PHYSIOLOGYNEWS

summer 2007 | number 67

Meetings

LifeSciences2007
8–12 July 2007, Glasgow, UK

Edinburgh

Belfast

Also featuring

A publication of The Physiological Society

Images of Belfast

Belfast Focused Meeting
Ion channels and the microcirculation
Queen's University Belfast
4-5 April 2007

photos by Heidi Adnum and Prem Kumar

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

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Contributions and Queries

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Cover photo

'The Red Rectangle' - a dying star sculpts ladder-like structures of gas and dust (NASA, ESA, Hans Van Winckel (Catholic University of Leuven, Belgium) and Martin Cohen (University of California, Berkeley).

PHYSIOLOGYNEWS

Action points

Grants

For full information on Members' and Affiliates' Travel Grants, Network Interaction Grants, Non-Society Symposia Grants, Vacation Studentship Scheme, Departmental Seminar Scheme, Centres of Excellence and Junior Fellowships visit: http://www.physoc.org/grants

Membership applications

Applications for Physiological Society membership are accepted throughout the year; applications are reviewed by the Membership Committee on a monthly basis and a decision is normally made within 15 working days of each deadline. For full details please visit: http://www.physoc.org/membership

Change of address

Members should inform the Administration Office of any changes of address, telephone, fax or email address. Changes can be emailed to: imagre@physoc.org

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Physiology News

Deadlines

Letters and articles and all other contributions for inclusion in the Autumn 2007 issue, No. 68, should reach the Publications Office (lrimmer@physoc.org) by 13 July 2007. 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 Board of *Physiology News* (see contents page for details).

Physiology News Online

Physiology News is now available on The Society's web site: http://www.physoc.org.

Guidelines for contributors

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

Format of articles

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

Length of articles

This will be determined by the subject matter and agreed with the Executive Editor.

Submission of articles

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

Illustrations and authors' photographs

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

References

Authors are requested to keep the number of references to a minimum – preferably no more than two or three. Please cite all references in the style of *The Journal of Physiology* (see *Instructions to Authors 2005* at http://www.physoc.org)

In this issue

Welcome to our biggest ever *Physiology News*, which should reach you as you get ready for LifeSciences 2007, the largest meeting yet for The Society since the changeover to a single main Summer get-together. In this issue we tackle the really important stuff about the meeting – where to eat and drink in Glasgow (p. 5), while on p. 4 Prem Kumar gives some background to LifeSciences 2007, particularly on why your poster session neighbours may be pharmacologists or biochemists.

Integration of the bioscience subjects is nothing new – the people attending Physiological Society meetings, and publishing in *J Physiol*, 100 years ago included what we would now call biochemists and pharmacologists (see e.g. p. 58).

We should never forget the common origins of our disciplines. And in an era when labelling is ever more opaque, it is all the more vital to look beyond the packaging to the common language – the science. For instance, Hodgkin and Huxley, in their mathematical description of the action potential, were what is now called 'systems biologists' 50 years before there was such a thing as 'systems biology' (see Denis Noble's piece on p. 34).

So enjoy the magazine. It has science, history, news and reports, opinion, humour, human interest, education, and more. We hope it has something for everyone. And we hope to see you reading it in the Rennie Mackintosh tearooms and the pubs of Glasgow. Just not during the plenaries, please.

Austin Elliott

Credit where credit's due?

Author order, and credits, on papers, seems to be one of those issues that won't go away.

Each year more papers published in physiology – and the biosciences generally – have multiple (by which I mean more than two) authors. As just one small example, the average number of authors on papers published by *J Physiol* was 2.5 in May 1987, 3.4 in May 1997, and 4.6 in May 2007.

So this shows there is more research collaboration going on. That's good – right?

Well, most universities these days have mission statements or the like declaring their enthusiasm for research collaboration. And easing collaboration has often been argued to be one of the drivers for mergers that create large super-departments.

However, there is a problem. Who, exactly, gets the credit for collaborative papers when push comes to shove?

This issue has been thrown into sharp relief in the UK by the Research Assessment Exercise, or RAE. In the RAE, now about to reach its 6th round with RAE2008, scientists are graded primarily on their four best research outputs – i.e. research papers. But what if these papers are all, or mostly, collaborations?

This issue is, of course, not new. It has been hashed over many times, notably in medical journals (e.g. 1), and the RAE panels have thought hard about it. To quote from one subject panel's published terms of reference and criteria (2):

'The sub-panel recognises that much of the research within its remit is collaborative and naturally leads to multi-authored research outputs.'

And the solution: 'Authors declaring co-authored outputs must demonstrate (in the 'Other relevant details' field) their clear and distinct contribution to the cited output. This contribution must be substantial.'

Which is fair enough. All you have to do is say: 'My lab did all the *in vivo* work and I co-wrote the paper.' But there is a sting in the tail:

'Where an author citing an output is not deemed to have made a substantial and distinct contribution to it, the output will be assessed as Unclassified for that author.'

The devil, as so often, is in the detail. The usable-ness of a collaborative research paper as one of one's RAE four will depend on the panel's judgement of what is a big enough contribution. Which it will be impossible to know in advance. And if it's not deemed enough, that paper will not count.

Given the consequent 'jeopardy factor' if a panel decides to throw out an author's paper for this reason – a poorer RAE rating for the scientist, and a reduction in the university's income for the next several years – word on the street is that some universities may not take the risk. So collaborative papers may be out, at least for some institutions.

All a bit Catch-22. But there is a wider point.

It seems obvious that this all goes back to the recurring problem with multi-author papers, namely being clear on who did, or contributed, what. Wouldn't it be easier if this information was in the final published version of the paper, in black and white, signed up to by all authors and there for any reader to see?

Some journals already do this as a matter of course – see the example from the open access *BMC Physiology* in the box – and even in journals that do not require it, extended 'Contributions' statements of this kind are becoming more common.

Apart from the RAE – so far just a British preoccupation, although lookalikes seem to be springing up in other places – these statements have other uses. If assessing what authors contributed to their published papers is part of the hiring process for jobs in science – as it certainly is in most cases – it is clearly fairer if this judgement is based on public information.

Succinct statements of 'who did what' might also decrease the amount of 'gift' or 'guest' authorship (see e.g. refs 1 and 3), another of those recurring problems of scientific publication. If all someone had done was provide an antibody, a cell line or a cDNA, they would have to be identified as doing so (and no more) in print. Even if it was 'met the authors at a conference and helped edit the paper by email'. For instance, some of the fall-out from the Hwang Woo-Suk stem cell affair concerned the role of 'guest' authors in both Korea and the US (4).

Finally, laying out who contributed what to the work might decrease the number of arguments about exactly who gets to be corresponding author, or last / senior author. To say nothing of first author.

All in all, it seems to me, statements of 'Authors' contributions' have a lot of positives, and few negatives. And in an era where the majority of *J Physiol* papers have four or more authors, and some have more than 10, they also seem inevitable.

So come on journals – how about it?

Austin Elliott

References

1 Sheikh A. (2000). *J Med Ethics* **26**, 422-426 (http://jme.bmj.com/cgi/content/full/26/6/422).

2 http://www.rae.ac.uk/pubs/2006/01/docs/dall.pdf

3 http://www.councilscienceeditors.org/editorial_policies/whitepaper/2-2_authorship.cfm

4 Cho MK *et al.* (2006). *Science* **311**, 614 –615 DOI: 10.1126/science.1124948.

From a recent paper in *BMC Physiology* (http://www.biomedcentral.com/bmcphysiol/), slightly anonymized:

Authors' contributions

ABC was responsible for the overall design of the study and the analysis of the results. DE provided technical guidance and advice in the development of the methods for… FGH performed most of the biochemical and routine histological analyses. IJK performed most of the hindlimb perfusions and the compilation of the cell death data. LM assisted with all phases of the study and wrote most of the manuscript.

LifeSciences2007

Glasgow hosts our joint meeting with the Biochemical Society and the British Pharmacological Society

The Physiological Society is involved in a major cross-disciplinary meeting this year from 9 to 12 July at the SECC in Glasgow. Planning for this meeting began as early as 2004, during Bridget Lumb's tenure as Meetings Secretary, when the basic principles of such a collaboration were established and agreed and it has been my pleasure to represent our Society's interests for the past year or so. Please take a look at http://www.lifesciences2007.org for the latest information. This is a good and detailed web site and has all the information you would want somewhere on it!

LifeSciences2007 replaces our annual main meeting and may be a seminal move for the way in which advances in physiology and allied subjects are disseminated in the UK. Our Society, the Biochemical Society and the British Pharmacological Society have joined forces to produce a joint Meeting that will showcase the best of international lifescience research with its mix of invited speakers and free communications. The Meeting differs from the model used, for example, by Experimental Biology (EB) in the USA, in that a central tenet of the Glasgow Meeting was that all talks/posters would be badged by scientific theme only and not by society. Thus, it is hoped that physiologists, biochemists and pharmacologists will all be sitting side by side in each lecture theatre or crowding around the same poster, a principle guided by a realistic appraisal of how we are all working in the 21st century. Information will be sought after the Meeting from many sources – especially from delegates and members of all three societies – to find out if the venture was a success and, if so, how to establish a series of such meetings in the future. Of course, my aim as Meetings Secretary will be to balance all views against the wishes of our membership and I will stay true to my principle of trying to establish increasing numbers of opportunities for physiologists to meet to discuss their science in an appropriate environment – whether this be at a themed meeting or workshop of around 100 participants or at a larger Meeting such as **LifeSciences2007** where we are expecting at least 2,000 delegates.

We will be hosting 61 symposia at **LifeSciences2007**. These were selected from 120 high quality applications and the Scientific Committee – with representatives from all three societies and industry – had a pretty difficult and absorbing time narrowing the numbers down. Although we were/are all life scientists, it was clear that differences do exist between our basic approaches and what a physiologist believes is essential is not, necessarily what a biochemist or a pharmacologist believes. Given that two physiologists would probably disagree over what is hot and what is not, I suppose that was expected, but we found a way to resolve all issues fairly. Once selected – primarily on scientific quality and timeliness - we were able to divide these symposia into a number of themes that are beautifully colour coded for clarity on the web site. There are many excellent sessions, but I bring your attention to one that might be of general as well as specific interest entitled 'Xtreme-Everest' that will be hosted by Michael Grocott (UCL). Exciting new physiology relating to human endurance will be described for the first time – within weeks of the remarkable expedition which is currently underway (late April 2007) and can be followed at: http://www.xtreme-everest.co.uk/index.php

Over 240 invited speakers have confirmed attendance and these include many young, as well as the more established, investigator. There are seven plenary speakers. Of these, 'our' people include Tom Bolton, Michael Joyner and Denis Noble speaking on smooth muscle excitation, blood pressure regulation and systems biology, respectively. Demand for these lectures is high and we have already had to introduce a ticketing system. I'm not sure whether a black market might arise from this – 'I'll give you two Joyner's for a Noble' – but it does demonstrate how much interest is being generated. In addition to the invited speakers, there are 599 poster and 96 oral communications – the latter due primarily to our Society's insistence. The delivery of oral communications is something that I am aware of as being high in the list of many Members' wishes for our meetings and we will always aim to get as many slots as we can in whichever style of meeting we host/part-host.

We have in place a social programme that runs in competition with the lure of Glasgow's own free-form entertainment (see some suggestions on p. 5) and the Tourist Board will be on hand to help delegates. More formally, we have organised an opening Welcome Drinks Reception at the Kelvin Grove Art Gallery, a Beer Science session (!), Glasgow Walking Tours – your choice of Historic Glasgow or Glasgow Horror – but, if Glasgow is undergoing development at the rate that Birmingham is, these two walks might even be combined! Finally, we are holding a Gala Dinner at the Hilton Hotel Glasgow, the admission price to include a decent meal with wine followed by our old favourite, the ceilidh. A chance to make new friends whilst falling over is not to be missed!

Last, but by no means least, there will be the Young Life Scientists Symposium 2007 *Advances in signalling*. This will be held on Sunday 8 July at Strathclyde University, with Phillip Hawkins (Babraham Institute, UK) and Nina Balthasar (University of Bristol, UK) as keynote speakers. This symposium is free to attend (for the young!) and includes a dinner on Sunday evening. These symposia are now an established part of our meetings and provide a fantastic opportunity for PhD students and postdocs to get together to talk science and interact socially. Further details on the YLS may be found at http://www.lifesciences2007.org/YLS2007/default.asp

The Scientific Organising Committee has worked very hard to get this meeting together as the three societies all have different ways of doing things. In the end, all abstracts were submitted through Scholar1 and it has been interesting to 'introduce' a new batch of scientists to the use of this software. I am particularly grateful to Nick Boross-Toby for securing a significant number of major sponsors. Please visit trade stands at all meetings as it really does help with maintaining support, and visit http://www.lifesciences2007.org/exhibition to see who is exhibiting at Glasgow. Pfizer Ltd and Merck have been extremely generous and we are very grateful to them.

Nick, Heidi, David and I look forward to meeting you at **LifeSciences2007** and hope that you have a great time there.

Prem Kumar
Meetings Secretary

Places to eat and sights to see

A random selection of restaurants, bars and attractions in Glasgow, recently voted the 'coolest city in Britain'

Places to eat and drink

Ashoka (19 Ashton Lane, Byers Road) – well prepared, tasty Indian food.

Babbity Bowster (16-18 Blackfriars Street) – traditional pub with good food at reasonable prices. Relaxed atmosphere, no background music.

Beer Café (78 Candleriggs) – specialises in beers from small foreign brewers.

The Bothy (Ruthven Lane) – contemporary Scottish food with staff in kilts.

Café Cherubini (360 Great Western Road) – good value breakfasts.

Dakhin (89 Candleriggs, Merchant City) – south Indian cuisine, ideal for vegetarians.

The Lab (26 Springfield Court) – a small bar and beer garden behind Princes Square.

Stravaigin (28 Gibson Street) – fresh seafood and local produce.

Ubiquitous Chip (12 Ashton Lane, Byers Road) – bar (upstairs) and restaurant (downstairs). Good selection of wine and whisky.

The Village (129 Paisley Road) – good value lunchtime specials to take-away or eat in the café-style (downstairs) or traditional (upstairs) restaurant.

Willow Tea Rooms (217 Sauchiehall Street/97 Buchanan Street) – the world famous tea rooms, designed by Charles Rennie Mackintosh in 1904, serving tea, snacks and Scottish savouries.

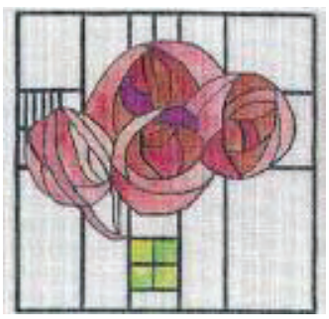
Places to party

13th Note (50-60 King Street) – music venue, bar and vegetarian café. Jukebox, DJs upstairs at weekends.

The Buff Club (142 Bath Lane) – soul and disco. All gigs on the ground floor, first band on stage around 2100.

Places to see

Gallery of Modern Art (Royal Exchange Square, Queen Street) – the second most visited contemporary art gallery outside London, with work by local and international artists.



Glasgow Cathedral and Necropolis (Castle Street) – the only medieval cathedral in mainland Scotland that survived the mid-16th century reformation. The site dates back to the late 6th century when St Mungo, the city's patron saint, was bishop of the ancient kingdom of Strathclyde. The current building is of 12th century origin, with its majestic tower added in the 1400s.

Glasgow Science Centre/Tower/IMAX (50 Pacific Quay) – a world of science and technology brought to life through hundreds of interactive exhibits and experiences.

House for an art lover (Bellahouston Park, 10 Dumbreck Road) – a modern recreation of one of Charles Rennie Mackintosh's competition designs. Good restaurant in the basement.

Hunterian Museum and Art Gallery (University of Glasgow) – William Hunter's substantial and varied collection, bequeathed to the University of Glasgow in 1783.

Kelvingrove Art Gallery and Museum (Argyle Street) – from decorative arts to archaeology and the natural world.

The Lighthouse (11 Mitchell Lane) – Scotland's first dedicated national centre for architecture and design, the conversion of Charles REnnie Mackintosh's 1895 Glasgow Herald newspaper office.

People's Palace and Winter Garden (Glasgow Green) – social history of Glasgow from 1750.

Pollok House and Country Park/The Burrell Collection (2060 Pollokshaws Road) – an impressive 18th century mansion filled with Spanish art, antique furniture, silverware and ceramics. A collection of over 9,000 works of art (medieval, tapestries, alabasters, stained glass, oak furniture and work by Degas, Cezanne, Epstein and Rodin) gifted to the city by Sir William Burrell. Country park with good paths through mixed woodland. Restaurants at both venues.

The Tall Ship at Glasgow Harbour (Stobcross Road) – the Glenlee, a 19th century schooner, restored to depict Glasgow's maritime heritage.

Places to shop

Buchanan Galleries Shopping Centre (Buchanan Street) – Glasgow's newest shopping centre with 80 shops and 2,000 car parking spaces.

St Enoch Shopping Centre (55 St Enoch Square) – the largest shopping centre and food court in Scotland and Europe's largest glass structure.

Prince's Square Speciality Shopping Centre (48 Buchanan Street) – designer boutiques, cafes and bars under an Art Nouveau glass roof.

The Barras Market (Gallowgate, nr Bell Street – Saturday mornings only) – covered and open stalls selling a huge range of goods from antique furniture to computer games.

Society Lectures at Glasgow

Tuesday 10 July

The Paton Lecture (0830-0930)
Denis Noble (Oxford, UK)
Claude Bernard, the first systems biologist and the future of physiology

Michael de Burgh Daly Prize Lecture (1330-1430)
Michael Joyner (Rochester, USA)
A sympathetic view of the sympathetic nervous system – human blood pressure regulation

Wednesday 11 July

Annual ReviewPrize Lecture (1330-1430)
Thomas Bolton (London, UK)
Smooth muscle excitation

X-rays, twitching muscles, and burning anodes

Gerald Elliott describes the experiments that led to the first x-ray diffraction pictures of contracting muscle

I arrived at King's College London as a Demonstrator in Physics in Autumn 1954. I had applied for the position during my final (undergraduate) year at Oxford because, in the aftermath of the DNA double-helix papers, I was excited by the idea of applying physics to biology. The Head of Physics at King's was JT Randall. He and HR Boot had revolutionised microwave radar by strapping the anodes of the cavity magnetron so that it oscillated only in the fundamental mode. This created the technology that JT regularly liked to tell us had won the war. Afterwards Randall had set out to solve biological problems, and his group was working on the structure of collagen in connective tissues. I think I must have impressed him at the job interview because I had done National Service as a subaltern in the REME (Royal Electrical and Mechanical Engineers) before going to university, so I was able to discuss the operation of the 3Mark7 anti-aircraft radar set. When I got back to Oxford I received a letter offering me the job; I took it to show my physics tutor, FV (Francis) Price. He gave me one of his best ever pieces of advice: 'take it quickly, Gerald, before they change their minds'. So I did.

Post World-War II muscle science originated that same year (1954) with

Gerald Elliott
(photograph by AJ Bron)

two classic papers in a single issue of *Nature* (Huxley & Niedergerke, 1954; Huxley & Hanson, 1954). These established the sliding-filament model for the contraction of striated muscle in which the two major muscle proteins, myosin and actin, are contained in separate thick and thin filaments. As all undergraduates are now taught, these filaments, which move past each other when muscle lengthens passively or contracts actively, give rise to the cross striations whose changes during relaxation and contraction were described definitively in these papers.

In the light of the 1954 papers, the overwhelming question became *how* the two sets of filaments move past one another and generate tension during contraction. This would require an answer compatible with physics and chemistry, though subtleties could surely have arisen in more than 3 billion years of biological evolution. Attention quickly focused on the cross-

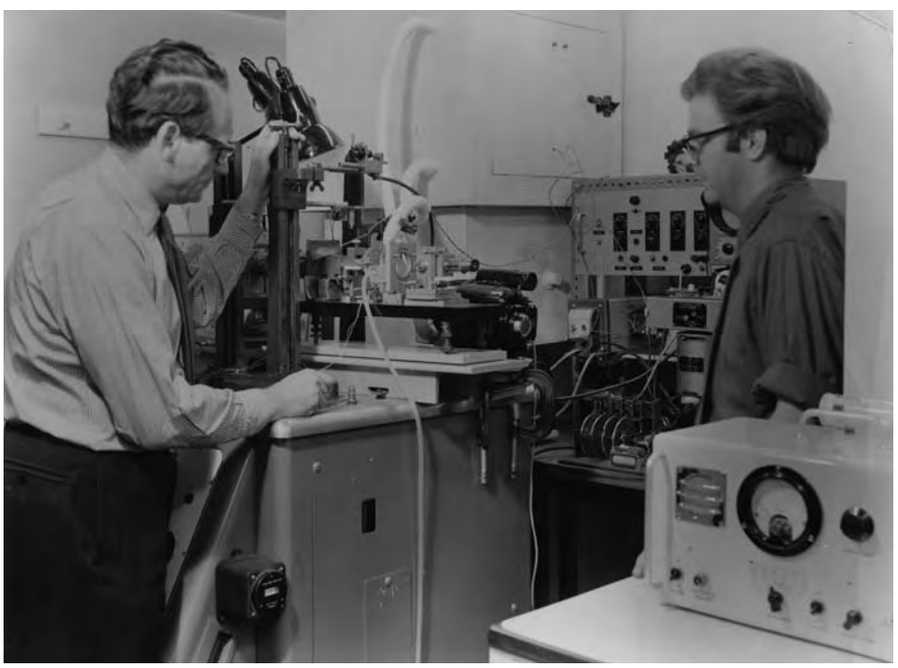


Figure 1 (right, top). Jack Lowy (left) and GE with the experimental set-up in the Drury Lane laboratory. The X-ray camera is central, and the film cassette would be placed on the vertical circular mount that is visible on the camera, the object of both our gazes. In front of the film mount is a short-range telescope (horizontally-mounted) that was used to place the muscle in the X-ray beam. In the background are the Ringer's reservoir, the stimulator and the end of the oscilloscope camera. The X-ray shutter control was held on a Palmer stand behind the camera. A second Palmer stand, in front of the camera, supported the transducer and the vertically placed toad sartorius muscle, but it is not easy to make out these details in this picture. On the table in front of GE the four circular wheels and micro-switches of the rotating master timing control are clearly visible. In the right foreground the Geiger counter used to check for radiation leaks can be seen (photograph by Zoltán Gábor).

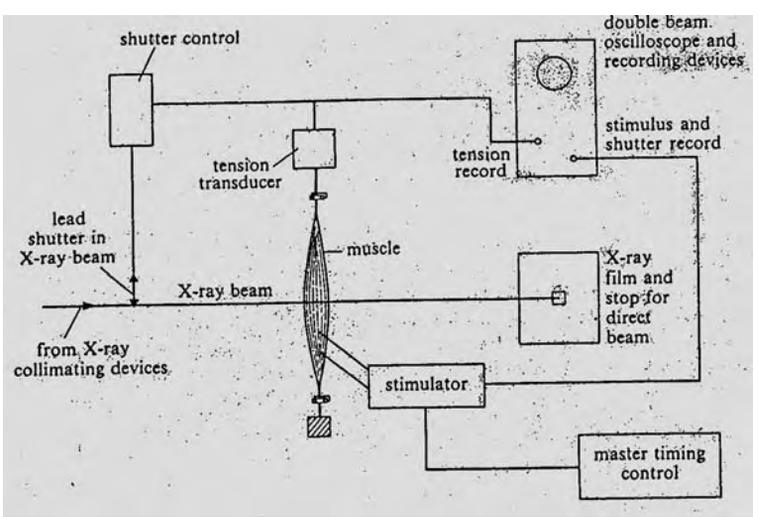


Figure 2. 1970s vintage diagrammatic representation of the experimental set-up (courtesy of the Open University).

bridges between the two sets of filaments, first observed in electron microscope thin sections by Hugh (HE) Huxley (1957), who had also done pioneering one-dimensional low-angle X-ray studies of living muscle (Huxley, 1952). Jean Hanson, a zoologist who had worked with Hugh in Boston and had shown him the elements of light microscopy, was now at King's using the electron microscope to study smooth muscles. She became effectively my PhD supervisor.

I started out with an electron microscope PhD project on the structure of paramyosin smooth muscles from lamellibranch molluscs, 'the muscles of mussels'. Unhappy with the electron microscope as a sole tool, I developed a two-dimensional low-angle X-ray focusing camera for use in biology. The camera was based on the mirror-focusing device of Kirkpatrick and Baez (father of the rather more famous Joan) and had been designed for use in metallurgy by Albert Franks (1955). In the late 1950s Roy Worthington arrived at King's from Australia as a postdoctoral fellow and he and I applied two-dimensional diffraction to striated muscles. We observed meridional diffraction patterns from both the thick- and thin-filament arrays in the low-angle X-ray diffraction patterns of a variety of muscles (Elliott & Worthington, 1959; Worthington, 1961; Elliott, 1964). The earliest public report of this work was my first ever communication to The Physiological Society in 1959 (Elliott & Worthington, 1959). We also saw layer lines; in insect muscle these had the periodicity of the actin filaments

Figure 4. A youthful Austin Elliott in his 1960s laboratory-visiting gear (photograph by GE).

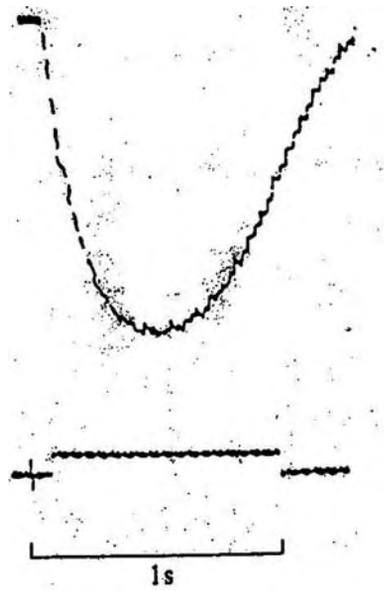


Figure 3. Oscilloscope tracing. The upper trace shows the tension response of the muscle, and the lower trace shows the stimulus and the opening of the X-ray shutter (Elliott *et al*. 1967).

(Worthington, 1961). In living striated vertebrate (frog and rabbit) muscle they had the periodicity of the myosin filaments, and were assigned to the cross-bridges which at the time we called the "myosin projections' (Elliott, 1964).

Early in the 1960s the time was ripe to attempt to get the first X-ray diffraction patterns from contracting striated muscle. By this time Roy Worthington had departed to take a position in the USA and I began to collaborate with Jack Lowy, a muscle physiologist whom Jean Hanson had met one summer at the Plymouth Marine Biological Laboratory and invited to King's. Barry Millman was also involved, a Canadian who had finished his Ph D on the physiology of molluscan muscles with Jack and then joined me to get X-ray pictures from these same muscles in the contracting state (Millman & Elliott, 1965). Molluscan muscles contracted for much longer periods than did vertebrate striated muscles. With the latter the basic problem was that it took an exposure of 1-2 hours to get a meridional X-ray pattern from living resting muscle with our cameras, and clearly it was not possible to keep the muscle contracting for that long. It was obvious we needed to develop a stroboscopic method.

I experimented with the Hilger micro-focus X-ray sets in the laboratory, the exact same ones Wilkins, Franklin and Gosling had used to take the famous wide-angle DNA pictures. These sets had a very finely focused X-ray beam, 40 microns square, but were limited to a beam current of 400 μA. With continuous running, any more current would punch a neat hole in the water-cooled copper anode.

One day I recalled a much-decorated REME sergeant instructor who had told me how he had driven a damaged truck without a cooling system out of the firing line in Normandy. 'It meant I had to replace the engine', he said, 'but at least I got to do it without the b*****s shooting at me.' I found that by shorting out the bias resistor one could overrun the X-ray tube to give a beam current of 3 mA without appreciable de-focusing. At this current the anode would hold up for 2 seconds before it punctured. This improvement factor was enough to make the experiment feasible; we could get our muscle twitch exposure in the first second or so, and then allow the anode to cool down for half a minute before stimulating the muscle again.

To run the equipment I built a system of cams and micro-switches that

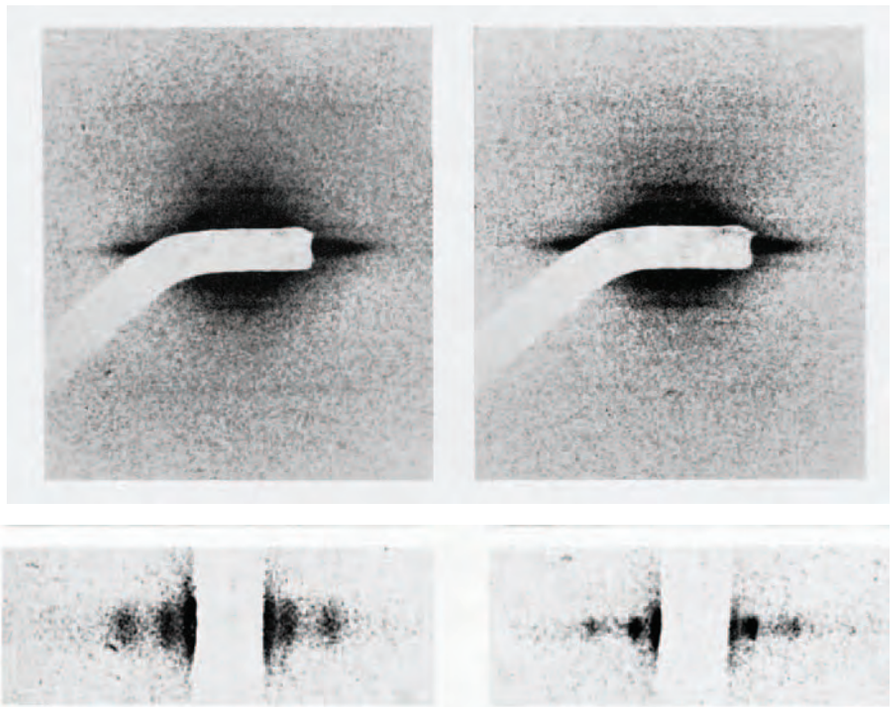


Figure 5. A typical set of X-ray diffraction diagrams; the upper pair show meridional patterns, and the bottom pair show equatorial patterns. On the left are patterns from contracting muscle; on the right, patterns from the same muscles at rest (Elliott *et al*. 1967). The details of the meridional patterns can best be appreciated by looking at the diagrams titled in the layer-line direction (photographic montage by Zoltán Gábor).

revolved once per minute and controlled the whole experiment through relays. In the photograph (Fig. 1) this controller is on the table next to the X-ray set, between Lowy and me. I was proud of it because I had fashioned all the cams myself. Back then laboratories had well-equipped student workshops and one's first task as a research worker was to learn how to operate a lathe and milling machine.

