG aggregates converts local *compression* into dissipated *tensile* strain.
Collagen fibrils (verticals) are bridged by antiparallel AGAG chains (zigzags) covalently linked to PG protein (filled circles) which associate with fibrils at specific binding sites. Arrow (top left) indicates a 'crimp' within the fibril which takes up some slack under tensile stress. Horizontal lines indicate ECM into which the fibrils are anchored.
Left, 'REST' shows AGAG chains, fibrils etc. in unstressed ECM.
In the right half; 'COMPRESSED', a compressive force (shown as a probe pressing in the direction of the arrow) impacts on the ECM. Tissue H_2O is displaced into neighbouring spaces where it forces elongation of the axis of the AGAG aggregates via slippage between the participants, with stretching of the collagen fibril crimp. Arrow (bottom) indicates reversal of the slippage to the resting state on releasing compressive stress, with return to the original position of the tissue H_2O (see text).

anions. The total swelling pressure inflates the fibrillar matrix. This was elegantly proved by precipitating the polyanions with cobalt hexammine or cetylpyridinium. The volume occupied by the precipitated polyanion is much less than that in solution, effectively removing it from the tissue. PG-rich tissues (e.g. cartilage or corneal stroma) then shrink, losing their turgor (Hedbys, 1961) and elasticity.

The anatomy

How do the fibrils co-operate with the PGs? They cannot move independently or the shape of a CT would depend on its stressful history and a permanent shape would be impossible. The fibrils must be in a constant orientation. Collagen fibrils in, for example, tendons and corneal stroma, are in beautiful parallel arrays but conventional electron histochemistry shows no interfibrillar connections, although there must be structures which hold the fibrils in permanent orientations.

In fact, collagen fibrils are tied together by PG AGAG bridges. These are in solution *in vivo* and consequently invisible. They were discovered when electron-dense reagents (e.g. Cupromeronic Blue) were developed to stain AGAGs for electron microscopy (Scott, 1985). Cationic Cupromeronic Blue binds electrostatically to polyanionic AGAGs, precipitating them while preserving much of the shape, size and orientation of the AGAG. The dermochondan sulphate-containing PG was called decoran because it decorates the collagen fibrils in a beautiful regular pattern. Similar PGs containing keratan sulphate are found in the corneal stroma. The PG protein is attached non-covalently to the fibrils and AGAG chains aggregate head-to-tail across the interfibrillar gap (Fig. 1).

Not only are the PGs bound to collagen fibrils, thus stopping them from wandering under stress, but they bridge and tie the fibrils together in register. The lengths of the AGAG chains correlate with the interfibrillar separation, being short (e.g. about 15 kDa in tendon) where fibrils are densely packed, rising to 50 kDa in cornea, where fibrils of constant diameter are precisely well separated, and to 350 kDa in the very dilute vitreous humour. The bridge-fibril interaction repeats regularly along the fibril, i.e. modularly. I therefore termed it the ‘shape module’. ECM cells unable to make the PG protein were unable to form shape modules and the ‘ECM’ they produced was totally disordered.

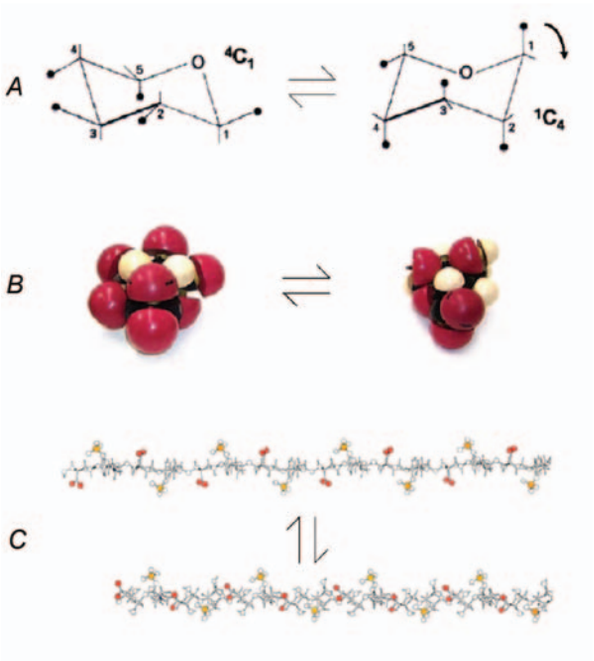

Figure 2. *A* L-iduronate (IdoUA) in C1 (left) and 1C (right) conformations. Filled circles are oxygen-containing groups (OH and COO^-). Arrow (top right) indicates the movement of the axial glycosidic bond towards the equatorial position in C1 under tensile forces acting along the polymer axis, thus converting 1C into the C1 alternative conformation. C1 IdoUA is wider than 1C along the polymer axis (*B* and *C*, below)
B Space-filling models of left) IdoUA C1, and right) IdoUA 1C, carboxylate at bottom. Red; oxygen, white; hydrogen. The dermochondan longitudinal axis runs left ↔ right through the equator of each. 1C is the more compact on this axis.
C Elevation views of stick-and-ball models of 2-fold helical dermochondan homopolymers comprising exclusively:- $^4C_1$ Ido UA (top); $^1C_4$ IdoUA (bottom). Carboxylate oxygens; red, sulphate ester sulphur; yellow.
Compact IdoUAs confer shorter chain lengths (bottom) for the same number of sugar units. Under tension these elongate to longer (top) configurations.
L-iduronate ‘springs’ in shape module dermochondan glycans enhance the elastic capacity of the AGAG bridge, which could stretch without slippage between glycans. (Based on Scott, 2003.)

Shape modules are found in all ECMs. They are ubiquitous and overwhelmingly dominant structures in animals as remote as holothurians. Thus, *animal shapes are stabilised by carbohydrate strings*, i.e. the AGAG chains in interfibrillar bridges.

The physiology

Although CT ECMs provide a robust shape, they must deform reversibly in response to internal and external stresses. 100% return to the original shape is mandatory, *post* stress. Clearly, 99.9% will not do. Shape module components must therefore be (or be part of) elastic structures.

Fibrillar elastic properties are well described. Elastin elongates reversibly (up to 200%), driven by hydrophobic and entropic interactions. It is ‘animal rubber’. Collagen fibrils are elastic to a much smaller degree, elongating reversibly by ~10%, probably by rearrangements of collagen molecules within the fibrils.

PG AGAG elasticity is relatively uncharted. Polymers entangle and disentangle, thus contracting and expanding, but there is little organised structure in this process and it is probably not significant in tissue elasticity.

Two new mechanisms were proposed (Scott, 2003), one depending on the spring-like behaviour of a specific sugar (L-iduronate) in the AGAG chain, and the other in which shape module AGAG bridges partially disaggregate under tensile stresses.

L-iduronate springs

The AGAG sugars (D-galactose, D-galactosamine, D-glucosamine and D-glucuronate) except one, (L-iduronate), are stable $^4C_1$ pyranose conformers (Fig. 2). L-iduronate occurs in three conformers ($^1C_4$, $^4C_1$ and $^2S_0$) with almost identical energies (Casu *et al*. 1988). One easily changes to another. Nevertheless, NMR shows that the $^1C_4$ and $^2S_0$ forms are preferred in aqueous solutions of dermochondan sulphate, which is characteristic of shape modules. Molecular modelling showed that $^1C_4$ and $^2S_0$ forms are more compact ('thinner') along the AGAG chain axis than the $^4C_1$ form, and a chain of $^1C_4$ forms could stretch by 7-10% under tensile stress to the $^4C_1$ forms. On release the chain would shorten as L-iduronate contracted to the preferred compact forms (Fig. 2).

For the first time this schema provides a mechanical *raison d'etre* for L-iduronate, which is formed by epimerisation in the polymer of D-glucuronate. This energy-expensive bit of biosynthesis was formerly without a rationale. It suggests why the content of L-iduronate varies greatly in dermochondans from different tissues.

Flexible tissues such as skin are characterised by large scale epimerisation (> 90%), whereas in rigid structures such as cartilage conversion stops at a much lower (~10%) level. Turgor in cartilages is high, so that L-iduronate may normally be in the fully stretched form, perhaps adopting compact forms under compression. L-iduronate/D-glucuronate ratios in corneal stroma are higher than in cartilage, consistent with the idea that cornea requires rigidity to maintain shape and hence its optical properties, but also elasticity to respond rapidly and reversibly to stresses associated with blinking and eye movements.

Atomic levers may control elasticity in L-iduronate-containing AGAGs

Direct physical evidence indicates that tensile stress changes sugar conformations. Using atomic force microscopes, single polysaccharide molecules were stretched while observing the relationship between lengths and applied force. Conversions from chair to boat forms were engineered by so-called 'atomic levers' (Marszalek *et al*. 1999), axial glycosidic bonds which, when pulled, forced the pyranose ring into a conformation in which the stress is transmitted in a straight line along the polymer chain. It is deducible from the work of Marszalek and co-workers that chondroitan 4 & 6 sulphates, hyaluronan and keratan sulphates will not stretch under tensile stress since their glycosidic bonds are equatorial rather than axial, offering no leverage to force conformational changes. Their sugars are fully extended anyway. On the contrary, the compact $^1C_4$ and $^2S_0$ forms of L-iduronate have axial glycosidic links. These atomic levers enhance the effect of tensile stress on the change between conformers (Fig. 2), thus improving intrinsic elasticity. Heparan sulphate, which is not an ECM AGAG, nevertheless should show L-iduronate elasticity, relevant to its role in flexible cell membranes.

L-iduronate confers small scale elasticity, but there are requirements for larger scale reversible deformabilities. Corneal stroma swells reversibly 5-fold and the spring mechanism cannot accommodate that. An intermolecular mechanism was proposed to deal elastically with large scale deformations (Scott, 2003).

The PG sliding-filament mechanism of shape module elasticity

AGAG bridge structure in the shape module is based on non-covalent interactions. Electron microscopy, NMR and molecular modelling indicate that the AGAG chains are probably layered on top of each other, head-to tail, which allows hydrophobic and H-bonds to form between neighbouring AGAGs (Fig. 1). This stringent lock-and-key complementarity repeats along the polymer chains. In the case of hyaluronan the H-bonds were shown by *rheo* NMR to break reversibly under shear stress. Thus aggregated AGAGs can be pulled apart in a slippage process, during which the low energy hydrophobic and H-bonds are broken. Slippage occurs along the AGAG bridge axis, in the direction of applied tensile stress. On releasing the stress slippage reverses as bonds originally present are reformed into the optimal (lowest energy) structure (Fig. 1).

In this scheme compressive forces are converted into tensile forces distant from the compression, disseminated throughout the tissue. The scheme predicts that when slippage exceeds a certain limit the tissue will disintegrate, probably irreversibly. Indeed, when corneal stroma swells beyond about 5-fold, the process becomes irreversible.

Coda

The requirement for elasticity in blood vessel walls, for example, is well recognised. The properties of elastic proteins (resilins, etc.) have been documented throughout animal biology. However, they are only part of the story. Their partners in shape-defining connective tissue must also be elastic if the tissue as a whole is to be elastic. The spring and dashpot properties of carbohydrates are now seen to complement elastin, etc. in providing elastic linkages in modular ECM structures, playing a vital role in maintaining shape.

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References

Arcadi JA (1952). Book Review of *Connective Tissues* (Transactions of the first conference of the Macy Foundation). *Bull Johns Hopkins Hospital* 90, 334-335.

Casu B, Petitou M, Provasoli M, & Sinay P (1988). Conformational flexibility: a new concept for explaining binding and biological properties of iduronic acid-containing glycosaminoglycans. *Trends Biochem Sci* 13, 221-225.

Hedbys BO (1961). The role of polysaccharides in corneal swelling. *Exp Eye Res* 1, 81-91.

Marszalek PE, Pang Y-P, Li H, Yazal JE, Oberhauser AF & Fernandez JM (1999). Atomic levers control pyranose ring conformations. *Proc Natl Acad Sci USA* 96, 7894-7898.

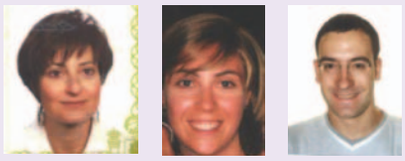
Scott JE (1975). Composition and structure of the pericellular environment: physiological function and chemical composition of pericellular proteoglycan (an evolutionary view). *Phil Trans Roy Soc Lond B* 271, 235-242.

Scott JE (1985). Proteoglycan histochemistry - a valuable tool for connective tissue biochemists. *Coll Rel Res* 5, 541-598.

Scott JE (2003). Elasticity in extracellular matrix 'shape modules' of tendon, cartilage etc. A sliding proteoglycan-filament model. *J Physiol* 553, 335-343.

Elastin and hypertension: is there a link?

Recent work has highlighted the association between a defect in elastin synthesis, large artery abnormalities and hypertension development. Studies in spontaneously hypertensive rats also point in the same direction and suggest a link between a defect in elastin organization and resistance artery remodelling



Silvia Arribas (left), Ana Briones (centre) and José González

The physiological demands of the arterial system in vertebrates require that arteries store energy during systole and release it during diastole, enabling the system to maintain a continuous blood flow. This function is made possible by vessel elasticity, which depends largely on the presence of elastin in the vessel wall. Elastin is synthesized by smooth muscle cells, and is secreted as the soluble monomer tropoelastin, which is then cross-linked in the tissue space and associated with microfibrillar proteins to form insoluble elastin matrix. In large vessels elastin is organized into concentric rings of elastic lamellae around the arterial lumen. Each elastic lamella alternates with a ring of smooth muscle, forming a lamellar unit that provides the compliance that arteries need to absorb and transmit hemodynamic forces (Parks *et al.* 1993).

The suggested link between a defect in elastin synthesis during early development and hypertension was proposed some time ago, and is based on the evidence that people with low birth weight tend to have higher blood pressure later in life. One mechanism that might underlie this association is impaired elastin synthesis when growth is retarded during a critical period of blood vessel formation in the fetus, leading to abnormal large artery compliance and finally higher blood pressure (Martin & Greenwald, 1997).

Hypertension is also present in a proportion of patients with two clinical conditions associated with elastin gene defects: supravalvular aortic stenosis (SVAS) and Williams-Beuren syndrome. Often individuals with these diseases are young children who are susceptible to peripheral vascular disease, myocardial infarctions or stroke (Milewicz *et al*. 2000).

A recent study of mice heterozygous for the elastin gene (eln+/-) – a murine model of SVAS – has provided good evidence for a link between elastin and hypertension (Faury *et al*. 2003). In this study, Faury and co-workers demonstrate that elastin haplo-insufficiency results in abnormalities in large artery mechanical properties which lead to a hypertensive phenotype from birth.

These data suggest that any factor that reduces elastin during a critical window of vessel wall formation and alters large artery compliance could have a modifying effect on the progression of, or susceptibility to, hypertension. As elastin is the dominant extracellular protein in large arteries, the effects of elastin defect on these vessels is to be expected. However, we were very surprised to find that elastin also plays a critical role in the mechanical properties and structure of resistance arteries, where this protein is very scarce. Small arteries play an essential role in blood pressure regulation by contributing to total peripheral resistance. In fact, increased peripheral resistance due to resistance-artery narrowing – termed 'inward vascular remodelling' – is a key determinant for the maintenance and progression of hypertension, and possibly its development (Mulvany, 2002).