The early 1970s vintage diagram (Fig. 2) shows how these first experiments were done. In the second when the X-ray tube was running at excess current the muscle was stimulated and produced tension in a twitch response. A transducer (RCA 5734, for those old enough to remember such things) sensed the tension, and at a predetermined level a fast relay with a lead shutter glued to the armature operated to allow the X-rays to pass through the muscle and produce a diffraction pattern on the X-ray film. When the tension fell the shutter closed. The tension, and the operation of the shutter, was recorded with a double beam oscilloscope and camera. Thus we got a continuous record of the time course and size of the contractions, as well as of the functioning of the shutter and stimulator (Fig. 3).

To get enough X-rays to produce measurable meridional patterns it was necessary to keep this experiment going, with one or two twitches per minute, for around 2 days. The muscle was vertical, and was irrigated with a constant drip of cooled Ringer's solution from a reservoir contained in the laboratory-built refrigerator that can be seen in the background in Fig. 1. A peristaltic pump returned the Ringer's solution to the reservoir. We found that a single toad sartorius muscle would keep going, stimulated twice per minute, for the time required. After 3 days the twitch tension would have fallen to about half its initial value, and the filament of the X-ray tube also tended to burn out at about the same time!

There were many other opportunities for the experiments to go wrong; for instance, the Ringer's circulation system needed frequent adjustment and was prone to block at the point of the dripping pipette, causing the muscle to dry out. We instituted a regime of

From then to now

As a postscript to this personal account, in the intervening four decades the synchrotron, which produces an X-ray beam containing many thousand times the number of quanta available from any static generator, has revolutionised diffraction studies of muscle and muscle proteins. The crystal structures of both the actin molecule and the enzymatic head of the myosin molecule (which constitutes the cross-bridge) have been published in atomic detail (see e.g. the review by Geeves and Holmes, 2005). Modern X-ray experiments have also been helped by the development of two-dimensional electronic X-ray recording devices that have made the film techniques that we used totally obsolete.

For an exciting index of the level of detail given in modern synchrotron diffraction studies of contracting muscle, any interested readers can consult a paper entitled 'Molecular dynamics of cyclically contracting insect flight muscle *in vivo*' published in *Nature* 2 years ago by a group that included Tom Irving, once one of Barry Millman's PhD students at the University of Guelph in Ontario (Dickinson *et al*. 2005). To quote from the abstract:

'To elicit stable flight behaviour and permit the capture of (X-ray) images at specific phases within the 5-ms wingbeat cycle, we tethered flies within a visual flight simulator. We recorded images of 340 μs duration every 625 μs to create an eight-frame movie, with each frame reflecting the instantaneous structure of the contractile apparatus.'

Despite such immense advances, there are still unknowns in the current view of the contractile event. Modern work has recently been reviewed by Geeves and Holmes (2005), who present what might be called the majority viewpoint. However, Roy Worthington and I (who are still collaborating 50 years after we first met at King's College) have pointed out a few physiological and biochemical lacunae in some aspects of the majority approach (Worthington & Elliott, 2005; Elliott & Worthington, 2006). The discussion continues.

laboratory visiting at night and during the weekends to deal with the inevitable glitches. Luckily the Drury Lane biophysics laboratory had an enclosed parking bay, so I could drive in and not waste time looking for a parking space (yes, parking in central London was already problematic back in the early 60s!). The current Editor of *Physiology News* would sometimes come with me on the weekend visits, and I cannot resist the temptation to include a picture of Austin in his 60s lab-visiting gear (Fig. 4). By the second half of the 60s Austin and his younger brother Gavin were taking turns to tag along – the latter, by the way, has had the good sense to avoid the family trade and is now a successful architect.

By Summer 1963 it was clear that, given time, the method would produce useful data on current questions. The critical ones were:

(i) whether the distance between the filaments, which depended on the sarcomere length, would adjust to a constant value during the contractile event;

(ii) whether the spacing along either the actin or the myosin filaments would change;

(iii) whether the layer lines, which arose from the myosin cross-bridges, would alter.

I reported our progress at a Royal Society meeting organised by AF and HE Huxley in November of that year (Elliott, 1964).

The event was overshadowed by the shocking news of the assassination of President JF Kennedy that we heard towards the end of the second day; this was just after I had given my paper. I had spent the previous summer (1962) working at the NIH in Bethesda, just up the road from the White House, and had often defended Kennedy's Social Services reforms from Americans, including many physicians, who disliked both the reforms and JFK himself. I felt his murder as a personal blow, therefore, as of course did many people around the world.

Dramatis Personnae

Jean Hanson: Elected to the Royal Society in 1967, Jean died in 1973 at the tragically early age of 52 from a rare brain event. Her life and work is described in her Royal Society biographical memoir, written by JT Randall (1975).

Jack Lowy: Jack originally came to England in 1938 as a refugee from Czech Moravia, and was a member of The Physiological Society for many years. He left King's College in 1969 to become founding Professor of Biophysics at the University of Århus in Denmark. After taking early retirement in 1977 following a heart attack he worked for 18 years as Visiting Professor at the Open University Oxford Research Unit, continuing to probe muscle structure by X-ray diffraction. He died in 2000 on Yom Kippur (Elliott, 2000).

Barry Millman: After some years at King's College Barry (another long-time Physiological Society member) returned to Canada as first Professor of Biophysics at the University of Guelph, where he is now Emeritus Professor.

Roy Worthington: Roy left King's College in 1960, initially moving to the University of Michigan in Ann Arbor. In 1969 he moved to Carnegie-Mellon University in Pittsburgh, where he is now Emeritus Professor of Physics and Biology. He is pictured here at his 1959 wedding to wife Alma (GE was best man, below).

Gerald Elliott: After narrowly failing to swap science for politics when I almost got elected to Parliament as part of Harold Wilson's big Labour majority in 1966, I spent some time in the USA as Professor of Chemistry at Carnegie-Mellon University in Pittsburgh, as well as several idyllic late 60s summers at the MBL in Woods Hole.

We very nearly stayed in the States permanently, but the great social upheaval of the time, together with the realisation that we were Europeans at heart, brought us back to the UK. In 1969 I became founding Professor of Physics at the Open University, where I set up a Biophysics Group at the OU Oxford Research Unit. As well as keeping working on muscle, I started a programme on the transparent front tissues of the eye (cornea and lens) that continues to this day (my first OU PhD student in this area was Julia Goodfellow, lately the Head of BBSRC). After retiring from the OU in 1996 I spent 9 years as a Distinguished (ancient?) Research Fellow in Vision Sciences at Cardiff University. I finally gave up paid employment at the end of 2005 (the eve of my 75th birthday) and am now an Honorary Research Associate in the Nuffield Laboratory of Ophthalmology, University of Oxford.

By the following year we had accumulated enough data to answer the first two of our questions, and to give indications on the third. We knew, though, that Hugh Huxley and his collaborators in the Cavendish laboratory at Cambridge had started to do similar experiments, and they had a rotating-anode X-ray tube, which gave them at least a ten-fold advantage in exposure time over us. We had asked Randall to buy us such an X-ray generator, as they were available commercially (though the Cambridge model had been built in-house), and were awaiting his verdict. The DNA helix events had left some friendly rivalry between King's and the Cavendish, and this time we thought we would like King's to be first. We therefore sent a paper off to *Nature,* reporting that the inter-filament distance did not readjust to a constant value, and that the spacing along the actin and myosin filaments did not change within the experimental error of our experiments. We decided not to comment on the layer line pattern in the first instance, because we could only see weak layer lines in our contracting muscle pictures (Fig. 5).

As it turned out, the Editor of *Nature* sent our paper to MF Perutz, the Head of the Cavendish, to referee. He was content to recommend publication but in the manner of the times he wanted a paper from the Cambridge workers to be written for inclusion in the same issue. Thus the earliest low-angle X-ray diffraction studies of living contracting striated muscle by Elliott, Lowy and Millman (1965) and Huxley, Brown and Holmes (1965) also came to be published back to back in a single issue of *Nature*. The Cambridge group reported that the layer-line intensities decreased during contraction (question iii). Our pictures had also shown this tendency, see above, though we would not have felt confident enough to report this *a priori*. We contented ourselves with supporting their observation in a footnote. We described our results in detail later (Elliott *et al*. 1967), but it was by then clear that until we got a rotating anode system we could not hope to compete with the extra intensity available in the Cambridge laboratory. After the *Nature* papers Randall ordered the new generator, but it had to wait for the next lab spending round, which seemed to take ages.

Many other aspects of the diffraction patterns from contracting striated muscle were described in detail in a wonderful paper by HE Huxley and Brown (1967) that has become a classic of the field. In the 1970s the synchrotron became the X-ray source of choice for muscle studies, largely through the work of Hugh Huxley and Ken Holmes, and early work using laboratory X-ray generators became the stuff of history.

Gerald F Elliott

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News

Research funding

The Department of Health has announced £45 million in funding for 29 research programmes in areas such as mental health, medicines for children, diabetes, neurodegenerative diseases and neurology. The funding has been allocated through the National Institute for Health Research programme grants for applied research funding schemes. It is hoped that this new government funding will improve health outcomes for patients in England, with particular emphasis on conditions that cause chronic distress to patients and that are a significant issue for the NHS to manage. It also aims to enable NHS trusts to tackle areas of high priority for patients.
http://www.gnn.gov.uk

Nutrition Society digital archive

The Nutrition Society has made its scientific publications archive freely available to the public. The archive contains more than 87,000 pages of peer reviewed papers on nutritional science. All the papers were originally published in the Nutrition Society's journals and proceedings from 1944 to the present day. The archive can be accessed via the Cambridge University Press web site.
http://journals.cambridge.org/nutrition

For more information about the Nutrition Society please visit
www.nutritionsociety.org

E-petition on science funding cuts

The government has slashed the funding of scientific research councils by £68 million. Those most affected include the Engineering and Physical Sciences Research Council (£29 million reduction in funding) and the Medical Research Council (£10.7 million reduction).

To add your vote to the e-petition calling for the government to review its decision, go to
http://petitions.pm.gov.uk/research

Open access – views from the US and the UK

American Physiological Society Executive Director Martin Frank considers whether the scientific societies in the US can afford free access for all, and the UK Physiological Society's Executive Committee responds to the 'public access' policies released into the public domain

For most investigators, the advent of the online journal world has relieved them of the burden of going to the library, paging through dusty volumes, and tracking the multitude of note cards taken about articles of interest. Our online world allows us to perform full-text searching with linkage to databases, technology resources, etc. all from a home or office computer. The American Physiological Society is one of the scientific societies that, together with HighWire Press, has contributed significantly to this new world by creating a platform that now houses some 3.7 million articles, including nearly 1.5 million free articles as of October 2006. The creation of this online library has occurred without compromising peer review and without a significant impact on the subscription prices charged by not-for-profit publishers.

Having created a relatively seamless online world, Open Access (OA) advocates are now asking publishers to sacrifice existing models of cost recovery to bring about a world in which articles can be obtained from multiple sources, i.e. not only the journal of record but also institutional and government repositories such as PubMed Central (PMC). Although competition is generally a good thing, this is a very different kind of competition. In journal publishing, competition has generally occurred between the commercial and society publishers or between high-impact journals. The question is whether publishers should also be forced to compete with the government in the dissemination of journal content. In other words, should the government and funding agencies dictate where researchers may publish based on the journal's willingness to allow the article to be deposited in PMC for display and access within 6 or 12 months? There are costs associated with this kind of competition, both to the government (to create the repository) and to the publisher (if government access supplants the journal of record).

The NIH and PubMed Central

The National Institutes of Health (NIH) has been the driving force in efforts to modify the publication policies of scientific journals. Although one can go back to Harold Varmus's ideas for E-Biomed to explore the origins of these efforts (Marshall, 1999), the current program was launched in May 2005 as a Public Access Plan designed to provide taxpayers with access to the scientific literature their tax dollars support. NIH grantees were asked to upload accepted, peer-reviewed manuscripts to a PMC repository where they would be displayed 12 months or less after publication. The goals of this program were to facilitate public access and NIH portfolio management and to create an archive of NIH-funded research. The 12-month time frame was a compromise that attempted to balance the needs of NIH with the needs of society publishers, many of whom publish high-impact journals in which a significant proportion of the content consists of NIH-funded articles. Since its launch, only about 4% of the expected number of articles have been uploaded to PMC. No doubt one of the reasons for some society authors was that they questioned the need for a duplicate publication site for their manuscripts when publishers already make the final articles freely available within the prescribed time frame.

Efforts to shift the time frame

The low compliance experienced under the NIH Public Access Plan has stimulated efforts by OA advocates to mandate participation and make articles publicly accessible after only 6 months. Some have been aggressively lobbying Congress, seeking the inclusion of language in the NIH appropriations or reauthorization legislation. They enlisted the assistance of Senators Cornyn (TX) and Lieberman (CT) to broaden the government's program by introducing S.2695, Federal Research Public Access Act (FRPAA) of 2006. This bill would require 11 federal agencies with more than $100 million in extramural research funding to arrange for grantees to deposit peer reviewed and approved manuscripts in government-approved repositories, where they would be made publicly accessible within 6 months of publication.

FRPAA advocates contend that a 6 month mandatory policy will help to protect the important role of journals and publishers in the peer-review process, the cost of which is presently covered by subscription revenue. At the same time, librarians contend that FRPAA will save them money by providing access to content to which they currently subscribe. However, librarians will only be able to save money by canceling subscriptions based on access from PMC. Thus journals would lose revenue, forcing publishers to identify other sources of revenue to support the peer-review and publication processes.

The NIH Public Access Plan was originally proposed as a 6 month mandatory plan. In supporting this view, NIH Director Elias Zerhouni wrote that the policy would not harm journals since "NIH-funded articles account for more than half of the total published articles for only 1%" of the 5,000 journals indexed by PubMed (Zerhouni, 2004). However, that 1% represents 50 journals, and nearly two-thirds were society journals, including six APS journals. Many society journals

publish articles supported not only by NIH but by the other federal agencies covered by FRPAA, as well as private funding agencies that are also advocating for a 6 month policy. As more of a journal's content becomes freely available within 6 months from PMC, the publisher's ability to support peer review through subscriptions will be diminished.

The threat to subscription revenue is real. According to Garfield (1998), in a study of the 100 journals with the highest cumulative impact, the impact of physiology journals showed more significant increases over time than other fields when comparing impact at 2, 7, and 15 years. Garfield contended that the shift was due to the 'innate character of physiological research.' The long-term value of physiological research is also reflected by the journal's cited half-life. Among the APS research journals (Table 1), the one that publishes studies employing emerging technologies is *Physiological Genomics*, with a cited half-life of 2.8 years. The *Journal of Applied Physiology*, which addresses more established areas of physiological science, has a cited half-life of 9.9 years. The ready availability of content from physiology journals within 6 months could encourage institutions to cancel subscriptions. This might save money for libraries, but it would force the Society to seek support for peer-review and publication costs from other sources.

Shifting to author pays

The most commonly mentioned open access payment model is an author pays model. A shift from a subscription model to an author pays model is actually what many OA advocates would prefer since free access would be immediate and not after 6 or 12 months. Many point to the Public Library of Science (PLoS) as an example of a publisher of high-impact journals that makes content freely available immediately through an author pays model. To do so, PLoS, which is subsidized by philanthropic sources, now charges authors a highly subsidized $2,500 per article to publish in *PLoS Biology* and *PLoS Medicine*. (Originally, the fee was $1,500.) At the APS and many other scholarly publishers, whose publication costs are not subsidized by philanthropic sources, the cost of peer review and publication averages $3,000 per article. If subscription revenue were lost, these costs would need to be recovered through higher author fees. A Cornell University study estimated that it would cost that institution $1.5 million more if researchers published exclusively in author pays rather than subscription-based journals (Davis *et al*. 2004). Using the same model, similar estimates have been made for other research-intensive institutions (Davis, 2005). Thus one unintended consequence of federal programs that mandate access could be that costs are shifted from a wide array of subscribers to a few research-intensive institutions.

Under an author pays model, library acquisition budgets could be reduced, allowing for the transfer of overhead funds to the Dean's office to assist with the payment of author fees, assuming that the NIH does not reduce overhead rates and that Deans do not invest the new-found income in other ways. Alternatively, author fees might have to come directly from the author's research grant or from funding agency supplements. In either case, this would effectively reduce funds available for research.

Many OA advocates proclaim that free access to federally funded research is a public good. However, I would contend that research directed toward the development of treatments and cures for disease is also a public good. *As scientists, we must actively work to make sure that research continues to be ....the public good on which the federal government and charitable foundations spend their money.*

Table 1. Impact factors and cited half-life of APS journals *

Journal	Impact factor	Cited half-life
Physiological Genomics	4.636	2.8
Am J Physiol - Endocrinology & Metabolism	4.456	6.0
Am J Physiol - Renal Physiology	4.263	5.8
Am J Physiol - Cell Physiology	3.942	5.8
Am J Physiol - Lung Cellular and Molecular Physiology	3.939	4.9
J Neurophysiology	3.853	8.0
Am J Physiol - Regulatory, Integrative, and Comparative Physiology	3.802	6.4
Am J Physiol - Heart and Circulatory Physiology	3.560	5.9
Am J Physiol - Gastrointestinal and Liver Physiology	3.472	6.2
J Applied Physiology	3.037	9.9
* ***J Physiol***	**4.272**	**9.2**
Exp Physiol	**2.054**	**5.4**

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Martin Frank
APS Executive Director

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Public access policies and The Physiological Society in the UK

Funding agencies have become increasingly engaged in the debate about the future of scholarly communication and the dissemination of peer-reviewed scientific articles. A number of such 'public access' policies have been released into the public domain, the most notable of which are the following:

The National Institutes of Health (NIH) Public Access policy

The policy can be viewed at: http://publicaccess.nih.gov/Enhanced_Public_Access.pdf

Research Councils UK (RCUK)

Position Statement on Access to Research Outputs. The statement can be viewed at: http://www.rcuk.ac.uk/access

Wellcome Trust position statement in support of open and unrestricted access to published research

The statement can be viewed at: http://www.wellcome.ac.uk/doc_WTD002766.html

The Physiological Society's response to the policies

The Physiological Society is committed to the principle of public access to research data published in *The Journal of Physiology* and *Experimental Physiology* as far as it is compatible with sustaining its scientific mission and the viability of its journals. Online access to review articles is free immediately on publication, with free access to all content 1year after publication. Institutions in designated developing countries are granted immediate free access to both journals. In addition, all the back issues of *The Journal of Physiology* from Volume 1 (1878) have been scanned through the generosity of the Wellcome Trust and are freely available electronically on PubMed Central. The Physiological Society has funded a similar digitisation programme for *Experimental Physiology* (formerly the *Quarterly Journal of Experimental Physiology*) and all back issues are freely available electronically on HighWire.

The Society has concerns that some "public access" policy proposals, which mandate early access to the content of its journals, could threaten their standards and their long term viability. Publication of research data is expensive. In the US, many journals impose submission and page charges. While funding bodies in the US allow for such publication costs in their grants, this is not common practice in the UK. The Society's journals do not, therefore, charge authors for submissions, the costs of publication being largely funded from subscriptions. If public access policies require authors to post their articles on open access web sites, before they are made available by the journals, this will jeopardise income from these subscriptions and would require the introduction of charges to the author.

The editorial boards of *The Journal of Physiology* and *Experimental Physiology* have voiced the additional concern that public access policies will erode the quality of the peer review process which, for both journals, is justifiably acknowledged to be amongst the most fair and thorough in the field of life science journals. Both boards believe that the costs of peer review must be met in order to maintain standards.

The Society has a number of further reservations about the feasibility and fairness of the author-pays model of journal publishing, that will be required if early open access is mandated. The Society is committed to continuing the publication of its journals as a means of promoting the advancement of physiology and will support open access publishing, but only if it is adequately funded. *The Journal of Physiology* and *Experimental Physiology*, through Blackwell Publishing, currently offer authors the option of paying an open access fee of £1,250/$2,500 to have their papers made available free on publication (http://www.blackwellpublishing.com/static/onlineopen.asp). The editorial boards are pleased that the Wellcome Trust has now provided explicit advice to institutions on how authors should apply for funds to cover open access fees. It is important to note, however, that the current level of the open access fee is insufficient to cover the costs of publication and peer review.

The Physiological Society and the editorial boards of *The Journal of Physiology* and *Experimental Physiology* believe that the costs of publishing research papers must be adequately funded so that the scientific community and the wider public can continue to benefit from effective peer review and publisher-funded enhancements. Income from the journals is also used by The Society to fund scientific meetings, grants and educational activities, all of which provide direct charitable support for physiology and physiologists. The Society urges funding agencies to address these issues before requiring authors to take actions that will jeopardize the income streams of The Physiological Society journals, which have traditionally provided these benefits.

APS Renal Research Recognition Award

Congratulations to Scott S P Wildman (Department of Physiology, University College London), pictured below, who was awarded the American Physiological Society Renal Research Recognition Award 2007 at Experimental Biology in Washington DC. The award is a result of his research contribution to the role of P2 receptors in the nephron. Scott is a recipient of a British Heart Foundation Intermediate research Fellowship.

Everything comes in cycles

Richard Naftalin (below) considers problems relating to primary and secondary active transport

In the 1950s and 60s there were still revanchist arguments about whether active transport existed at all! Gilbert Ling believed that protoplasm had specific adsorptive properties with stereoselectivity for K^+ over Na^+ that explained the asymmetric ion distribution in cells without any need to invoke an energy-consuming process (Ling & Ochsenfeld, 1976) However active transport of Na^+ across frog skin was unequivocally demonstrated by Ussing (Ussing & Zerahn, 1951).

E J Harris, my mentor in the Biophysics Department at University College London, was one of the first to show that Na^+ is pumped out of muscle via an energy-consuming process (Edwards & Harris, 1957; Harris. 1957). Post & Jolly (1957) also showed that ion distributions across the cell membranes depended on an energy-consuming process. This work stimulated Skou to characterise the sodium pump as an ATPase enzyme, which underwent reversible phosphorylation (Skou, 1998). Ron Whittam's (Whittam & Ager, 1964; 1965) and Garrahan & Glynn's (1967) demonstration that active movements of Na^+ and K^+ are coupled stoichiometrically to ATP breakdown and running the pump backwards produced net ATP synthesis (Lew, Glynn & Ellory. 1970) consigned the Na^+-K^+-ATPase pump, firmly into the conceptual camp of an enzymatic 'mechanical' carrier protein for three decades.

Peter Mitchell, the inventor of chemiosmotic theory, put the dilemma of coupling of proton flow to ATP synthesis very well in his review paper (Mitchell, 1977). He wrote that the consensus view of energy transformations in the 1950-60s was in terms of chemical group transfer reactions, as with substrate level phosphorylation of ATP; so the bioenergetics people were looking for 'a chemical intermediate' which could explain chemical transduction of

electron and proton transport with ATP synthesis. Mitchell's hypothesis, which stated that no such intermediate existed and that the energy transformation was the result of coupling of the proton electrochemical potential gradient to the ATP synthetic process, was quite revolutionary and was met with varying degrees of incredulity. One of the problems then was that there was no concrete proof – just a diagram! This omission was set to rights by Boyer's and Walker's structural solution of the ATP synthase (Boyer, 1997; Stock *et al*. 2000).

Coupled transport became the physiological *zeitgeist* of the late 60s. Robert Crane's view that sugars were transported up their concentration gradient, as a result of energy transduced from a Na^+ gradient across intestinal brush borders, was readily accepted, perhaps as a result of Mitchell's efforts (Crane, 1962). The apotheosis of Crane's work came about 10 years later with the unequivocal demonstration, using brush border membrane vesicles, that the only source of energy for uphill sugar accumulation was the electrochemical gradient of Na^+ across the membrane (Murer & Hopfer, 1974).

Another important example of coupling of flows was the demonstration that water flows are coupled to net Na^+ movement in intestinal epithelia, as a consequence of osmotic coupling resulting from active Na^+ accumulation into a 'central compartment' (Curran 1960), later located in the extracellular and lateral intercellular spaces.

The other important strand to understanding of coupled flows in biology came from theoretical work on irreversible thermodynamics as applied to biological flows (Kedem & Katchalsky, 1961). Two important aspects of their work have not been fully assimilated. They recognized that coupling of flows across biological membranes, as in artificial membranes, arises from frictional interactions between the flowing components within the membrane. Coupling between separate molecules is not the result of static 'thermodynamic' interactions, as where electrical and chemical potentials generate a combined electrochemical potential within a single ion. This goes against the grain of current thinking, which assumes that the cotransported molecules are yoked together by the carrier within the membrane phase, so that their separate thermodynamic potentials merge stoichiometrically to generate a multicharged complex whose single electrochemical potential generates coupled solute flows across the membrane.

Problem with primary active transport

A second important aspect of irreversible thermodynamic theory is the recognition that the scalar (non-vectorial) nature of the energy dissipation from chemical reactions, such as ATP hydrolysis, must be transformed into a vectorial force to generate vectorial ion flow (Kedem, 1961). All the circulating carrier models of active transport by P-type ATPases, are presumed to impart concentrative energy to the transported ligand by the agency of the protein levering, via ATP-dependent alterations in the relative affinities of Na^+ and K^+, the bound ligands between the E1 and E2 states of the enzyme. This is presumed to transform the scalar energy of ATP hydrolysis to a vector force generating concentration work between the intra and extracellular solutions (Jardetzky, 1966; Lauger, 1984; Apell, 2004; Artigas & Gadsby, 2006).

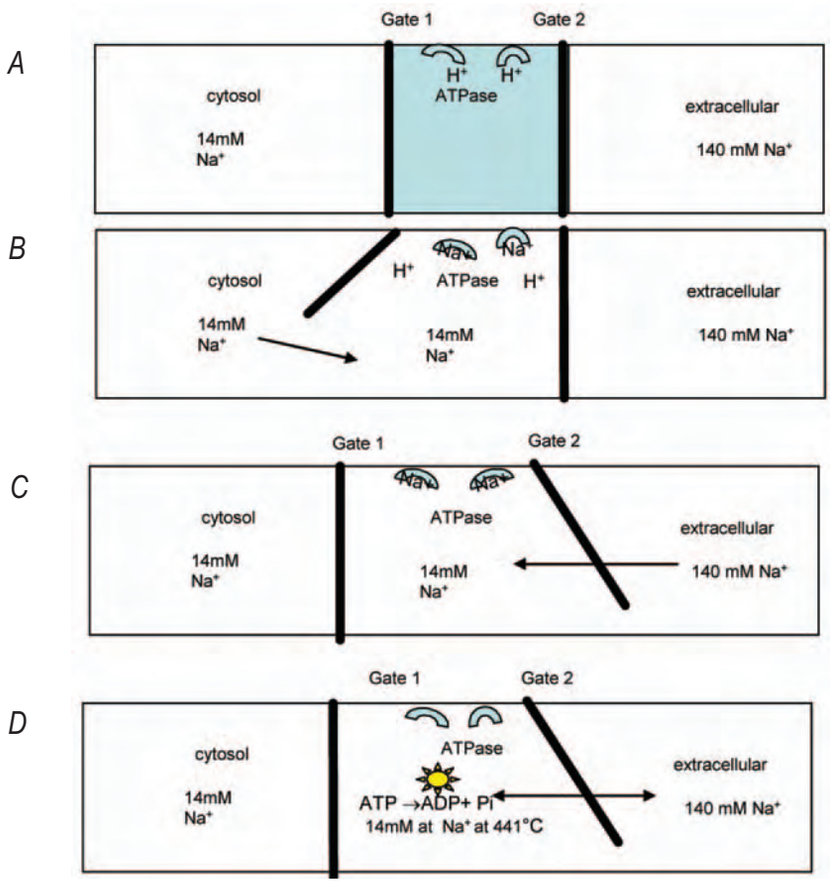

Figure 1. Modification of the Artigas & Gadsby (2006) model to account for ATPase-dependent activation of ion mobility and gating on the basis of Apell's findings (2004). *A*, State 1, empty gate ATP-Pi bound. *B*, State 2, gate 1 opens spontaneously allows Na+ entry from cytosol which activates ATPase generates local heat. *C*, No energy transfer from ATP hydrolysis net Na+ transference from extracellular to central compartment. *D*, State 3, Energy transfer from ATP hydrolysis to caged Na+ raises molecular temperature from 37o to 441oC within central compartment thus energizing Na+ and gate 2 permits 10-fold uphill flow of Na+ into extracellular fluid.