Our recent data demonstrate a link between altered elastin organization in mesenteric resistance arteries (MRA) from spontaneously hypertensive rats (SHR, a model of human essential hypertension) and vascular remodelling (Briones *et al.* 2003). Fluorescent confocal microscopy enables examination of relatively thick intact tissues without the need to cut sections. We have taken advantage of this and of the autofluorescent properties of elastin

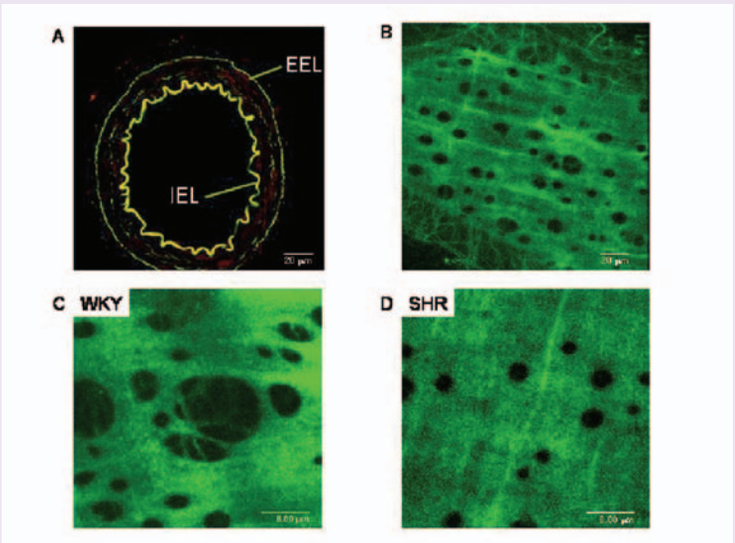


Figure 1. The autofluorescent properties of elastin allows for visualization of the distribution of this protein with fluorescent laser scanning confocal microscopy. Transversal ring of a rat mesenteric resistance artery (MRA) showing elastin distribution in external elastic lamina (EEL), internal elastic lamina (IEL) and some isolated fibres in the media (Fig. 1*A*). Figure 1*B* shows a confocal projection of the fenestrated IEL as well as some isolated fibres through the wall. Below, higher magnification projections showing the differences in elastin distribution in the IEL of a normotensive WKY (*C*) and a hypertensive SHR (*D*) rat (after Briones *et al.* 2003).

(excitation 488nm, emission 515 nm) to determine the organization and distribution of this protein in intact MRAs from SHR and from the normotensive reference strain, WKY, fixed at physiological pressures. In MRA elastin was restricted to a thin internal elastic lamina, a loose network of elastin fibres in the adventitia (external elastic lamina) and some fibres in the medial layer (Fig. 1). SHR MRAs showed altered internal elastic lamina organization with smaller fenestra when compared to the normotensive strain (Fig. 1).

However, elastin content, estimated from fluorescent intensities, was similar between strains. This altered elastin organization was associated with reduced lumen diameter and with increased stiffness in adult SHR, as shown by the leftward shift of the stress-strain relationship and larger β values (Fig. 2).

The importance of elastin organization in resistance arteries is emphasized by the fact that elastin digestion with elastase for 1 h induced a dramatic increase in lumen size and in β value, suggesting that elastin has an unanticipated role in overall small artery dimensions and mechanical properties. More interestingly, elastase abolished the structural and mechanical differences between hypertensive and normotensive vessels, supporting the hypothesis of a link between inward remodelling and a defect in elastin organization (Fig. 2) (Briones *et al.* 2003).

Our recent data demonstrate that this defect occurs between the 2nd and 4th week of life, when SHR rats are in the pre-hypertensive phase and small artery dimensions are still similar between strains (González *et al*. 2003). These data suggest that abnormal elastin organization occurs prior to inward remodelling, and might contribute to it.

All these data point to the potential importance of elastin or proteins related to elastic fibre assembly and organization, and the genes that control these, as critical issues in understanding vascular remodelling in essential hypertension.

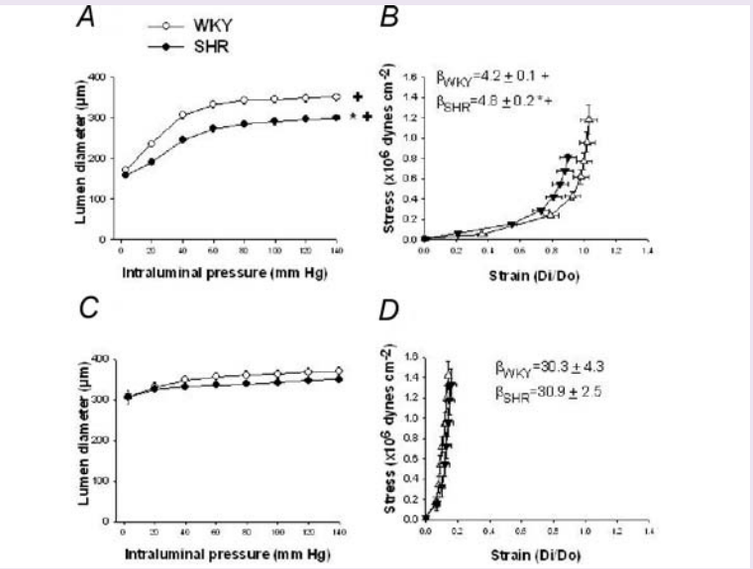


Figure 2. Lumen diameter-pressure *(A,C)* and stress-strain curves with β values (*B,D*) in pressurized mesenteric resistance arteries from normotensive WKY and hypertensive SHR rats in the absence (*A,B*) and in the presence (*C,D*) of elastase (0.062 mg/ml, 60 min). *p< 0.05 when compared to WKY rats; +p< 0.05 when compared to arteries incubated with elastase; 2-way ANOVA. Elastase incubation abolished the structural and mechanical differences between strains. β is a parameter directly proportional to Young's Elastic Modulus and an indicator of arterial stiffness (after Briones *et al*. 2003).

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References

Briones AM, González JM, Somoza B, Giraldo J, Daly CJ, Vila E, González MC, McGrath JC & Arribas SM (2003). Role of elastin in spontaneously hypertensive rat small mesenteric artery remodeling. *J Physiol* 552, 185-195.

Faury G, Pezet M, Knutsen RH, Boyle WA, Heximer SP, McLean SE, Minkes RK, Blumer KJ, Kovacs A, Kelly DP, Li DY, Starcher B & Mecham RP (2003). Developmental adaptation of the mouse cardiovascular system to elastin haploinsufficiency. *J Clin Invest* 112, 1419-1428.

González JM, Somoza B, Fernández-Alfonso MS, Gálvez B, Abderrahim F, González MC & Arribas SM (2003). Elastin re-organization and vessels stiffness is an early event in the development of vascular remodeling in resistance arteries from SHR. *Hypertension* 42, 387.

Martin CN & Greenwald SE (1997). Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. *Lancet* 350, 953-955.

Milewicz DM, Urbán Z & Boyd C (2000). Genetic disorders of the elastic fibre system. *Matrix Biology* 19, 471-480.

Mulvany MJ (2002). Small artery remodeling in hypertension. *Curr Hypertens Rep* 4, 49-55.

Parks WC, Pierce RA, Lee KA & Mecham RP (1993). Elastin. *Advances in Mol Cell Biol* 6, 133-182.

The leaky mitochondrion

Mitochondria use proton gradients to make ATP. Sometimes, however, proton 'leak' uses the gradient to produce heat instead. Here, Kent Sahlin and co-workers argue that the balance of these two consequences of fuel oxidation might be under physiological control



Kent Sahlin (left), Michail Tonkonogi (centre) and Maria Fernstrom

The mitochondrion is the power plant of the cell, where the energy derived from oxidation of fuels is converted to ATP, i.e. oxidative phosphorylation. The process involves pumping of protons across the mitochondrial membrane and the proton gradient this forms drives the synthesis of ATP. Although the major part of oxygen utilization is used for ATP synthesis (coupled respiration), the mitochondrial membrane is leaky and protons may leak back through the membrane. Proton leak will increase oxygen consumption (uncoupled respiration, UCR) and the energy will be dissipated as heat instead of being trapped as 'useful energy', i.e. ATP (Fig. 1). UCR has been estimated to about 40% of basal metabolic rate in the rat (Brand *et al.* 1994) and will reduce the efficiency of oxidative phosphorylation, which is typically measured as the yield of ATP per consumed oxygen (i.e. P/O ratio).

Although the term leak indicates deficiency in system design, UCR may have, or may be associated with, important physiological functions e.g. thermogenesis and body weight regulation, prevention of oxidative stress, prevention of mitochondrial damage induced by fatty acids (FA) and control of oxidative phosphorylation.

The mechanism of UCR in skeletal muscle is not known but is currently an extensive field of research. Proton leak may occur through membrane proteins (e.g. uncoupling proteins, UCP) or by non-specific transmembrane flux, which is dependent on the lipid composition of the mitochondrial membrane (Brand *et al.* 2002). In brown adipose tissue proton leak is mediated by UCP1 and has a well described role in thermogenesis. A homologous protein (UCP3) has been found in skeletal muscle but the significance of UCP3 as a determinant of UCR and basal metabolic rate is under debate. Concentrations of UCP3 increase in parallel with plasma FA during fasting and high fat diets and it has been suggested that the role of UCP3 is to protect mitochondria from an overload of long chain FA (Hoeks *et al.* 2003). The high UCP3 content in fast-twitch glycolytic muscles, where the capacity for FA oxidation is reduced, supports such a role (Hoeks *et al.* 2003).

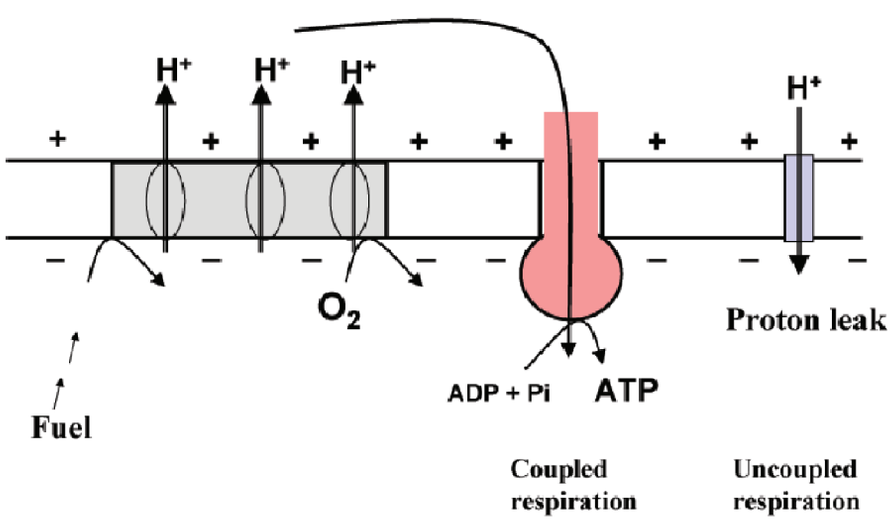

Figure 1. Schematic diagram of mitochondrial inner membrane indicating the mechanisms of coupled and uncoupled respiration. The driving force for proton influx is due to both an electrical and a concentration gradient over the membrane.

There is also evidence that UCP3 functions as a mild uncoupler and as such reduces formation of reactive oxygen species. Markers located near the UCP2 and UCP3 gene are strongly associated with basal metabolic rate (Bouchard *et al.* 1997) and suggest a role of UCR in thermogenesis.

An approximate measure of UCR is the oxygen consumption of isolated mitochondria and permeabilized fibres under non-phosphorylating conditions. It has been known for a long time that FA stimulate UCR in isolated mitochondria and that the effect varies between different forms of FA (Matthias *et al.* 1999). An intriguing finding is that endurance training increases the sensitivity of UCR to FA (Tonkonogi *et al.* 2000). FA-evoked UCR increased two-fold after 6 weeks of training (Fig. 2). Diet-induced thermogenesis is increased in endurance trained subjects in proportion to their VO_2max (Lopez *et al.* 2000). It is possible that this relates to the increased mitochondrial uncoupling in the presence of FA and that endurance training may prevent obesity during overfeeding.

A well known adaptation to endurance training is an increased mitochondrial biogenesis. This would in itself increase UCR provided proton leak per mitochondrial volume remains constant. However, UCR, measured in the absence of FA, was reduced after endurance training when related to

mitochondrial volume (Fernstrom *et al.* 2004). The decrease in UCR was paralleled by reduced levels of mitochondrial UCP3 protein. The training-induced reduction in UCR may be a mechanism to avoid unnecessary waste of energy due to increased mitochondrial volume and may also increase efficiency during exercise. This idea is supported by the observed inverse correlation between mechanical efficiency during cycling and UCP3 protein in human skeletal muscle (Schrauwen & Hesselink, 2003).

UCR may contribute to the hitherto unexplained excess oxygen consumption during and after heavy exercise. During prolonged exercise at a constant work rate there is a slow increase of VO_2 (oxygen drift) indicating a decreased mechanical efficiency. UCR, measured in permeabilized muscle fibres (i.e. after removal of the muscle cell membrane), was increased after prolonged exercise (Tonkonogi *et al.* 1998), and the oxygen drift may relate to increased uncoupling. However, UCR measured in isolated mitochondria (with and without FA) as well as UCP3 protein both remained unchanged after prolonged exercise (Fernstrom *et al.* 2004).

Measurements of UCR in muscle fibres and in isolated mitochondria have a number of limitations. During the preparation of mitochondria, potential changes in UCR may be reversed due to reversal of the exercise-induced perturbation of the cellular environment (e.g. acidosis, Ca-overload and hyperthermia). Only the effect of structural changes that remain in the permeabilized fibres and the isolated mitochondria will be observed. In vivo UCR may therefore be entirely different from that measured in vitro under standardized conditions.

UCR may also relate to intermittent opening of large protein pores in the mitochondrial membrane (i.e. permeability transition pores; PTP). PTP opening is stimulated by Ca-overload, oxidative stress and energetic stress, i.e. conditions prevailing during high-intensity exercise. Contrary to our expectations, mitochondria isolated from muscle samples taken immediately after prolonged exercise were more resistant to Ca^{2+}-overload than before exercise. Again this may not give a correct picture of what is occurring in the working muscle since the exercise-induced increase in mitochondrial Ca^{2+} is likely to disappear during the preparation process. However, the results suggest that endurance exercise has a protective role and increases Ca-tolerance of mitochondria.

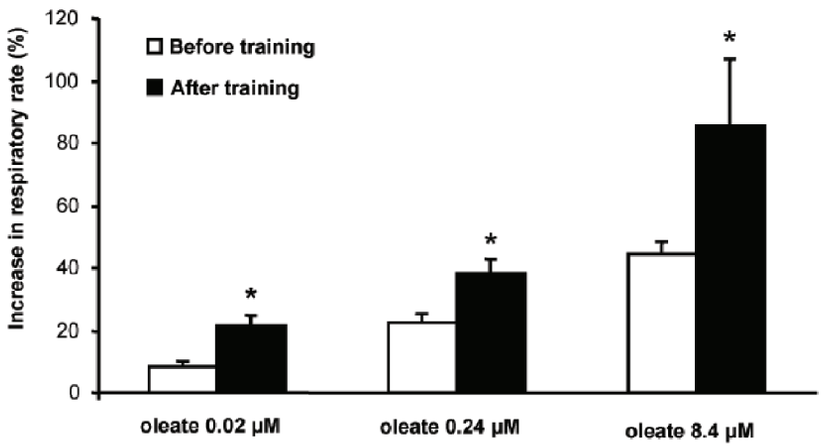

Figure 2. Effect of increased concentrations of long chain fatty acid (oleate) on uncoupled respiration in isolated mitochondria obtained before and after 6 weeks of endurance training. Measurements were performed with pyruvate/malate in the presence of oligomycin (inhibitor of ATP synthase) and atractyloside (inhibitor of adenine nucleotide translocase).

Irrespective of the role of UCP3 in skeletal muscle, UCR is an important factor in muscle energetics and as such important for body weight regulation and work efficiency. The reduced efficiency in oxidative phosphorylation associated with UCR may impair performance during exercise. However, transition from rest to exercise reduces proton leak, and futile proton cycling (pump and leak) may be a mechanism to control oxidative phosphorylation during rapid transitions in energy flux. UCR is thus a parameter under physiological control and can be both upregulated (overfeeding) and downregulated (exercise). The leaky mitochondrion and the physiological role of proton leak deserve further attention.

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References

Bouchard C, Perusse L, Chagnon YC, Warden C & Ricquier D (1997). Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. *Hum Mol Genet* 6, 1887-1889.