Although the affinity ratio of the carrier for Na^+ and K^+ is changed from 28 to 710 by raising ATP from 0.1 to 4mM (Homereda *et al*. 1991) this only represents energy transferred to the carrier protein itself and not to the mobile ligands.

It has become apparent that the two gates enclosing the central occlusive compartment of the Na^+- K^+-ATPase can open simultaneously during the normal pump cycle (Apell 2004; Artigas & Gadsby 2006). Thus, the hypothesis that ions contained within the central compartment are prevented from running down their concentration gradients by fixed mechanical properties of the gates is no longer tenable. If the chemical reaction only transfers energy to the transporter to alter its gating properties and affinities towards the transported ligands, this will not raise the kT energy of ligands within the occluded central cage to permit further isoergonic transfer from the central compartment to the higher energy state of ions within the *trans* solution. A pore pump model incorporating direct energy transference from ATP hydrolysis to increase ionic motion out of the central occlusion site is required (Fig. 1).

Normally, in isothermal isobaric conditions, it is assumed that ligand thermodynamic activity is controlled exclusively by changes in electrochemical potential. However, in an enclosed chamber such as envisaged between the gates of the P-ATPase, it is possible for thermal energy to be transferred from the exothermic reaction of ATP hydrolysis to increase the local activity of mobile ligands within the cage. The local scalar chemical reaction will provide a vectorial pump drive if polarized selective escape mechanisms for K^+ and Na^+ are available via the gates.

For net uphill Na^+ movement, from the cage, through the gate, into the *trans* (external) solution, where the ligand reaches a steady state concentration ≈ 10-30 fold higher (140mM) than in the *cis* solution (14mM), requires an equivalent increase in kT energy of the transported molecule sufficient to raise the 'temperature' within the central cage from 37°C to 441°C .

To obtain energetic equivalence between the caged ions Na^+_1 and those in the external solution at higher concentration Na^+_2,

$$RT_{1a} \, Ln \, Na^+_1 = RT_2 \, Ln \, Na^+_2$$

the temperature of Na^+_1 has to be raised to T_{1a}

Hence, if Na^+_2 / Na^+_1 = 140 mM/14 mM and T_2 = 310°K, then T_{1a} = 714°K.

Assuming the occluded compartment has a spherical volume with radius ≈ 3-4 Å, then hydrolysis of 1 molecule of ATP ≅ 83 zJ (1 zeptoJoule = 10^{-21}J) will generate sufficient heat to power net Na^+ diffusion 'uphill' against of 30-fold concentration gradient. Given a thermal conductivity of 0.6 W/m/°K, a density of 1000 kg/m^3 and heat capacity of 4 kJ/°K/kg, this heat would be dissipated over a distance of 1 nm in ≈ 5- 10 ps.

Problem with secondary active transport

The early papers on cotransport clearly differentiated between its kinetic and thermodynamic elements (Heinz & Patlak, 1960; Vidaver & Shepherd, 1968). The law of mass action requires rate equations describing ligand movements of Na^+ and any organic solute G, complexed to a carrier, (Na_2.G.C); where n Na^+ atoms and one molecule, G, are bound to the carrier (C), that there will be separate term(s), $k_{ij} \propto [Na]^n$ and [G] and ($[Na]^n$. [G]) within the rate equation.

Even if it assumed that the only mobile carrier forms are (Na^+_2.G.C) and C within the *membrane phase*, the thermodynamic work done to transport Na^+ and G between the *solutions* bathing each side of the membrane relates only to the chemical, or electrochemical potential differences of the ligands existing between the

external solutions, where the ions and solute G remain totally dissociated. The external work ΔW done in transporting ligands Na^+ and G from the left solution (l) to the right solution (r) across the membrane is:

$$\Delta W = RT\ Ln\ (Na_l/Na_r) + F\Delta E + RT\ Ln\ (G_l/G_r) + RT\ Ln\ (J_{Narl}/J_{Nalr}) + RT\ Ln\ (J_{Grl}/J_{Glr}) - \Delta\Phi$$

J_{ilr} is the flux of component i, from left to right side (mole.cm^{-2} s^{-1}) and ΔΦ is the work dissipated in irreversible losses, F is the Faraday constant and ΔE the transmembrane potential, R is the gas constant, T temperature degrees Kelvin, G the concentration of glucose inside or outside.

When the concentrations of transported ligand (glucose) rises in the internal solution, so that the net glucose flux tends to zero, a steady state will be reached and in Kimmich's words 'for a transport system entirely driven by the transmembrane potential difference in the electrochemical potential for Na^+ the following relationship must be obeyed' (Kimmich, 1981):

$$RT\ Ln\ ([G_i]/[G_o]) \leq (RT\ Ln\ ([Na]_o/[Na]_i) + F\Delta E)n$$

This 'thermodynamic' mechanism with assumptions of equality of thermodynamic driving forces controlled by a fixed stoichiometric coefficient 'n', where n is an integer between 1 and 4, quickly acquired mechanistic significance. In chicken enterocytes, the accumulation ratio inside/outside of 3-O methyl-D glucose >70 for a Na^+ gradient of approximately outside/inside = 10. It was deduced that the thermodynamic potential driving uphill intracellular sugar accumulation requires the stoichiometric coefficient n $\cong$ 2 moles Na^+ per mole of sugar and carrier:

$$\text{e.g. } [Glucose]_{in}/[Glucose]_{out} = [Na]^2_{out}/[Na]^2_{in}$$

The carrier model of cotransport assumes that no significant energy loss within the carrier transport process and that no accompanying leak of uncomplexed ligand occurs, *irrecoverable* work = ΔFx = 0 and n is the 'stoichiometric coefficient of Na^+ binding within the mobile carrier complex. In theory, at static head, no net flux of either the driving solute Na^+, or the driven solute glucose occurs. Any leaks that do occur i.e. uncoupled

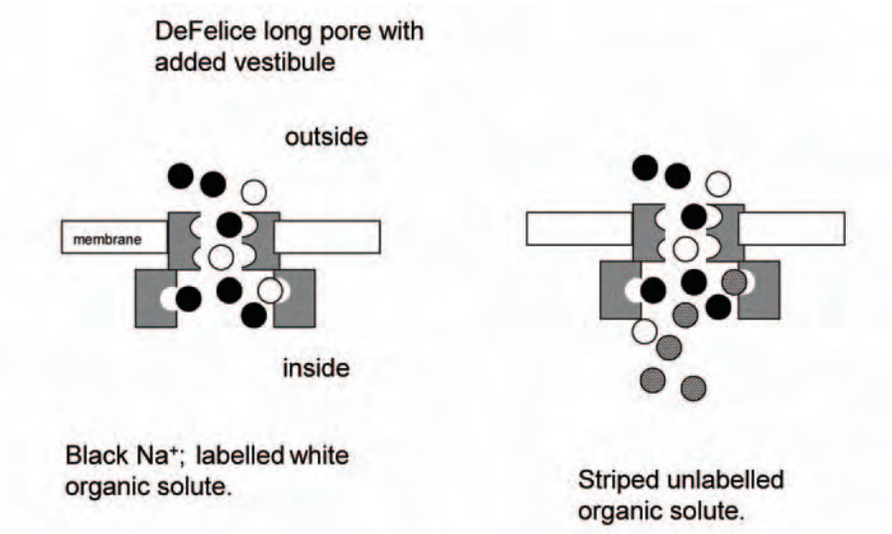

Figure 2. Competition for the vestibular sites by high concentrations inside speeds unidirectional rates of cotransport of solute.

Na^+ or glucose movements and even in the isolated transporter they do (Parent *et al*. 1992, Loo *et al*. 1998) are assumed to be outside the cotransport process and can be factored out of the central assumption of the cotransport model- that there is a static complex between the mobile ligands and carrier lasting the duration of passage across the transporter.

Another way of viewing cotransport is by use of the phenomenological equations originated by Onsager (1931):

$$J_1 = L_{11}X_1 + L_{12}X_2 \qquad (1)$$
$$J_2 = L_{21}X_1 + L_{22}X_2 \qquad (2)$$

In this case J_1 represents Na^+ flux via a membrane containing Na^+ glucose cotransporters and X_1 is the conjugate driving force of Na^+, the electrochemical potential gradient of Na^+ = (RT Ln ($[Na^+]_{out}/[Na^+]_{in}$) + F Δψ); L_{ii} are straight coefficients defining the linear relationship between the driving forces X_i and their conjugate flows J_i. J_2 represents glucose flow via the Na^+-glucose cotransporter within a brush-border membrane, its conjugate force X_2 = RTLn ($[G]_{out}/[G]_{in}$) and L_{22} is the straight coefficient relating glucose flow to its chemical potential gradient. The cross coefficients L_{12} and L_{21} relate the flows J_1 and J_2 to their non-conjugate forces i.e. X_2 and X_1 respectively.

If glucose accumulates on one side to an extent where net glucose movement = J_2 = 0,
Then in equation 2: where J_2 = 0, i.e. the static head for glucose transport;

$$L_{21}X_1 = -L_{22}X_2$$
$$L_{21}/L_{22} = -X_2/X_1 = \Delta\mu_{(in\text{-}out)glucose}/\Delta\mu_{(out\text{-}in)Na}$$

The ratio of thermodynamic forces $X_2/X_1 = \Delta\mu_{glucose}/\Delta\mu_{Na}$ between the internal and external solutions for glucose and between the external and internal solutions for Na^+ is similar to the stoichiometric coefficient 'n', as defined by Kimmich. However, this phenomenological treatment, unlike that of Kimmich, requires no assumptions about the constancy of n, or that at static head that *both* J_{Na} = $J_{glucose}$ = 0. Equations 1 and 2 indicate that the static head condition only applies to the driven solute (glucose) and continuous leakage of the driving solute, Na^+ is a necessity, rather than an irrelevant inconvenience.

The extent of glucose accumulation at static head relates directly to the cross coefficients, L_{ij}, which in turn relate to the frictional interactions between Na^+ and glucose within the transporter. The thermodynamic forces in the external solutions driving Na^+ and glucose transport are unaltered by the extent of Na^+-glucose interaction within the

transporter and may explain why Na^+ leak current across SGLT1 is reduced by glucose (Loo *et al*. 1998; Parent *et al*. 2000).

A perceived disadvantage of phenomenological equations is their generalized nature: no mechanism other than a frictional interaction is specified and is therefore considered to be too vague to be useful – in contrast to the highly specific cotransporter models. However, the phenomenological equations have the advantage of transparency in illustrating the underlying physical principles governing coupling.

Alternative models for cotransport in which direct energy transference within a pore-like transporter between adjacent molecules have been postulated (Hodgkin & Keynes, 1955; Su *et al*. 1996; DeFelice, Adams & Ypey, 2001). These alternative mechanisms gives reasonably satisfactory simulations of the observed data both for Na^+- 5HT cotransport and Na^+-glucose transport.

Collision models have the major advantage that they do not make assumptions about large protein conformational changes, allow for dissipative losses (leaks) to generate the flows, and are more consistent with the known structures of biological transporters.

Acknowledgement

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Hyaluronan, the guardian of joints

Most body fluid cavities are sealed by a tight epithelium or mesothelium, whereas the joint lining is almost devoid of cell junctions, 20-30% of its interface being 'naked' intercellular matrix. How then is the hyaluronan-rich, lubricating synovial fluid retained? When intra-articular pressure is raised (e.g. by flexion), ultrafiltration of the tangled hyaluronan chains by the synovial extracellular matrix creates a dynamic, reversible concentration polarisation layer with sufficient osmotic pressure to oppose further fluid loss. In addition HA reflection also raises the fluid's viscosity and reduces the energy-expensive replacement synthesis rate

Joint diseases lack the dramatic, life-threatening quality of cardiac disease, which may be why synovial joints have been largely neglected by physiologists. Or perhaps the neglect is due to the seeming simplicity of joints; after all, where is the intellectual challenge in a door hinge? This was very much my own response when joint physiology was mooted as a post-doctoral research area by my supervisor, Charles Michel. Thirty years on the naivety of this response is clear, for the apparent simplicity of joint function is achieved through a complex interplay of physiological, biophysical and biochemical mechanisms. This article describes one aspect of this, namely how joints manage to retain their lubricating fluid even though all the components of the fluid can, to a greater or lesser degree, seep out through the cavity lining.

Synovial fluid for lubrication and cartilage nutrition

Synovial joints contain a microscopically thin layer of highly viscous synovial fluid with two primary functions (Fig. 1). First, it transports glucose, amino acids and oxygen by convection-diffusion from the synovium to the articular cartilage, which is avascular but cellular and metabolically active. This mode of nutrition limits the thickness of viable articular cartilage to a few millimetres (Zhou *et al*. 2004). Second, synovial fluid lubricates the moving surfaces, limiting mechanical inflammation and reducing wear of not only the weight-bearing cartilage-on-cartilage interface but also cartilage-on-synovium and synovium-on-synovium interfaces. The lubricants include the high-load, boundary lubricant lubricin (glycoprotein) and the low-load, hydrodynamic lubricant hyaluronan (see below).

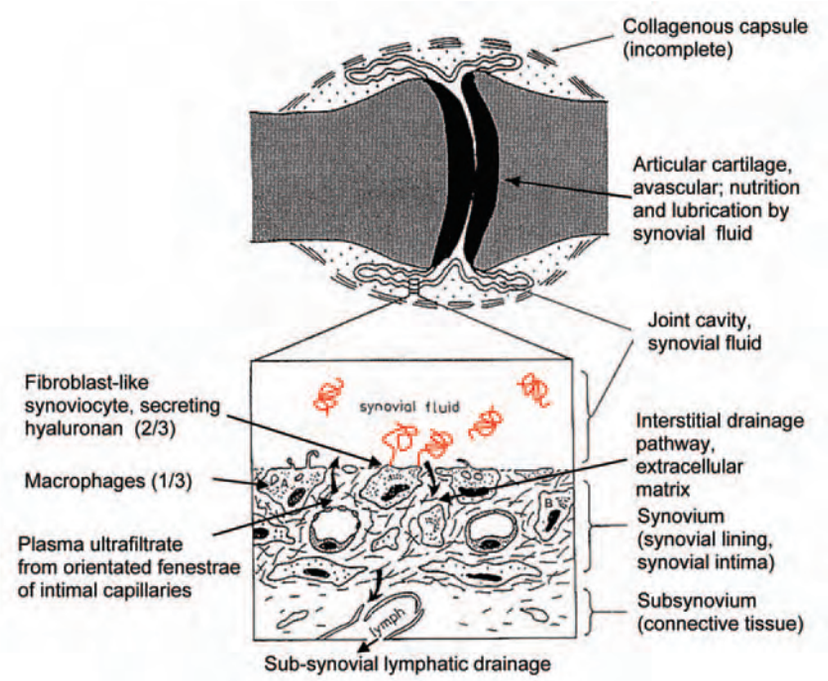

Figure 1. Structure of synovial joint and synovium. HA chains (red) are synthesized and extruded directly into the extracellular compartment by a transmembrane synthase in the synoviocyte membrane; no Golgi packaging, vesicular storage and release is involved.

Synovium produces synovial fluid

Synovial fluid is produced by the vascular joint lining or synovium, as William Hunter (surgeon-obstetrician, St George's Hospital) recognized in 1743. Synovium is a thin, discontinuous layer of mesoderm-derived cells bounding the joint cavity. Unlike the epithelium or mesothelium lining more familiar cavities such as the cerebrospinal space and pleural cavity, synovial cells form few junctions and are separated by µm-wide, matrix-filled gaps. As a result 20-30% of the fluid interface is 'naked' extracellular matrix (ECM) (Fig. 1). To cope with the inherent leakiness of this structure, a novel way of regulating the joint fluid content has evolved, involving the creation of a transient, reversible obstacle to fluid outflow (but not inflow) – a point we will return to. The water, nutrients, electrolytes and plasma proteins of synovial fluid come from the capillaries a few µm below the surface in accordance with established principles of capillary physiology (Levick, 1995). The highly water-permeable fenestrations of these capillaries are, appropriately, grouped on the side facing the joint cavity. The synovial lining cells themselves comprise 2/3rd fibroblast-like synoviocytes (FLS) and 1/3rd macrophages. The wide intercellular spaces are the sole drainage pathway from the joint cavity, allowing a turnover of fluid that has entered the cavity from the capillary fenestrations.

The FLS secrete the lubricants hyaluronan (HA) and lubricin into the plasma ultrafiltrate to create the highly viscous synovial fluid (*syn-ovium* refers to the egg-white consistency).

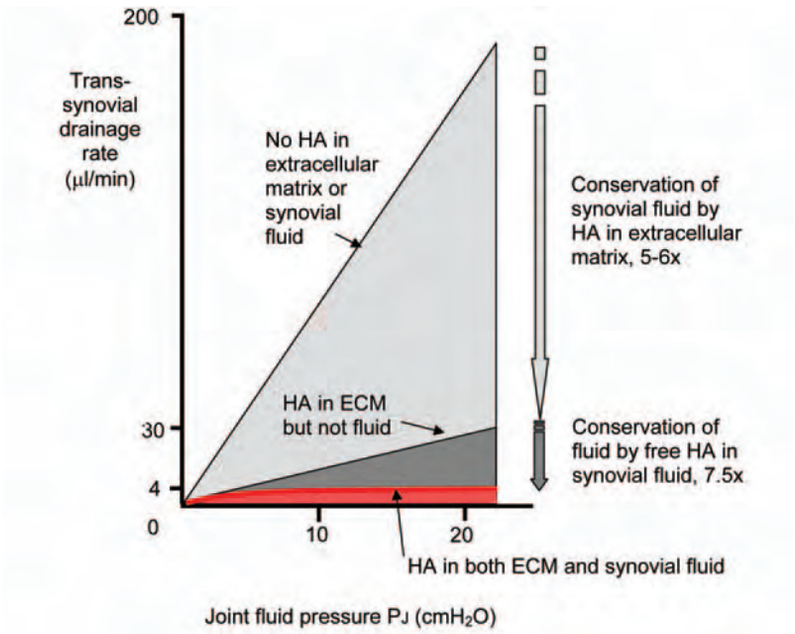

Figure 2. Summary of contributions of HA in synovial ECM and cavity fluid to prevention of joint fluid depletion. Based on studies of trans-synovial flow of infused Ringer solution, with or without HA at 3.6-4.0 mg ml^{-1} (2000 kDa) in cannulated knees of anaesthetised rabbits; for data points see Figure 5. Fluid absorption from the infusion reservoir was recorded by drop counter, intra-articular pressure was regulated by infusion reservoir height and pressure was measured through a second cannula and transducer. Matrix HA was removed by treatment with *Streptomyces* hyaluronidase (top line). When HA was present in both the intra-articular fluid and ECM, raising pressure increases outflow only slightly (outflow buffering). The combined effect is a 45-fold reduction in fluid escape rate.

Joint angle affects intra-articular pressure, hence fluid turnover

Synovial fluid pressure varies with joint angle, being low in extension (a few cmH_2O subatmospheric) and higher in flexion (2-5cmH_2O). As a result capillary ultrafiltrate enters the cavity during extension and drains out through the interstitial pathway into subsynovial lymphatics during flexion. Joint movement thus acts as a pump to promote synovial fluid turnover. But here we meet a puzzle – when the turnover time is measured in rabbit and non-arthritic human knees, the turnover time for synovial fluid water and albumin is ~2 h, whereas that for synovial fluid hyaluronan (calculated as mass/secretion rate) is 12-36 h. This indicates that hyaluronan may be selectively retained inside the cavity – with profound consequences for fluid exchange, as we shall see.

The hyaluronan molecule – more voluminous than a virus

Hyaluronan is a ~3-6 million Dalton unbranched polymer of repeating acetyl glucosamine-glucuronic acid disaccharides. It is 'spun' from the FLS plasmalemma by the transmembrane enzyme HAS2. The µm-long chain adopts an extended, random coil configuration encompassing a vast, hydrated domain of radius100-200 nm (cf. albumin 3.6 nm). The resulting high, shear-dependent viscosity is enhanced further by overlap of adjacent molecular domains at concentrations of >1mg ml^{-1}. HA is a universal component of ECM, including synovial ECM (0.8mg per ml extrafibrillar space), but its concentration in synovial fluid is particularly high, 2-4 mg ml^{-1} in human and rabbit knees. Both the concentration and chain length are reduced in arthritic effusions, lowering the viscosity.

Fluid leaks out from flexed joints

Trans-synovial fluid movement has been studied chiefly in the cannulated knees of anaesthetized rabbits. When the cavity is infused with physiological saline or albumin solution, trans-synovial fluid escape rate increases markedly with pressure (Fig. 2). The reader's knee and hip joints have probably been in a flexed position for some time while reading this article, raising the intra-articular pressure and fluid loss. There is only ~50-100 µl synovial fluid in a rabbit knee and 500-1000 µl in a non-arthritic human knee, so how do we avoid squeezing out this tiny volume during flexion? The answer seems to lie largely in the action of HA at two locations – the synovial lining ECM and the synovial fluid.

Extracellular matrix HA dramatically increases hydraulic resistance

When the soluble HA is washed out of the joint cavity experimentally, plots of trans-synovial flow-*v*-pressure show that the synovial drainage pathway, the ECM, has a substantial hydraulic resistance (Fig. 2, middle line). This has been confirmed using a servo-null micropipette to probe the trans-synovial pressure gradients. This high resistance underpins the fluid-encapsulating role of synovium. If the ECM is depleted of bound HA using *Streptomyces* hyaluronidase, the hydraulic permeability increase 5-10 fold, showing that matrix HA plays a key role in maintaining a low hydraulic permeability (Fig. 2, upper line). The magnitude of the effect is remarkable because HA is only a minor component of the matrix (0.3 mg $(g\ tissue)^{-1}$). It may be that the µm-long HA molecule serves as the 'string' to which the other, major matrix components such as proteoglycans are attached (string of beads analogy). Cutting the string disrupts the matrix organization and hence its hydraulic resistance.

Synovial fluid HA buffers fluid outflow

The free HA in synovial fluid causes a second major reduction in joint fluid escape, though not through its viscosity. When a HA solution is infused into the rabbit knee cavity, trans-synovial flow no longer increases steeply with pressure. Raising the intra-articular pressure now causes such small increases in fluid loss that the relation is almost a plateau (Fig. 2, bottom curve). This buffering of outflow depends on the HA concentration and molecular size and is reversed by HA washout. It also depends on the synovial ECM, being abolished by a collagen-sparing protease, chymopapain.

The quasi-plateau shows that the opposition to trans-synovial filtration increases in proportion to the applied pressure, and this led to the idea of outflow buffering through concentration polarisation. The idea is that the vast HA molecules experience partial ultrafiltration (molecular reflection) during initial filtration at the synovial ECM surface, creating a concentrated HA 'filter-cake' at the interface (Fig. 3). The osmotic pressure of this layer provides the force that opposes filtration. The greater the filtration pressure, the more concentrated the layer and the greater the osmotic force countering fluid escape – the hydraulic equivalent of a rectifying ion channel. Fluid can still enter the joint from the capillaries without hindrance, but its egress over a much bigger surface area is hindered by HA concentration polarisation.

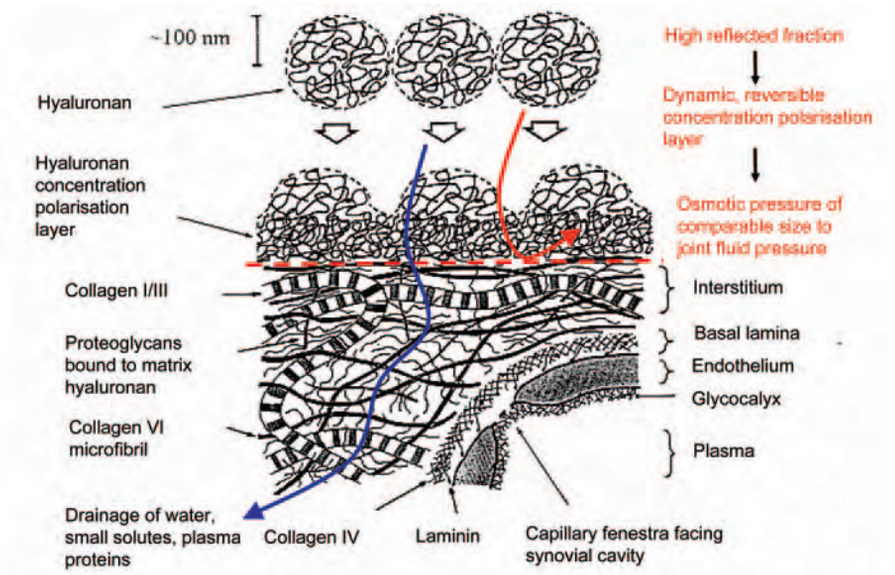

Figure 3. HA concentration polarisation hypothesis. Outflow buffering is explained by the osmotic pressure of reflected, concentrated HA at the interface with the interstitial drainage pathway. An increase in intra-articular pressure transiently raises outflow, but the accumulating, reflected HA then reduces outflow to only slightly more than before. Smaller solutes such as plasma albumin are not significantly reflected by the ECM (pore radius 33-59 nm).

Extracellular matrix as a molecular sieve

Does the joint lining reflect HA, as postulated above? When HA solutions were infused into anaesthetized rabbit knee joints and filtered under pressure across the joint lining, the intra-articular HA concentration increased and the subsynovial and lymph HA concentrations decreased, relative to the infusate (Fig. 4), indicating molecular ultrafiltration. Moreover, the proportion of HA molecules reflected fell with increasing filtration rates, a phenomenon characteristic of concentration polarisation (Lu *et al*. 2004). The reflected fraction depends on HA concentration and molecular size, just like the outflow buffering curves (Fig. 5). Depending on the conditions >90% of HA molecules are reflected and retained in the cavity. Since the cells lack tight junctions, the ECM must be the molecular sieve. In keeping with this, proteoglycan depletion by chymopapain almost completely abolishes HA reflection by rabbit synovium (Fig. 4) and increases the calculated interstitial pore radius from 33-59 nm to 192-336 nm.

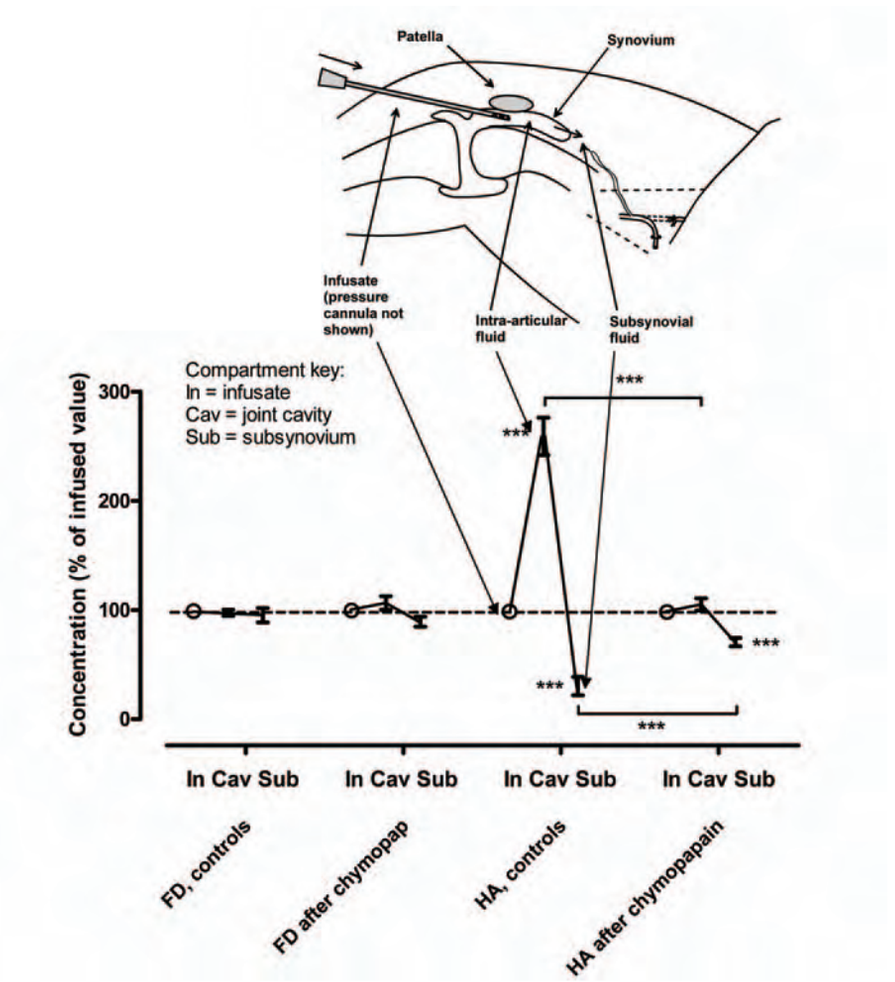

Figure 4. Demonstration of HA molecular sieving by synovium. An infused solution of hyaluronan (HA, 2000 kDa) and fluorescein dextran (FD, 20 kDa) is filtered across the synovial lining into the subsynovium and joint lymphatics. Analysis of fluid samples shows increased concentration upstream and reduced concentration downstream for HA but not FD. Removal of ECM proteoglycans and glycoproteins by protease (chymopapain) greatly reduces the ultrafiltration of HA. From Sabaratnam S *et al* (2007).