Brand MD, Chien LF, Ainscow EK, Rolfe DF & Porter RK (1994). The causes and functions of mitochondrial proton leak. *Biochim Biophys Acta* 1187, 132-139.

Brand MD, Pamplona R, Portero-Otin M, Requena JR, Roebuck SJ, Buckingham JA, Clapham JC & Cadenas S (2002). Oxidative damage and phospholipid fatty acyl composition in skeletal muscle mitochondria from mice underexpressing or overexpressing uncoupling protein 3. *Biochem J* 368, 597-603.

Fernstrom M, Tonkonogi M & Sahlin K (2004). Effects of acute and chronic endurance exercise on mitochondrial uncoupling in human skeletal muscle. *J Physiol* 554, 755-763.

Hoeks J, Hesselink MK, van Bilsen M, Schaart G, van der Vusse GJ, Saris WH & Schrauwen P (2003). Differential response of UCP3 to medium versus long chain triacylglycerols; manifestation of a functional adaptation. *FEBS Lett* 555, 631-637.

Lopez P, Ledoux M & Garrel DR (2000). Increased thermogenic response to food and fat oxidation in female athletes: relationship with VO(2 max). *Am J Physiol Endocrinol Metab* 279, E601-607.

Matthias A, Jacobsson A, Cannon B & Nedergaard J (1999). The bioenergetics of brown fat mitochondria from UCP1-ablated mice. Ucp1 is not involved in fatty acid-induced de-energization ("uncoupling"). *J Biol Chem* 274, 28150-28160.

Schrauwen P & Hesselink M (2003). Uncoupling protein 3 and physical activity: the role of uncoupling protein 3 in energy metabolism revisited. *Proc Nutr Soc* 62, 635-643.

Tonkonogi M, Harris B & Sahlin K (1998). Mitochondrial oxidative function in human saponin-skinned muscle fibres: effects of prolonged exercise. *J Physiol* 510, 279-286.

Tonkonogi M, Krook A, Walsh B & Sahlin K (2000). Endurance training increases stimulation of uncoupling of skeletal muscle mitochondria in humans by non-esterified fatty acids: an uncoupling-protein-mediated effect? *Biochem J* 351, 805-810.

The contribution of steric and electrostatic factors on distribution of macromolecules in the interstitial space

There is more to the space between cells than you might think - it is a crowded (if you're a macromolecule) and highly charged environment. Helge Wiig and colleagues discuss the possible consequences

Helge Wiig (top), Christina Gyenge (centre) and Olav Tenstad (above)

The present article focuses on the interstitium, which is the physical and biochemical environment of cells. The interstitial space consists of connective and supporting tissues of the body and is located outside the blood, lymphatic vessels and parenchymal cells. A schematic of a generic interstitium is provided in Fig. 1*A*. Although the composition and structure of this physiological space varies from tissue to tissue, there are basic characteristics and functions that are representative of interstitia of most tissues. Essentially the interstitium can be divided into two phases: the interstitial fluid and the structural molecules of the interstitial or the extracellular matrix. As a generalized description, the interstitial extracellular matrix can be thought of as a three-dimensional 'meshwork' composed of a complex aggregation of protein fibres and carbohydrate polymers.

The presence of numerous interstitial macromolecules, particularly glycosaminoglycans (hyaluronan and proteoglycans) and collagen-based species, results in macromolecular crowding of the interstitial space. Consequently, the fluid space available for other species diffusing through the interstitial media is less than the total interstitial fluid volume, i.e. a given interstitial solute will distribute itself in the fluid space outside the meshwork or, alternatively, through those spaces of the meshwork that have dimensions larger than that of the solute. This phenomenon of geometrical, or sterical interstitial exclusion was first described by Ogston and Phelps (1961) and refers to the fact that two solid structures cannot occupy the same confined volume at the same time, as illustrated in Fig. 1*B*. The steric exclusion phenomenon is relevant only for species with high hydrodynamic sizes such as proteins and not for small molecules such as water, small ions and nutrients.

In addition to the steric exclusion, due to the fact that glycosaminoglycans are negatively charged at physiological pH values, electrostatic factors might also be involved in selectively excluding other negatively charged macromolecules transported through the interstitium (Fig. 1*C*). Data from the lung have indicated that fixed negative charges in the interstitial matrix significantly reduce the space available for anionic lactate dehydrogenase 1 (pI ~ 5.0) as compared to the cationic lactate dehydrogenase 5 (pI ~ 7.9) (Taylor & Parker, 2003), suggesting a charge effect on macromolecular probe distribution.

The magnitude of the excluded volume has important consequences in the dynamics of transcapillary exchange. Due to exclusion, the effective protein concentration in the interstitium is much higher than the value that would be estimated if it were assumed that all the fluid in the interstitium was available. As stated in the review by Aukland and Reed (1993), the physiological importance of the exclusion phenomenon is two-fold; its

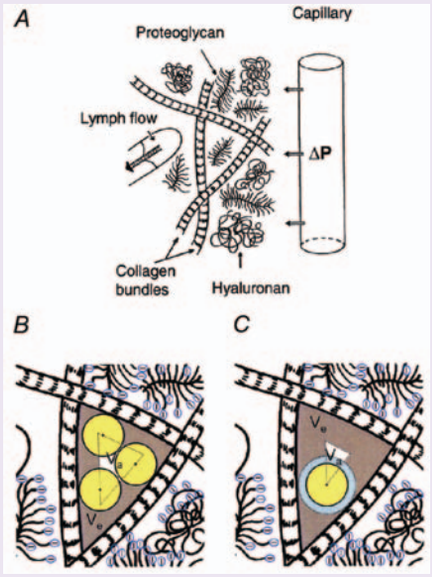

Figure 1. The interstitium and the exclusion phenomenon

A, schematic model of the interstitium. The interstitial space is located outside the blood and lymphatic vessels as well as parenchymal cells and may essentially be divided into two phases: the interstitial fluid and the structural molecules (e.g. neutral collagen and negatively charged hyaluronan and proteoglycans) of the interstitial or extracellular matrix. There is a net filtration pressure across the capillary resulting in a net fluid filtration into the interstitium. When the tissue is at steady-state an equal amount of fluid is removed by the lymphatics back into the circulation.

B, sterical exclusion. The presence of structural molecules results in macromolecular crowding of the interstitial space. Consequently, the fluid space available for other species diffusing through the interstitial media is less than the total interstitial fluid volume since two solid structures cannot occupy the same confined volume at the same time, a phenomenon called geometrical or sterical interstitial exclusion. The centre of the spherical molecule (yellow) can only access the area inside the dotted line, the available volume V_a, and is excluded from the area outside the dotted line, V_e.

C, sterical and charge exclusion. Due to the fact that glycosaminoglycans are negatively charged at physiological pH values, in addition to sterical factors, electrostatic factors are also involved in selectively excluding negatively charged macromolecules that are distributed in and transported through the interstitium. Therefore, a negatively charged probe will have a higher apparent radius (light blue) than an uncharged one. The case illustrated shows that, when the combined effect of steric and electrostatic factors is considered V_e is higher than in the case where only steric factors were accounted for (see Fig. 1B).

increase results in a more rapid approach to a new steady-state after a change in transcapillary fluid flow and less transfer of interstitial protein to plasma for a given capillary hyper-filtration. Interstitial exclusion thereby influences plasma volume regulation. Furthermore, the study of exclusion phenomena provides information regarding the organization of structural elements of the interstitium. Our recent *in vitro* and *in vivo* experiments involved testing the electrostatic exclusion hypothesis and, moreover, quantifying the magnitude of volume exclusion provided by these fixed negative charges in a given interstitium.

Together the interstitial fluid in skin and muscle accounts for almost 60% of the total body interstitial fluid volume (Aukland & Reed, 1993). Therefore these organs are of major importance for fluid balance studies, and have been the focus of our recent studies on the effects of charge on the distribution volume. Albumin is the most abundant plasma protein, and is an important determinant of plasma and interstitial colloid osmotic pressures.

Previous studies have stressed the importance of steric exclusion and shown that albumin is excluded from a large fraction of most interstitia (Aukland & Reed, 1993). Because of its pI of ~5 the albumin molecule has a net negative charge at physiological pH. Accordingly, this substance is a highly relevant candidate to use as probe in interstitial exclusion studies.

An important step for conducting our studies was the possibility of modifying the net charge of the anionic albumin to more positive values. Once this task was achieved we performed an *in vitro* study involving fully swollen rat dermis (Wiig *et al.* 2003). By quantifying the contribution of negative charges to the volume exclusion of albumin we found that a decrease in the net charge of albumin results in an increase in the interstitial distribution volume of this species. Thus, we demonstrated a substantial influence of negatively charged tissue elements on albumin distribution, amounting to about 40% of the albumin exclusion effect.

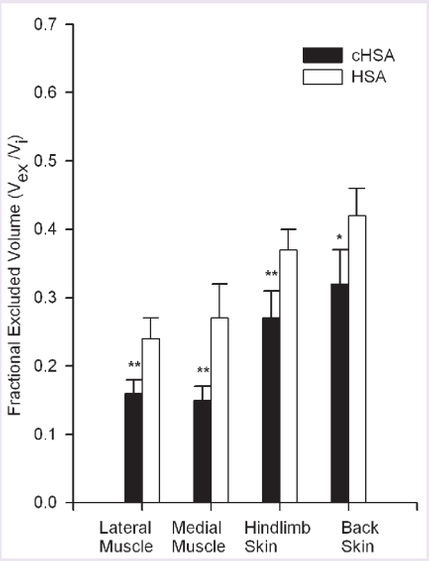

Figure 2. Fractional excluded volume of cationized human serum albumin (cHSA) and human serum albumin (HSA) in lateral and medial muscle and hindlimb and back skin (data from Gyenge et al, 2003). Cationization of albumin significantly affected its exclusion volume in skin as well muscle. From these data we were able to estimate that on average, the contribution of fixed negative charges to albumin exclusion from skeletal muscle and skin interstitia is in the range of 25-40%. The number of rats is, n = 9. Values are given as means ± SE. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the corresponding tissue involving uptake of native albumin.

The *in vivo* experiments tested our hypothesis on albumin exclusion in skin and muscle of normally hydrated rats (Gyenge *et al.* 2003). A prerequisite for this type of study is to establish a steady-state tissue tracer concentration, which was done by continuous intravenous infusion of normal and cationized (net charge close to neutral) albumin for 5-7 days. Another requirement is to isolate fluid representative of interstitial fluid, and for this purpose we sampled fluid from wicks implanted in the tissues of interest.

As evident from Fig. 2, once again a net change in the charge of albumin significantly affected its exclusion volume in skin as well as muscle. From these data we were able to estimate that, on average, the contribution of fixed negative charges to albumin exclusion from skeletal muscle and skin interstitia is in the range of 25-40%, a contribution much higher than previously believed.

The findings discussed above may also have implications for therapy. With the emergence of new therapeutic tools like monoclonal antibodies, the distribution of macromolecular probes in tumours is of importance. Therefore, studies of exclusion phenomena are of considerable interest in tumours since the interstitium is a major barrier to drug delivery in this tissue (Jain, 1997). In preliminary studies in rat mammary tumours we have shown a significant effect of fixed negative charges on interstitial distribution of albumin, an effect that has to be considered when studying uptake of macromolecular therapeutic agents.

In conclusion, it thus seems that a greater exclusion of more negative proteins is a general phenomenon occurring in normal as well as pathological tissues.

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References

Aukland K & Reed RK. (1993). Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol Rev* 73, 1-78.

Gyenge CC, Tenstad O & Wiig H. (2003). In vivo determination of steric and electrostatic exclusion of albumin in rat skin and skeletal muscle. *J Physiol* 552, 907-916.

Jain RK. (1997). The Eugene M. Landis Award Lecture 1996. Delivery of molecular and cellular medicine to solid tumors. *Microcirculation* 4, 1-23.

Ogston AG & Phelps CF. (1961). The partition of solutes between buffer solutions and solutions containing hyaluronic acid. *Biochem J* 78, 827-833.

Taylor AE & Parker JC. (2003). Intersitial exluded volumes: the effect of charge. *J Physiol* 553, 333.

Wiig H, Kolmannskog O, Tenstad O & Bert JL. (2003). Effect of charge on interstitial distribution of albumin in rat dermis in vitro. *J Physiol* 550, 505-514.

Affiliate Members

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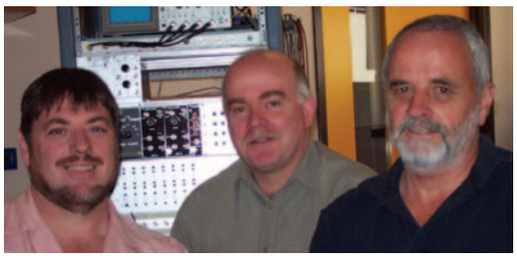
Don't forget to send in your own contributions for *Physiology News* to Laura Blackburn at:

lmlb2@cam.ac.uk

What limits jaw movements during vigorous head movements?

Tim Miles, Stan Flavel and Mike Nordstrom explain the mechanisms that keep the mandible in a fairly stable posture during exercises like running and jumping, in which the head moves vigorously up and down



Stan Flavel, Michael Nordstrom and Timothy Miles

Ever wondered why your teeth don't bang together when you run or jump, or even when you nod your head violently? We all know that this does not happen, and that it would be very uncomfortable if it did. Recent studies have demonstrated that the mechanisms that maintain mandibular posture under static conditions are different from those that restrain it when the head is moving.

The mandible, of course, hangs below the skull, hinged at the temporomandibular joints and supported against gravity by soft tissues including the muscles of the face, the jaw-closing muscles and various ligaments. Consider first how it is supported when its owner is sitting or standing quietly with the head upright in the so-called rest position or postural position of the mandible. This position is important in clinical dentistry and maxillofacial surgery because it is reproducible within a millimetre or two, and therefore can be used to establish the normal face height in situations such as the construction of artificial dentures in patients who have no remaining natural teeth. If the dentures are made too high, the teeth will click together when the subject speaks and eventually pain may develop in the masticatory muscles. If the dentures are too low, the face will have an unattractive, collapsed appearance. Surgeons also use the rest position of the mandible as a reference point for establishing the height of the face when they are repairing the facial bones following injury, or repositioning the maxilla and/or mandible for cosmetic reasons.

It has often been postulated that, when the head is still, the mandible is supported in its rest position by a stretch reflex in the jaw-closing muscles, i.e. the mass of the mandible stretches muscle spindles in these muscles, which send signals to the brain to excite the motor neurones innervating the jaw-closing muscles, pulling the jaw upwards again (see Woda et al. 2001 for review). A problem with this notion is the fact that it is extremely difficult to demonstrate any activity in the jaw-closing muscles when the mandible is in its rest position. We have recently shown that there is an extremely low level of activity in the jaw-closers at rest, but that this is not the result of a stretch reflex. In fact, the muscle activity is pulsatile and alternates with pulses of low-level activation of the jaw-openers (Jaberzadeh et al. 2003).

How, then, is the position of the mandible maintained in the more challenging situation in which the head moves up and down during walking and running? Figure 1 shows that in a subject who walks briskly (1.4 $m.s^{-1}$), not only does the head move up and down with every step, but the mandible moves up and down relative to the rest of the skull (i.e. the jaws open and close with each step). The downward mandibular movements must stretch the jaw-closing muscles; however, there is no burst of activity in the jaw-closer electromyogram (EMG) with each step that would indicate that the subsequent upward jaw movement was the result of a stretch reflex. This is seen clearly when the EMG signal before and after each landing is averaged for hundreds of steps (upper panels of Fig. 2).

Not surprisingly, the head movements are more vigorous when subjects run. The greater impact of landing causes the mandible to move more briskly downwards relative to the skull. This movement triggers a burst of EMG in the masseter which is evident in both

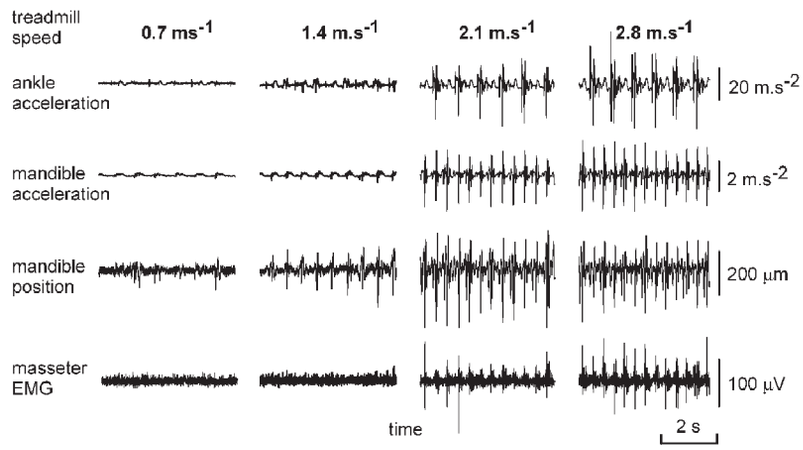


Figure 1. Kinematic and jaw muscle EMG records recorded from a subject who was walking and running at various speeds on a level treadmill. The ankle accelerometer was on one leg only and therefore indicates only every second step. Note that although the jaw moves slightly during walking, the burst of EMG with every step began only when the subject began to jog at 2.1 $m.s^{-1}$. *Reproduced from The Journal of Physiology*.

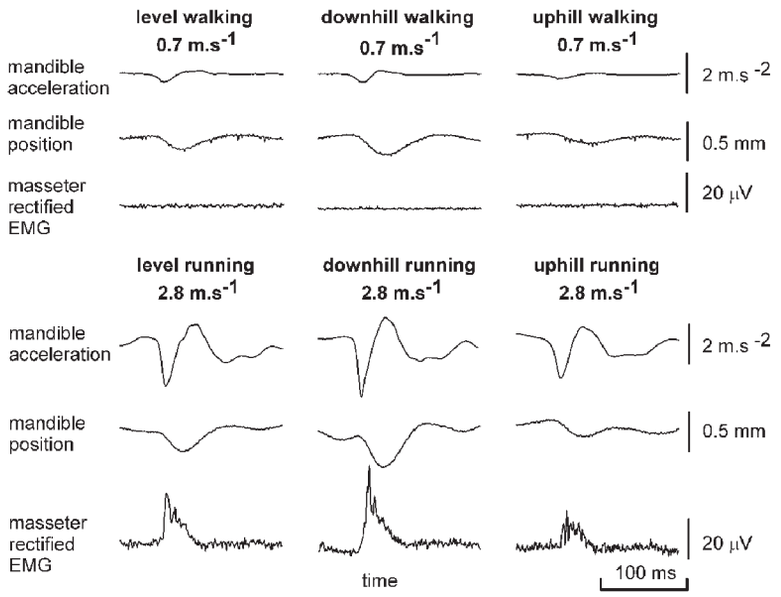


Figure 2. Averaged kinematic and jaw muscle EMG records recorded from a subject who was running at various speeds on a treadmill whose surface was horizontal, inclined downwards and inclined upwards in different trials. The jaw movements and EMG responses were greatest in the downhill running which made the subject land on his heels, and least in the uphill running because he landed on his toes. *Reproduced from The Journal of Physiology*.

the raw data in Fig. 1 and the averaged data in the lower panels of Fig. 2. This muscle activity then causes the mandible to move briskly upwards again. When subjects run uphill, they land on their toes. This gentler landing results in a slower, smaller movement of the mandible, which is often insufficient to trigger a reflex response. However, during downhill running, they must land on their heels, and the larger mandibular movement that results always triggers a reflex response in the jaw-closers.

To eliminate the possibility that the EMG response was the result of a vestibular reflex, our subjects also hopped down from a step and landed on one heel, both with their teeth clenched together to prevent their jaws from moving when they landed and without clenching. If the reflex arises from the vestibular system, it should still be present when the teeth are clenched. Landing on the heel (which feels pretty uncomfortable, incidentally) evokes strong reflex activation of the jaw-closing muscles when the teeth are unclenched. However, clenching the teeth abolishes the reflex (Miles et al. 2004). Thus we can be confident that the reflex is not vestibular, and, given its latency, is a stretch reflex.

The amplitude of this reflex response does not increase linearly with the amplitude or velocity of stretch. Instead, the amplitude of the EMG increases during brisker jaw movements even when the maximum amplitude and/or velocity of downwards jaw movement decreases or remains constant. This is because the downward movement of the mandible stretches the jaw-closing muscles and evokes reflex muscle excitation which then restrains the downward jaw movement. That is, when the stretch is sufficient to evoke reflex EMG activity, the muscle activation then prevents further downward jaw movement. Stronger stretches evoke more muscle excitation which result in smaller maximal downward jaw movement.

Thus, at rest, the mandible is supported not by reflex activity but by the visco-elasticity of the soft tissues in the masticatory system. This mechanism is sufficient to support the mandible during walking. However, the brisker downward movements of the mandible that occur when one lands on one's heel during running evoke stretch reflexes in the jaw-closers that actively maintain the posture of the mandible. Even during running, the mandible usually moves up and down less than a millimetre, and the teeth do not crash together. This is a unique demonstration of how a stretch reflex operates to maintain posture under entirely natural conditions.

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References

Jaberzadeh S, Brodin P, Flavel SC, O'Dwyer NJ, Nordstrom MA & Miles TS (2003). Pulsatile control of the human masticatory muscles. *J Physiol* 547, 613-620.

Miles TS, Flavel SC & Nordstrom MA (2004). Control of human mandibular position during locomotion. *J Physiol* 554, 216-226.

Woda A, Pionchon P. & Palla S (2001). Regulation of mandibular postures: mechanisms and clinical implications. *Crit Rev Oral Biol Med* 12, 166-178.

Fino

Fino, a very dry, pale sherry drunk like a white wine, is staging something of a resurgence. Gonzalez Byass, the producers of Tio Pepe, have commissioned Roger Linden, head of the Division of Physiology at King's College London, to investigate why the drink is so versatile. His preliminary study suggests that when you sit down to dinner your mouth may have been assaulted by all sorts of contaminants, from toothpase and coffee to exhaust fumes. Roger Linden and his team are investigating the effect on the taste buds of the five basic tastes - sweet, salt, bitter, sour and umami (Japanese, approximately, for 'rich in flavour'). Preliminary results suggest the intensity of salt and sour are perceived as less and the taste of bitter perceived as greater. It helps explain why fino's slate-dry, nutty, savoury taste is good with smoked fish and salted nuts, and can be drunk with 'difficult' foods such as Asian and Indian cuisine. Research continues ...

Extracted from an article by Glyn Tucker published in the *Cambridge Evening News* on 12 June, 2004.

Making old muscles young again – a therapeutic role for β_2-agonists?

James Ryall and co-workers ask whether β_2 anabolic enhancers might help prevent muscle loss in the elderly

'Exercise alone will not prevent sarcopenia. Other strategies, including muscle anabolic agents, must be considered'. Above, left: James Ryall, Above, right: David Plant and Gordon Lynch, left.

What is sarcopenia?

Some of the most serious consequences of ageing are its effects on skeletal muscle. 'Sarcopenia' (derived from the Greek words 'diminishing flesh') is the term widely used to describe the slow but progressive loss of muscle mass with advancing age. Sarcopenia is characterised by a loss of both muscle quantity and quality that leads to a gradual decline in strength and a slowing of movement. The loss of strength is so dramatic it can remove an elderly person's ability to carry out the tasks of daily living and rob a person of their functional independence, whilst also increasing the risk of sudden falls and fractures. As the number and proportion of older persons in the population continues to escalate, sarcopenia will have a dramatic impact on the quality of lives, and place ever-increasing demands on public health systems.

The loss of muscle mass and strength is thought to be due to the progressive atrophy and loss of muscle fibres associated with a loss of motor units, and a reduction in muscle 'quality' due to the infiltration of fat and other non-contractile material such as connective tissue. In some instances, there is a preferential loss of fast muscle fibres, leaving muscles with a greater proportion of slow fibres which, along with alterations in intracellular calcium handling, contributes to the slowing of movement. Motor unit remodelling (whereby denervated fast fibres subsequently become reinnervated by slow motoneurons) can also account for these changes. These age-related changes in skeletal muscle can be attributed to a complex interaction of many factors that affect neuromuscular transmission, muscle architecture, fibre composition, excitation-contraction (E-C) coupling, and metabolism (Plant & Lynch, 2002).

The goal of any strategy for treating sarcopenia, or any condition where muscle wasting is indicated, is to preserve and, ideally, increase muscle size and strength (Lynch, 2002a). One way to slow the progression of sarcopenia is through regular exercise, particularly training programmes involving resistance exercise or strength training (Singh, 2002). Although resistance training is clearly the best exercise to improve functional strength, and there are many health benefits associated with this form of training that can improve quality of life in the elderly, it must be recognised that exercise alone will not prevent sarcopenia. Also, there is a general reluctance to exercise, particularly in the elderly, and this further highlights the need for other therapeutic approaches. Elite Master's level athletes who train and compete year in and year out for a large portion of their lifespan, simply do not perform at the same level when old that they did when they were younger. Clearly, there are other factors that contribute to the preservation of muscle quantity and quality.

Other approaches that are complementary to exercise also need to be considered. Given the magnitude of the growing public health problem related to sarcopenia, there is enormous interest in the development and evaluation of therapeutic strategies for preventing or ultimately reversing age-related muscle wasting and weakness.

Hormone replacement therapy for treating sarcopenia?

Ageing is associated with a so-called 'somatopause', defined as the decrease in circulating levels of anabolic hormones, including (but not limited to) growth hormone (GH), insulin, insulin-like growth factor I (IGF-I) and testosterone. These hormonal changes are thought to be responsible, at least in part, for the age-related loss of muscle mass and strength. Numerous clinical trials on older adults have focused on increasing circulating levels of hormones back to adult levels, as a way to combat sarcopenia. However, recent evidence from the US Women's Health Initiative (combined Hormone Replacement Therapy) trial indicated that, for postmenopausal women, the risks for side effects (such as heart disease) from combined oestrogen and progestin appear to outweigh the benefits. Therefore, at this stage, it appears that this approach has only limited therapeutic applicability. In the absence of successful hormone replacement therapies, other muscle anabolic agents have been proposed to treat sarcopenia.

Role for β_2-adrenoceptor agonists

Although traditionally prescribed for alleviating bronchospasm in the treatment of asthma, β_2-adrenoceptor agonists (β_2-agonists, such as the most widely described, clenbuterol), when given systemically at higher doses, have potent anabolic effects on skeletal muscle. β_2-agonists cause muscle hypertrophy via a cyclic AMP-dependent mechanism that results in an increase in protein synthesis and a decrease in protein degradation (Fig. 1). This hypertrophic effect, combined with a known lipolytic action, has proved desirable for those working in the livestock industry trying to improve meat quality and yield. Unsurprisingly, β_2-agonists have also been used/abused by those engaged in competitive bodybuilding and soon after by other

athletes competing in strength- and power-related sports.

As a therapy for treating muscle wasting and weakness β_2-agonist administration has produced promising, although not entirely conclusive, results in many animal-based studies and some studies involving human patients (for review, see Lynch 2002b). When given to both young and old rats, the clenbuterol-induced increase in muscle mass was equivalent regardless of age, supporting the hypothesis that β_2-agonists could be an effective intervention for countering sarcopenia (Carter *et al.* 1991). However, treating aged rats with a micromolar dose of clenbuterol, comparable to that used to treat asthma, did not prevent the age-related loss of muscle mass and strength (Chen & Alway, 2000). A more powerful β_2-agonist, and/or at a higher dose, may therefore be necessary for treating sarcopenia effectively.

We have recently shown that, at an equimolar dose to clenbuterol, another β_2-agonist, fenoterol, has a 10-15% greater anabolic effect on rat fast-twitch (EDL) and slow-twitch (soleus) muscles (Ryall *et al.* 2002). In a follow-up study we found that after just 4 weeks of daily administration, fenoterol completely ameliorated the age-related loss of muscle mass and strength in aged (28 month old) rats. The fenoterol-induced increase in mass and strength was attributed to hypertrophy of existing fibres and not to an increase in fibre number. To our knowledge, this was the first study to demonstrate complete restoration of both muscle mass and strength to (adult) control levels following β_2-agonist administration (Ryall *et al.* 2004).

One advantage of treating sarcopenia using β_2-agonists rather than other anabolic agents relates to the ability of some β_2-agonists to cause a shift in muscle fibre type proportions, typically from slow- to fast-twitch. Thus, powerful β_2-agonists, such as fenoterol, are able to not only prevent, or reverse, the loss of muscle mass and strength, but might also help retain a higher proportion of fast-twitch fibres that will attenuate (in part) the characteristic slowing of contraction in aged mammals.

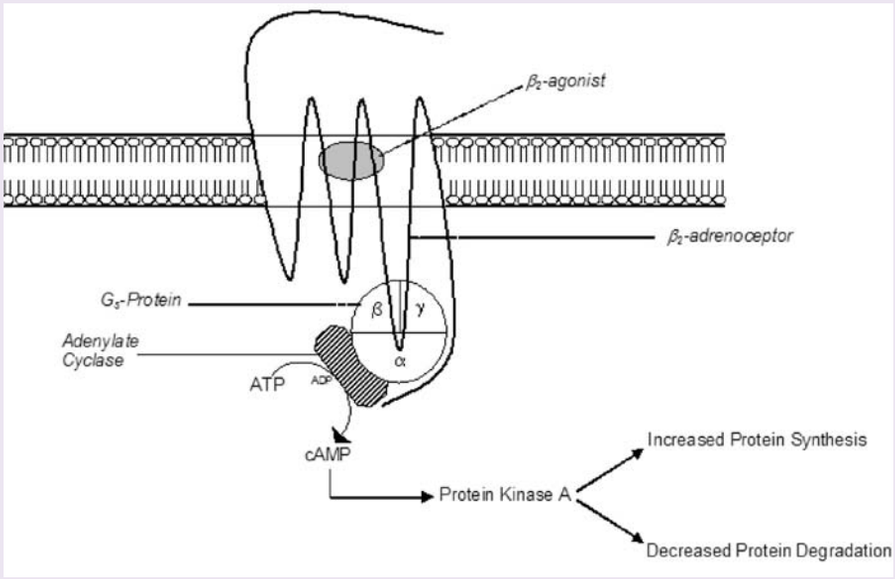

Figure 1. β_2-adrenoceptor signalling pathway responsible for skeletal muscle hypertrophy after β_2-agonist stimulation. Binding of the β_2-agonist to the adrenoceptor stimulates production of cyclic AMP by adenylate cyclase. This, in turn, activates protein kinase A leading to muscle hypertrophy via mechanisms controlling both protein synthesis and protein degradation

Too good to be true?

Despite the positive attributes of β_2-agonists for treating sarcopenia, they have several deleterious side-effects, especially when administered in high doses. Given that β_2-agonists act via the β_2-adrenoceptor, and there exists a population of β_2-adrenoceptors in the heart, it is difficult (perhaps impossible) to separate the hypertrophic effect on skeletal muscle from that on the heart. Cardiac hypertrophy has been observed in nearly all studies that have examined the effects of β_2-agonist administration on skeletal muscle. Most of these studies have employed high doses in order to produce skeletal muscle hypertrophy and therefore, not surprisingly, they have also resulted in significant (and potentially deleterious) increases in heart size and, in some cases, fibrosis. This has so far limited the clinical potential of β_2-agonists for sarcopenia, and also for other muscle wasting disorders (such as muscular dystrophy and sepsis). It is also important to note that many athletes are not aware of these potentially deleterious effects of chronic high-dose β_2-agonist administration. For developing an effective therapy for sarcopenia, one must prevent or reduce these detrimental effects on tissues other than skeletal muscle, and the challenge is to devise treatments that utilise different β_2-agonists that are capable of eliciting skeletal muscle hypertrophy at extremely low doses, and following only a short treatment duration.