The concentration polarisation and outflow buffering can be modelled mathematically as a quasi-steady state phenomenon (providing a simple,

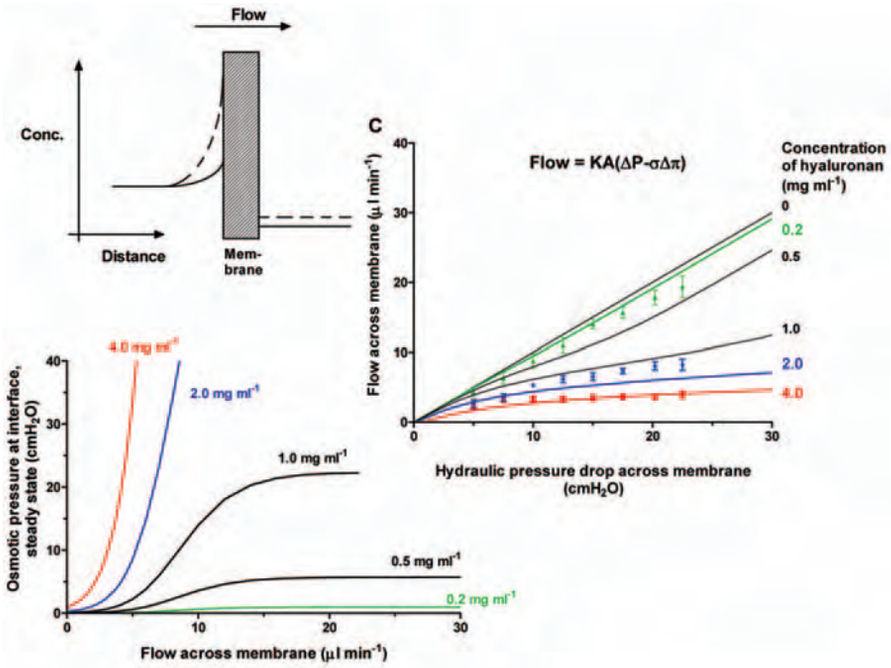

Figure 5. Predictions of non-steady state model of concentration polarisation (lines) with experimental results (points with SEM bars). *Top left*; HA concentration profile at partially reflecting ultrafilter for low (solid line) and high filtration rates (dashed line). HA accumulation is calculated from the rate at which molecules are washed towards the filter, the rate at which they diffuse back down the concentration gradient, and the rate at which they pass through the filter. *Bottom left*; calculated osmotic pressure at interface as a function of filtration rate for different bulk HA concentrations. The extremely steep rise for bulk HA concentrations >1 mg ml^{-1} is due to the highly nonlinear osmotic pressure-concentration relation. *Right*; trans-synovial filtration rate calculated from osmotic pressure difference $\Delta\pi$, hydraulic pressure difference ΔP, membrane area A and hydraulic conductance K. The theoretical outflow buffering at 4 mg ml^{-1} hyaluronan (a typical normal value) approximately matches the experimental observations. Model parameter values of Lu *et al*. (2005).

analytical solution) or more accurately as a non-steady state phenomenon (requiring computer simulation). Using experiment-derived parameters the models provide a good fit to the HA reflection results and outflow buffering curves over a range of HA concentrations (Fig. 5). The non-steady state model can be extended to describe the closed joint, where the boundary conditions differ from the open-ended infusion system used experimentally.

HA reflection and HA secretion

HA reflection has a wider importance besides its role as a guardian of joint fluid. Concentration polarisation boosts local viscous lubrication. Also, reflection prolongs the intra-articular working life of HA by an order of magnitude, and thus conserves energy-expensive replacement by synthesis. HA synthesis is a current growth point in the field. It is clear that mechano-sensitive regulatory pathways exist that couple HA secretion to joint usage; and, at least in culture, a Ca^{2+}-PKCα-ERK1/2 pathway contributes to stretch-stimulated HA secretion (Momberger *et al*. 2006). The mechanosensitive, ERK-mediated HA production is also crucial to joint cavitation during embryogenesis (Lewthwaite *et al*. 2006).

Implications for arthritis

Arthritic joints contains a reduced concentration of HA of reduced chain length. As a result, the fluid viscosity is reduced and outflow buffering is lost (Fig. 5). Metalloproteinase activity is high, so HA loss is presumably increased, which may help explain the high plasma HA levels seen in rheumatoid arthritis. Loss of outflow buffering will increase the fluid escape rate, helping to offset the increased fluid input from inflamed synovial microvessels (joint effusion). This 'beneficial' effect, however, is at the expense of increased loss of lubricant and the formation of periarticular oedema, which probably causes the characteristic morning stiffness of arthritis. The subsynovial oedema is due to poor coupling between subsynovial lymph drainage and trans-synovial filtration rate.

Intra-articular injections of HA are now a popular form of treatment for osteoarthritis in the USA and Japan. There is evidence of moderate benefit in moderately severe disease, but the mechanism of action over weeks/months is by no means clear.

Summary

The joint cavity lacks a tight cellular lining. Instead a biophysical hydraulic 'rectifier' has evolved, namely a reversible HA concentration polarisation layer. This greatly slows the fluid loss from the cavity through the extensive, relatively fast-draining ECM pathway in flexed joints (higher intra-articular pressure), while allowing unimpeded, slow plasma ultrafiltration into the cavity from superficial fenestrated capillaries in extended joints (low intra-articular pressure). Thus HA concentration polarisation compensates for the absence of the ion pump-based regulatory mechanisms found in many other fluid cavities; and reduces by an order of magnitude the need for replacement HA synthesis.

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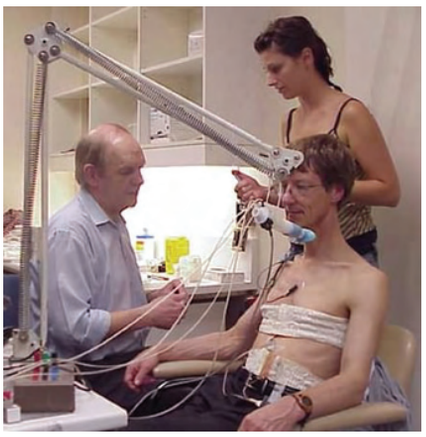
Neuromechanical matching of central respiratory drive: a new principle of motor unit recruitment?

Compared with the muscles involved in human dexterity or those involved in locomotion, the muscles responsible for ventilation receive little attention. Inspiration is achieved largely by contraction and shortening of the diaphragm and expiration is largely passive during 'quiet' breathing. The muscles lying between the ribs are easily ignored: they come in layers and the angulation of their muscle fascicles varies topographically along an intercostal space. Apart from the diaphragm, the function of the respiratory muscles was a subject of debate for many years (Campbell *et al.* 1970). Yet, it is now clear from careful electromyographic studies, that several other muscles acting on the chest wall contract rhythmically during quiet breathing – these include the scalene muscles, the parasternal intercostal muscles and the external intercostal muscles. Hence, there is a group of 'obligate' inspiratory muscles in quiet breathing. At the other extreme, if respiratory drive becomes sufficiently high, for example with a high concentration of inspired carbon dioxide, central respiratory drive potentials are observed in hindlimb motoneurones (Kirkwood *et al.* 2005).

Based on the Maxwell reciprocity theorem, Wilson & De Troyer (1992) showed that the mechanical advantage of a particular muscle acting on the chest wall (i.e. the change in pleural pressure produced by the muscle per unit muscle mass) could be assessed by measuring the length change of the muscle during inflation of the respiratory system. Thus, a muscle with a high mechanical advantage shortens more than a muscle with a low mechanical advantage. Using this method, these investigators then measured the distribution of mechanical advantage among the intercostal muscles in the different interspaces (De Troyer *et al.* 2005).



André De Troyer (left) and Anna Hudson prepare Simon Gandevia for investigation.

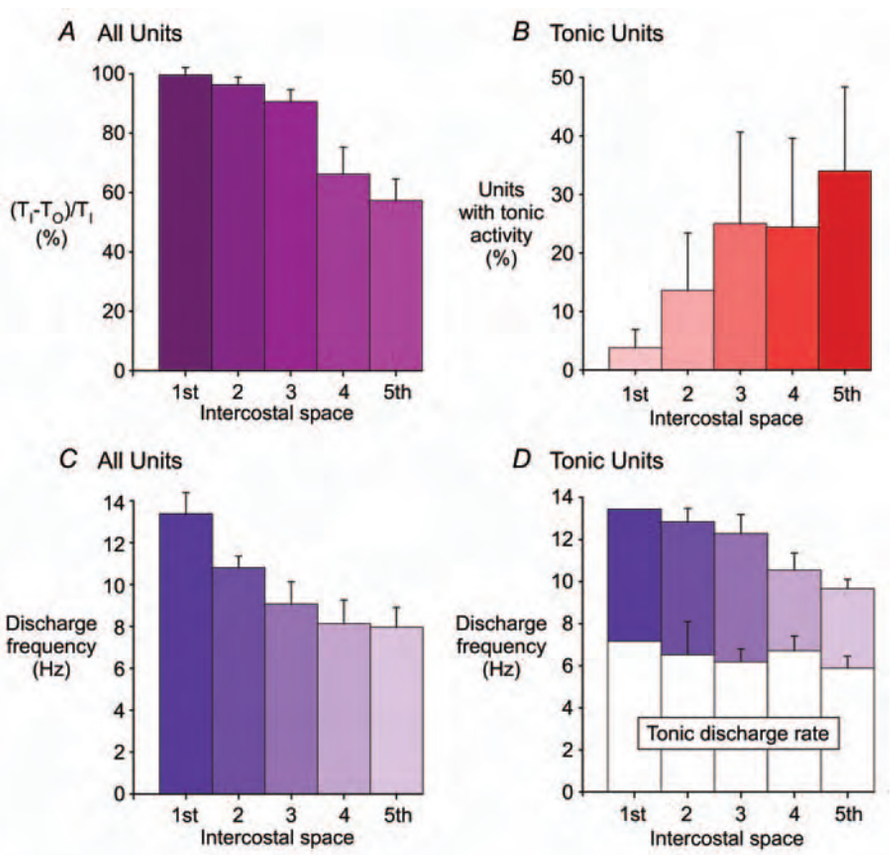


Figure 1. Topographic distribution of activity in the parasternal intercostal muscles. *A*, The duration of phasic inspiratory activity indicated relative to total inspiratory time (T_I) varied with interspace. The onset of inspiratory activity (T_O) was early in the cranial interspaces and delayed in the 4th and 5th spaces. *B*, Tonic activity was more common in caudal interspaces. *C*, The average inspiratory discharge rate (mean ± SEM) of all single motor units in the parasternal intercostals was highest in the first interspace (darkest bar) but decreased in a caudal direction (lighter bars). *D*, The units with tonic activity (panel B) had a similar discharge rate during expiration for all interspaces. However, as for the entire population (panel C), the inspiratory modulation of these units was greatest in the cranial interspaces (dark bars).

They subsequently evaluated the distribution of neural drive among these muscles during spontaneous breathing in anesthetized dogs, and these studies showed that muscles with a low mechanical advantage for inspiration become active relatively late in inspiration and contract weakly, whereas those with a high mechanical advantage become active early in inspiration and contract strongly. This immediately raises two questions: how is such a scheme organized to match the topographic drive to respiratory effectiveness in different regions of muscle, and, does such a scheme operate in awake animals and in humans? In dogs, this matching mechanism contains a 'hard-wired' element because it persists after denervation of the diaphragm and the chest wall (De Troyer *et al.* 2005). Hence, while afferents from these respiratory structures can influence inspiration, their activity is not necessary to generate a neural output that contains some 'neuromechanical matching'.

We applied methods of motor unit sampling that we had previously used to record from populations of motor units in healthy subjects and patients with respiratory disease (e.g. De Troyer

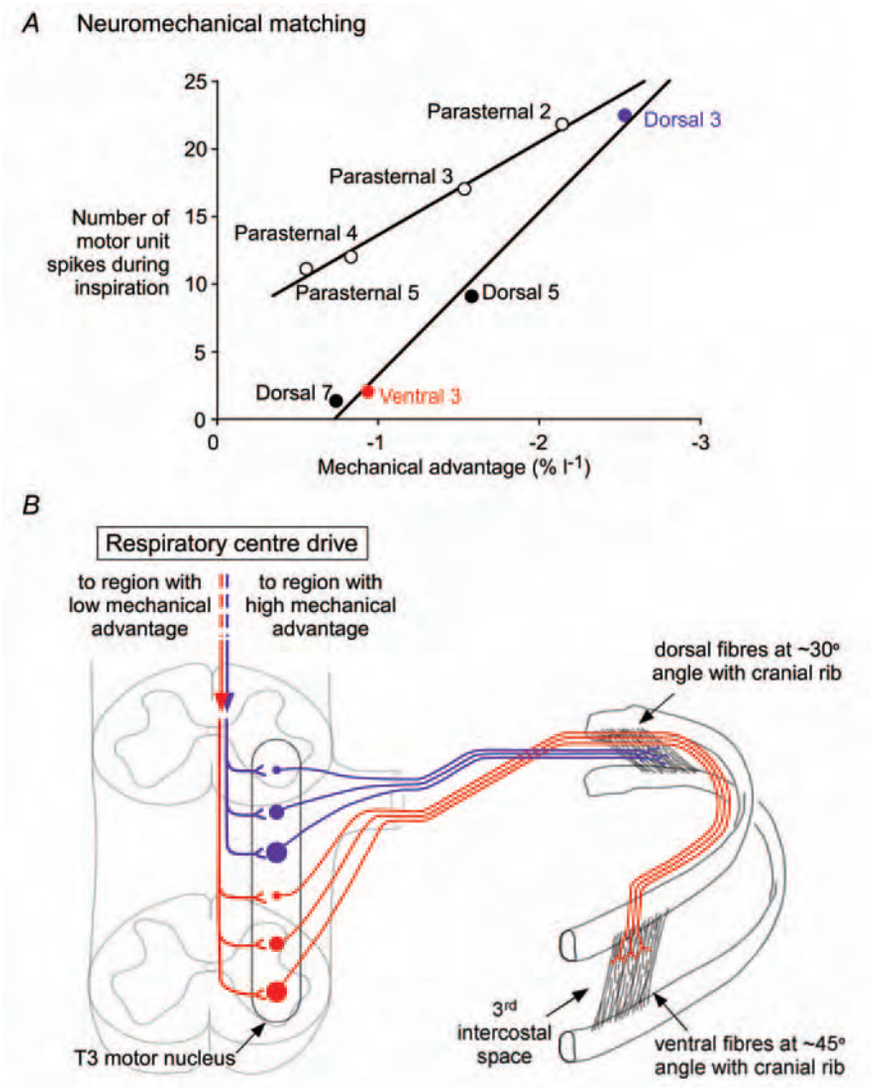


Figure 2. Neuromechanical matching in the human intercostal muscles.
A, Mechanical advantage is plotted against the mean number of motor unit spikes during inspiration (i.e. neural drive) for different regions of the parasternal (open circles), and external (closed circles) intercostal muscles. There is a strong linear relationship between drive and mechanical advantage for both muscles ($r^2 = 0.99$).
B, A mechanism for 'neuromechanical matching' in the intercostal muscles. During quiet breathing, descending drive from medullary respiratory centres depolarises motoneurones in the motor nucleus innervating the dorsal and ventral regions of the 3rd external intercostal muscle. Descending command recruits specific 'dorsal' (purple) or 'ventral' (red) motor units which sequentially activate the corresponding regions of muscle. The 'dorsal' units are recruited early, and reach high discharge frequencies, whereas the 'ventral' units are recruited later and reach lower frequencies. There may be recruitment of motoneurones according to Henneman's size principle within the 'dorsal' and 'ventral' pools of motoneurones, but not across the entire motor nucleus.

et al. 1997), to determine how the central nervous system drove the intercostal muscles during quiet breathing in awake seated humans (De Troyer *et al.* 2003; Gandevia *et al.* 2006). With the assistance of ultrasound and auditory feedback to the experimenter, monopolar recording electrodes are manipulated within regions of the intercostal muscles to record the activity of many single units. The timing and rate of their discharge is calculated. There are major topographical differences in the activity of motor units in the external intercostal muscles in quiet breathing: motor unit activity occurs early in inspiration in the 3rd dorsal external intercostal (after an average of ~10% of inspiratory time), but it occurs later in the 5th dorsal external intercostal (after about a third of inspiratory time) and is usually absent in the 7th dorsal external intercostal. The level of peak inspiratory motor unit discharge has a corresponding gradient: higher firing rates in the 3rd dorsal external intercostal compared with lower ones in the 5th dorsal external intercostal (11.9 ± 0.3 Hz versus 6.0 ± 0.5 Hz; mean ± SEM). Units discharging tonically, but still with an inspiratory modulation, are more frequent in the lower dorsal external intercostal spaces.

We then applied this approach to the full set of 5 parasternal intercostal muscles, with all spaces being studied in one session. Again, a marked topographical distribution of inspiratory activity occurs with a similar cranio-caudal gradient. Activity in the first three parasternal intercostals commences after ~10% inspiratory time, with an average peak single motor unit discharge of 13.4 ± 1.0 Hz in the first space (Fig. 1). However, activity starts after about a third of the inspiratory time in the lower two spaces and single motor unit discharge reaches only 8.0 ± 1.0 Hz in the 5th parasternal intercostal. Again, tonic activity is prominent in the lower spaces where inspiratory mechanical advantage is lower (Fig. 1*B*).

These results are intriguing for several reasons. First, within some spaces, such as the 3rd external intercostal space, there is also a diminution in the mechanical advantage along the intercostal space, from posterior to anterior, and this is paralleled by a reduction in neural drive (Fig. 2*A*). If, as has robustly been shown, there is an orderly recruitment of motoneurones according to Henneman's size principle (Henneman, 1957), then another principle may be superimposed (Fig. 2*B*). This would be recruitment according to the mechanical advantage of the muscle fibres innervated by the motoneurone. How and why would such a matching strategy be laid down in development? Does this principle of neuromechanical matching occur for structures apart from those in the trunk where muscles have a large lateral dimension relative to the lengths of their muscle tendon units? Is it adaptable?

Second, how might the descending drives from the medulla achieve the neuromechanical matching? The topographic and temporal disparities among the intercostals are unlikely to be achieved by the motoneurones receiving a near-synchronous descending drive (via 'central

respiratory drive potentials'). Some motoneurones firing tonically do not increase their inspiratory discharge until after many of their neighbours have begun to discharge phasically. This implies that the temporal dispersion in the activation times within a subset of motoneurones innervating a particular region of an intercostal muscle may reflect dispersion in timing of the descending inspiratory drive or sculpting of descending drive at a spinal level. Further, we do not yet know if similar neuromechanical matching accompanies voluntary inspiratory efforts.

Finally, the results highlight how little we know about the total daily activity patterns of the intercostal muscles. Their function includes trunk movement as well as phasic and tonic stabilization of the spine associated with upper and lower limb movement. This may explain why the very limited data on fibre-type for these muscles does not seem to match their activity patterns during quiet breathing, and why their inspiratory activity may be governed by a unique strategy.

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Parleys in phosphate

In my opinion the simplest tool ever devised to measure the active transport in the intestine is the everted gut sac! Segments of intestine can be turned inside out and made into sacs by tying both ends, after filling with a physiological solution containing the substance whose transport one wishes to study. The sacs are incubated in the same solution under physiological conditions for a fixed period. If an active transport of the substrate under study occurs, its concentration inside the sac builds up while the outside solution loses the substrate. Using this method my graduate student Mary and I studied the phosphate transport in the mouse intestine. Easy availability, less food consumption and cheaper cost were the considerations in choosing the mice. Mary, returning to research after many years of teaching, had a way of handling men and mice alike. Both need to be caged, she often used to say! We noted that the duodenal segment showed the maximum transport which dropped to the minimum in the adjoining jejunal segment only to rise again in the ileum to reach the submaximal level (Mary & Rao, 2004)

Hence it was a bit of consternation to see two papers (Radanovic *et al*. 2005 and Marks *et al*. 2006) that showed duodenum to be the least effective segment in transporting phosphate in mice. While it is easy to take shelter under headings like differing experimental conditions and newer technologies, I thought it may not be a bad idea to delve deeper into these investigations.

The pattern of increasing transport of phosphate from jejunum to ileum is common to all the three studies quoted above. Duodenal transport in our studies seems to be quite high when compared to others who used brush border membrane vesicles or in situ ligated loop to monitor the transport of phosphate. Both detected mRNA responsible for the synthesis of NaPillb in the mouse duodenum, even though the transporter itself is almost unnoticeable. And both mention that modes other than the sodium dependent NaPillb mediated transport, may exist. Such possibility is fortified by a recent report (Williams & De Luca, 2007) showing that phosphate transport *in vivo* does not require sodium! It is therefore most likely that the everted sacs we employed might have used all the transport modalities, sodium dependent and independent, to push phosphate uphill into the serosal compartment.

In our study we found that duodenum while transporting phosphate maximally also shows a gender difference, being higher in males. This difference has not been reported in either of the two differing papers since they have used only male mice for experimentation. Gender difference has been reported in rats with reference to intestinal calcium transport (Uhland-Smith & De Luca, 1993). Moreover, oestrogen (Colin *et al*. 1999) and testosterone (Hope *et al*. 1992) seem to stimulate it, specifically in the duodenum. Therefore, it is not unreasonable to assume that sex hormones may be responsible for the observed differences in phosphate transport too. Then how do we account for the poor duodenal transport of phosphate reported in the two studies, since the age and weight of mice employed in all the three studies is about the same. An old publication (Mirskaia & Crew 1930) gave me the hint. Time of onset of puberty and sexual maturity show a lot of variability in mice! A recent publication (Miller *et al*. 2002) also endorses such variability. Since I have become a 'social scientist' (if you wish further enlightenment on this term, refer to *Physiology News* **54**, 33-35) may I appeal to Marks *et al*. to reinvestigate the effect of sex hormones on the duodenal transport. In mice of course!

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Blood vessels signalling to neurones through nitric oxide

Most neuroscientists interested in information processing in the brain have synapses foremost in their minds, while conceding that glial cells situated nearby have important roles to play. Conceptually remote from this picture are the network of blood capillaries, but recent evidence suggests that, by releasing nitric oxide, they too influence neuronal function

Giti Garthwaite (top left), John Garthwaite (above) and Katalin Bartus (left).

Looking through a microscope at a histological section of brain, even at high magnification, the microvasculature usually passes unnoticed. Yet, dotted around are huge numbers of unimposing structures, only about 6 μm in diameter and mostly empty – the capillaries. When assembled in 3-dimensions, however, the capillaries form a complex labyrinth of tubing (Fig. 1*A*, *B*), any single part of which is maximally a typical cell diameter (25 μm) away from any neuronal or glial element. Of course, this plumbing arrangement is optimised for delivering and collecting dissolved gases needed for, or produced by, neuronal and glial cell metabolism. Now, it seems, the maze of microvessels also supplies its own dissolved gas, nitric oxide (NO), to affect the functioning of the brain.

The famous experiments of Robert Furchgott revealing the existence of an endothelium-derived relaxing factor (Furchgott & Zawadzki, 1980) and the subsequent identification of the factor as NO helped elevate the status of the endothelium from simply being the passive inner lining of blood vessels and introduced a new concept of cell-to-cell signalling that, in different ways, quickly became of importance to physiology and pathophysiology throughout the body. Endothelial cells manufacture NO from L-arginine using the eponymous endothelial NO synthase (eNOS) that is complexed with other proteins within specialised invaginations of the cell membrane. In the brain, eNOS is concentrated in the capillary network which lacks smooth muscle and is constituted largely of endothelial cells. The NO-synthesising enzyme within the neural tissue itself is mainly located in neurones (hence, nNOS) and is targeted to synapses where it binds to scaffold proteins that also link to the NMDA subclass of glutamate receptors found in most excitatory synapses. A third isoform, the inducible NO synthase, is not normally present but can be expressed in many different cell types during inflammatory conditions (Alderton *et al*. 2001).

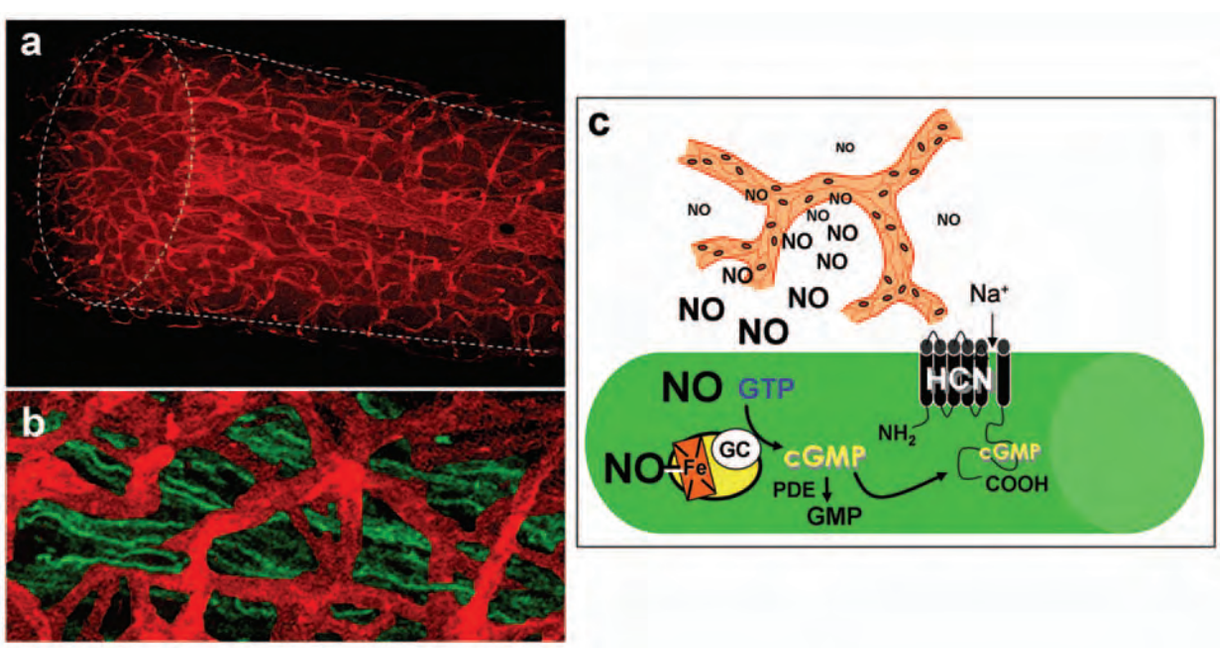

Figure 1. Signalling from blood vessels to axons in optic nerve. *A*, Whole-mount preparation of 10-day old rat optic nerve immunostained for eNOS. The dotted line depicts the shape of the nerve, including its cut end (ellipse). *B*, Composite illustration of the relationship between axons (green, neurofilament-200 immunostaining) and capillaries (red, eNOS immunostaining) in the optic nerve. C, Schematic of the proposed mechanism whereby NO generation from eNOS in the capillary circulation persistently depolarizes axons by raising the levels of cGMP which then acts on hyperpolarization-activated cyclic nucleotide-regulated (HCN) ion channels.

The physicochemical properties of NO set it apart from other signalling molecules. Firstly, it lacks chemical specialisation, although it does possess an extra (unpaired) electron, making it a radical. Some other radicals are chemically reactive and can cause damage to cells, but NO is stable in physiological concentrations, which are probably around a nanomole or less. Secondly, like oxygen and carbon dioxide, NO diffuses very quickly through membranes, obviating the need for a specialised release mechanism and endowing it with the ability to act on neighbouring cells within microseconds of its manufacture. Finally, NO binds avidly to haem groups possessing vacant coordination sites. This property has been exploited to provide highly sensitive NO detectors within cells, initiating physiological NO signal transduction. The relevant haem groups are attached to proteins possessing intrinsic guanylyl cyclase activity and, unlike the haems of haemoglobin, they exclude oxygen, allowing NO to bind without becoming oxidised. The binding of NO triggers a conformational change in the protein that propagates to the active site, resulting in the formation of cyclic GMP (cGMP) from GTP. In this way, NO concentrations of 1 nM or less can be detected and transduced into greatly amplified cGMP concentrations (Garthwaite, 2005).

The conventional mechanism for NO formation in the brain is activation of synaptic NMDA receptors by the neurotransmitter glutamate. The physical association of nNOS with NMDA receptors and the high permeability of NMDA receptor channels to calcium ions, on which nNOS activity depends (via calmodulin), combine to explain this special relationship. NMDA receptors are well-known for their role in the initiation of synaptic plasticity and studies on many different areas of the central nervous system have found that NO participates in certain forms of long-term potentiation (e.g. in the hippocampus, cerebral cortex, cerebellum and spinal cord) or depression (e.g. in the cerebellum and striatum). Here, NO probably operates only very locally, its sphere of influence being limited to the dimensions of a single synapse (Garthwaite, 2005).

Evidence that NO made in blood vessels provides signals to neurones came from two related studies. The first was carried out on a synapse-free stretch of central white matter, the optic nerve, in which axons run from the retina to the visual centres of the brain (Fig. 1*A*, *B*). Our curiosity was raised by two unexpected observations. The first was that optic nerve axons are greatly enriched in NO receptors so that exposure to NO causes cGMP to accumulate in them to high levels. The second observation was that, through cGMP, exogenous NO causes the axons to depolarize by a few millivolts. For these findings to have any physiological meaning, there would need to be a local source of NO. Sure enough, when NO synthase activity was blocked, the axons hyperpolarized by a few millivolts, implying that NO was continuously being formed in sufficient amounts to keep them depolarized. The source was eventually identified as eNOS in endothelial cells. Moreover, increasing or decreasing the activity of eNOS caused opposite changes in the membrane potential of the axons, signifying a dynamic coupling between endothelial cell activity and axon function (Garthwaite *et al*. 2006). The voltage responses in the axons were brought about by alterations in the activity of a class of cyclic nucleotide-regulated ion channels (Fig. 1*C*). Among other functions, these channels help maintain reliable conduction during high frequency axonal firing.