Recently, new generation β_2-agonists have been approved by the Food and Drug Authority (FDA) for the treatment of asthma. These β_2-agonists, specifically salmeterol and formoterol, have been developed specifically to have an increased duration of action for the treatment of asthma, and a greater β_2-adrenoceptor selectivity (the predominant skeletal muscle subtype) compared with existing β_2-agonists. These new β_2-agonists have the potential to elicit skeletal muscle hypertrophy at very low (micromolar) doses, due to their extended duration of action, whilst at the same time minimising any unwanted side-effects, due to the greater level of selectivity. Their efficacy and safety for application to muscle wasting disorders remains untested.

To sum up, there is a considerable need for therapies that can slow the effects of aging on muscle function. Strategies

are needed to restore muscle size and strength in the frail elderly so that quality of life can be maintained or improved. Physical activity must continue to play an important role in all these therapeutic approaches; however, it must be realised that exercise alone will not *prevent* sarcopenia.

Consideration must therefore be given to other strategies including muscle anabolic agents. Although β_2–agonists show considerable potential as an intervention for sarcopenia, much research is needed to test their efficacy and safety, especially the need to separate skeletal muscle and cardiac effects. These issues need to be addressed before they can be recommended for clinical application.

Acknowledgements

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References

Carter WJ, Dang AQ, Faas FH & Lynch ME (1991). Effects of clenbuterol on skeletal muscle mass, body composition, and recovery from surgical stress in senescent rats. *Metab Clin Exp* 40, 855-860.

Chen KD & Alway SE (2000). A physiological level of clenbuterol does not prevent atrophy or loss of force in skeletal muscle of old rats. *J Appl Physiol* 89, 606-612.

Lynch GS (2002a). Novel therapies for sarcopenia: ameliorating age-related changes in skeletal muscle. *Expert Opin Ther Pat* 12, 11-27.

Lynch GS (2002b) Beta-2 Agonists. In *Performance-Enhancing Substances in Sport and Exercise*, ed. Bahrke M & Yesalis C, pp 47-64. Human Kinetics, Champaign Illinois.

Plant DR & Lynch GS (2002). Excitation-contraction coupling and sarcoplasmic reticulum function in mechanically skinned fibres from fast skeletal muscles of aged mice. *J Physiol* 543, 169-176.

Ryall JG, Gregorevic P, Plant DR, Sillence MN & Lynch GS (2002). β_2-agonist fenoterol has greater effects on contractile function of rat skeletal muscles than clenbuterol. *Am J Physiol* 283, R1386-1394.

Ryall JG, Plant DR, Gregorevic P, Sillence MN & Lynch GS (2004). β_2-Agonist administration reverses muscle wasting and improves muscle function in aged rats. *J Physiol* 555, 175-188.

Singh MA (2002). Exercise comes of age: rationale and recommendations for a geriatric exercise prescription. *J Gerontol* 57, M262-M282.

Affiliate News

Patricia de Winter unravels the mysteries of molecular biology at the Annual Molecular Techniques Workshop in Cork

For the uninitiated in the mysteries of molecular biology, attempts to comprehend a paper that includes the use of such techniques can lead to the feeling that the authors are speaking a completely different language to which the reader is not privy. I was one of these poor unfortunates until I was lucky enough to be accepted onto the 7th Annual Molecular Techniques Workshop held at University College, Cork. Recently the workshop organiser, Patrick Harrison, sent out a feedback form to those who had attended the workshop in 2002 and 2003, with a view to obtaining longer-term information on how the course has affected the subsequent research of its attendees. Most physiologists who attend are Affiliates rather than Members so this information is useful because it provides some evidence of the course's effectiveness in training young physiologists for future practice.

A short feedback form (four questions) was sent to 31 course participants in early May 2004. Thirty forms successfully reached their destinations and 18 (60%) were returned completed. Nine respondents are currently in post-doc/research associate positions, six are at various stages of their PhD and two are in permanent academic positions. Fourteen people (47% of respondents) are currently using techniques learnt on the course, and the others, predominantly PhD students, have not yet done so but will seek, or have obtained, a post-doc position that will involve the use of molecular techniques. Five participants have published at least one paper or have papers in press/preparation that involve techniques learnt on the course.

Finally, participants were asked how their attendance on the course has changed their research. Responses to this question were varied, but some major themes were predominant: most people stated that they have gained the confidence they needed to try using the techniques in their own labs, even in labs where these techniques are not used routinely; and that the course provided them with a good grounding in molecular techniques. Some people stated that they can access and critically assess literature which was previously unintelligible and that attending the course broadened their research base. In some cases the focus of their research is now more molecular biology-orientated. Certainly, respondents felt that the knowledge they had obtained would continue to serve them well in the future.

To end on a personal note, I attended the workshop in 2003, during the latter half of my PhD and, although I have as yet been unable to employ any of the techniques I learnt, I do hope to be able to do so when I return to the world of work this autumn. For me, the boost in confidence was extremely important. Not only do I feel that I will be able to tackle RNA/DNA isolation, restriction enzyme digests, PCR, transfection etc. without my former feeling of trepidation, but I no longer look at articles containing strings of ATGC and phrases such as 'we cloned a cDNA' in utter horror.

Finally, I would like to add that the workshop was extremely well-organised and evidently founded on an excellent knowledge of the subject. Some weeks after the course, Patrick Harrison sent us all a superb written report that he had compiled. It detailed all our results with a rationale for the procedures and clear explanations of what we had expected to find. I am certain that none of us can thank him and the other course staff enough for their dedication, patience and willingness to impart their expertise.

Patricia de Winter
Birkbeck, University of London

Maternal stress in pregnancy may affect the cardiovascular system of the child

Prenatal stress of the mother has been shown to have long term effects on the cardiovascular reactivity of the child. When pregnant rats were mildly stressed in the last week of pregnancy, their adult offspring had increased systolic blood pressure responses to mild stress. Natalya Igosheva and Vivette Glover explain

Natalya Igosheva

Our recent work (Igosheva *et al*. 2004) suggests that if the mother is stressed while she is pregnant this may permanently alter the cardiovascular reactivity of her child. This is probably due to fetal programming.

This concept has become well known through the work of David Barker (Barker, 1995). He and his colleagues have shown that being small at birth is a risk factor for later cardiovascular disease and associated illnesses such as diabetes. Barker has proposed 'the fetal origins of adult disease hypothesis'. This states that the physiological, neuroendocrine or metabolic adaptations that enable the fetus to adapt to changes in early life environment result in a permanent programming (or re-programming) of the developmental pattern of proliferation and differentiation events within key tissue and organ systems and have pathological consequences in later life. Barker and his group have mainly looked at the nutrition of the fetus to explaining the possible mechanisms underlying these findings.

A second field of work, originally in animal models, has shown that if the mother is stressed while she is pregnant this alters the later behaviour of her offspring. In particular, the offspring show an increased stress response when exposed to a new stressor (Weinstock, 2001).

In animal studies it is possible to control for possible confounders, such as alterations in parenting behaviour after prenatal stress, by cross-fostering all the offspring to new unstressed mothers (Schneider *et al*. 1999).

In both rats and monkeys the underlying mechanisms are starting to be understood. It has been shown that the exposure to prenatal stress re-programmes the main stress response system of the offspring. The function of the hypothalamic-pituitary-adrenal axis, which makes the stress hormone cortisol, is permanently altered, and this is associated with changes in the neurochemistry of the hippocampal region of the brain (Matthews, 2002). The link between antenatal maternal anxiety and child behavioural problems has recently also been shown in humans. The children of mothers who were particularly anxious while pregnant (in the top 15%) were found to be at double the risk for later behavioural problems such as attention deficit/hyperactivity (O'Connor *et al*. 2002)

Our recent research (Igosheva *et al*. 2004) suggests that these two areas of study, prenatal stress and cardiovascular

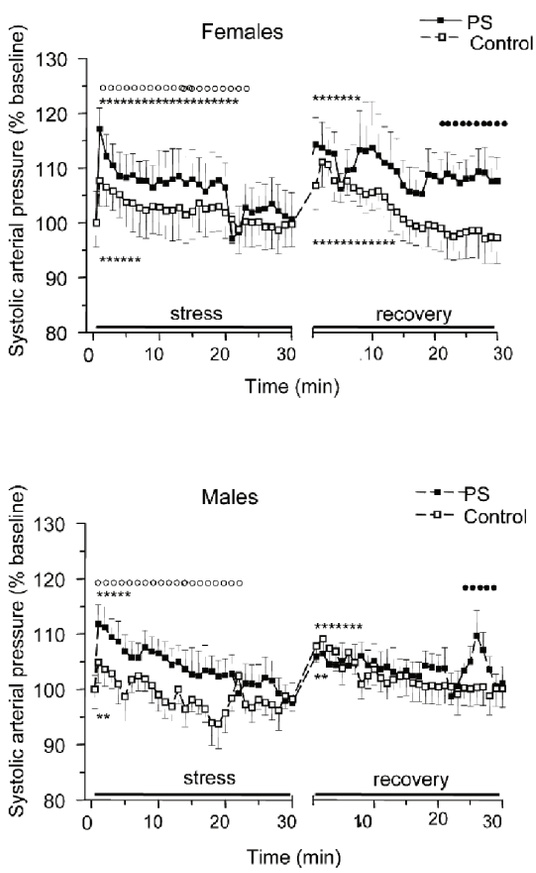

Figure 1. Changes in systolic arterial pressure (SAP) during 30 min period of restraint and for 30 min following return to the home cage in control and prenatally stressed female and male rats. Prenatally stressed (PS) rats showed an increased peak of SAP responses following the restraint stress and an extended duration of SAP responses during both acute stress and recovery. There were sex-related differences in SAP responses upon return to the home cage with PS females showing a higher SAP increase than PS males. Data are expressed as percentage changes from baseline values to the response for both stress and recovery periods. Control offspring,▫, $n = 7$; PS, prenatally stressed offspring,▪, $n = 7$. Values are given as means – SEM. ∗ – $P < 0.01$ vs. basal values; ● – $P < 0.05$; o – $P < 0.01$ vs. prenatally stressed group. Adapted from Igosheva *et al.* (2004)

function, may be more linked than previously realised. In this study, pregnant rats were mildly stressed during the final week of their pregnancy, by exposing them to light and restraint. A control group were studied in parallel with no such prenatal stress. All the newborn pups were then cross-fostered to new mothers and all these offspring were studied when they were 6 months old. The basal characteristics of all the offspring were the same. However, when subjected to a new mild stress, those animals whose mothers had been exposed to stress during pregnancy showed a much larger increase in systolic blood pressure than did the controls (Fig. 1). Their blood pressure also took longer to recover back to normal. Prenatally stressed rats also showed a greater increase in blood pressure variability compared with control animals during exposure to restraint stress, more prolonged heart rate responses to acute stress and delayed recovery. Most of these effects were more pronounced in the females than in the males.

Thus it seems that prenatal stress may also have a permanent effect on the cardiovascular system. As prenatal stress can also cause smaller babies, it is one possible mechanism underlying the Barker findings. Such a pattern of haemodynamic responses to stress would have significant consequences for adult cardiovascular health if it also occurs in humans. Much more research will clearly be needed to establish this. However, it may be that a new way to prevent the development of predisposition to a range of health problems will be to ensure that pregnant mothers have as stress free a time as possible.

Natalya Igosheva
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Acknowledgments

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References

Barker DJ (1995). Fetal origins of coronary heart disease. *BMJ* 311, 171-174.

Igosheva N, Klimova O, Anishchenko T & Glover V (2004). Prenatal stress alters cardiovascular responses in adult rats. *J Physiol* 557, 273-285.

Matthews SG (2002). Early programming of the hypothalamo-pituitary-adrenal axis. *Trends Endocrinol Metab* 13, 373-380.

O'Connor TG, Heron J & Glover V (2002). Antenatal anxiety predicts child behavioral/emotional problems independently of postnatal depression. *J Am Acad Child Adolesc Psychiatry* 41, 1470-1477.

Schneider M L, Roughton EC, Koehler AJ & Lubach GR (1999). Growth and development following prenatal stress exposure in primates: an examination of ontogenetic vulnerability. *Child Dev* 70, 263-274.

Weinstock M (2001). Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Prog Neurobiol* 65, 427-451.

Physiology in Belarus

The 50th anniversary of the Institute of Physiology of the National Academy of Sciences

Over the last few years I have visited Belarus and the Institute of Physiology of the National Academy of Sciences of Belarus on several occasions. At the end of the last year the Institute (which has been headed since 1984 by Valeri Gourine – a member of the Physiological Society and a personal friend) celebrated its 50th anniversary.

The Institute was founded on 7 September, 1953 on the base of the Institute of Theoretical Medicine of the Byelorussian Academy of Sciences. Since the late 1950s the Institute had been engaged in studies of the structural and functional organization of autonomic ganglia, the role of afferent pathways in local and systemic reflexes, neurohumoral mechanisms of vestibulo-visceral reflexes, causes of disturbances of those processes of nerve myelination that lead to multiple sclerosis, and the effects of ionising radiation on biochemical processes. In the 1960s the Institute transformed into an international scientific centre.

Valeri Gourine, Director of the Institute of Physiology of the Byelorussian Academy of Sciences

The major directions of research in the 1980s and 1990s were general activities of the nervous centres which regulate the functions of internal organs, mechanisms of body temperature regulation, compensatory and recovery processes in tissues under the influence of physiologically active substances, and neural and humoral mechanisms of function regulation under stress. At present the major directions of research at the Institute are central and peripheral mechanisms of thermoregulation, the control of body functions during hypo- and hyperthermia and fever, physiology of the autonomic nervous system and physiological mechanisms of adaptation to environmental factors.

Currently the Institute of Physiology is the leading scientific institution that coordinates physiological research in the Republic of Belarus. The staff of the Institute consists of 72 full-time researchers working in seven laboratories and three working groups. There is a postgraduate course and a council for defence of dissertations in physiology and related disciplines. The research conducted at the Institute conforms to modern trends in physiological sciences and to priority directions of basic medical studies.

I hope all members of the Physiological Society will join me in sending our congratulations to all at the Institute and wish them good luck in the next 50 years of their development

Bill Winlow

Cardiovascular Physiology

Warsaw International Workshop for Young Physiologists through the eyes of the organisers...

The 6th International Society Workshop for young physiologists dedicated to cardiovascular physiology was held in Warsaw from 13-16 May, 2004. The Workshop was organised by Andrzej Beresewicz, David Eisner, Gerrit Isenberg, Alex Verkhratsky and Susan Wray, and held at the Medical Institute for Postgraduate Education. As usual, the Workshop programme included lectures in the morning and laboratory sessions in the afternoon.

Young researchers discuss their projects (above) and Gerrit Isenberg enjoys a compulsory ride at the Farewell Picnic (below)

The Workshop commenced with a reception in the 'House of Science' where participants were welcomed by International Secretary David Eisner and Andrzej Beresewicz. Altogether 42 young scientists were selected from more than 80 applications; the selection was based on abstracts submitted.

The participants came from 13 different countries (registrants in brackets): Poland (14); Ukraine (12), Slovak Republic (8), Czech Republic (6), Russia (4), Hungary (2) Norway (1), Estonia (1) Belorussia (1) Turkey (1), Israel (1) Romania (1) and the UK (1). Students were exposed to a series of lectures presented by Mark Boyett (Leeds, UK), Bohdan Lewartovski (Warsaw, Poland), David Eisner (Manchester, UK), Alex Verkhratsky (Manchester, UK), Susan Wray (Liverpool, UK) Maria Fiora Wendt-gallitelli (Halle, Germany), Barbara Casadei (Oxford, UK), Gerrit Isenberg (Halle, Germany), Gerd Hassenfuss (Goettingen, Germany) and Andzej Beresewicz (Warsaw, Poland). These lectures covered various aspects of cardiovascular physiology and pathophysiology.

The lectures were followed with practical demonstrations which worked amazingly well, and no 'visitor effect' was encountered. In parallel, speakers ran 'meet the expert' sessions, which allowed for close interactions with students. Young researchers discussed their own projects as well as various theoretical and practical questions. These sessions worked very well, although they required a substantial effort from both sides. On the second day the parallel afternoon sessions were focused on research ethics, fraud and publication. Although not a 'how to commit fraud' session, it had the full attention of all the students!

Each registered student presented a poster, and the poster session was very lively. The three best posters were chosen by a small evaluation committee and the winners were given the opportunity to deliver 10 min oral presentations at the beginning of the last session. In addition, the winners were presented with Physiological Society certificates and a small cash award. The poster winners were Alexander Bondarenko (Bogomoletz Institute of Physiology, Kiev, Ukraine), Ole-Jakob How (University of Tromso, Norway) and Thomas Simunek (Charles University, Prague, Czech Republic).

On the second evening the whole crowd was driven to the outskirts of Warsaw where everybody was subjected to a compulsory ride (see photo) on rather domesticated and forgiving horses. This was followed with a session of archery and a magnificent rustic dinner which was crowned with a roasted wild boar. Dances and informal communications followed, yet the local organisers once more proved very careful indeed and at precisely 23.30 the bus delivered happy participants to their abodes.

Overall the meeting was an undeniable success, as corroborated by all the positive feedback from participants. (A student account, written by Natalie Middleton who represented Britain, follows). For this success the Society and all participants and lecturers are very much indebted to the absolutely flawless organization provided by Andrzej Beresewicz and his wonderful team of Warsaw colleagues.

The full programme of the workshop is available at:
http://www.physoc.org/international/warsaw2004/programme
where an extended collection of photos can also be viewed in due course.

Andrzej Beresewicz, David Eisner, Gerrit Isenberg, Alex Verkhratsky, Susan Wray

...and through the eyes of a student

Natalie Middleton

As the sole delegate from the UK, I was clearly a minority amongst the diverse characters from Central and Eastern Europe that attended the Cardiovascular Physiology Workshop in Warsaw. However, they were a friendly and inclusive bunch and I was immediately made to feel part of the group at the 'Welcome Reception'. This ambience suffused the Workshop from beginning to end, helping to make the event not only an invaluable learning tool and opportunity to meet experts in the field, but also an enjoyable and highly entertaining experience!

The theme of the workshop was 'Signal Transduction in the Cardiovascular System'. An international panel of physiologists imparted their expert knowledge in a series of lectures, which underpinned the experimental laboratory demonstrations interspersed throughout the programme. Specific areas that were addressed varied from David Eisner's engaging talk on aspects of cardiac excitation-contraction coupling, to the concluding lecture on 'Signal transduction mechanism of ischemic preconditioning' presented by the Workshop organiser – Andrzej Beresewicz.

As a PhD student in exercise physiology coming from a sport science background, attending this workshop gave me a different perspective on my own research, and enhanced my understanding of cardiovascular mechanisms. Through presentation of my recent findings on 'Impact of marathon running on markers of cardiac damage', I was particularly interested to hear novel opinions and ideas in this area. As the workshop was intended primarily for young scientists, the lectures and demonstrations were pitched specifically at this type of audience. The visiting lecturers were approachable and supportive, with a genuine interest in the students' research topics, and all were enthusiastic in providing suggestions for future work. I believe this attitude was fundamental in creating a rapport and facilitating interaction between the experts and the students.

During the allotted poster presentation sessions, there was also ample opportunity for discussion with other students, which increased my awareness of the current cardiovascular physiology research being carried out across Europe. This time also served as a chance to establish new contacts and foster potential collaborations for future research projects.

The laboratory demonstrations introduced a practical element to the Workshop, and ranged from the recording of single myocyte shortening to investigating the Langendorff-perfused guinea-pig heart. Although these demonstrations were beyond the scope of my own research, they were fascinating to watch, and were accompanied by clear explanations which reinforced my physiological knowledge. I am sure these sessions also sparked a few ideas for future experiments in many of the students!

A further dimension of the workshop, and one that was particularly successful, was the 'Meet the Expert' sessions – an informal discussion between a selected expert and a small group of students. Being unfamiliar with experimental animal models, I found a discussion on nitric oxide and genetic knock-out mice with Barbara Casadei extremely informative. This also stimulated debate on experimental problems encountered in the lab and some possible solutions. This session also gave an insight into the cutting-edge research work of an expert in the field, providing students with valuable information and a role model we could aspire to.

Despite inclement weather, the Farewell Picnic was thoroughly enjoyed by all. We experienced generous Polish hospitality, and there was no escaping the horseriding which was apparently mandatory! Memorable images over the 4 days were captured by an ever-present photographer, and CDs with photos on were presented to delegates on the final day as a memento of the Workshop. I thought this was an excellent idea and this gesture reflected the great deal of time and effort that the organisers had invested in the Workshop.

I am now looking forward to the next Physiological Society meeting, and hope that it will be as interesting and worthwhile as I found this one. As for Poland, with many invites from my new Polish acquaintances to visit, I will no doubt be back in the near future!

Natalie Middleton
Sport Science, Brunel University
Middlesex, UK

Andrzei Beresewicz presents the poster winners with Society certificates and a small cash prize

Ella and the Ben Fund

Ella's parents explain how the Society's Benevolent Fund has helped her to cope with deafness caused by pneumococcal meningitis. The picture shows Ella on the day that her cochlear implant was switched on

Our daughter Ella contracted pneumococcal meningitis just before her first birthday in January 2003. As a result she sustained profound hearing loss as well as some movement and coordination problems and latterly epilepsy. Soon after her deafness was diagnosed she was fitted with powerful hearing aids. At this time we were living in a flat with polished floorboards, and were advised by Ella's teacher of the deaf that these conditions would be detrimental to the development of her listening skills using her aids.

The Physiological Society Benevolent Fund very kindly provided funds for us to have a carpet fitted to help lessen the echo in our living room. However, Ella derived no benefit from these hearing aids and was subsequently assessed for a cochlear implant which was implanted at Great Ormond Street Hospital later that year. This incredible technology allowed her to begin to hear and identify environmental sounds before turning to her name and beginning to understand a few words. We have no doubt that the improvement in the acoustics in our living room has made this easier for her.

Since her implant Ella has been attending the Elizabeth Foundation once a week, a charity supporting pre-school deaf children. Here she takes part in nursery activities aimed at developing listening and communication skills. Again the Benevolent Fund has helped out with a generous gift which will be used to pay travel costs and donation fee to the Foundation. As Ella's mother has had to give up work to care for Ella these gifts have been very greatly appreciated and we would like to thank the donors to the Benevolent Fund for their incredible generosity.

P.S. from the Chairman of the Trustees

The Trustees of the Benevolent Fund are so grateful to Ella's parents for sharing their story. Because we respect the confidentiality of our beneficiaries it's difficult to show Society Members that the Fund really works. Perhaps the doubters who thought it was 'some sort of slush fund for old physiologists' now see what a difference it can make.

The Fund was established in 1976 when the Committee was asked if the Physiological Society could help the widow of a young Member whose son had special needs. Itself a charity, the Society may not give away its money but it can support the administration of the Ben Fund. This means that *all* donations to the Fund can be used to help what the Trust Deed calls those in 'necessitous circumstances' whose work, at any level, has contributed to the 'advancement of physiology'. 'At any level' means just what it says: tea ladies, technicians, teachers – essential members of any physiologically involved department – are all eligible for help, as are their dependants.

Calls on the Fund are erratic – some years are happily free of trauma and tragedy, others are not. When the 1976 Committee put its hands in its collective pockets the £100 produced made all the difference but, nowadays, £100 doesn't go that far. In the last 12 months the Fund has paid out £5,250. Some of our beneficiaries may merit further help.

We would like to be in a position to give *worthwhile* support whenever genuine need arises. To do this we need to keep up our income. Some Honorary Members are generously donating what would have been their Society subscription. While big donations are always very welcome, small donations from lots of you – say £5.00, which equals £6.40 with Gift Aid – could make a welcome difference to our bank balance without having much effect on yours.

Ann Silver

There is a donation form opposite, and also on the Society web site (http//:www.physoc.org). ***Alternatively, you'll be able to make a donation at the Ben Fund stand at the King's College Meeting in December.***

The Benevolent Fund of the Physiological Society

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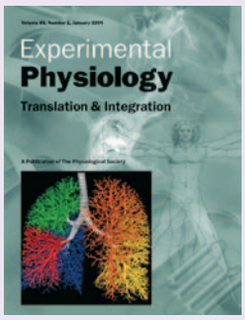
Experimental Physiology at FASEB

Awareness, by physiologists worldwide, of the mission and focus of *Experimental Physiology* is of paramount importance for its future success. This is why the recent FASEB meeting in Washington, USA was a valuable opportunity to advertise the journal, crucially to an American audience. Washington greeted us with some lovely warm weather in April, together with an excellent venue. The new convention centre is huge and in general the facilities are good. If I have a criticism it is the lack of social facilities close to each of the lecture rooms and the fact that the large open space meant 'bumping' into colleagues was less probable.

The poster display and company stands were excellent and Blackwell Publishing did a terrific job in displaying EP. Some 150 free copies of EP (89.1) went like hot cakes and the



lunch reception got a lot of visitors around the stand, although no doubt some attended because of the 'booze'. There was a great deal of real interest and I think the message regarding what we are trying to achieve with EP got across. People were encouraged to get their libraries to subscribe especially since they can get *The Journal of Physiology* and *Experimental Physiology* at a reduced cost, and both are available electronically. Many thanks have to go to Alison Brown (Blackwell) for organising a very successful reception and looking after the stand so well. David Paterson and I fielded lots of questions about 'why EP?' and what we are trying to achieve. Most enquirers were enthusiastically supportive of the main aims regarding the publication of papers using state of the art technology to understand how genes/molecules determine or alter function of organs or systems.

Whilst in Washington we also had a meeting with our North American Editors. Eleven attended and included Mike Hogan, Julian Paton, Nandurai Prabhaker, Mohan Raizada, Peter Hunter, Jere Mitchell and John West, as well as myself, David Paterson and Alison Brown and Robert Harrington of Blackwell. There was a strong wish by all to see the journal succeed even though the inevitable question came up 'why do we need to publish in EP?' Although there are presently no other journals with quite the same objectives, *The Journal of Physiology* does at present occasionally publish papers in areas that I would hope will become more the province of EP.

Clearly the impact factor is important and we need to show a sustained increase in it. Whilst we seem to be on the right track, it was felt we could do more. One important suggestion was to capture papers in the area related to understanding biological complexity by modelling regulatory processes. We are at a stage where the tools are becoming available to take on the challenge of making quantitative predictions to physiological outcomes from genomic knowledge. The article from Peter Hunter and Denis Noble's group (Crampin *et al.* 2004) in the January issue of EP (89, 1-26), which Society Members would do well to read, is a good indication of the importance of this area that might be termed computational physiology.

Our North American colleagues were very supportive of our efforts to get EP moving forward and upward, not only because of its aims, but because they considered its great history made it imperative. On this point we are presently trying to choose the best 10 papers published in EP over about 100 years. I would welcome any suggestions from Members.

John H Coote
Chair, Experimental Physiology

Public engagement

Dear Editor,
I welcome your recent editorial discussing the importance of public engagement, but also recognise the difficulties. We are all overworked and are rewarded for little that does not contribute to external reviews of teaching and research – a serious mistake in my view. But interactions with the public or school children can take only a few hours each year, is one of the best ways to improve our presentation skills and is fantastically enjoyable.

Yet – such activities carry little recognition on our cvs or for RAE. One thing that does count is competitive prizes...so why doesn't the Society offer a couple of prizes for science communication, perhaps one for an early career scientist and one for a senior scientist? I am sure that industry or other funders would support this and it would send an important message from the Society.

Nancy Rothwell
University of Manchester

Nano – or anon

Dear Editor,
David Miller's exposition on picograms and nanograms (*Physiology News*, **55,** 21) took me back to 1953 when nanograms were still uncommon in biological usage. Volume 38 of *The Quarterly Journal of Experimental Physiology* contains a paper by JH Gaddum, CO Hebb, Ann Silver and AAB Swan on the action of 5-HT in the perfused lung. The legend to Fig. 4a, showing the assay of 5 H-T, contains the words 'Small figures give doses in nanograms (ng.) of the base'. The assays were done in Gaddum's lab and Catherine Hebb had left for Canada before he wrote the legend. (No requirement then for all authors to certify they'd seen the final version.) Soon after the paper appeared Catherine was phoned by a colleague who said 'Catherine, what's a nanogram?' 'An anagram?' she replied, 'why, that's what you get in crossword puzzles.'

Ann Silver
Honorary Member, Cambridge

Nociception in vertebrates: anatomy, electrophysiology, genomics and behaviour

The aim of this symposium, held in Edinburgh from 31 March to 2 April, was to bring academics, veterinarians and clinicians together to discuss the latest research on nociception and pain in mainly animal models. Speakers gave an up to date and fascinating insight into all of the major vertebrate groups including fish, amphibians, birds, mammals and the most highly evolved vertebrate – the human.

From left: John Harris, Dale Sandercock, Mike Gentle (back), Bruce Lynn, Craig Stevens, Lynne Sneddon, Johnny Roughan (back), Dorothy McKeegan, Sue Fleetwood-Walker and Carlos Belmonte

Lynne Sneddon (Liverpool) discussed research on the modern fishes as well as their predecessors, the lamprey and hagfish, and demonstrated that by comparing results from the lower vertebrates, many nociceptor properties are evolutionary conserved. Bruce Lynn (UCL) gave an excellent insight into the various types of nociceptors that could be characterized by their anatomy and electrophysiological properties. Mike Gentle (Roslin Institute) provided us with a fascinating insight into the pathology of arthritis in birds and the associated pain-related behaviours. Sally Lawson (Bristol) and Richard Morris took us deeper into the CNS and examined the highly complex role of dorsal root ganglion cells and spinal neurons. John Harris (Nottingham) discussed how information from the spinal cord innervates reflex responses to painful stimuli.

Chronic pain is currently a major clinical problem in humans and Hermann Handwerker (Erlangen) gave an enlightening presentation that demonstrated the complex interplay of peripheral and central pathophysiological mechanisms. Andrea Nolan (Glasgow Veterinary School) demonstrated that chronic pain also occurs in many animal models showing a need for the integration of clinical and fundamental science. Carlos Belmonte (Alicante) and Dorothy McKeegan (Roslin Institute) took us to the trigeminal nerve and discussed how corneal nociceptors are stimulated by cold and nasal nociceptors show amazing sensitivity to noxious gases, such as ammonia, in mammal and bird models respectively. Pain assessment is problematic, as is the efficacy of analgesics but Johnny Roughan (Newcastle) and Julie Fitzpatrick (Glasgow) showed us that it is possible to assess pain by using behavioural indicators. Mechanisms of nociception and pain can be assessed at the molecular level and Craig Stevens (Oklahoma) demonstrated that opioid receptors in amphibians are strikingly similar to mammalian receptors. Sue Fleetwood-Walker (Edinburgh) examined glutamate receptors and their involvement in spinal cord sensitization in chronic pain. Mouse knockouts and their use in dissecting not only the molecular substrates of pain but the complex 'feeling' of pain was elegantly discussed by Steve Hunt (UCL). Finally, Turo Nurmikko, Director of the Pain Institute in Liverpool, displayed amazing images of brain activity from fMRI and PET scans demonstrating the cerebral coding for the location, type and intensity of pain.

This was truly a fascinating symposium with an integrative flavour encompassing techniques in neuroanatomy, electrophysiology, behaviour and genomics. I am grateful to the Physiological Society for their generous contribution by supporting my application for a Non-Society Symposium Grant.

Lynne U Sneddon
University of Liverpool
Liverpool, UK

Transfer News/Promotions

R Angus Silver has been made Reader at the Department of Physiology, University College London.

Craig Smith (currently a Royal Society University Research Fellow) has been appointed Senior Lecturer in Molecular Physiology in the School of Biological Sciences at the University of Manchester.