The second study originated in trying to understand the role played by NO in synaptic plasticity in the hippocampus where we were puzzled to find that there needs to be a continuous low-level of endogenous NO in order for the synapses to become enduringly potentiated when exogenous NO is given at the same time as a weak synaptic stimulation. Again, eNOS present in blood vessels was pinpointed as the source of the continuous NO supply (Hopper *et al*. 2006). These findings lead to a picture in which NO derived from eNOS provides a global 'enabling' signal to the hippocampal synapses, priming them to respond to discrete nNOS-derived signals when NMDA receptors become active.

Although many details of the underlying mechanisms are missing, the notion that endothelium-derived NO affect brain neurones has circumstantial support from various quarters. For example, mice lacking eNOS show defective synaptic plasticity not only in the hippocampus but also in the cerebral cortex and striatum. Furthermore, they also exhibit various neurochemical and behavioural phenotypes (e.g. much reduced male aggression) and decreased neurogenesis (for references, see Garthwaite *et al*. 2006; Hopper & Garthwaite, 2006).

Many fascinating questions remain about the function of this line of communication. Are blood vessels persistently contributing to neuronal excitability in regions other than the optic nerve? Does the pathway provide a new link between the periphery and central nervous system and help explain, for instance, how hormones (e.g. oestrogens) or physical exercise, both of which increase eNOS activity, influence brain function? Could the loss of eNOS activity known to be caused by β-amyloid contribute to the symptoms of Alzheimer's disease?

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Do birds experience visual illusions?

An object or motion we see does not always exist in the real world due to misinterpretations of retinal images by the brain. Where and how visual illusions occur are debated, and their elucidation would bridge psychology and neuronal activity

Psychologists and physiologists hold a view that the visual illusion is subjective perception of something that is seen but lacks physical counterpart. For example, movie is actually the visual illusion of motion and continuity of a sequence of stationary photographs. Visual illusions reflect how visual information is processed and thus may act as a 'window' into the mysteries of the brain.

An object is segregated from background by its shape defined by contours or edges. Real contours are defined by 'first-order' visual cues such as luminance or colour contrasts; some other contours cannot be directly seen but inferred by the brain from 'second-order' cues such as textures and relative motion and thus called illusory contours. In experimental studies, a gap formed between two groups of gratings and phase-shifted abutting gratings are usually used as illusory contours because they are inferred from textures but not directly seen from luminance contrasts (Fig. 1). Motion is also a main physical attribute of an object. If you stare at running water in a waterfall for minutes and then shift your gaze to objects on the bank, you will see the objects moving upwards. This phenomenon is called the waterfall illusion or motion after-effect (MAE) in general (Anstis *et al*. 1998).

Figure 3. Yu-Qiong Niu, Shu-Rong Wang, and Rui-Feng Liu (from left to right) have a discussion on visual information processing in the pigeon's brain.

In recent years, the response characteristics of visual neurons to illusory contours and motion have attracted a great deal of attention (Eagleman, 2001; Nieder, 2002). It is known that cortical neurons in monkeys and telencephalic neurons in owls can detect illusory contours. On the other hand, MAE also occurs in cortical areas sensitive to visual motion. All this suggests that visual illusions may be exclusively detected in cortical neurons (Anstis *et al*. 1998). However, whether visual illusions are also processed in subcortical areas remains unknown and how to explain the neuronal mechanisms underlying MAE is intensely debated (Eagleman, 2001; Nieder, 2002).

The bird visual system provides a good model to study the neuronal mechanisms underlying visual illusions for two reasons. First, visual neurons in the pretectal nucleus lentiformis mesencephali (nLM) can detect real edges in gratings moving through their receptive fields (Fu *et al*. 1998), implying that they may also be able to detect illusory contours. On the other hand, nLM and its mammalian counterpart, the nucleus of the optic tract, are both involved in generating optokinetic nystagmus to stabilize retinal images so that their neurons should detect anything in motion. It is interesting to note that the excitatory (ERF) and inhibitory receptive field (IRF) of pretectal neurons in pigeons overlap in visual space but possess opposite directionalities (Fu *et al*. 1998; Cao *et al*. 2004). This opponent RF organization implies that after-responses of pretectal neurons to cessation of visual stimulus motion in one direction would create illusory motion in the opposite direction.

In our experiments, pretectal ERF and IRF were mapped with a computer and color-coded (Fig. 2). Real and illusory contours were generated and moved by the computer as visual stimuli presented to the pigeon, and electrophysiological responses of single nLM neurons to these stimuli were recorded with micropipettes filled with a solution containing sodium acetate for

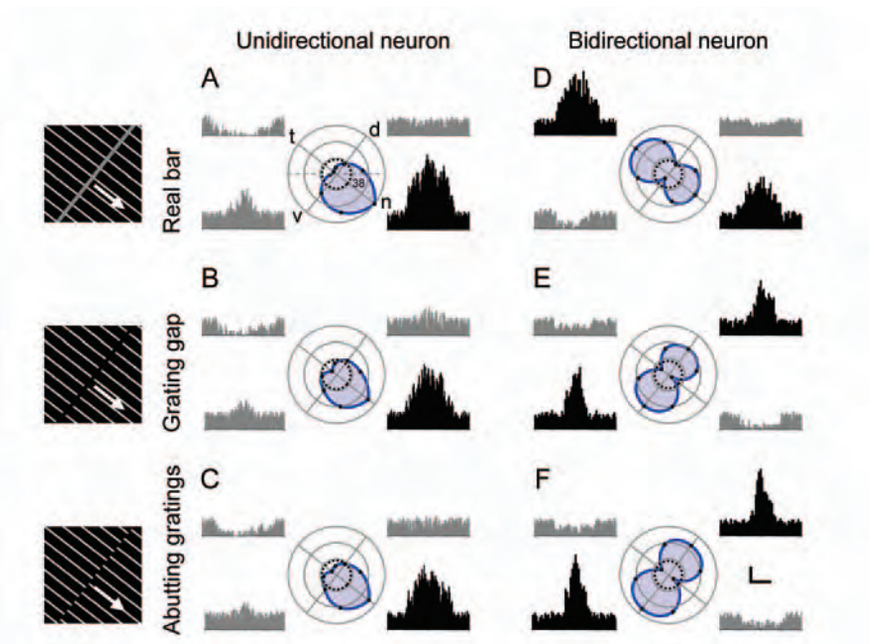

Figure 1. Directional tuning curves in two pretectal neurons (A-C, D-F) for real (AD) and illusory contours (BE, CF). Histograms are obtained from firing rates evoked by motion in eight directions spaced by 45 deg but only those in four orthogonal directions are depicted for clarity. The preferred directions are identical in unidirectionals (A-C) but changed by ~ 90 visual degrees in bidirectionals (D-F) for real versus illusory contours.

recording neuronal spikes and pontamine skyblue for dye-marking recording sites. Firing spikes in pretectal neurons were collected on-line and analyzed off-line with the computer (Niu *et al*. 2006).

All the 204 neurons recorded in the pigeon's nLM responded vigorously to visual motion. They could be divided into two groups according to their selectivity for the direction of motion: one group contains 195 unidirectional neurons that fired maximal rate to motion in one (preferred) direction and minimal rate in the opposite (null) direction, and the other group includes nine bidirectional cells which were characterized by maximal rates in two opposite directions and minimal rates in the two directions orthogonal to both preferred directions.

The pretectal neurons examined in our study all responded similarly to real and illusory contours in terms of firing patterns and rates. The preferred directions in the unidirectionals were identical for real and illusory contours whereas those in the bidirectionals were changed by ~ 90 visual degrees for real versus illusory contours (Fig. 1). It seems that pretectal bidirectionals can discriminate real from illusory contours but unidirectionals cannot. On the other hand, some nLM neurons produce excitatory responses to visual motion and inhibitory after-responses to cessation of prolonged motion in the preferred directions, or inhibitory responses to visual motion and excitatory after-responses to cessation of prolonged motion in the null direction (Fig. 2). These after-responses to cessation of prolonged motion in the preferred (null) direction were similar to the visual responses to real motion in the null (preferred) direction. They had threshold duration of motion and persisted longer as motion duration was increased. These characteristics are quite similar to those of MAE reported by the subjects in our psychological experiments. Because the ERF and IRF of a pretectal cell overlap in visual space and possess opposite directionalities, after-responses to cessation of prolonged motion in one direction may create illusory motion in the opposite direction. To histologically verify the exact locations of recorded neurons, 25 recording sites were marked with dye and all were localized within the pretectal nucleus (Niu *et al*. 2006).

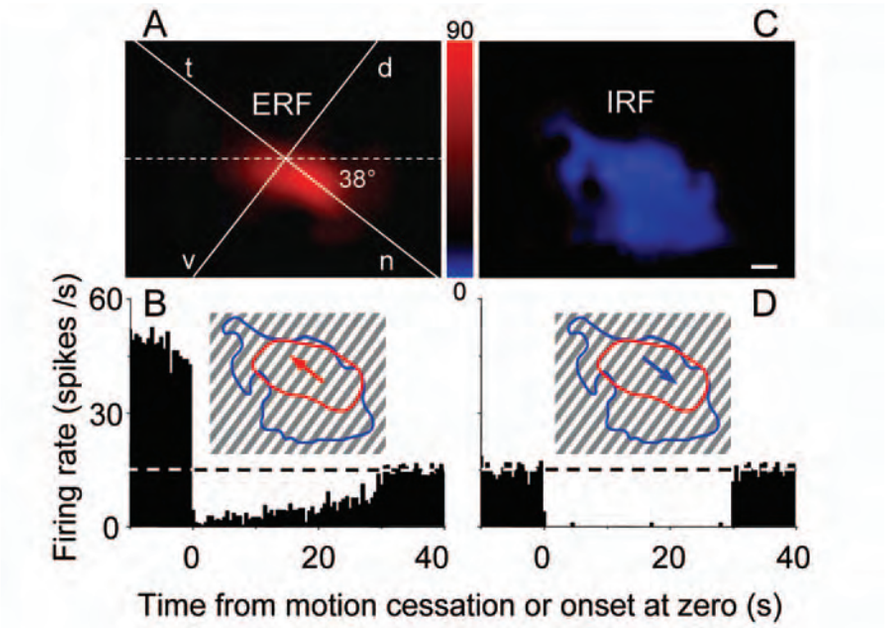

Figure 2. Excitatory (ERF) and inhibitory (IRF) receptive fields and their opposite directionalities in a pretectal cell may underlie motion aftereffect. Inhibitory after-responses of this cell to cessation of prolonged motion in the preferred direction (A, B) approximate its visual responses to real motion in the null direction (C, D). ERF (red) and IRF (blue) are computer-mapped and color-coded with a scale (spikes /s) between A and C.

It appears that illusory contours and motion could be detected at the earliest stage of central information processing and processed in bottom-up streams from subcortical areas to the telencephalon, and that the waterfall illusion or motion aftereffect may result from functional interactions of ERF and IRF with opposite directional selectivity in visual neurons.

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Meetings memories

When the calendar for Scientific Meetings followed a fixed pattern, those in certain places tended to embrace certain traditions (including dense November fog at the Mill Hill Meeting). If the March Meeting at University College London coincided with the Oxford and Cambridge Boat Race, this was usually shown on a TV screen in the big Class Room. Similarly, when Sir Bryan Matthews held the Chair in Cambridge he arranged that, if relevant, the Test Match score would be chalked up on a blackboard in the Lecture Theatre. Those in the audience who had no interest in cricket would be puzzled by a technician who nipped in periodically to write mysterious numbers under 'A' [Australia] and 'B' [Britain]. This practice, which continued for a while after Sir Bryan's retirement, was in operation at the time that Abby Fowden gave her first Communication to The Society. Just as she came to the end, the latest score was posted. She found the enthusiastic applause most gratifying.

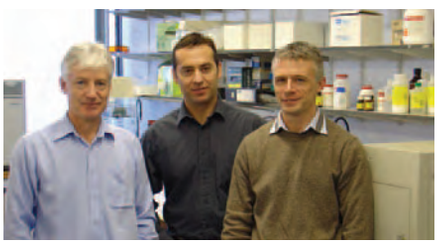
Ann Silver

Adaptive responses to starvation in humans: important role for skeletal muscle PDK4

In humans, adaptations to fasting have evolved in order to survive periods of limited food resources and thus starvation. One important aspect of the adaptive response to fasting is a reduction in skeletal muscle carbohydrate utilization in order to spare glucose for those organs and tissues with an obligatory requirement for it (e.g. central nervous system). Muscle pyruvate dehydrogenase kinase (PDK4), the enzyme that impairs the rate-limiting step in glucose oxidation, is of major importance in mediating the starvation-induced shift in metabolic fuel utilization

The physiological and metabolic responses to fasting have been studied extensively in many species over the last 150 years. William North published his studies on the effects of starvation and exercise on nitrogen metabolism (in which he acted as his own subject and observed an increase in whole body nitrogen excretion under both conditions) in June 1878, in the second ever issue of *The Journal of Physiology* (North, 1878). In the same journal Pembrey and Spriggs (1904) observed that during fasting in rats the respiratory quotient (and thus glucose oxidation) decreased quickly within the first few days of fasting and remained constant during the prolongation of fast. In 1932, Goldblatt and his co-workers in St Thomas's Hospital, London presented evidence of carbohydrate (CHO) intolerance after starvation in healthy men (Goldblatt & Ellis, 1932). Specifically, in response to ingestion of a standardized glucose load following a 39h fast, as opposed to overnight fast, they observed an augmented blood glucose response and lower respiratory quotient and CHO oxidation rate. The starvation-induced impairment in CHO oxidation persisted even after injection of 10 units of insulin prior to glucose ingestion, prompting the authors to suggest that this may be an important adaptation which facilitates glycogen repletion in tissues (such as skeletal muscle and liver) that may have incurred a fall in glycogen content during the previous period of starvation. To our knowledge, this was the first experimental evidence of fasting-induced insulin resistance in humans.

Although these questions are still pertinent today, limited progress has been made since those pioneering studies in elucidating the precise



Ian Macdonald (left), Kostas Tsintzas and Andy Bennett

mechanism(s) controlling the starvation-induced switch in substrate utilization and the development of insulin resistance in humans. Studies on humans from our laboratory in the 1990s using insulin clamps and stable isotopes demonstrated a reduction in whole body insulin sensitivity and a shift in basal (non-insulin) and insulin-stimulated substrate utilization from CHO to fat after 36-72h of starvation (Mansell & Macdonald, 1990; Webber *et al.* 1994). Importantly, starvation resulted in a marked reduction in glucose uptake by the forearm muscle both in the basal state and during insulin infusion, indicating profound muscle insulin resistance (Mansell & Macdonald, 1990).

What is the mechanism by which skeletal muscle downregulates its glucose oxidation? Some elegant animal studies performed by Mary Sugden and her coworkers in the late 1980s and early 1990s showed that suppression of skeletal muscle pyruvate dehydrogenase complex (PDC) activity plays a major role in the down-regulation of glucose oxidation in response to starvation (Sugden *et al.* 1993). Skeletal muscle PDC activity is inhibited by phosphorylation of the complex by pyruvate dehydrogenase kinase (PDK). The more recent identification of at least four different forms of PDK (PDK1-4) in skeletal muscle has intensified the research on their role as potential molecular regulators of glucose oxidation under a number of nutritional and pathological conditions of altered glucose homeostasis, including starvation and refeeding.

Therefore, our most recent study using insulin tolerance tests and muscle biopsies attempted to elucidate the intramuscular mechanisms underlying the adaptive response to fasting for 48h and to subsequent refeeding with a CHO-rich diet for 24h in healthy humans (Tsintzas *et al.* 2006). Our findings confirmed the previously demonstrated starvation-induced development of insulin resistance (as whole body insulin sensitivity decreased by ~42% after fasting) and demonstrated that this persists even after 24h of refeeding (as insulin sensitivity recovered by only half of the reduction upon refeeding) (Fig. 1*A*). Similarly, starvation decreased and refeeding increased skeletal muscle PDC activity, although there was a tendency for the latter to be lower after refeeding when compared with the pre-starvation value (Fig. 1*A*). As PDK is a major regulator of PDC, we also measured the expression of all four isoforms of PDK identified in human skeletal muscle. Starvation increased muscle PDK4 gene and protein content (without affecting the other three isoforms), whereas refeeding reversed these responses (Fig. 1*B*). We concluded that the selective increase in protein content of PDK4 in human skeletal muscle is an important adaptation to starvation, which most likely contributes to the long-term control of PDC activity and thus reduction of CHO oxidation under those conditions. In addition to these changes in factors affecting glucose oxidation, we also observed a decrease

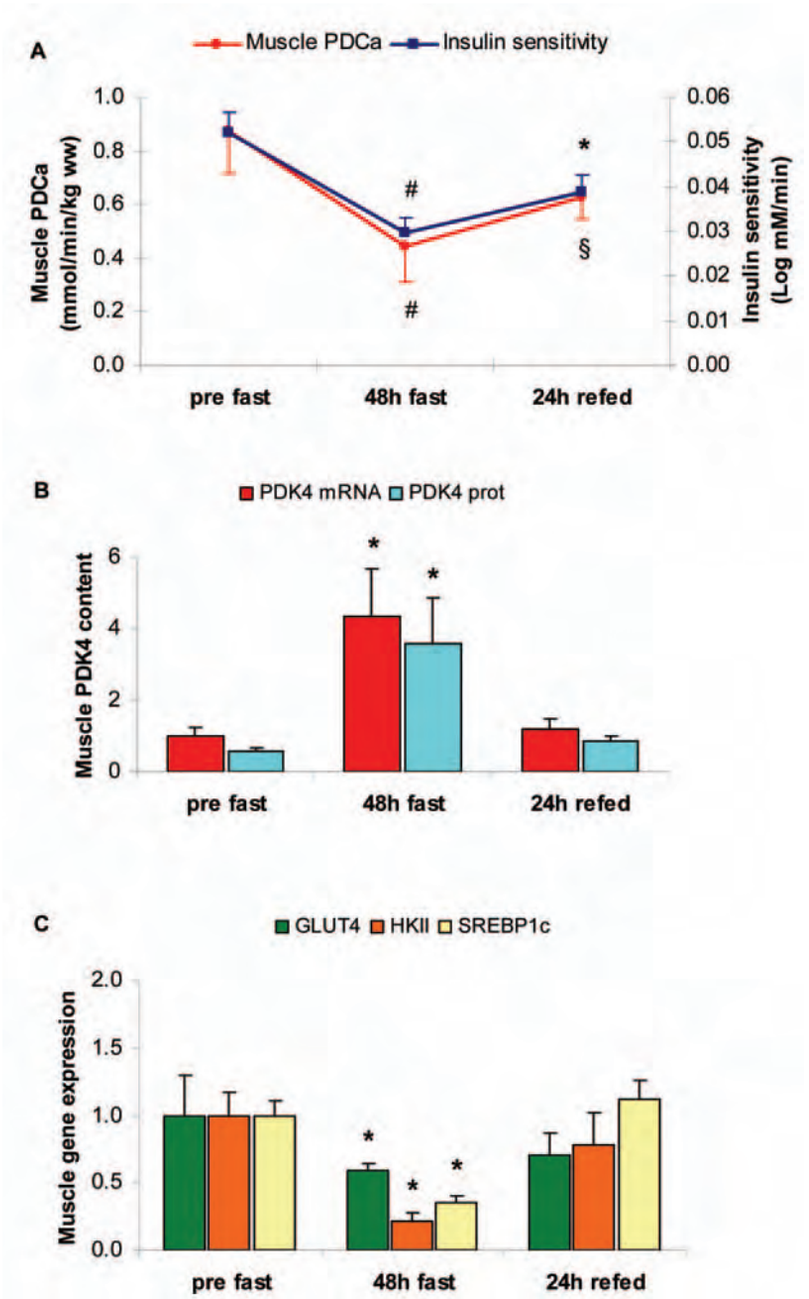


Figure 1. Whole body insulin sensitivity and skeletal muscle PDC activity (**1A**), PDK4 mRNA and protein (**1B**) and GLUT4, HKII and SREBP1c mRNA (**1C**) responses to 48 h starvation (Fast) and 24 h refeeding (Refed) with a high CHO diet. Values are mean ± SEM; n = 10 for insulin sensitivity and all mRNA data and n = 9 for PDCa and PDK4 protein data. $^{\#}P < 0.01$ from pre-fast; $^{*}P < 0.05$ from pre-fast; §$P < 0.10$ from pre-fast.

in the expression of key genes involved in muscle glucose uptake and phosphorylation: hexokinase II (HKII) and SREBP1c (a transcription factor that mediates the effects of insulin on HKII) were downregulated by 5- and 2.5-fold, respectively, after 48h of starvation whereas refeeding completely reversed these responses (Fig. 1*C*). Although muscle GLUT4 content also decreased by ~2-fold in response to starvation, its reversal was incomplete by refeeding and closely matched the slow recovery of insulin sensitivity (unpublished observation) (Fig. 1*C*).

What is the mechanism by which starvation increases PDK4 content in human skeletal muscle? We have recently shown for the first time in healthy humans that insulin can suppress PDK4 gene expression in human skeletal muscle (Chokkalingam *et al.* 2007) whereas elevated levels of plasma free fatty acids (FFA) can elevate the muscle PDK4 content (unpublished observation). Since circulating insulin concentrations decrease whereas FFA levels increase during starvation, these may be important regulators of PDK4 expression in muscle during starvation.

However, in contrast to what one may expect, starvation or refeeding failed to alter the content and/or activate (by phosphorylation) important muscle insulin signalling proteins (including IRS1 and 2, Akt/PKB, FOXO1) (Tsintzas *et al.* 2006). Our findings also showed that starvation and the concomitant increase in circulating fatty acids did not affect the expression of transcription factors [peroxisome proliferator-activated receptors (PPARs) and their coactivator PGC1α] and key genes involved in muscle fatty acid uptake and oxidation, namely fatty acid translocase (CD36), carnitine palmitoyltransferase 1 (CPT1) and long-chain acyl-CoA dehydrogenase (LCAD) (Fig. 2). Collectively, these findings suggest that, in contrast to results from animal and *in vitro* studies, an increase in skeletal muscle PDK4 content in fasted humans may occur in a novel manner distinct from the PPARs and insulin signalling pathways. Future studies should examine whether changes in intramuscular substrate availability/flux are responsible for the adaptive changes in glucose metabolism during fasting in humans.

In summary, it has been known for a long time that healthy humans adapt to fasting by increasing fat and reducing carbohydrate utilization in skeletal muscle in order to spare glucose for those organs and tissues (e.g. brain) with an obligatory requirement for it. We have shown for the first time that during starvation in humans, unlike rodents, regulation of fat metabolism does not require an adaptive response at the level of gene expression, implying a much greater capacity for fat oxidation than is utilized in the overnight fasted state. In contrast, changes in the content of key genes involved in glucose uptake (GLUT4), phosphorylation (SREBP1c and HKII) and oxidation (PDK4) are required to switch off glucose utilization by muscle

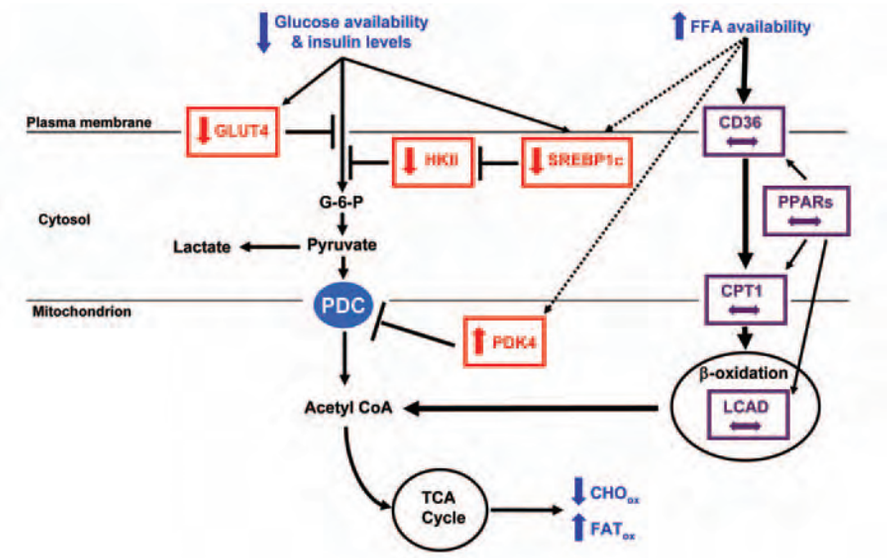


Figure 2. Simplified diagram of the adaptive response to starvation showing a shift in substrate utilization from CHO to fat in human skeletal muscle. The increase in fat availability and oxidation during starvation does not require changes in the expression of genes (CD36, CPT1 and LCAD) and transcription factors (PPARs) involved in fat metabolism (shown in purple). On the other hand, genes involved in glucose uptake (GLUT4), phosphorylation (HKII and SREBP1c) and oxidation (PDK4) are switched off (shown in red). Physiological and metabolic responses are shown in blue. Arrows indicate direction and magnitude of responses to starvation. Dashed lines indicate potential interactions.

tissue (Fig. 2). This may represent an important aspect of the molecular basis of the development of insulin resistance in metabolic conditions characterized by energy restriction.

Acknowledgments

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Ernest M Wright, professor of physiology at the David Geffen School of Medicine at UCLA and a Society Member, has been elected to the German Academy of Sciences Leopoldina in recognition of his scientific achievements in the field of transport proteins which carry essential molecules in and out of cells.

Founded in 1652, and officially renamed after Emperor Leopold I in 1677, Leopoldina is the world's oldest society of scholars in the natural sciences. The number of members below the age of 75 is limited to 1,000. Three quarters of its members come from Germany, Austria and Switzerland, and the remainder from more than 30 other countries. There are currently 34 Nobel Prize winners and, in total, there have been 163 members awarded this honour. There are 28 sections of the Leopoldina ranging from mathematics to medicine.

The antique equipment show

Have you ever noticed a pile of redundant equipment in a cupboard under the stairs or in that old store room that has been neglected for the last decade or so? Have you ever wondered why these old pieces of equipment are gathering dust in the corner? Were you thinking of putting them in the skip? All too likely as 'old-style' physiology departments merge into mega departments with relocation, refurbishment and redundancy. But someone at some time must have wanted to keep it so why not find out what it is for, who used it and whether it really is worth keeping?

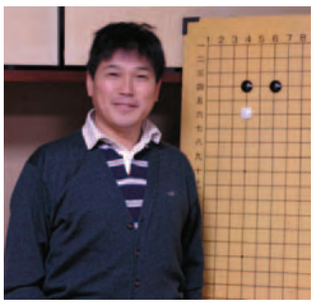
One of the functions of the The Physiological Society's History and Archives Committee is the preservation of interesting pieces of equipment that may be significant in illustrating and archiving the history of physiology and of The Society. Pieces of equipment could be one of a kind or crucial to important experiments which advanced the subject. The Committee ensures that the existence of the equipment is documented and is currently making arrangements to collect and store important pieces of equipment.

Apparatus used in the 1960 Hodgkin & Horowicz paper on the effect of sudden changes in ionic concentrations on the membrane potential of single muscle fibres. *J Physiol* **153**, 370-385.

So before you consign a dusty relic to rubbish please try and find out a little bit about it, who used it, and for what, and its approximate age. If you think it might be of historical interest please contact Simon Kellas (skellas@physoc.org) in the first instance and he will get in touch with a member of the History and Archives Committee who will take your enquiry further. Remember we need to preserve our history, and with a little bit of time and care we can all contribute to this process.

Saffron Whitehead
History and Archives Committee

Multi-electrode array recording of gut pacemaker activity



Shinsuke Nakayama

After swallowing, peristaltic waves flowing throughout the gastrointestinal (GI) tract automatically transport food towards the rectum. It is well known that the neural circuit of the enteric nervous system is responsible for this activity: namely, the mechanical pressure of the food in the lumen simultaneously stimulates excitatory motor neurones of the anterior gut and inhibitory motor neurones of the posterior gut via interneurones, thereby co-ordinating proximal contraction and distal relaxation.

The existence of special pacemaker cells for GI motility has been recently recognised. Interstitial cells expressing c-Kit (Maeda *et al*. 1992), a receptor tyrosine kinase, are distributed throughout GI tract and are referred to as interstitial cells of Cajal (ICCs) due to histological resemblance. The mechanisms underlying spontaneous electrical activities in the GI tract have remained unclear for a long time, but, with the progress of ICC studies, are now beginning to be identified.

Network-forming ICCs in the myenteric region (ICC-MyP) are thought to generate primary pacemaker potentials (Dickens *et al*. 1999). It is hypothsised that spontaneous Ca^{2+} oscillations in ICCs (Torihashi *et al*. 2002), produced by co-ordinated actions of intracellular Ca^{2+} release channels and Ca^{2+}-permeable channels in the plasmalemma (Aoyama *et al*. 2004; Liu *et al*. 2005; Nakayama *et al*. 2005), periodically activate plasmalemmal Ca^{2+}-dependent ion channels, e.g. Ca^{2+}-activated Cl^- channels and non-selective cation channels, thereby activating smooth muscle cells connected electrically (Nakayama & Torihashi, 2002). Furthermore, distinct types of ICCs (intramuscular ICCs: ICC-IM) act as intermediates between ICC-MyP and GI smooth muscle, amplifying ICC-MyP pacemaker activity (Fig. 1).