Brian Robertson (Department of Physiology and Pharmacology at the University of Strathclyde) has been appointed to the Chair in Neurobiology (Eberhard Buhl Chair of Biomedical Sciences) at the University of Leeds

Congratulations to ...

Nancy Rothwell (University of Manchester) and **Graham Dockray** (Liverpool University) who have both been elected Fellows of the Royal Society. More coverage in the next issue.

and, on a personal note, to ...

Our Editor **Austin Elliott** and Anita on the birth of Sophie Marie (7 lbs) on 5 June.

Send your transfer and promotion news for publication to lrimmer@physoc.org

BIOSCIENCES FEDERATION

Policy activities

April saw another flurry of consultation documents, including two of particular note: the Commons Science and Technology Committee follow-up inquiry into the Research Assessment Exercise and the Treasury consultation on a 10 year investment framework for Science and Innovation. The main points of the first were to ask for more details regarding the organisation of the panel and sub-panel structure, to urge against admin-heavy procedures and to criticise the failure to integrate the review with the ongoing assessment of dual support. The response to the Treasury document was long and complex highlighting, among other points, the need for academic freedom, the need for more funding for basic research and for improvement to the career structure for scientists. All consultation document responses are available at http://www.bsf.ac.uk/recent.htm

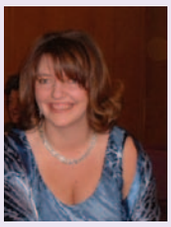
Future activities

I hope to see those of you working in or near Exeter at the British Association Festival of Science in September. The Federation's session is entitled 'Fundamental research; what's the point?' and features talks on fish hormones, deep sea vents and the first breath of life. The session will take place at Exeter University on 9 September. Contact Sai Pathmanathan (spathmanathan@physoc.org) for free tickets. There will be a symposium on the commercialisation of bioscience on 12 October at the Royal Society, and for younger scientists three careers conferences, at King's College London (6 Nov), Leeds University (20 Nov) and Glasgow University (27 Nov) (see separate advertisements for all events). For information on these and other events, keep an eye on the website at www.bsf.ac.uk.

Maggie Leggett

Moving on

I can hardly believe the time has come to say goodbye. As you read this, I shall be preparing to take up a position at the BBSRC. Much as I resigned a while ago – and am now serving a seemingly endless notice period – I have still not accepted the fact that one day I shan't be working for the Society.



Time to say 'goodbye' - Maggie Leggett

The past 5 years have been, above all else, great fun. I joined you with a career history in teaching and research, and had at that point no idea that a job could actually be enjoyable. However, devising schemes with various committees, organising events for academics, school children and members of the public, writing booklets and designing posters, overhauling policy and arguing with committees that the status quo really did need to change has been quite exciting. And, of course, there is the eating and drinking for which the Society is famous. Lying by a pool in Budapest, dancing the night away in some dive in Cambridge and getting decidedly the worse for wear on a boat in Bristol – can this really be work?

It's not all been great. I remember the sinking feeling when I realised that, as

a result of some ambiguous wording on my part, Members who shared the same name also got to share a bedroom in a hall of residence at a Society meeting. Luckily no Member has the surname 'Leggett', but there were some people who were, quite rightly, distinctly unimpressed. And there have been a few of you over the years who have shouted at me for not knowing your requirements for dinners or rooms stated on registration forms you or your secretaries forgot to send. Or who were just bored and cross and wanted someone to have a go at. But these minor irritations are a small price to pay for a fun and rewarding job.

The Society has developed considerably in the last 5 years, and it's been interesting to be part of that change. The governance structure, membership application procedures, the organisation of Meetings and the grant schemes have all been overhauled. Personally I think the Society is at the beginning of a long process of complete transformation, which might be driven both by changes in the way scientific research is published and the demands of government. I imagine in the future there will be one administration running a number of individual societies. As a Member this might be indistinguishable to you from the current system, although it could reduce some flexibility. However, it should be far more streamlined and less expensive. Of course, I could be influenced by my secondment to the Biosciences Federation, but there is undoubtedly duplication between offices of all the various societies and an ever increasing need to reduce administration costs.

I am joining the BBSRC as 'Head of Public Engagement' – involving the public in how they allocate money to particular research areas. I shall, of course, need to retain close links with the academic community, and hope that I come into contact with many of you again. If anyone would like to get in touch I am sure the London office will forward emails to me. Those with whom I have worked closely are really too numerous to name, although I would like to mention a few who not only have been really supportive of me but also have given up their time to the Society for little or no reward. Rob Clarke, Prem Kumar, Chris Fry, Jeremy Ward, Dafydd Walters, Bridget Lumb, Malcolm Hunter, Stan White and Graham McGeown all come under this category, although there are many more who deserve gratitude. Good luck to you all; I shall watch the progress of the Society with interest.

Maggie Leggett

Publications Office

Jill Berriman, Managing Editor on the Society's journals, left the Publications Office at the end of June, after 13 years with the Society, to join her husband, John, who is now working in NewYork. In her various roles with the Society – Copy Editor, Senior, and then Chief, Production Editor and, since 2001, Managing Editor – Jill was involved in format and content changes to both *The Journal of Physiology* and *Experimental Physiology*, the introduction of in-house DTP and electronic submission of manuscripts and, at the end of 2003, a move to Blackwell Publishing after almost 125 years with Cambridge University Press. Our very best wishes for a bright new future go with Jill and John.

James Ryder

1974-2004

It is with great sadness we report that James Ryder, a promising and enthusiastic young physiologist, passed away peacefully in his sleep in May 2004.

James had recently taken up a lectureship in the Department of Sports Science at the University of Hull and was becoming established as a lively member of Lars McNaughton's team in the department. Although only in Hull for a short period, he had established excellent teaching and research relationships with his students, colleagues in the Department and across the University.

James had completed his University of Liverpool PhD at University College Chester, supervised by David Cotterrell, on a multidisciplinary investigation into markers of physical fitness of elite young soccer players . He had presented his work on the physiological aspects of fitness in his young soccer players to the Society on a number of occasions. He had also done a year as a post doctoral research co-ordinator working on the physiological responses to exercise in post-myocardial infarct patients before moving to Hull.

James graduated with a BSc in Sports and Exercise Science from South Bank University as a student of Susan Ward's. He then read for an MSc in Sport and Exercise Science at University College Chester completing his dissertation on the effects of hydroxy-methyl-butyrate supplementation on muscle damage following eccentric exercise, work which he had presented to the European College of Sports Science.

As a young academic embarking on a teaching and research career in exercise physiology he will be much missed by family, friends and colleagues in Chester and Hull. Our heartfelt condolences go to his parents, Ken and Sue Ryder.

David Cotterrell[1]
Lars McNaughton[2]

[1]University College Chester
[2]University of Hull

The Society also reports, with regret, the death of Oliver Holmes and G Eric Lamming, OBE since the last issue of the magazine.

AAAAAAARRRRRRR ... RAE

'Universities UK chairman Ivor Crewe warned the (House of Commons) Science and Technology Committee that game-playing (by universities) was likely to be worse rather than better in the next RAE.' (*THES*, 21 May)

As we approach the halfway point between the last and the next Research Assessment Exercises (RAEs), we offer a helpful glossary of RAE-related terminology.

(For non-UK based readers: just be grateful this doesn't apply to you.)

RAE 2008: Self-explanatory.

Research-active:
1. **(**As used by academic scientists): a staff member who participates in, and publishes, scientific research.

2. (As currently used by UK University and departmental managers): a staff member who is considered to be **RAE-returnable.**

RAE-returnable:
1. (Original meaning): a staff member who produces research output, typically peer-reviewed research papers.

2. (In recent RAEs, and in some less research-intensive universities): a staff member who, over the RAE period, has produced four peer-reviewed papers.

3. (Current meaning in research-intensive Universities): a staff member who is likely to be graded 5* or better. Hence: a staff member who is first or corresponding author on four or more peer-reviewed papers, who has £50K pa (or better £75K pa) research grant income, and who has been an invited speaker at (at least) one international conference each year.

Non-RAE returnable: any staff member whose personal research profile is deemed unlikely to score in the category their university has decided their **Unit of Assessment** has to get (see **RAE-returnable**).

Unit of Assessment: whatever subject category we can return this lot in where they will score the best rating.

Honorary (Senior) Lecturer: staff member over 50 deemed **non-RAE returnable** and 'persuaded' to take early retirement to get them off the books so they need not be returned in **RAE 2008**.

Senior Teaching Fellow: staff member under 50 deemed **non-RAE returnable** who has been re-badged as 'Teaching Only Staff' so s/he need not be returned in **RAE 2008**.

Teaching Fellow (also 'Demonstrator'): junior teaching-only staff member, typically doing 20+ contact hrs/wk, hired to take teaching load off **RAE-returnable** staff.

'Research-led teaching' (also 'teaching in a research-rich environment'): teaching done by **RAE-returnable** staff.

Service teaching (colloq./slang 'community service'): teaching done by **Senior Teaching Fellows, Honorary Senior Lecturers, Teaching Fellows** and other **non-RAE returnable** staff.

Pre-RAE readiness personal interview: expect a kicking.

RAE Review Team leader: chief hatchet man.

Pre-RAE mid-term review: expect more kicking.

Colleague (RAE meaning): competitor.

Encouragement, support, help, mutual interest, scholarship: not in RAE dictionary.

Keep your head down.

Mark Cain

Only too believable?

I would like to add my voice to that of the 'harassed doctor working in general medicine' who complains about the lack of physiological knowledge of undergraduates and junior medical staff (*Physiology News*, **55**, 46). I have been involved in teaching anaesthesia for over 25 years. We deal directly, and daily, with patients (both healthy and many with pre-existing cardiovascular, respiratory, renal, hepatic and CNS impairment) undergoing surgery of varying degrees of severity and extent. This involves the physiological and pharmacological manipulation of, predominantly, the cardiovascular and respiratory systems to ensure they survive the attentions of our surgical colleagues who are attempting to cure their presenting illness – cancer, fractures or whatever. As such it amounts to a very real exercise in applied physiology and pharmacology with the aim of maintaining homeostasis in the face of volaemic, thermal, nociceptive and mechanical insults. Consequently physiology and pharmacology constitutes a major proportion of our examination syllabus, as it does for many of the other specialties.

I, too, have noted a decline in the basic science knowledge of our students but do not know whether this is the result of Problem Based Learning (PBL) as suggested by your correspondent. I had always assumed it was due to the replacement of the biological sciences in the undergraduate curriculum with such subjects as social science, psychology, management and the formal teaching of 'team working', 'communication skills' and empathy.

Something I have also noticed recently is that graduates from non-UK universities (mainly Indian) have a better knowledge of physiology than our local people. This impression is supported by a recent article in *Hospital Doctor* (June 24) highlighting an increasing failure rate amongst UK graduates in the Part I (basic sciences) exam for membership of the Royal College of Obstetricians and Gynaecologists. Graduates of Indian universities also performed better than the locals. This was ascribed to a decrease in the teaching of basic physiology and pharmacology in UK medical schools.

The knowledge required to pass the basic science exams for the various specialties is not much more than what used to be required of undergraduates, but they do have to know it very well. They have to be able to 'manipulate' it, and be able to apply it to rapidly changing situations, such as the hypovolaemic patient 'resuscitated' with 5% dextrose (sugar and water) referred to by your 'harassed' correspondent. They also have to persuade the (pre-PBL trained) examiners that they can do so. Like the Obstetricians and Gynaecologists I believe their failure to do so reflects lack of sufficient exposure to basic physiology, taught by physiologists, in their formative undergraduate years. How else can they learn that we have salt and water circulating in our veins and not dilute Coca Cola?

Iain Campbell
Department of Anaesthesia, Wythenshawe Hospital, Manchester, UK

The synaptic organization of the brain

Edited by Gordon M Shepherd. 2004, Oxford University Press. 719 pp, £42.50.
ISBN 0-19-515956-X

This fifth edition of Shepherd's well-respected text is still worth having on the bookshelf. It is not a complete brain book, since it concentrates on 'the brain regions best understood for their synaptic organisation and functional correlates'. This means, for example, that you get two out of 12 chapters on the olfactory system and nothing on the brainstem, which strikes me as a bit odd, in spite of the pre-amble. Nevertheless, the regions covered are dealt with systematically and clearly. Each chapter has a similar structure, with sections on the neuronal elements present, their synaptic connections and basic circuits, their intrinsic membrane properties, synaptic actions and dendritic properties, and the functional properties of the circuits. This is all done clearly and thoroughly, packing a great deal of information into a small space.

Having said that, the book does have its weaknesses. The general context tends to be assumed rather than given, expecting a considerable degree of background knowledge on the part of the reader for many aspects of the discussion to make sense. The fact that this volume is not a complete brain book also means that there are many loose ends dangling from the text, again requiring additional sources of information if the reader wants to tie them off. For example, if you turn to the index while reading about the cerebellum, you will find no entries under 'olive', 'pons' or 'medulla'. There is also a tendency to skate over the functional properties rather too rapidly to make full sense of the great deal of detailed anatomical and cellular information included. Instead of explanation, the presentation sometimes seems more like a bit of neurophysiological prestidigitation. 'Roll up! Roll up! Ladies and gentlemen, watch closely as I place all these neurones, their connections, the properties of their dendrites and the shape of their action potentials inside this hat. I shake it gently and wave my wand like so. And look! Here we have a thalamus! A miraculous part of the brain which allows us to love, feel, move, see and a whole lot of other stuff too. Hold that for me madam, if you will. There. Now, watch closely everyone while I make a retina…'

Of course, this is the stuff of neuroscience. Skilled practitioners have been using the approach for years to extract large amounts of money from granting bodies, as well as occasionally illuminating the way parts of the brain may work. But to some extent this book, like many multi-edition texts, is beginning to show its age a little. In places, the density of its specifics is starting to overwhelm the path from the synaptic building blocks to the integrative aspects which got us all interested in the first place. Another slight oddity is the reference list, occupying 145 out of 719 pages, or over 20% of the book. It is certainly a worthwhile compilation and fulfills the editor's wish to 'prize a scholarly depth behind our understanding'. But why isn't it on the web, along with the other useful web resources listed on page xi? That might also have helped reduce the price, which strikes me as a bit heavy for a paperback.

The synaptic organisation of the brain is a classic text, and still very much worth having and reading for anyone interested in the details of neuroscience. I'm sure it will have many more editions. But I think a bit of an overhaul in terms of coverage and context would make it a substantially better book.

John A Lee

The neuropsychology of vision

Edited by Manfred Fahle & Mark Greenlee. 2003, Oxford University Press. 344 pp, £ 65.00
ISBN 0-19-850582-5

Since we are highly visual animals, it is hardly surprising that the mechanisms underlying our conscious experience of seeing have occupied a prominent place in brain studies. But it is difficult to know whether it is correct to add a statement such as 'and so the processes underlying vision are among the best understood aspects of cerebral function'. On the one hand they have certainly been very intensively studied, but on the other 'vision' is probably the most complex thing we do. Recent studies have revealed not just one or a few cortical areas devoted to aspects of visual processing, but over 40 areas distributed around the occipital, parietal and temporal lobes, and together accounting for as much as a third of the entire cortical machinery of our brains.

An originally unexpected finding is that most of these cortical areas represent the entire visual field, giving rise to

Other books received. Reviews will be carried in future issues of *Physiology News*

Free radicals: enzymology, signalling and disease. Edited by C Cooper, M Wilson & V Darley Usmar (Portland Press, £65.00. ISBN 1-85578-161-1)

Long term potentiation: enhancing neuroscience for 30 years. By Timothy Bliss, Graham Collingridge & Richard Morris (Oxford University Press, £65.00. ISBN 0-19-853030-7)

Textbook of endocrine physiology, 5th Edition. By James E Griffin & Sergio R Ojeda (Oxford University Press, £18.99. ISBN 0-19-516566-7)

Basic and clinical neurocardiology. By Andrew J Armour & Jeffrey L Ardell (Oxford University Press, £50.00. ISBN 0-19-514129-6)

MCQs and EMQs in physiology. (Hodder Arnold, £12.99. ISBN 0-340-81191-9

multiple cerebral representations of our visual surroundings. This in turn indicates that different cortical regions are specialised to analyse different aspects of the visual world around us, and it follows that our experience of 'seeing' is actually the subjective correlate of parallel processing in many, partly independent brain regions. The evidence supporting this interpretation comes from many different sources, ranging from single cell studies in animals to detailed analysis of the often strange subjective experiences of patients who have suffered damage to various areas of their brains through trauma, tumour or stroke. Fahle and Greenlee have done a good job in pulling together contributions which span this spectrum of approaches, so that anyone with an interest in this area is likely to find new and interesting information in the book. Chapters cover anatomy and physiology, non-invasive imaging and neurophysiological techniques, lesion studies in monkeys and humans, psychophysical studies including blindsight, agnosia and neglect, and finally a discussion of recovery and rehabilitation in cerebral visual disorders. Thinking clearly about vision requires a multi-disciplinary approach and this volume is a welcome one-stop-shop which collects up to date and well referenced discussions covering many important topics in the field.

John A Lee

The central nervous system

Structure and function, Third Edition. By Per Brodal. 2004, Oxford University Press. 515 pp, £49.50
ISBN 0-19-516560-8

The third edition of Per Brodal's book is beautifully produced and continues the traditions of excellence set up by the four editions of the same name published by his father Alf Brodal between 1949 and 1982. The book is clearly and concisely written and manages not to lose the reader in anatomical detail, while explaining physiological mechanisms. The diagrams are well thought out and text boxes are used to highlight physiological, pharmacological or anatomical details, when required, without perturbing the flow of the main text.

The book is aimed at medical students and successfully links basic and clinical science together. It includes new chapters on the control of eye movements and the vestibular system (entitled 'the sense of equilibrium') and all the remaining chapters have been thoroughly revised and updated to include data from molecular biology to clinical psychology.

This makes the book refreshingly easy to read and I would recommend it as an informative text for medical students with interests in the CNS.

Bill Winlow

Molecular biology of the neuron

Second Edition. Edited by RW Davies and BNJ Morris. 2004, Oxford University Press. £65.00
ISBN 0-19-850998-7

This edition of *Molecular Biology of the Neuron* updates the first edition that was published in 1997 and includes many of the scientific advances made since that time. There are 16 chapters, each written by leading research workers.

In their preface the editors state that 'this book provides a platform for knowledge, which allows new advances to be put into context, without being an expert'. Having dipped into many of the chapters, I must concur with their viewpoint. It is always difficult to ensure a uniform standard of writing from a diverse group of authors, but these editors seem to have achieved it. Thus, each of the chapters provides a useful review of current knowledge of molecular data relating to neurons.

The book is clearly aimed at the neuroscience community and will be appreciated by those in clinical, systems or cellular neuroscience, since there are chapters dealing with information flow through the nervous system and others considering neuronal development and disease. I found the chapters on protein trafficking and synapse to nucleus calcium signalling particularly illuminating, but it is unfair to pick out particular chapters for mention, since all of them are well constructed. Perhaps in the next edition, the publishers could improve the book even more by the use of colour to enhance the diagrams and tables.

Bill Winlow

Ions in the brain

Normal function, seizures and strokes. By George C Somjen. 2004, Oxford University Press. £59.50
ISBN 0-19-515171-2

In these times of publicity-seeking scientists making escalating scientific claims of dubious credibility, it is indeed refreshing to come across a scientific author who states that he *doesn't* know how the brain works.

It is particularly enlightening when that author, George Somjen, whose name will be familiar to anyone with even a passing interest in the electrophysiological properties of the brain, is a giant in the field who has spent the last 30 years attempting to unravel the complexities of ion disturbances associated with brain malfunction.

In the present book Somjen has taken on the task of describing the regulation of ions in the brain, and how that regulation is disrupted during brain malfunction.

The book is laid out in four sections: the first describes the regulation of ions in the normal brain and how these ions affect neuronal function, with the subsequent three sections each containing several chapters that describe alterations in ion levels

associated with epilepsy, spreading depression and ischaemic stroke.

As would be expected, Somjen highlights his own input to the topics discussed, but never to the exclusion of others, and I was gratified to see the respective key contributions of my two mentors in Seattle – Wayne Crill and Bruce Ransom – duly acknowledged. Each chapter has a summary of key points, but contained within each chapter are helpful 'in conclusion' paragraphs summarising the previous topic.

Interspersed throughout the text are technical notes bringing the reader's attention to various key points which make understanding of the text easier, e.g. naming of the hippocampal layers, explanation of current clamp etc. As the book is the work of a sole author it has a pleasing consistency of style that is lacking in the usual edited book chapter format, and my only criticism is that the figures are standard black and white that would not be out of place in a 1980s textbook. However, as most of the figures are reproductions of diagrams from published papers this is understandable, and in only a few cases where there are multiple overlaid traces on a graph is the lack of colour detrimental.

The first section on regulation of ions in the brain is fairly detailed, but I feel a novice would probably be better directed to a standard textbook (such as Kandel) as a lot of preliminary knowledge is assumed, e.g. by page 9 of the chapter on regulation of brain ions we are introduced to the GHK equations. To obtain the maximum benefit from this book, it would be advisable for the reader to get the concepts of ion gradients, equilibrium potentials and the effects of ion transporters/exchangers, etc. securely under their belt. In this section the description of the effects of osmotic stress is particularly clear. I would argue that a better illustration of the effects of reversibility of the Na^+-Ca^{2+} exchanger on $[Ca^{2+}]_i$ is Fig. 7 of Stys *et al*. (1992) *J Neuroscience* **12**, 430-439, rather than the one shown (Fig. 2-4), particularly in light of the final section on ischaemic stroke and the toxic accumulation of excess $[Ca^{2+}]_i$. However, Fig. 2-5 which illustrates the corresponding $[H^+]$ versus pH should be memorised by all.

I found the section on epilepsy the most satisfying, as there is an extensive review on the various theories of seizure generation with exhaustive evidence provided. As a rewarding corollary, the section on mechanisms of action of anti-convulsant drugs suggests that, if we know how ion channel malfunctions contribute to epilepsy, then targeting drugs to specific ion channels can result in a successful preventative therapy. A computer model of seizure mechanisms is provided, but the model is based on an individual neurone. This may seem surprising given the widely assumed positive feedback mechanism whereby localized excitability recruits more distant neurones, but Somjen does describe multi-unit models and convincingly argues that a one neurone model can simulate seizure discharges. As an aside I had always thought the depolarisation $\rightarrow$ increased Na conductance $\rightarrow$ further depolarisation cycle illustrated in Fig. 9-8 was known as the Hodgkin cycle, rather than the Katz cycle as stated in the text.

The section on spreading depression is perhaps the least interesting, mainly as it has not yet been positively associated with any neurological condition; indeed whether spreading depression actually occurs in humans is unknown. Thus its importance in neurological dysfunction is questionable, although it is a fascinating phenomenon. As with the epilepsy section all angles of mechanisms are covered in great detail. The final chapter illustrates a model of spreading depression based on the epilepsy model, and as I read this chapter it became very apparent that it shares a remarkably similar mechanism to a slow action potential. The model is not empirical as 'appropriate adjustments' were made, and although the chapter is entitled 'Solving the puzzle of spreading depression by computer simulation', sadly this is not the case, as the model merely reproduces the experimental data and suggests possible scenarios that could result in onset of spreading depression. One interesting aspect raised is that spreading depression may be related to incomplete buffering of elevated $[K^+]_o$, which immediately suggests it may occur in areas with low astrocytic density.

The final sections on stroke describe the mechanisms of ischaemic cell death. Ischaemia can result in both necrosis and apoptosis and there are multiple theories of how cells are killed, each of which shares to varying degrees the same combination of the key factors - Ca^{2+} influx, glutamate receptors, and zinc. I was disappointed that there was no mention of the effects of ischaemia in white matter, as in all cortical strokes, which are generally considered to affect only grey matter, the underlying white matter is also affected. Stokes can also occur purely in white matter areas, e.g. lacunar infarcts, and given the lack of synapses and neuronal cell bodies in these areas a different array of non-neuronal mechanisms has been described. It is disheartening to note that there is no chapter on the mechanism of neuroprotective drugs in stroke, as there are currently no clinically effective neuroprotective strategies. This is particularly frustrating as the contribution of ion channels to ischaemic damage is broadly understood, but unfortunately successful laboratory results have not been translated into effective clinical applications.

All in all this is an excellent book which is a must read for all interested in the fields covered. While not claiming to tell us how the brain works, Somjen sticks with the facts and describes in exquisite detail what happens when the brain malfunctions, which is surely how successful strategies for combating devastating neurological conditions will ultimately be devised. A worthy testament to an exemplary scientific career.

Angus Brown

THE JOURNAL OF PHYSIOLOGY

SYMPOSIA

Structure/function correlates in neurons and networks: *a symposium in honour of the late Eberhard H. Buhl*

at the University of Leeds, UK
Friday, 10 September 2004

Organisers: **Brian Robertson, Miles Whittington and Fiona Le Beau**

Speakers:

Peter Somogyi (Oxford, UK)
The anatomy of network activity related spike timing in the hippocampus

Vincenzo Crunelli (Cardiff, UK)
Cellular thalamic correlate of the alpha rhythm

Ole Paulsen (Oxford, UK)
Mechanisms underlying cholinergically-induced network oscillations in the hippocampus in vitro

John O'Keefe (London, UK)
Phasor coding in hippocampal place cells

Hannah Monyer (Heidelberg, Germany)
Transgenic models to study synchronous oscillatory network activity

Roger D Traub (Brooklyn, NY, USA)
Electrical coupling between principal cell axons and their role in generating very fast (>70 Hz) and gamma (30 – 70 Hz) frequency network oscillations

Istvan Mody (Los Angeles, CA, USA)
Plasticity of GABAergic inhibition

Alex Thomson (London, UK)
Selective connectivity in neocortical circuits

Katalyn Halasy (Budapest, Hungary)
Neuropeptides involved in the regulation of food intake in lateral septum of rat

Gianmaria Maccaferri (Chicago, IL, USA)
Interneuron diversity and hippocampal network dynamics

Gabor Tamas (Szeged, Hungary)
Signal interactions in identified cortical networks

Ivan Soltesz (Irvine, CA, USA)
Structure of cortical microcircuit theory

Kai Kaila (Helsinki, Finland)
Anion regulation, GABA actions and network oscillations in the developing hippocampus

Stuart Cobb (Glasgow, UK)
Cholinergic modulation of hippocampal cells and circuits

André Fisahn (Stockholm, Sweden)
In vitro gamma oscillations – overview, underpinnings, differences

Roland Jones (Bristol, UK)
Differential control of transmitter release in subpopulations of neurones in the entorhinal cortex

For further information go to:
www.jphysiol.org

or email:
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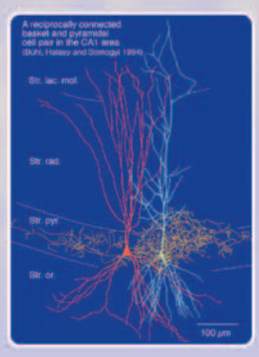
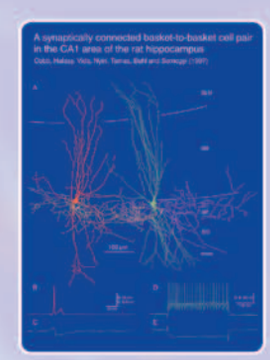
Full information and registration details at
www.fbs.leeds.ac.uk/buhl





The Journal of Physiology is published by Blackwell Publishing on behalf of the Physiological Society in 24 fortnightly issues in eight volumes. For further information go to **www.jphysiol.org**.
The 2004 subscription price is £2090/$3724
(including online access to the current and all available back files).

Blackwell Publishing

YOUNG PHYSIOLOGISTS SYMPOSIA
Channels to networks

University of Leeds
September 2004
For further information contact Helen Garner
Email: bmshlg@leeds.ac.uk

King's College London
17 December 2004
Joint with the Chilean Physiological Society
Theme free symposium, abstracts on all topics welcome
For further information contact Charlotte Waters (KCL)
Email: charlotte.waters@kcl.ac.uk
or Paola Casanello (Chile)
Email pcasane@med.puc.cl

Further information about these events will be circulated via email and published on the website.

BA FESTIVAL OF SCIENCE
The responsibility of being a scientist

University of Exeter
6-10 September, 2004

Website: http://www.the-ba.net

ADVERSE REACTIONS TO DRUGS AND CHEMICALS: STUDIES FROM MOLECULES TO MAN

University of Liverpool, Liverpool, UK
9-10 September, 2004

Joint meeting of the British Pharmacological and Toxicology Societies.

email: meetings@bps.ac.uk
Website: http://www.bps.ac.uk

EUROPEAN COUNCIL FOR CARDIOVASCULAR RESEARCH
9th Annual Meeting

Nice, France
1-3 October, 2004

The ECCR was formally established in 1997 to create within Europe a unique forum for active clinical and pre-clinical researchers in the important field of blood pressure and cardiovascular research. The annual meeting covers a broad range of subjects, including genetics of cardiovascular diseases, vascular biology, cardiac and renal aspects as well as modern strategies of prevention and therapy in cardiovascular disease.

Website: http://www.eccr.org

INTERNATIONAL WORKSHOP IN CELL PHYSIOLOGY
Transport mechanisms across cell membranes: channels and pumps

Russian Academy of Sciences and the Sechenov Institute of Evolutionary Physiology and Biochemistry, St Petersburg, Russia

13-17 October, 2004

Focusing on the molecular mechanisms of membrane transport and experimental approaches. Sponsored by the Physiological Society and intended primarily for young scientists from Central and Eastern European countries and from other European countries. The programme will include lectures, poster sessions and laboratory demonstrations.

Website: http://www.physoc.org/international

INTERNATIONAL WORKSHOP IN ION CHANNELS
Ion channels: from physiology to pathology

Universidad de Sevilla
7-9 February, 2005
This International Workshop will focus on the general aspects of ion channel molecular physiology and channelopathies. The meeting is sponsored by the Physiological Society and intended for young scientists from the UK, Spain and Eastern European countries, though participants from other countries are also very welcome. Up to 40 applications will be accepted and preference will be given to applicants who will present a poster. Interested applicants should submit a short cv and register online by 1 September, 2004.

Website: http://www.physoc.org/international/seville2005

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.

Website: http://www.IUPS2005.org

Journal of Physiology symposia will take place at the IUPS on 4 and 5 April. Full details will be available at www.jphysiol.org

THE PHYSIOLOGICAL SOCIETY
CHANGE OF ADDRESS

The Physiological Society Administration Office has moved to:

PO Box 11319
London WC1X 8WQ

Noticeboard

Notices for the Winter 2004 issue of *Physiology News* should reach the Publications Office by 20 September, 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.

THE PHYSIOLOGICAL SOCIETY
Meetings

2004

UNIVERSITY COLLEGE CORK AND ANNUAL GENERAL MEETING
1-3 September (Wed-Fri)
Abstract period closed

UNIVERSITY OF BRISTOL
4-5 September (Sat-Sun) (Focused meeting)
Abstract period closed

UNIVERSITY OF OXFORD
1-3 October (Fri-Sun) (Focused meeting)
Abstract period closed

KING'S COLLEGE LONDON
18-20 December (Sat-Mon)
Joint meeting with the Chilean Physiological Society

Opening date for receipt of abstracts	20 September
Closing date for receipt of abstracts	29 September

2005

SEVILLE, SPAIN
10-13 February (Thu-Sat)
Sponsored symposia in association with the Spanish and Dutch Physiological Societies

IUPS, SAN DIEGO, CA, USA
31 March-5 April (Thu-Tue)

UNIVERSITY OF BRISTOL
20-23 July (Wed-Sat)

Opening date for receipt of abstracts	1 February
Closing date for receipt of abstracts	15 March

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

LIFE SCIENCE CAREERS

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www.bsf.ac.uk/careers

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