Similarly to the essential co-operation of enteric neural activities, it is important to understand how network-forming interstitial cells produce total pacemaker electrical activity along the GI tract. However, electrophysiological recordings generally require expert techniques, particularly for simultaneous membrane potential recordings from multiple sites. It may take years for such a research project to achieve reliable results.

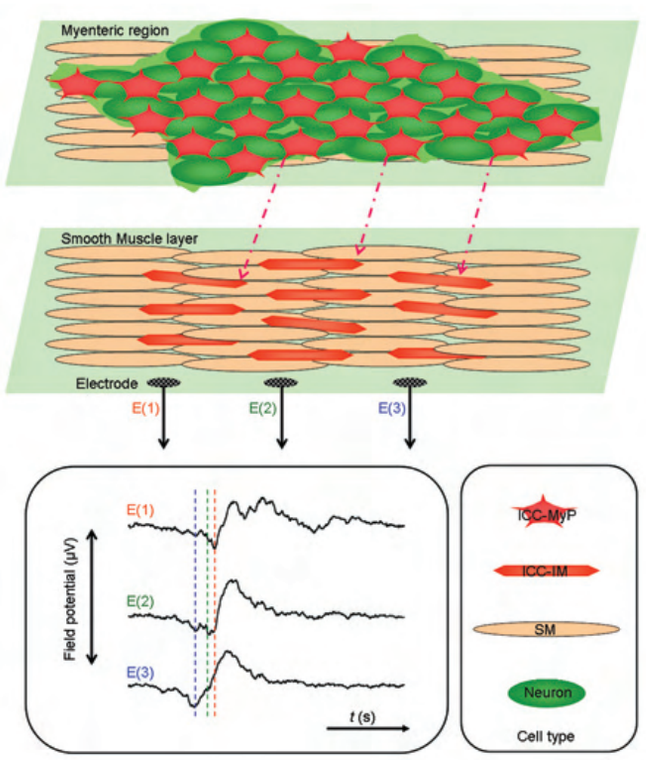


Figure 1. Diagram showing the distribution of ICCs in the myenteric region (top panel) and smooth muscle layer (middle panel). In the myenteric region ICCs (ICC-MyP) are distributed in a network structure. These cells produce primary pacemaker potentials which drive smooth muscle contraction via intramuscular ICCs (ICC-IM). Lower panel shows an example of phase-shifting pacemaker activities recorded via extracellular electrodes (8 x 8 planar electrodes).

In our paper, a convenient method to enable spatio-temporal analyses of ICC pacemaker electrical activity was introduced (Nakayama *et al*. 2006). The top panel of Fig. 2 displays a recording chamber with 64 planar electrodes (8 x 8 grid with a polar distance of 300 μm, corresponding to a ~2 x 2 mm recording area) on the bottom plane, where the smooth muscle tissue isolated from the stomach of guinea-pig is mounted. The field potentials were simultaneously measured over the recording area via a multi-channel amplifier. This system has been applied to record spike-like fast electrical activities in brain slices. To record slowly oscillating GI pacemaker activity, it is important to apply an appropriate high-pass filter (Brock & Cunnane, 1987). In this recording, we normally applied 0.1 Hz, because a high-pass filter of 1 Hz or higher greatly reduces the signal intensity. Moreover, to differentiate ICC pacemaker electrical activity from smooth muscle activity, it is necessary to add dihydropyridine (DHP) Ca^{2+} antagonists to the medium. This drug and analogs selectively block L-type Ca^{2+} channels, which make a major contribution to phasic smooth muscle contraction accompanied by depolarisation (Huang *et al*. 1999; Dickens *et al*. 1999).

A typical ICC pacemaker electrical activity (field potential) recorded from

the gastric antrum consisted of an initial, fast negative potential (red arrow in the middle panel of Fig. 2) followed by a slowly decaying component (green double arrow).

Potential images reconstructed from the multi-channel recordings revealed a phase shift of ICC pacemaker activity clearly resolvable in the small recording region. Spontaneous electrical activity frequently propagated from the oral to the anal end in the longitudinal direction. Furthermore, a phase shift in the circular direction was observed in many preparations. TTX had little effect in the phase shift, suggesting that the ICC network itself works through phase-shifting Ca^{2+} oscillations. Presumably, this mechanism plays an important role in producing smooth peristaltic waves to squeeze the luminal content and complete emptying of the GI tract.

Approximately 20 years ago, an attempt to measure the distribution of GI pacemaker activity using multiple glass electrodes was made in our lab under the supervision of Emeritus professor Tadao Tomita. We aimed to elucidate the interaction of GI pacemaker activity, and we got several interesting results. For example, some of the results suggested that the slow component of the field potential is produced by the interaction of electrical activities in a relatively wide area rather than by the local pacemaking cells just beneath the recording electrode. However, the data obtained at that time were not published because field potentials acquired from a small number of recording sites were not considered to be reliable enough.

Later the arrayed 8 x 8 planar electrode system became available. Thus, some of our speculations become a reality, which was a satisfactory result. It was demonstrated that the slow component also involves the influence of an initial fast component generated from a region with rather long distance (>1000 µm) as well as the plateau and repolarising components of spontaneous electrical activity produced by the nearest pacemaker cells to the recording electrode (Nakayama *et al*. 2006).

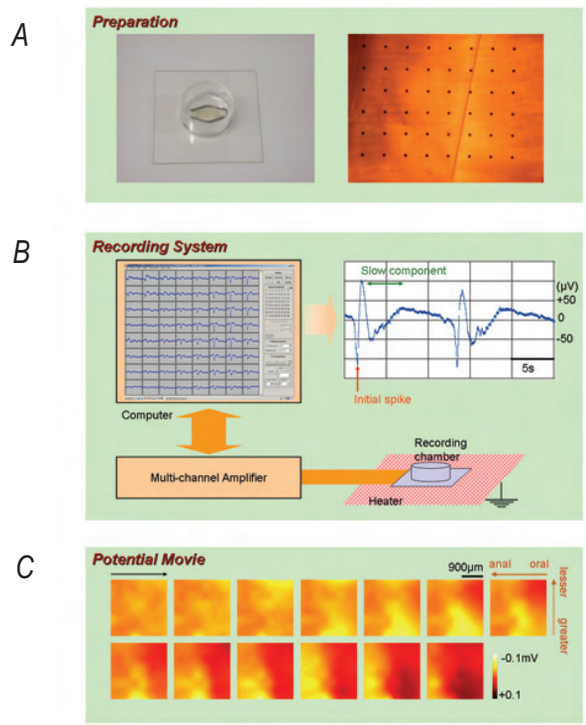


Figure 2. 8 x 8 planar electrode study. Preparation (*A*): Micrographs show a guinea-pig stomach muscle preparation fixed in a recording chamber with an 8 x 8 array of planar microelectrodes. Left panel: camera; right panel: inverted microscope. The circular muscle layer side was down. Recording system (*B*): Field potentials were recorded through a multi-channel amplifier through high-pass filtering at 0.1 Hz. The recording chamber on a heating device was continuously perfused with physiological solutions. Potential image (*C*): An example of a series of potential images (at 250 ms intervals) constructed from 8 x 8 multi-electrode recordings. Spontaneous electrical activity is propagating from the oral to anal end with the velocity of 0.8 mm s^{-1}.

Numerous diseases are known to impair GI movement (Sanders, 2006). For example, diabetes mellitus, a very common disease in western countries and now Japan, as well, is frequently complicated with GI dismotility, which makes it difficult to control the postprandial blood-glucose concentration.

Measurements of the interaction and coupling of pacemaker activity are likely to provide a useful index in such diseases. As a model of GI dismotility, the pacemaker electrical activity in GI tracts of W/W_v mice, in which ICCs reduce in number, are now being measured in our lab. Some of the recordings and analyses are expected to be communicated in the near future.

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Who stole physiology's clothes?

'This application of mathematics to natural phenomena is the aim of all science'

Given the current fashion for 'systems biology', for which I found over 3 million hits on Google as I wrote this article, you might be forgiven for thinking that the author of this quotation is a 21st century theoretical physicist looking for new fields of application for his formidable mathematical talent. So many people are entering systems biology from the physical sciences today, including the complete range of physics, engineering, mathematics, computing and chemistry. You may also be sceptical. Is biology, and physiology in particular, ready for the development of a theoretical arm, as happened long ago in physics, chemistry and engineering? In fact the writer was the great grandfather of **experimental** physiology, Claude Bernard, and the quotation is to be found in his classic Introduction à l'étude de la Médecine Expérimentale, published in 1865. Claude Bernard has a strong claim to be regarded as the first systems biologist. His concept of control of the internal environment of the body was clearly a systems level property and he foresaw that one day such processes would be the subject of mathematical analysis. His book was extremely influential in establishing physiology as an empirical science, but we should not forget that he himself was also a great theoretical thinker, establishing the claims of integrative physiology against the reductionist claims of chemistry and physics.

But, with a few exceptions, including most notably the Hodgkin-Huxley analysis of the nerve impulse, physiology has not really taken to Claude Bernard's theoretical vision as enthusiastically as it did to his experimental insights. We may now be paying a price for that neglect. For the vast majority of the beneficiaries of the trend towards systems biology have been outside (and usually below) the domain of physiology. Yet physiology is the very essence of integrative understanding of the logic of living systems (Boyd & Noble, 1993; Noble, 2006). We should be the peak, the crowning glory, of the systems approach.

The Joint Report of the Academy of Medical Sciences and the Royal Academy of Engineering (2007) is a wake-up call. The working group of engineers and medical scientists who produced the Report were chaired by Sir Colin Dollery (at GlaxoSmithKline) and Dick Kitney (at Imperial College). It is an unashamed call for the physiological end of systems biology to be developed and funded. Moreover, it warns that 'Growth in systems biology is threatened by the serious decline in UK capacity in its underpinning disciplines, including many of the physiological, pharmacological, engineering, mathematical and physical sciences.' It also says that 'Ensuring that the UK secures an internationally competitive position in systems biology requires substantial new investment by government and industry, together with a change in attitudes and working practices in the universities. Central to success will be the coordination of activities across academia, industry, research funders, the NHS and government. Systems biology will inevitably become an approach that pervades scientific research, in much the same way that molecular biology has come to underpin the biological sciences. It will transform the vast quantities of biological information currently available into applications for engineering and medicine.'

The basis of this prediction is that the diseases we are now attempting to understand and treat are multi-factorial diseases requiring research that tackles the complexity of multiple factors interacting to produce physiological function or particular pathologies.

In financial terms, the Report recommends an investment of £325 million over a period of 10 years. This is not an impossible aim. It corresponds to just over £30 million per annum.

As a physiologist who now attends more international meetings on systems biology (usually to give their plenary lectures) than straightforward meetings on physiology, I have encountered different forms of scepticism within physiology. The first is that the subject is not new. After all, many physiologists were 'systems' oriented long before the fashion developed. With this sentiment I agree, and that is also why I started this article with the quotation from Claude Bernard. Nevertheless, it is a little too complacent. Science often reinvents itself. The identification of a discipline called systems biology has attracted physical scientists and mathematicians in large numbers in a way that physiology itself has done to a much smaller degree. Many of these are bringing new insights and skills to bear on biological problems. We ignore that development at our peril. For if we do ignore it, then others will indeed steal our clothes. Many have already done so. Systems biology should not be simply an extension of bioinformatics and genomics.

The second form of scepticism is that the subject lacks an agreed definition. With that criticism I also agree. But it is surely up to us to define what it might mean, at least in our own area. That is what I have tried to do in my little book, The music of life (Noble, 2006). In some ways, that book is a successor to The logic of life (Boyd & Noble, 1993) which was produced for the 1993 IUPS Congress held in Glasgow. I remember a plenary lecture by Sydney Brenner at that Congress in which he threw out a challenge: that after the sequencing of the genome, the ball would be in the court of physiologists to go on to meet the next challenge. Having broken Humpty-Dumpty into his billions of molecular fragments, how could we put him back together again and understand his function as an integrative system? My view is that this necessarily requires a multi-level interdisciplinary approach, which is also the view expressed in the report.

The full 60 page Report can be downloaded from the web sites of the two Academies and I strongly recommend my colleagues in The Society to read it. If its recommendations are acted upon, it could be the saviour of some areas of physiology that have declined in recent decades.

Denis Noble
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Physiology is the bedrock of Human Enhancement Technologies (official)

On 1 March 2006 the UK Parliament's Select Committee on Science and Technology launched an inquiry into the use of human enhancement technologies (HETs) in sport. This interesting term is intended to cover anything that improves human performance in the context of sport, be it legal or illegal. They set out with the dual purpose of helping UK sports people to find the best legal methods, but in reality the great bulk of their work covered 'doping'. Their aim was that the 2012 Olympic Games should be remembered as a major sporting event in which doping did not detract from its success. They set out to 'horizon-scan' future illegal HETs and methods for countering doping while trying to seek to evaluate mechanisms by which UK athletes can be supported by legal mechanisms by which an athlete's performance may be enhanced.

I sent written evidence to the Select Committee on behalf of The Physiological Society and was invited to attend for a grilling by them. In addition, Ron Maughan was taken on as scientific adviser to the enquiry, so physiology was well represented.

There are several pertinent elements for physiologists in the Committee's report and I can recommend it as useful reading for anyone who wants to understand how doping is regulated in UK sports and what are the limitations in this that physiology might help illuminate.* The report's first conclusion is that 'It is important to increase research into potential illegal HETs. It is also important to increase research into normal physiology to enable better understanding, and hence detection, of doping and the effects different HETs have. The development of a blood profiling passport would contribute to such research.'

This is very useful for encouraging physiological research, because it

London 2012 provides physiology with a huge opportunity.

concedes that merely detecting illegal substances using knowledge of analytical chemistry and drug metabolism always leaves detection one step behind new substances. In contrast, defining physiological performance in terms of the normal human spectrum indicates the likelihood of doping, even when the substance is currently undetectable. By approaching it in this way, and by following the longitudinal progress of performance in physiological terms as well as banking blood samples, all as part of the 'Athlete's passport', the deterrence to cheating is increased.

The committee were, however, well aware of the factors currently limiting good quality exercise physiology research. They picked up very strongly that there is no great research council remit for this type of research, that funding is hard to come by, that the incentives to a scientist to pursue a career in these areas are not strong and that the RAE's insistence on impact factors was a final nail in the coffin. They, therefore, went on to urge the government strongly to take action on all of these points, particularly in relation to Research Council funding. Not that Government will necessarily listen, but having the evidence and conclusions written down always helps in the long run.

What exactly constitutes 'legal' human enhancing technologies is, of course, a wonderful topic for discussion. To be 'illegal' a procedure has to have two out of three of 'enhance performance', 'pose a health risk' or 'violate the spirit of sport'. Each of these concepts, independently, is very leaky, so we are in for a long haul to reach agreement. Nevertheless, the Committee had an interesting go at this, concluding that 'Better understanding of legal mechanisms for enhancing performance is required. Better horizon scanning of new developments (e.g. in medical research) is required. There is a need for increased funding for sports science. There is also a need for better translation of research from other disciplines into sport.'

Sounds like another good case for physiology to me. Indeed, what was striking about the whole exercise was the importance of understanding the processes that define the limits of human performance in sport and, therefore, what can be done to alter these limits either inside or outside the criteria for legality. In this physiology appeared as the essential conduit for marshalling inputs form other disciplines, particularly immunology, genetics and pharmacology.

My own conclusions were that, even if the Government ignores this report (a likely scenario given some of its criticism of Government agencies such as UK Sport), we should seize and build on some of the arguments that it contains to push for physiological research.

Finally, we should recognize the opportunities that sport gives to promote physiology to the public. Having the Olympics in London in 2012 provides physiology with a huge opportunity. As a start to this *J Physiol* will have an Olympic year Special Issue on exercise physiology in early 2008 (the Beijing Olympic year), edited by Michael Joyner, there will be a symposium on drugs in sport at this year's Life Sciences 2007 meeting in Glasgow (*Drugs and human performance: mechanisms of action and efficacy of detection*, organized by Stephen Harridge for the Human Physiology SIG) and the planned joint meeting with the Chinese physiologists in Beijing in autumn 2008 will provide another excellent opportunity. We must build on this momentum.

Ian McGrath

* http://www.publications.parliament.uk/pa/cm200607/cmselect/cmsctech/67/6702.htm

BA Festival of Science 2006

Vicky Cowell, representing a patient's charity that supports biomedical research, was an enthusiastic speaker at our Society's session at the BA Festival in September 2006. She has kindly agreed that we can reproduce the article that she produced on it for her charity's magazine *HOPE*.

Liz Bell

Physiological Society session on regenerative medicine. The future for patients in the 21st century

Liz Bell, Head of Policy and External Affairs at The Physiological Society recently told *HOPE*, the magazine for Patient's Voice for Medical Advance: 'The Physiological Society has been organising sessions for young people at the BA Festivals of Science for some years, and it was felt that *Stem cells as they relate to possible advances in regenerative medicine* was a particularly good topic to tackle at the September 2006 Festival at the University of East Anglia in Norwich. This area could have a big impact on society in general in terms of offering potentially revolutionary treatments for patients, so The Society put together a programme of speakers to include scientists working in the area, a social scientist working as part of an Economic and Social Research Council programme on the potential impacts of stem cell technologies, and an advocate for patients potentially on the receiving end of all this. The session played to a packed house of young people who stuck into a lively debate with our speakers. Young people are clearly very interested in how these technologies might help their health issues throughout their lives'.

The Chair of the session was Huseyin Mehmet, a Reader in Developmental Neurobiology at Imperial College London. His job was to welcome and introduce the speakers, to try and keep us to the set time limits for our talks (not an enviable task!) and to stimulate the audience question and answer session after the individual presentations.

The Physiological Society's session on regenerative medicine played to a packed house of young people who engaged in a lively debate with the speakers.

Recently, despite an incredibly hectic work schedule, Huseyin Mehmet very kindly agreed to give *HOPE* an overview of the three presentations from Chris Mason, Andrew Webster and Vicky Cowell and to answer questions about this stimulating subject.

'Chris gave us a clinical perspective of the current state of play regarding clinical therapies, including bone marrow transplants, skin grafting and retinal replacement. He also discussed the exciting work going on at University College Hospital, where heart patients are being treated with an experimental stem cell transplant. He stressed that although clinical applications of stem cell research are encouraging we are still a way off from universal therapies using stem cells.'

'Andrew gave a very entertaining talk with his unbiased critique of the current climate in terms of moral and ethical dilemmas in stem cell biology. These primarily focus on issues surrounding the use of embryonic tissue. It was clear from his presentation that there are a broad range of views and opinions across Europe. In the USA the current legislation is confusing. It is a popular misconception that embryonic stem cell research is banned in the USA. However, this is not the case. The truth is that such research is banned using federal funding – but by using private funds there are no limits and the embryo has very few rights. In my opinion this is a far more worrying scenario than in countries like the UK where (even with its shortcomings) the HFEA controls and regulates research using embryos.'

'Last, but not least, was Vicky, who gave us a perspective that is often over-looked in these discussions – *How do patients with debilitating conditions feel about stem cell research*? After all, if animals have strong advocates why can't patients, who are equally deprived of voices? Vicky gave three real life examples of people with life-threatening diseases who might be future beneficiaries of stem cell research. Her accounts were both moving and informative, particularly when she discussed the case of her own daughter who has cystic fibrosis.'

Q As Chair of the session what was your impression of the audience reaction?

HM As someone who is particularly interested in the public dissemination of science, and especially in persuading young people to engage in research, I was extremely pleased to see so many well-informed teenagers in the audience – mainly 16 and 17 year olds. This is most encouraging because it suggests that schools have highlighted this subject and that the youngsters are genuinely interested. In terms of audience participation this was the best I have ever encountered.

Q What is your opinion about the general future of stem cell research?

HM Over the past few years there has been unprecedented publicity and excitement surrounding stem cell biology. While the British media have been more cautious than most, it is inevitable that the public will be disappointed at the speed of progress. In my view it is far more likely that stem cells will become invaluable resources for the pharmaceutical industry – for example in drug testing. Nevertheless, the potential of stem cell biology in providing a real therapeutic alternative to the use of drugs should not be discarded as an impossible dream. I am sure that in the future this ultimate goal of stem cell research will be realised.

Vicky Cowell
Director, Patient's Voice for Medical Advance

NC3Rs Parliamentary event

Scientists impress MPs with work to minimise the use of animals in scientific research and testing

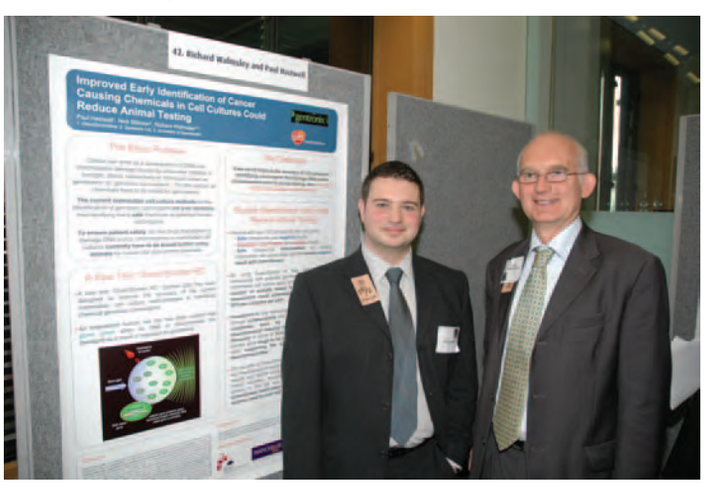
Phil Willis MP presents the Replacement Prize to Kelly BéruBé and Tracy Hughes (above) and David Lynn from the Wellcome Trust visits Richard Walmsley, Paul Hastwell and Nick Billinton's prize-winning poster (below).

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), which provides a UK focus for the promotion, development and implementation of the 3Rs in animal research and testing, held a showcase event in Portcullis House on 28 February. The event, hosted by Phil Willis MP, chair of the House of Commons Science and Technology Committee, featured 50 projects that applied the 3Rs to research using animals in academia and industry, and the researchers brought posters to illustrate their successful work to the invited guests which included MPs and Peers. Prizes of £2,000 were awarded to the best posters as selected by a panel of 15 judges with representatives from the RSPCA, the Wellcome Trust, ABPI, BBSRC, academia and industry, chaired by Lord Turnberg, chair of the NC3Rs Board. The event was sponsored by the Association of the British Pharmaceutical Industry (ABPI) and the Wellcome Trust.

The Replacement Prize was won by Kelly BéruBé and Tracy Hughes (Cardiff University's School of Biosciences), for their work to find a possible alternative to animal testing in the field of inhalation toxicology. By developing 3-D cell cultures of lung tissue from human cells or 'human tissue equivalents of respiratory epithelia', they were able to give them the appearance and behavioural characteristics that closely resembled those found in the human airway, and accurately mimicked the human responses to tissue damage. This innovation could eventually replace the use of animals in toxicity testing of airborne materials.

The Refinement Prize was won by Claire Rourke (GlaxoSmithKline), for her work investigating a novel way to give laboratory rodents doses of drugs for testing. Currently, a tube is inserted down the throat of the animal, but it was found that the animals could be trained to voluntarily drink from a syringe that contained the drug, with some sugar added for taste.

The Reduction Prize was won by Richard Walmsley (University of Manchester), Paul Hastwell (GlaxoSmithKline), and Nick Billinton (Gentronix Ltd) for their work in improving the identification of cancer causing chemicals using cell cultures. They developed a cell line that glows green when exposed to chemicals that damage genetic material. Because this test is much more accurate than the existing cell culture tests, far fewer chemicals have to be tested in animals, which are currently still necessary to see whether chemicals actually have the potential to cause cancer.



Phil Willis said: 'My colleagues and I were tremendously impressed with the outstanding range and quality of research on display. The research demonstrated that not only could alternatives to animal models be developed but the quality of scientific outcomes could be improved.'

Vicky Robinson, chief executive of the NC3Rs, said: 'We were overwhelmed by the interest that our event 'Showcasing the 3Rs' generated. Not only did we get a huge number of researchers keen to communicate their work to a wider audience, but we also had an impressive turnout from MPs, who obviously felt that finding out more about this type of work was central to being informed about the issue of using animals in research when talking to their constituents.'

Liz Bell

For more information on the work of the National Centre for the 3Rs please visit www.nc3rs.org.uk

For this issue Thelma Lovick asked three lecturers in physiology at the Universities of Durham, Bristol and Aberdeen to spill the beans on the events that lead them to embark on their chosen careers

Natural curiosity proved to be an exciting journey for Susan Pyner, right

What turned me onto physiology? Probably it was like most of us: a natural curiosity to know how our bodies work. That is still the case today, although now I have put a more scientific bent on it and am looking to understand the complex integration that goes on between the various body systems in order to maintain a normal (healthy) body.

My undergraduate studies were the real starting point for focusing this curiosity on the discipline of physiology. They gave me an introduction to the function of living things with the added bonus of being able to demonstrate that function on ourselves. Recording the ECG, the effect of exercise on heart rate, determining respiratory volumes and capacities etc. – these were practicals that I'm sure we have all experienced in one form or another and probably still use in our teaching repertoire. Good as they were though, they only provided partial satisfaction for my curiosity and I wanted more.

I was accepted for an MRC-sponsored PhD studentship to work in John Coote's laboratory in the Physiology Department at the University of Birmingham. The department provided training in integrative physiology and fostered my enthusiasm further. The combination of the people in the department and the environment they created made this, for me, an excellent place to work and to play. I commenced my studies looking at the electrophysiological properties of sympathetic preganglionic neurones (SPN) in the neonatal rat spinal cord slice. However, what started out as a minor aspect of the study, a histological investigation into the neuroanatomical organisation of SPNs, turned out to provide the foundation for the direction my scientific career would eventually take. The function of a system depends upon how that system is constructed. Thus, I have taken a path that allows me to investigate system construction in parallel with system function by applying neuroanatomical studies to understand cardiovascular control.

Staying excited about physiology during the PhD years was also partly due to Birmingham being such a good place to 'play'. Apart from gaining my PhD I learnt to ballroom dance courtesy of the University's active lifestyle programme, I swapped squash for tennis and played a bit of cricket for the department and yes, even with me as a team member, they were still very successful!

For me a major contribution for staying 'happy' with physiology has been the people I have met and worked with and the resulting friendships. Their excitement, determination and enthusiasm to form collaborations have all had a big part to play. I will not mention names but just offer my thanks to you all. Conference attendance at national or international levels is also in the mix for making physiology a good way to spend your life. Highlights so far (apart from the science of course) include snorkeling off the Great Barrier Reef, wine and cheese tasting in Akaroa, bungee chair challenge in Auckland, eating fish and chips on Bondi Beach and climbing up the inside of the Statue of Liberty. I wonder what Glasgow will offer this summer!

Where has being 'turned on to physiology' brought me to today? A lectureship at Durham University, a position I took up nearly 6 years ago. For those of you who may not know, Durham University has two campuses: the main one here in Durham and Queen's Campus at Stockton situated about 26 miles to the south on the banks of the river Tees. My office and lab are sited in Durham so a modicum of travel is involved for teaching and, yes, this does present certain challenges, not the least of which is how to expose the students to the research labs and what goes on inside them. This is not a situation unique to myself, but is faced by my colleagues who also contribute to the courses at Queen's Campus.

How does physiology fit at Durham? Well for me it's combining the teaching of physiology and neuroscience in our biomedical science and medical programmes at Queen's Campus with my research looking at the neural mechanisms controlling cardiovascular function. There are still plenty of opportunities to enthuse about physiology and the relevance of integrative physiological investigations in this day and age, when so much attention is directed towards stem cell and proteomic studies. Apparently I have achieved this at least once and to blow my own trumpet I quote from a feedback form (anonymous of course): 'a pleasure to be taught by someone who obviously loves their subject'. Praise indeed.

All in all a curiosity to know how the body works has proved to be a fun and exciting journey that still, for me, has not reached an end. I hope I can stay 'turned on to physiology'. Ask me again in a few years time.

It was a bit strange to go back in memories and time when I was asked to write this article. It has been a long road that finally brought me to the School of Medical Sciences at Foresterhill in the University of Aberdeen to start my independent research career in the field of muscle differentiation.

This route started almost 15 years ago in 1993 when I spent my first term in the teaching laboratories in the chemistry department of the University of Hanover. For the first 2 years we – some 40 few biochemistry students – shared lectures and practicals with 160 chemistry students. There was one difference, however. In addition to 60 hours of inorganic, organic and physical chemistry each week, we had to do zoology, botany and microbiology in the holidays as well! I remember well one of my professors commenting on the overcrowded timetable: 'it is impossible to manage to study biochemistry'.

Initially therefore, physiology was quite far away from my thoughts, as it would have been just one more topic to worry about. However, I think subjects such as botany or zoology initially opened my mind to seek the links between 'structure and function', which finally brought me to physiology. Muscle and nerve physiology particularly caught my interest. To cut a long story short, after the normal period of 6 years I graduated in biochemistry. For the exams, we had a choice of topics to be examined in, in addition to the main topic. This topic could be from a broad spectrum of biological subjects and I chose physiology.

You should note that in Germany physiology is not a subject you can study separately. Normally it is part of medicine and a field of medical specialisation. Therefore most of my teachers, in lectures and practicals, were medics and there always was a clear medical link. There was one clear rule: medics and biochemists don't favour each other. It was a bit like the Scottish and the English, as I have experienced here in Aberdeen, close but still too different.

It's been a long road ... which brought Michael Scholz, below, to physiology

In the beginning physiology was just a side-track along with my biochemistry studies. However, just before the start of my 6 month project for my Diploma (Master) I was offered work as a research assistant and the opportunity to do a PhD in the Physiology Department of the Hanover Medical School working on 'signalling controlling skeletal muscle fibre type transition'. Amongst about 30 graduates in this year I was the only one who went to 'face the medics' to do physiology. The main intention was to bring my experience in cell culture, transfection and cloning techniques to facilitate the step to 'molecular physiology'. Nowadays the term 'molecular' is widely employed, everything has to be somehow 'molecular'. But in those days it described the development of classical physiology to embrace approaches that looked more and more down to the molecular level, a perfect opportunity for me.

I spent 6 years in the Physiology Department, finally completing my *Dr rerum naturalis* in physiological biochemistry. This was something I definitely didn't expect when I did my first physiology practical. But on the other hand those practicals stimulated my interest in physiological functions such as muscle contraction and neuronal signal transduction that are generated by such a well orchestrated cooperation of various biochemical and molecular principles. I applied what I learned as biochemist: cloning, cell culture, protein assays, transfection, the whole biochemical tool box. In turn, I learned a lot about physiological systems, particularly skeletal muscle. The complex signalling events that control its growth, differentiation and plasticity became the area where I tried to combine my previous studies and experiences with physiology.

So it was not really one single event or moment that turned me to physiology, it was more a series of experiences and events that finally made me a physiologist. My initial ambition to become a biochemist, which was born at school during chemistry and biology classes, was extended as a result of my interest in the broader picture of 'how things work together' in the human body. Today both subjects are not separate and I struggle to say whether I do 'biochemical physiology' or 'physiological biochemistry'. I've come along way from those early beginnings but actually I think the journey has only just started and I can hardly imagine where it might take me in the end!

... and Helen Kennedy is delirious about hair cells

What turned me on to physiology? Well I've been wondering how to answer that and I guess the truth is it's just rather grown on me. As an A-level student I really enjoyed biology, and in particular medical microbiology with all those interesting life cycles and funny tropical diseases. Based on this I decided to do microbiology at the University of Bristol. I certainly wasn't a hard-working student at the beginning because, unfortunately, the degree didn't inspire me and by the end I was certain of one thing, there was no way I was going into research. Nevertheless, 18 months later, after a year of a job I hated, working in the NHS as a diagnostic virologist at Glasgow Royal Infirmary, I was back in Bristol applying for hopefully more interesting and exciting jobs in science.

I was really lucky and was invited to interview by Roger Thomas in the Department of Physiology. The job was for a research assistant position, with the possibility of doing a PhD. I felt completely out of my depth and spent the next few weeks finding out about physiology and the amazing things you could do with garden snails. I will never forget the day of the interview. I was interviewed by Roger and I was like a rabbit in the headlights. I tried hard to demonstrate my newly found knowledge and enthusiasm for the garden snail, whilst being absolutely transfixed by his home-made rainbow-coloured tie, which had some weird scientific thingy on it as well as his own name. I was transfixed, not by the physiology, but by the tie! But I was genuinely enthusiastic, and Roger later told me that's what had swayed him to offer me the post.

So off I went, headlong into a PhD, wondering quite what had happened. The first 18 months were a little slow but then I got my teeth into a project looking at calcium regulation using fluorescent dyes. The PhD was hard, lots of fun and Roger was an enthusiastic supervisor who was willing

Helen Kennedy was transfixed by Roger Thomas' home-made, rainbow-coloured tie with some weird scientific thingy on it, as well as his own name (pictured below).

to give me lots of scientific freedom and rein me in when necessary. When Roger left for Cambridge University I decided to try and broaden my scientific knowledge, but still keep my calcium signalling interests from my previous research. I went to the Biochemistry Department to work for Guy Rutter, looking at gene transcription and insulin signalling in pancreatic beta-cells. It was a very productive time but slowly a realisation hit me. Biochemistry was not for me. I wanted to get back to physiology and that, for me, was a defining moment. From then on I became very focused on finding a research area that really excited my interest and where I could apply my existing knowledge.

Hearing research was strong at Bristol, with Corne Kros, Matthew Holley, Jonathan Ashmore, Mike Evans and Nigel Cooper all in the department. Many exciting discussions quickly informed me that despite their complex physiology and the undisputed pivotal role for calcium, almost nothing was known about mammalian hair cell calcium signalling. Bingo. That was it. I was absolutely hooked. By day I worked in biochemistry but the rest of the time was spent writing my fellowship application to study calcium signalling in mammalian hair cells, a system I had never worked on and using techniques of which I had only the most basic knowledge. Fortunately I had the support of two excellent physiologists, Bob Meech and Corne Kros, and when my career development fellowship from the Wellcome Trust was awarded there was no looking back. In 2003 I spent more than a year working with Robert Fettiplace on hair cell transduction and then in the summer of 2004 I took up a lectureship in the Physiology Department at Bristol.

And now I pause for thought and wonder whether what I've written has actually answered the question? Maybe it has. There was no single thing that turned me on to physiology, it grew on me slowly, and I didn't even know it was there until I left to do biochemistry. I'm glad I got back to physiology, I love the research I do and, in fact, my enthusiasm for hair cells is bordering on the delirious. What's more, throughout my research career I have always enjoyed teaching physiology, and I hope that some of my enthusiasm has rubbed off because I really do think physiology is fun.

So thank you to all of you who helped and inspired me along the way, and a special thank you to Roger Thomas for giving me my initial break, even though at the time I didn't know I wanted it, and to Bob Meech who has helped all along the way, and still does.

Physiology News needs a Deputy Editor

We are looking for someone to take on the role of Deputy Editor of The Society's magazine. If you would like to know more, please contact Editor Austin Elliott (austin.elliott@manchester.ac.uk) or Executive Editor Linda Rimmer (lrimmer@physoc.org).

Affiliates – send us your images of physiology

If you have a stunning confocal image or original trace, let us publish it. If you have an unusual photo of an eminent physiologist from bygone days, or even one from the present day, we'd like that too. If you have made an ingenious bit of kit or you've found something at the back of a cupboard and you don't know what it does, let us see a photo of it. It's all about sharing your experiences. **We will pay £50 for any images published**.

What's it like doing a summer project?

Thelma Lovick asked some of last year's recipients of Vacation Studentships how they got on

Last year the Vacation Studentship Scheme provided subsistence expenses that enabled 41 students to spend periods of up to 8 weeks working in the laboratory of a host Member of The Physiological Society.

What do the students get out of it and, importantly, what do we as a society get for our money? To try and find out I e-mailed 15 of last year's recipients at random and asked if they would provide me with a few paragraphs about their experiences that could be published in *Physiology News*. I emphasized that I was as keen to know about the bad experiences as the good. Of these 15 e-mails, 12 were delivered but to my surprise, only four triggered a response. The experiences of these four students, which were all very positive, follow below.

But what about the others? If this had been a questionnaire a 33% response rate would be deemed quite good (if totally unrepresentative). But it wasn't. Did the others have such an awful time that they couldn't commit it to print? Did they just mess about and waste the money? Or is having your photo in *Physiology News* just not considered cool? Makes you wonder doesn't it?

Graham Patterson experienced the loneliness of the long distance electrophysiologist in Dundee

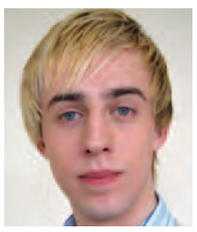
I am currently in my 4th year of medical school in Dundee and, whilst I thoroughly enjoy my course, I feel that there's less focus than I'd like on the basic sciences like physiology and pharmacology, which are obviously of paramount importance in the understanding of medicine. So when I was offered a place to study pharmacology as part of an intercalated degree between my 3rd and 4th years of medical school, I literally jumped at the chance. As part of this honours degree course, I carried out a research project to look into the modulation of seizure activity within the rat brain. Having thoroughly enjoyed the neurology section of my medical course, I was keen to participate in a project that would be both medically relevant and keep me interested, so when a project related to epilepsy was suggested, it seemed perfect for me. In addition, I knew I could work well with Bruno Frenguelli who was supervising the project; he had taught us briefly during our medical course and it was pretty obvious he knew his stuff!

The main aim of my work was to investigate the therapeutic potential of certain ATP receptors within the brain, in terms of their influence over seizure activity. This was achieved by preparing fresh slices of rat hippocampal tissue each day and placing them in a chamber constantly perfused with artificial CSF. Application of electrodes to the slice allowed me to elicit regular seizure activity *in vitro* and then, by adding different ATP (P2Y and P2X) receptor antagonists to my perfusion solution, I was able to uncover the modulatory effect that ATP had over seizure activity.

Fortunately, my project showed some promising results and so, with the support of a Vacation Studentship from The Physiological Society I was able to continue my work over the following summer. This great opportunity not only allowed me to further my research and build upon my previous findings, but also gave me a flavour of what full-time lab work involves. While I was working under the guidance of a very experienced neuroscientist, the work I carried out each day was done on a very independent basis; all solutions, drugs and animal material was collected and prepared by myself. I even got to organise my own work hours to an extent, which is pretty good going for a summer job, I'm sure any student would agree!

Having a large amount of responsibility thrust upon me was initially a little daunting (to put it mildly!) but with time I soon became confident in what I was doing and I'm now extremely grateful that I was allowed to work so independently as it's given me a huge confidence boost. Working alone on my project also gave me a good dose of reality; since the majority of laboratory based research is very much a solo act. This was great at times, but also led to much frustration, since you have only yourself to blame when things go wrong! Also, if you are working alone on something and only you knows how to operate the equipment, then the work you're doing very much controls how you spend your time; breaks are taken in a very opportunistic manner, and you don't dare make any plans for early in the evening as inevitably something will go wrong and require you to stay that extra hour! Despite all this, I'd definitely return to lab-based research in the future; it's an exciting area of medicine that I can't wait to become part of and the experience of my vacation studentship has only reinforced this feeling!






Graham Patterson (top, left), Jantinder Minhas (top, right) and Ben Hunt (above, left) and Harry Leitch

Harry Leitch got into kisspeptins in Cambridge

I'm a 3rd year medical student at the University of Cambridge and during my summer studentship I worked in Bill Colledge's Reproductive Physiology group in the Physiology, Development and Neuroscience Department. I came into contact with Bill through lectures – he lectured me in endocrinology in 1st year – and also through practical classes in physiology, reproduction and histology in 1st and 2nd year. His work with kisspeptins was brought to my attention by my supervisor in reproductive physiology in the 2nd year and I kept a keen eye on his publications after that. Bill's group is investigating the role that the GPR54 receptor and its ligands, kisspeptins (encoded by the kiss1 gene), play in reproduction, primarily through the use of transgenic mouse models.

In 2006 I represented Scotland in the Commonwealth Games in Melbourne and so was forced to drop down a year. I thus had a long summer between the Games and restarting the 3rd year and I thought it would be interesting to spend this time researching. I contacted Bill about the possibility of doing a project with him; he seemed happy to have me and he suggested I apply for a summer vacation studentship from The Physiological Society.

Of course, the first few weeks were a very steep learning curve but, in their own way, very rewarding. I seemed to make reasonable progress, despite a predictably large number of initial comedy errors, and quickly gained at least reasonable proficiency in a range of techniques; including cell culture, molecular cloning, PCR, immuno-histochemistry and cutting sections using a cryostat and vibrotome.

Bill and the rest of the lab provided expert tutelage and I hope their patience was rewarded at least in part by the data I was able to produce. Indeed, by the end of my stint I felt like a part of the team; the benefits I gained in terms of skill acquisition and experience were obvious, but I also hope that I contributed to the work of the lab as a whole during my stay.

Overall it was an excellent experience and has further inspired me to apply to the MB/PhD programme at Cambridge clinical school. This would provide me with a good balance between clinical training and further academic research. Hopefully this will allow a natural progression to a career in academic medicine, which is my ultimate goal. In fact I had such a good experience that I write this now from Bill's lab where I have spent the last two terms working on my part 2 project; the idea for which evolved during my summer studentship all those months ago!

Jantinder Minhas got blotting fatigue in Nottingham

During my second year of study for a degree in Human Genetics at Nottingham University I heard about The Physiological Society Vacation Studentship scheme from a member of staff during a tutorial. I have always been interested in the medical application of my biological background and having spoken to a few friends they persuaded me to apply to do a project during the summer vacation with Helen Budge in the Academic Division of Child Health at QMC Nottingham. Helen is undertaking research into maternal nutrient restriction and the role of IL-6 in obesity. This is a topic that has been very much in the public eye and receives a great deal of media attention.

I was awarded a studentship from The Physiological Society and met up with my supervisor and she explained the project outline and the techniques I would be using. The project sounded incredibly exciting and offered the chance to work independently on a novel research project.

The project was deemed a success and some of my results will be used for part of a research paper. However, there were times where results did not turn out as planned and the thought of another Western Blot would drive me crazy! My supervisor and the Senior Technician Vicky Wilson helped me at times when I did have trouble though. The support of the Department and a little bit of determination allowed me to complete my project to a high standard. I enjoyed working within the laboratory environment and feel it necessary to thank all those who were involved in my project, as they helped me realise my full potential.

The experience was superb and I would recommend it to all students within the area of biological sciences, particularly those considering going on to do a PhD. It gave me a hunger to pursue a career in research and also gave me confidence that I could complete research on a novel topic. In addition, the team ethic within the Department made the thought of a PhD all the more appealing.

Ben Hunt baked in the tropical heat of Birmingham

I began the final year of my undergraduate BMedSc degree course at the University of Birmingham with fair intentions of moving on to a graduate entry medicine course. However, the final component of my course was to carry out a 10 week laboratory based project, on which my dissertation was based. Our lab had previously shown that activity-related dilatation of cerebral arterioles in brain slices was compromised during the recovery period following brief exposure of the tissue to mild ischaemic conditions. We knew that connexin43, the astrocytic gap junctional protein, became dephosphorylated at this time but we didn't know whether this was a direct effect of ischaemia on the gap junctions or whether it was secondary to a more general demise of the tissue. My task was to look for histological signs of apoptotic or necrotic damage in brain slices that had been subjected to transient ischaemic conditions. The project got off to a good start and after solving such issues as a contaminated pH electrode, my thesis was completed and handed in just about on time.

The fact that I would frequently go to sleep 'seeing cells' was probably a sign that I should have gotten out while I had the chance! However, the reality was that I had thoroughly enjoyed my lab project, wanted more and was now considering the possibility of studying for a PhD. In view of the timescale associated with applying for funding, my supervisor, Thelma Lovick, suggested that a Vacation Studentship might serve me well as an introduction to the realities of a research life and would also allow me to consolidate the data that I had produced during my undergraduate project. After a short and well-deserved break, our grant application for a Physiological Society Vacation Studentship was approved and I returned to the lab in late June.

It turned out to be the hottest summer since records began and our air conditioning unit was somewhat over the hill, so it was quite a challenge to keep the solutions and myself at a reasonable temperature! Despite this, we managed to collect some good data, which then formed the basis for the abstract of a poster that was presented at the annual meeting of the Society for Neuroscience in October 2006. The data has also been included in a full paper just submitted.

The Physiological Society Vacation Studentship allowed me to gain an insight into academic life without the ever-looming deadline of my dissertation and this helped to confirm that I really wanted to study for a higher degree. Importantly, it also provided me with additional hands-on experience that other applicants for PhD studentships may not have possessed.

Since completing my vacation studentship, I was offered a PhD studentship with David Lambert at the University of Leicester, which I took up in January 2007.

When science isn't what it seems — fraud is born at the bench

Recently, a spate of high-profile cases of scientific fraud have reached the pages of *Nature*, *Science* and other high-impact journals. Foremost amongst these was the fabricated claim by Woo-Suk Hwang (2005) that he had created human embryonic stem cell lines by somatic nuclear transfer in his South Korean Laboratory. Every so often a 'big news' fraud story hits the scientific press, a great furore ensues and everything dies down again…until the next time (the last century was peppered with scientific frauds (Charles Dawson's 1912 Piltdown Man and Jaques Beveniste's 1988 *Nature* paper on the memory of water being notable examples)). So are these cases isolated and rare or does fraud pervade the scientific community?

In the following article which reports Raymond Tallis's *Sense About Science* lecture it is suggested that scientific method 'fosters honesty rarely observed elsewhere'. I agree that it may do so, but the other side of the coin is that this reliance on the integrity of individuals leaves science open to the abuse of trust (Elliott, 2005). Gerald Wiseman (2006) asserts that science is self-cleansing; fraudulent work, once revealed as such, is seldom cited and disappears from the literature. Once revealed as such … so what happens to 'the one that got away'? Does undetected fraudulent work continue to be cited and its perpetrators acquire acclaim? The test of scientific credibility is reproducibility, so this in itself, and the peer-review system, should weed out data either undeserving of publication or of subsequent citation. The system isn't perfect but it's hard to think of a better one.

Technological advances such as software to detect manipulated images, or the introduction of another layer of bureaucracy, may deter some, but not the most determined cheats. The character of a swindler cannot be altered by introducing more layers of surveillance (Bentley, 2006) but this, I believe, refers to the hardened fraudster, the scientific equivalent of a forger who seeks ever more sophisticated means to produce counterfeit currency.

My own opinion is that prevention is better than cure. Fraudsters are bred at the bench and supervisors who demonstrate sloppy scientific habits, either deliberately or through ignorance, beget students and post-docs who inherit their traits (Kreutzberg, 20004). And it's the seemingly little things that count, for that is where it starts: inappropriate statistical analyses, omission of data points, selection of which data should go into that paper, biased results arising from inadequate controls. Outright fabrication of data is relatively rare; whereas fraudulent treatment of data is generally much more subtle.

So what can those us of at the lower echelons of the scientific hierarchy do to ensure good science? It is my strong belief that leading by example is the best place to start – both good and bad science are infectious, so endeavour to perpetrate the good sort. What should you do if you come across fraud? This is a tricky area as there can be serious repercussions for whistleblowers. First, a thorough understanding of your institution's policies is essential – this is what Walter DeNino had when he discovered data manipulated by his boss, Eric Poehlman, an expert in obesity who was subesquently found guilty of scientific fraud in a US criminal court (Powell, 2006). Second, find a trusted and experienced researcher to see if they come to the same conclusions, advises Margaret Dale, dean for faculty and research integrity at Harvard Medical School (Powell, 2006). Third, try to find out if other colleagues harbour the same suspicions and if they, too are willing to act. There is some safety in numbers. Finally, an accusation of scientific misconduct is very serious and a step best taken only with absolute certainty and backing from others where possible.

Patricia de Winter
Post-doctoral Research Fellow, UCL

References

Bentley P (2006). Bureaucracy won't change the character of a cheat. *Nature*. **439**, 782-783.

Elliott A (2005). Currency fraud or just sharp practise? *Physiology News* **61**, 3.

Hwang WS *et al* (2005). Patient-specific embryonic stem cells derived from human SCNT fibroblasts. *Science*. **308**, 1777-1783.

Kreutzberg GW (2004). Scientists and the marketplace of opinions. *EMBO Rep* **5**, 330-332.

Powell K (2006). Misconduct mayhem. *Nature* **411**, 122-123.

Wiseman G (2006). Science fraud from patchwork mouse to patchwork data. *FASEB J* **20**, 587-589.

Noticeboard

SOCIETY MEETINGS/ INTERNATIONAL WORKSHOPS

Manchester, UK
5–6 September 2007
Focused Meeting *Cardiac electrophysiology*: *with a special celebration of the centenary of the discovery of the sinoatrial and atrioventricular nodes*

Bratislava, Slovakia
10–14 September 2007
Joint Meeting of The Physiological Society, the Slovakian Physiological Society and FEPS

Lviv, Ukraine
18–23 September 2007
International Workshop *Molecular physiology of membrane transport and cellular signalling*

Bristol, UK
17–18 December 2007
Focused Meeting *Renal cortex: physiological basis of glomerular and tubular diseases*

Cambridge, UK
14–16 July 2008
Main Annual Meeting

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

INTERNATIONAL STEPS FOUNDATION

Washington DC, USA
15-16 November
The International STEPS Foundation celebrates 40 years of volunteer work with the 8th Olympiad of the Mind, *Brain research: improving global harmony,* hosted by the National Academies in Washington DC.
http://www.steps-peace.org

PPTR 2009

Matsue, Japan
23-26 July 2009
The 3rd International Symposium on Physiology and Pharmacology of Temperature Regulation (PPTR).
http://www.med.shimane-u.ac.jp/gakkai/1stcircluar.pdf

Notices for the Autumn 2007 issue of *Physiology News* should reach the Publications Office by 13 July. Please send contributions to lrimmer@physoc.org

Sense About Science

In March *Sense About Science* held its 2nd Annual Lecture. *Sense About Science* is an independent charitable trust that promotes good science and evidence for the public. This year's speaker was Raymond Tallis, Emeritus Professor of Geriatric Medicine at the University of Manchester, philosopher, author and poet. Amongst the prestigious attendees were Robert Winston, reproductive biologist and Emeritus Professor at Imperial College London, Derren Brown psychological illusionist and self-declared sceptic, and *Guardian* columnist Ben Goldacre.

Raymond Tallis's lecture, *Longer, healthier, happier – human needs, human values and science'*, addressed a number of topical issues: the need for science to win public trust, the reasons behind public suspicion of science, and also the prevalence and popularity of 'junk science'. He highlighted the necessity of science policy to be backed by good science. The public believe that science is not delivering or that, if it is, the benefits are outweighed by the risks. Tallis outlined and rebutted these suppositions; science has increased human life expectancy, particularly as a result of improvements in treating infectious diseases and cancers. Given these benefits, why is there so much public scepticism towards science? It is likely to be the result of science underplaying its successes and emphasising its failures.

A celebration of its achievements is key to public appreciation of science, but what have we to celebrate? Tallis explained that science is global – we have a medium where we can disseminate knowledge worldwide, without cultural bias. Its model of building on old knowledge in a self-critical and self-correcting manner fosters honesty rarely observed elsewhere. Historically, science has had to overcome cultural beliefs about the nature of health and disease, here the knowledge and expertise acquired has led to improvements in how we care for our bodies.

The public does not trust science because they have trouble understanding the terminology and numbers. As science is intrinsically neutral, something some would consider 'amoral', it could be regarded as destructive, a view often associated with the over-sentimentalising of Mother Nature. Those people who are suspicious of science are more likely to be accepting of junk science. Junk science exploits the unknowns of true science, using emotional 'pulls' and anecdotes as fact. It is biased, directs suspicion at science and scientists, and accuses science of inhumanity. Authority is given to well-known people, resulting in inaccurate claims about science being made by celebrities or policy makers. The public believe them without understanding what they've been told.

Raymond Tallis's conclusion was that in order to improve the public perception of science, it is necessary to highlight its benefits and expose junk science as fraudulent, thus rendering it less attractive to the general public, in his words: 'Tough on unreason, tough on the causes of unreason'.

Selina Pearson
Affiliate Member, University of Birmingham, UK

Physiology for schools – practical help for practical activities

It is widely recognized that practical-based teaching is an essential part of learning in schools. As well as supporting the delivery of core content, good practical activities are motivational and help develop key skills. Unfortunately, many teachers find it difficult to develop new, or even revise existing, practical activities. These difficulties may arise because teachers have limited time and resources, or lack specialist subject knowledge. There are also, usually erroneous, fears that things can't be done for health and safety reasons. In response to this need, and through our programme *Physiology for schools*, The Society has begun an initiative to develop resources for practical teaching within the A/AS level biology related curriculum.

Recently, we explored the views and needs of educators and will present these finding at LifeSciences 2007 (*Opportunities for, and obstacles to, the development of bioscience practicals in the A-level curriculum*). With feedback from this workshop, we have developed an example resource that is intended to enable teachers in developing practical sessions. This example is included below. We are very keen for Members of The Society to help us develop similar resources targeted at physiological (in its broadest sense) aspects of the A/AS level curriculum. If you would like to help us and have an idea for such a resource, or would simply like more information on this initiative, then please do contact us.

Doug Corfield
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Donna Brown
(dbrown@physoc.org)

Investigating lung function – practical activities based around the measurement of peak expiratory flow rate

The physiology and pathophysiology of breathing are core components of A-level biology-related curricula. Lung function can be investigated through practical activities based around a simple measurement of peak expiratory flow rate (PEFR). This measurement can be made using a cheap hand-held device which can be incorporated into classroom teaching, at a number of levels, to cue the learning of core content (Box 1) and to address key skills (Box 2). The aim of this guide is to introduce teachers to the measurement of PEFR and to suggest ways in which this could be used in the classroom as an aid to learning.

Background to peak expiratory flow rate (PEFR) and its use

PEFR is a clinical measure of lung function that is particularly useful to monitor disease progression. The speed at which air can be exhaled from the lungs depends on the driving pressure generated by the expiratory muscles and by the resistance to air flow; a greater driving pressure will result in a greater flow, a greater resistance will lead to a reduced flow. As the PEFR manoeuvre is performed using maximum force, it measures the maximum flow rate during expiration; measurements are expressed in litres per minute. Performed correctly, there

Box 1. The lungs and lung function identified within the OCR AS/A level GCE Human Biology

5.1.3 The lungs

5.1.3.1 Investigating lung function: real world context

- It is important for health professionals to monitor various aspects of lung function in order to prevent or diagnose and treat lung disease
- Measurements of lung function are also carried out on athletes in training (see also A2 Module 2866)

Learning outcome

- describe the use of a spirometer and peak flow meter to measure tidal volume, vital capacity, forced expiratory volume per second (FEV_1) and peak expiratory flow rate (PEFR)

should be very little variation in repeated measurements of PEFR in an individual, at any one time.

Resistance to airflow in any tube is proportional to the length of the tube and is inversely related to the radius of the tube raised to the 4th power. This means that if you halve the radius of the tube, resistance will increase 16 fold. The small airways in the lung can be treated as a series of tubes connected in parallel. Changes in the radius of the small airways are common in lung disease. In children and adolescents, the most common form of lung disease is asthma. During an asthma attack the small airways narrow, the resistance to airflow increases and so PEFR falls. PEFR can therefore be used to track the severity of the disease in individual patients; a peak flow meter can be given to the patient to use at home and is often used along with a symptoms diary.

For people without lung disease, there will be differences, between individuals, in the force that their respiratory muscles can generate and differences in lung and airways size. Hence PEFR will differ between people. The most important predictors of PEFR are, age, height, sex and ethnicity.

Measuring and interpreting PEFR

Mechanical peak flow meters (right) cost around £10 each and can be obtained from a number of manufactures. More expensive electronic meters are also available. Although intended for individual patient use, the mechanical meters can be shared by different individuals, most easily by using disposable cardboard mouthpieces. Alternatively, the plastic mouthpiece can be cleaned between each use.

All peak flow meters will include instructions but, briefly, to take a peak flow reading you should:

- stand up
- slide the pointer down to the bottom of the scale at the end where you blow in
- lightly hold the meter horizontally, keeping your fingers away from the pointer
- breath in as deeply as you can
- holding your breath, place the mouthpiece into your mouth and seal your lips tightly around it
- blow out as hard and as fast as you can for a second or more - as if you were blowing out candles on a birthday cake. Be careful not to block the mouthpiece with your tongue or teeth
- look at the pointer and note your reading
- reset the pointer to the bottom of the scale
- do this three times and record the highest reading.

Source Vitalograph Ltd

Charts predicting PEFR, based on age, height and sex, can be downloaded from the manufacturers' web sites.

Suggestions for activities based around the measurement of PEFR

A classroom demonstration by the teacher as a cue to discussion. For example:

- How does the body move air in and out of the lung?
- What diseases affect the lung?
- How can we measure how bad lung disease is?

A simple experiment

Each pupil measures their own PEFR:

- Line the children up, side by side, in rank order of their PEFR
- What patterns do you see as you look along the line (height, sex)?
- Does this fit with your expectations?

Choose a child to represent an asthmatic

- How would their PEFR, as represented by their position in the line, change as their asthma worsened/improved, with treatment/without treatment?
- Move the pupil up and down the line to illustrate this

Box 2. OCR AS/A Level Key Skills

Activities using the measurement of peak expiratory flow can be tailored to address Level 3 Key Skills including: Communication, Application of Number, IT, Working with Others and Problem Solving. The ease with which large sets of original data can be generated using PEFR allows students to address Key Skills related to the Application of Number, specifically:

N3.1 Plan and interpret information from two different types of sources, including a large data set

N3.2 Carry out multi-stage calculations to do with amounts and sizes; scales and proportion; handling statistics; rearranging and using formulae. You should work with a large data set on at least one occasion.

N3.3 Interpret results of your calculations, present your findings and justify your methods. You must use at least one graph, one chart and one diagram

Relate the measured responses to the predicted reference values
- Does a scatter plot of measured vs predicted form a straight line?

Project option
- As PEFR is simple to measure it is an ideal tool with which to gather larger data sets. Such data sets allow pupils to explore different factors that may affect PEFR including the effects of:
  - age, sex, ethnicity or puberty
  - anthropomorphic factors e.g. height, chest wall diameter

As part of any project, the pupils will be able to analyze and present data using descriptive statistics (means, confidence intervals, predicted ranges) and regression analyses. Hypothesis testing studies to test, for example, the effects of exercise and training on PEFR could be performed and data analysed using Student's t-test.

Ethical and health and safety considerations

PEFR is a clinical measurement of lung function. A pupil may be concerned if his or her measurement is lower than the reference predicted value. However, the reference values provided here do not include the range for the normal values, only the mean. For PEFR, the normal range is quite wide (indeed PEFR is best used to track changes within individuals) and so both 'high' and 'low' values are to be expected. If, however, a pupil has a low PEFR value and reports other respiratory difficulties, then you should suggest the individual consults the school nurse or their GP.

The PEFR manoeuvre is entirely safe. On occasion, the deep breaths necessary for the measurement may induce transient light headedness. If this happens the individual should stop the manoeuvre.

As the PEFR manoeuvre only requires the subject to breathe out through the meter, any risk of cross-infection is minimal. Some models of peak flow meter (e.g. Clement Clark Wright Mini peak flow meter) have a one way valve built into them to prevent breathing in; these can be used with a simple reusable or disposable cardboard mouthpiece. Others, without this valve (e.g. Vitalograph) are best used with a disposable cardboard mouthpiece, that incorporates a one way valve. Except when a meter is used by only one individual, a new or sterilised mouthpiece should be used for each participant.

References and further resources

Manufacturers of peak flow meters and mouthpieces

Vitalograph Ltd, Maids Moreton, Buckingham, MK18 1SW.
Phone: +44(0) 1280 827110; Fax: +44(0) 1280 823302; Email: sales@vitalograph.co.uk
http://www.vitalograph.co.uk/products/mechanical_peak_flow_meters.html

Clement Clarke International Ltd, Edinburgh Way, Harlow, Essex CM20 2TT
Phone: +44(0) 1279 414969; Fax: +44(0) 1279 456304; Email: resp@clement-clarke.com
http://www.clementclarke.com/shop/index.html?cat_branch=shop/peak_expiratory_flow/

Background information on lung diseases

All about asthma http://www.asthma.org.uk/all_about_asthma/index.html

NHS direct health encyclopedia: *Asthma*
http://www.nhsdirect.nhs.uk/articles/article.aspx?articleId=36&sectionId=22401

NHS direct health encyclopedia: *COPD*
http://www.nhsdirect.nhs.uk/articles/article.aspx?articleId=540

PEFR further information and reference values

http://www.peakflow.com

Simple table of reference values
http://www.vitalograph.co.uk/pdf_library/productleaflets/asthma_copd/pfm_pred_values.pdf

More extensive information & reference values
http://www.vitalograph.co.uk/spirometry_normal_values_guidelines.html
http://www.peakflow.com/top_nav/normal_values/index.html

New Society membership categories

Associates
Associate membership is offered to individuals with a background in physiology who do not qualify for Ordinary, Affiliate or Undergraduate Associate membership; for example: university staff engaged in learning and teaching, employees of industry and physiology graduates in other spheres.

Undergraduate Associates
Undergraduate membership is set up through individual universities/institutions under the umbrella of The Physiological Society although individual applications will be accepted.

School and College Associates
School and College Associate membership is available to secondary and college educators.

For more information about all of these membership categories please visit: http://www.physoc.org/membership or call Irrum Magre on 020 7269 5726.

Council Election 2007

The Council of Trustees is legally responsible for the overall governance, management and policy of The Society, ensuring that the charitable objectives for which it has been set up are met. The Trustees are also the Directors of The Society. Elections to the Council take place annually and Trustees are elected to serve for a period of 4 years. This year there are two vacancies and the results of the election will be announced at the AGM on Wednesday 11 July.

The election will take the form of a simple ballot. Members have two votes and the two candidates with the most votes will be elected. For the first time, voting will be done through The Society's web site, using a secure online voting system. As well as reducing costs, it is hoped to significantly increase Member participation in the election process. Voting will open on Monday 11 June and close on Thursday 5 July.

Copy deadlines mean that full information is not available at the time of writing, but further information and details will be communicated to Members through The Society's web site, by email, and in the letter from the Chair of the Executive Committee accompanying the AGM papers.

Memorable technicians

Behind many Memorable Members of The Society there were memorable technicians. Jock Austin (right) was a remarkable example, and is remembered here by two Society Members who knew him well

Memorable technicians – a long-gone breed who, having left school, possibly at 14, with no formal qualifications, were nevertheless sometimes indispensable sources of knowledge and advice. Jock Austin, David Whitteridge's technician, was a remarkable example. His attachment to physiologists, as well as to physiology, is exemplified by the Godparents he and his wife, Pam, chose for their four children – seven of the eight being Members of The Physiological Society, and the eighth a Member's wife.

Two members of The Society have written short pieces about him; Ann Silver's was written for *Physiology News*, Iain Donaldson's was an obituary written – under strict constraints of space – for the *Edinburgh University Bulletin* shortly after Jock's death. There is a little repetition of material between the two pieces but, since their styles are different, it has seemed better to reproduce them as they are rather than to try to fuse them.

A reminiscence of Jock Austin by Ann Silver

Jock's association with David began in 1944. While serving in The Glider Pilot Regiment he received head injuries in a glider crash. To improve the movement in his paralysed hand, he was sent by St Hugh's Hospital to the Oxford Anatomy Department to help the carpenter, Percy Paede (later his father-in-law). Jock's doctor was the neuroanatomist Graham Waddell, and it was at his suggestion that Jock moved next door to join David in the University Laboratory of Physiology. There, under David's tuition, he revealed a flair for electronics and a strong bent for neurophysiology. In 1950 he moved with David to Edinburgh where in his unique, idiosyncratic way he inventively supported (and sometimes infuriated) David and the other electrophysiologists in the Department. Postgraduates and Honours students will always remember Jock's help and advice with gratitude. I spent the first year of my PhD building an enormous electophysiological apparatus under his guidance. His slight dysphasia (another legacy of his brain injury) coupled with my pretty tenuous grasp of electronics, meant that I sometimes soldered the wrong components together. The dysphasia also meant that he was better at making things than explaining how they worked – which they almost invariably did. His custom-built apparatus was invaluable in the days before much electrophysiological equipment was commercially available (ex-War Department components were, however, very cheap). The nerve stimulator that Jock made for class use was eventually marketed by Electrophysiological Instruments, a company that he co-founded. Later he was involved in developing a masking device that, by preventing stammerers from hearing their own voices, helped them overcome their problem. He helped Morrel Draper and David in a collaboration with Peter Ladefoged of the Phonetics Department. The object was to record potentials from intercostals of subjects making different speech sounds. Initially there were difficulties because some of those present during the experiments fainted at the sight of the needle electrodes approaching a bare chest. There is a story, possibly apocryphal, that the fainting rate was less when the subjects were female.

It wasn't only in the lab that Jock came up with practical solutions. He once removed an obstruction in a Hoover tube by forcing it out with water from the garden hose. Remarkably, when he had a slight stroke and temporarily lost his speech he immediately realised what had happened, attracted his wife's attention, and typed out 'stroke' on his computer. He brought up his children to be equally practical, expecting the girls as well as the boys to turn their hands to welding, lambing and any other jobs required on the Caithness croft to which he first retired. When asked why he was only luke-warm about a particularly pleasant future son-in-law he dismissed him as 'too wee to be much use about the croft'. Eventually work on the croft became too onerous so Jock and Pam moved to the old Austin family home in Portmahomack, Ross-shire. It was there that he died, fittingly in his workshop.

A short obituary of Jock Austin (1921-1994) by Iain Donaldson

W T S Austin – universally known as Jock – died on 29 July. Born in Forres, he was a glider pilot in the second World War. He crashed and received a very severe head-injury; the dominant cerebral hemisphere was damaged and he was speechless and paraplegic.

Treated in the Radcliffe Infirmary in Oxford by Hugh Cairns he had to learn to speak again – aided, to his disgust, by an *English* speech therapist. His recovery was remarkable; even more so his achievements in spite of persisting dysphasia and loss of fine control of his right hand. He became David Whitteridge's laboratory technician and moved with him when he took the Chair of Physiology in Edinburgh in 1950.

Although electrophysiology was well established by then, there was no commercial apparatus; everything had to be home-made. In the mid 1950s the first task of a new lecturer in neurophysiology was to build his equipment (or, rarely, to have it built for him) as a minimum, an oscilloscope, electronic stimulator and amplifiers. These were made largely from ex-WD components under the direction of Jock who built the prototypes and the Professor's models which others then copied or, at their peril, modified. Jock's particular genius was in seeing exactly what was needed for a new experiment then going off and making it. These gadgets were exactly adapted to their tasks, always ingenious, often idiosyncratic. One of the legacies of the head-injury was an inability to draw out circuits or to work out how to wire rotary switches (from the back) so that their actions when operated from the front of the instrument panel would be predictable. With the famous 'Jock boxes' which all the neurophysiologists used – a cubic metre of oscilloscope, amplifiers and stimulator, all home-made and perched on a stand above huge power supplies, lead-acid accumulators and high-tension batteries – one's first experiment was always to discover which knob did what when turned which way. It never occurred to any of us to dare to relabel them; that would have been lèse-majesté.

In the Professor's laboratory it was unthinkable to do an experiment without Jock's presence along the corridor if not in the lab; he was often called to kick into life (sometimes literally) one of his devices and always to supply and apply the match sticks with which to wedge the amplifier HT wires into the battery – since it worked when he did it and was less responsive to the ministrations of others. Jock's contribution to the renowned work of David Whitteridge was essential, enormous, and most willingly acknowledged.

He contributed greatly also to the work of the Department's students, undergraduate and postgraduate, playing a large part in the practical classes for the Honours Class.

With his wife, Pam, he started a small firm to build and sell his instruments; they were to be found in many laboratories but the company was never big enough to be very successful and, in the end, its most popular designs were acquired by Palmer who continued to sell them, in larger numbers, shinier boxes and at higher prices, for years, until the arrival of small laboratory computers and large-scale integrated circuits changed everything.

In latter years Jock's health was not good but, when he retired in 1978, it was to farm a croft near Lybster in Caithness, as always with Pam, and to continue to build boats, as he had for years, and sail them from his cottage in Portmahomack. Four years ago they retired for the second time, to Portmahomack. There he died suddenly but peacefully, in his workshop, in his 73rd year.

The world has moved on and we shall not see his like again; a career like Jock's who was not 'trained' and fitted no category of the 'blue-book', would no longer be possible. Nor would the present safety-at-work – should it be safety-or-work? – regulations allow students to build, modify, and rebuild their equipment, and in the process thoroughly understand it, as we did with so much benefit under Jock's guidance. Science is the worse for that. He is survived by Pam his wife, their two sons and two daughters and many grandchildren.

Do you recall a memorable Society Member or a technician who worked with one? Send your suggestions for future articles in this series to lrimmer@physoc.org

Equipment donation

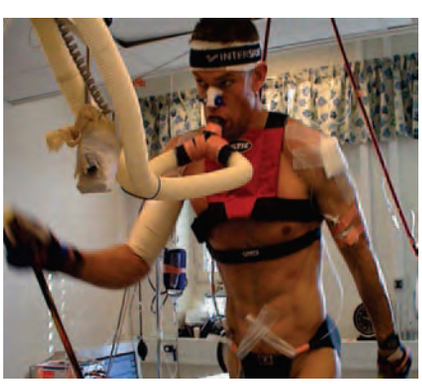
Marcela Nadal at the New York University School of Medicine will be setting up a laboratory in Argentina in September and is looking for donations of scientific equipment (particularly for electrophysiology).

If anyone is able to help please contact Marcela at nadalm01@med.nyu.edu

The Journal of Physiology symposia

The Journal of Physiology sponsored two symposia at Experimental Biology 2007 in Washington, DC. The first, *Obesity and the central nervous system*, co-sponsored with the CNS section of the American Physiological Society on Monday 30 April, attracted some 500 participants. The four speakers – Barry

Levin (New Jersey Medical School), Mary Dallman (University of California, San Francisco), Greg Morton (University of Washington, Seattle) and Steven Heymsfield (Merck Research Laboratories, Rahway) – clarified the latest research on the brain's role in obesity,



Around 300 participants turned up for the second symposium, *Exercise hyperemia: are there any answers yet*? on Wednesday 2 May, despite the early start, to hear Bengt Saltin (Copenhagen), Phillip Clifford (Milwaukee), Janice Marshall (Birmingham, UK), Dirk Duncker (Rotterdam) and Michael Joyner (Rochester) speak.

Proceedings of the symposia are due to be published in *The Journal of Physiology* on 1 and 15 September.

The Journal of Physiology

The Journal of Physiology and its association with the space shuttle Columbia

> ***'Mankind is led into the darkness beyond our world by the inspiration of discovery and the longing to understand'***
>
> **(President George W Bush speaking of the loss of Columbia)**

The 15 March 2007 issue of *The Journal of Physiology* (**579**, 799-810) contains an article by Iwasaki *et al*. entitled 'Human cerebral autoregulation before, during and after spaceflight'. This is the seventh in a series published by *The Journal* detailing the research carried out on flight STS-90 of the space shuttle Columbia. That flight was from 17 April to 3 May 1998 and was the 90th shuttle mission and 25th for Columbia. Two of the co-authors of the article were the payload specialists on the flight, James A Pawelczyk and Jay C Buckley Jr. The payload was Neurolab, a module for research into the effects of microgravity on the nervous system.

Altogether *The Journal* has published nine articles detailing research carried out on the space shuttle Columbia. Historically the most important of these was the one published in the 1 May 2006 issue (**572**, 829–838), by Iellamo *et al*. with a Perspectives article by James Pawelczyk (607–608). It presented the research carried out on the last flight of the shuttle. The crew themselves participated in the research and tragically died before the study was completed. *The Journal* published the results obtained, with a moving title-page footnote paying tribute to the crew.

That flight, STS-107, launched on 16 January 2003 and was the 113th shuttle mission and 28th of Columbia. They took a bicycle ergometer into space, and concluded that the muscle metaboreflex is enhanced during dynamic exercise in space and that the potentiation of the muscle metaboreflex affects the contribution of the vagally mediated arterial baroreflex to control of heart rate.

Flight STS-90 (above), the 25th mission of Columbia, was launched on 17 April 1998. Flight STS-107, the 28th and last mission of Columbia (opposite). Photos from NASA (http://www.nasa.gov)

In the words of the NASA web site:

'*Communication with the crew and loss of data occurred ... while Columbia was at a Mission Elapsed Time (MET) of 15 days 22 hours 20 minutes 22 seconds. The vehicle broke up while travelling at 12,500 mph (Mach 18.3) at an altitude of 207,135 ft over East Central Texas resulting in the loss of both vehicle and crew*.'

Jonathan Goodchild
Senior Production Editor, The Journal of Physiology

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Forthcoming *Journal of Physiology* Symposia at IBRO World Congress of Neuroscience, Australia 16 July (Brain adaptations for a successful pregnancy); 19 July (The cortex, interneurones and motoneurones in the control of movement).

The Journal of Physiology symposium at the 51st Biophysical Society Annual Meeting

The inner harbour in historic Baltimore, Maryland was the setting for a satellite symposium sponsored by *The Journal of Physiology* on *Regulation of ion channels and transporters by phosphatidylinositol 4,5-bisphosphate (PIP_2)*, held in conjunction with the 51st Biophysical Society Annual Meeting. This symposium brought together top scholars in the field to speak on many aspects of this topic, from the use of optical indicators of phosphoinositides and their concentrations inside cells, to the role of PIP_2 in regulation of K^+, Ca^{2+} and Trp channels, new techniques of PIP_2 manipulation in individual cells, the influence of phosphoinositides on trafficking of membrane transport proteins, and the use of structural and cellular models of PIP_2 synthesis, hydrolysis, and action on ion channels. The organizing chairs of the symposium were Nikita Gamper (University of Leeds, UK) and I (Mark S Shapiro, University of Texas Health Science Center, San Antonio, TX). Brian Robertson (University of Leeds) served as organising Editor from *The Journal of Physiology*.

One focus of the symposium was the physiological roles of phosphoinositides in regulation of M-type K^+ currents (made by the KCNQ/Kv7 family of channels) by $G_{q/11}$-coupled receptors. David Brown (Professor of Pharmacology, University College, London) gave a stimulating and comprehensive talk on neural control of M current that featured new probes for measuring and controlling phospho-inositides in neurons. He also elegantly highlighted the distinct signalling mechanisms used by different agonists, a recurring theme of this symposium. Bertil Hille (Wayne E Crill Endowed Professor of Physiology and Biophysics, University of Washington) reviewed the wide-ranging actions of PIP_2 on myriad ion channels, and was one of two speakers to feature the novel chemically-induced dimerization (CID) technique of manipulating PIP_2 levels rapidly (on the time scale of a patch-clamp experiment). He provided an exquisitely detailed glimpse into the signalling pathway linking muscarinic stimulation to modulation

David Brown (above) elegantly highlighted the distinct signalling mechanisms used by different agonists, a recurring theme of the symposium. Baltimore by night (below).

of KCNQ K^+ channels. Tamas Balla (Head, Section on Molecular Signal Transduction, National Institutes of Health) also spoke on the CID technique, highlighting its use in the study of Trp channels. He gave an exciting look at the use of a variety of different phosphoinositide-binding domains fused with fluorescent proteins to localize not only PIP_2, but also PIP_3 and singly phosphorylated PI(3)P and PI(4)P. Localization of particular lipids to golgi membranes was particularly noted.

I and Thomas Voets (Laboratory of Ion Channel Research, University of Leuven, Belgium) gave well-received talks on regulation by PIP_2 of two other important families of ion channels by PIP_2. Thomas spoke elegantly on modulation of TRP channels by PIP_2, voltage and Ca^{2+} ions, focusing on the TRPM4 and TRPM8 types of channels. He showed how PIP_2 binding to the 'pleckstrin homology' domain within TRPM4 favors opening by shifting the voltage- and Ca^{2+}-dependence of the channels towards more physiological ranges. My talk centered on regulation of high-threshold (N- and P/Q-type) voltage-gated Ca^{2+} channels by different $G_{q/11}$-coupled receptors. Following themes brought up by David Brown and Leslie Loew (Professor of Cell Biology, University of Connecticut Medical Center), I showed work suggesting that receptor specificity in the modulation of Ca^{2+} channels is conferred by receptor-specific stimulation of PIP_2 synthesis, compensating for PIP_2 depletion by phospholipase C activity. Indeed, mine was but one of several presentations illustrating the complexity of lipid-mediated signaling pathways. Donald Hilgemann (Professor of Physiology, University of Texas Southwestern Medical Center) is widely credited as the first to realize the regulatory role of PIP_2 on ion channels. His expert talk explored the dual role of phosphoinositides in the control of cardiac membrane transporters, the first being direct activation of transport proteins such as the Na^+/Ca^{2+} exchanger, and the second, indirect inactivation of the exchanger by PIP_2-mediated endocytosis.

Another welcome focus of the symposium was the use of physical and chemical models. Both Leslie Loew and Diomedes Logothetis (Professor of Physiology and Biophysics, Mount Sinai Medical School) emphasized powerful models in the understanding of PIP_2 regulation of channels, but of different model types.

Leslie Loew showcased the 'virtual cell' modelling environment of the Center for Cell Analysis and Modeling, of which he is Director, using it to perform kinetic analyses of receptor-activated phosphoinositide turnover. The analysis indicates the requirement for receptor stimulation of PIP_2 synthesis, concomitant with its hydrolysis, in order to account for the amount of IP_3 which such receptor stimulation produces. Diomedes Logothetis utilized structural models of inwardly-rectifier K^+ channels of bacteria and mammals. Drawing from his lab's work in the study of PIP_2 regulation of a variety of distinct K^+ channels, as well as published crystal structures of the channels, he indicated the likely channel residues that make intimate contact with the lipid molecules, and that are responsible for the diverse sensitivities of these channels to PIP_2 abundance in the membrane. His talk emphasized the likely importance of both electrostatic and hydrophobic interactions between protein and lipid. Finally, co-chair Nikita Gamper summarized the day's action with a crisp postscript. All the talks were enlivened by stimulating question and answer sessions, with debate and discussion by all present.

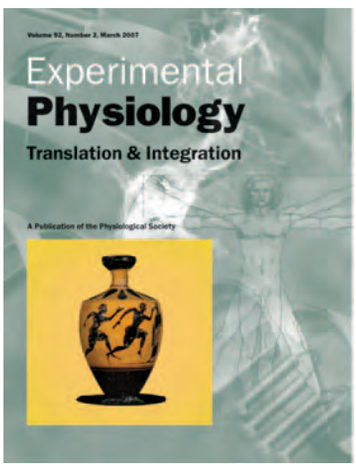
After the end of the scientific sessions, the speakers, organizers and selected guests were treated to a marvellous dinner at the *Black Olive* restaurant, a local favourite in Baltimore's Little Italy section. The scientific discourse continued unabated as the delicate wine flowed and the lovely Greek cuisine was brought forth by the attentive staff. Notwithstanding a potentially catastrophic incident with the credit card, a hearty vote of appreciation was given to the Editors and staff of *The Journal* for an enlightening event.

Mark S Shapiro
Associate Professor of Physiology, University of Texas Health Science Center, San Antonio, TX, USA.

More images of Baltimore appear on the inside back cover.

Experimental Physiology
Translation & Integration

Muscle-energetic and cardio-pulmonary determinants of exercise tolerance in humans



The March issue of *Experimental Physiology* contains reports from the Symposium *Muscle-energetic and cardio-pulmonary determinants of exercise tolerance in humans* which took place at The Physiological Society's meeting in London last summer.

Recognising that reduced exercise tolerance is a cardinal feature of ageing and many cardiorespiratory and metabolic disease states, the purpose of this symposium was critically to address putative mechanisms that might contribute to fatigue during whole-body exercise.

Tony Sargeant presented a critical analysis of the relative contributions of different skeletal muscle fibre-types to force generation and fatigue in the context of the muscles' power-velocity relationships. The energetic basis for the support of force generation was then addressed by Kevin Conley, who focused on the phosphorylative coupling efficiency of human skeletal muscle mitochondria in vivo (i.e. the P:O ratio) with ageing. The emphasis on ageing was continued by David Poole in the context of the skeletal muscle microcirculation and its role in supporting O2 delivery and consumption. Brian Whipp extended the scope of these considerations to how the functional linkages between muscle and pulmonary dynamics for pulmonary CO_2 exchange differ from those for O_2 exchange. Finally, the expression of the gas exchange responses as requirements for pulmonary ventilation was addressed by Susan Ward, with an emphasis on potential constraints and limitations.

In conclusion, these wide-ranging physiological-system perspectives illustrate the multi-factorial nature of exercise intolerance, and have the potential to provide insights into possible means of ameliorating the functional declines associated with chronic sedentarity, ageing and disease.

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Hydromineral neuroendocrinology

The May issue contains reports from a symposium entitled *Hydromineral neuroendocrinology* which took place last summer at the International Congress of Neuroendocrinology in Pittsburgh, USA.

The four symposium reports address key issues in the central control of body fluid homeostasis. The ongoing research described ranges widely from cellular and molecular neurobiological investigations to whole animal studies.

The first two papers present intriguing new data regarding the neural cells that sense changes in osmolality, and the molecular mechanisms by which they do so. The first paper, by Bourke *et al.* (2007), describes a detailed series of studies of osmosensory neurons in the OVLT. The second, by Liedtke (2007), describes in greater detail molecular studies of the TRPV family of cation channel proteins, and presents evidence supporting roles for TRPV1, TRPV2 and TRPV4 channels in the transduction of osmotic stimuli in mammals.

The last two papers focus on aspects of sodium homeostasis. Although cerebral osmoreceptors plainly provide control over thirst and AVP secretion, there is evidence that putative cerebral sodium receptors make an independent contribution to body fluid homeostasis. However, it has never been clear whether cerebral sodium receptors actually exist. Noda (2007), summarizes a series of recent experiments that demonstrate the existence of such receptors and characterize some of their properties. Daniels *et al.* (2007), address the cellular mechanisms by which AngII, acting in the brain, stimulates thirst or salt appetite in rats.

Collectively, these four papers present exciting new results at the forefront of studies of the how the brain controls body fluid homeostasis.

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BIOSCIENCES FEDERATION

The Biosciences Federation is seriously concerned about the loss of practical skills across the full range of the biosciences. That is, from ecology to *in vivo* pharmacology and from taxonomy to biochemistry. The biosciences are practical subjects and yet, in our schools and universities, the amount of practical experience that students acquire continues to diminish. This decline is likely to continue because we have lost and are losing teachers with practical skills.

For my A-levels we went out into the fields and threw metre squares 'randomly' on patches of grass and then proceeded to count the number of certain plants and insects within the square. Many of you will have had a similar experience at school or university and will probably remember, as I do, the enjoyment of these outings – and not just for getting your square around someone's neck! But this is now a rare educational activity. And the loss of training in field work is important because, for example, the subtle change in the distribution of lichens is an indicator of climate change. We have lost many lichenologists, and many of those who remain are close to retirement. To embark on a project in the field in this area now requires more than the usual attention to the competence of your supervisor: you could find yourself working on wrongly identified lichens.

The same is true for scientists with *in vivo* skills. Once again, I have fond memories of tracing dogfish cranial nerves – well, perhaps not so fond because I was not addicted to formaldehyde! But it was an introduction to animal work and developed a real awareness of how nerves pass through tissue and bone. The work brought a three dimensional understanding of line drawings and excited interests that I suspect would not have been ignited without this experience. Some will argue that a prosected dogfish can provide nearly all these educational elements – it is a debate that those involved in medical education know well. Nonetheless, some practice on cadavers seems preferable to the alternative for veterinarians, doctors and those using animals for research. Today, the pharmaceutical industry has great difficulty in recruiting in this area because few are qualified for the work.

Of course, not all bioscientists need to throw metre squares and cut up dogfish in order to make a research or teaching career in one of our disciplines. However, they are likely to need to make up reagents correctly and this is not a skill that one can anticipate today in all graduate students. The point is, the decline in practical skills threatens the strength of the biosciences.

How has the present situation arisen? There is no single answer to this question, but the expansion of university bioscience courses is an important component of the answer. With doubling, trebling and quadrupling of student numbers in the biosciences, it has often proved too difficult to find and pay for the space and staff to enable practical work of a high standard to continue. Indeed, as you will know, many courses are structured to minimise the need for practical training. It is possible today to do an Honours degree in Pharmacology and, if you are predicted to obtain a lower second class degree, your Honours project will be in the library. Graduates lacking practical skills will not usually attempt to find the time for more practical work when teaching in secondary schools.

What can be done to reverse this deteriorating situation? Clearly, motivation and money are needed. Motivation comes from need and leads to money. The ecological and *in vivo* examples given above were chosen because they are in areas where the need is real and so is the possibility of extra resource. We do not think that we can usefully argue for an all-embracing single step solution to this problem, but we do think that we can target areas and work with others to achieve change. Indeed, we are quietly achieving significant success through the work of our Animal Science Group and our Education Committee. The loss of practical skills is now part of the national agenda and resolution of particular needs is being discussed in a positive way with government and those involved with education.

Richard Dyer
Chief Executive Officer

NEWS

Consultations

The Society will be working with the Biosciences Federation to formulate a response to the EC Consultation on the European Research Area (ERA), in particular the Green Paper *The European Research Area: new perspectives*.

For more information on the EC consultation exercise and the Green Paper please visit http://ec.europa.eu/research/era/consultation-era_en.html

If you have any comments that you would like to submit to the BSF ERA Task Force please contact Liz Bell (ebell@physoc.org) as soon as possible.

The Society has also recently responded to the HEFCE *Review of the Teaching Fund Method* through the BSF. The BSF response can be found at: http://www.bsf.ac.uk/responses/HEFCE_teachfund.pdf

Better regulation initiative

Scientists working under the Animals (Scientific Procedures) Act have an opportunity to contribute to initiatives to improve the efficiency of the regulatory framework. Researchers with experience of the system are encouraged to get involved, for example, in the Home Office consultation process or in relaying experiences of the new low maintenance approach for PPL applications. For more information please visit: http://www.physoc.org/downloadfiles/RDSNewsSpring07.pdf

Athena SWAN Charter

The Athena SWAN Charter is a scheme for UK universities and their science, engineering and technology (SET) departments that recognises, celebrates and disseminates excellence in SET employment in higher education. It aims to assist the recruitment, retention and progression of women in SET. For more information please visit http://www.athenaswan.org.uk

For news updates visit our online noticeboard at http://www.physoc.org/news

The spice of life

We have reached that part of the British university year, the end of undergraduate teaching, when one's thoughts often turn to life outside, or even beyond, work.

This probably reflects a combination of extra time to take stock – no more dashing to lectures and tutorials, tripping over the ever-increasing numbers of students in laboratories and corridors, and trying to hide from tutees who want to know if you can write them another reference for the postgraduate law conversion course – and a good dose of mid-life crisis, at least in the Cain Empire.

Add to this that, as I speak, a university near you will doubtless be launching another early retirement or voluntary redundancy scheme and you can see how the prospect of an alternative career might loom large in my middle-aged mind.

But a career doing what? Most people younger than 60 will probably not want to do nothing. And it would be nice to keep doing something vaguely scientific, if only to make use of the accumulated know-how cluttering up the brain.

After some thought, I reckon I have come up with the answer: Alternative Medicine – and more specifically, selling herbal, or natural product, 'therapies'.

No-one looking at the shelves in Boots, or even in your local supermarket, could fail to be impressed by the amount of alternative remedies on sale. And as for the Internet – don't get me started.

And they're using our work to sell the stuff. Really.

Yes, a recurring theme in selling alternative remedies is to make them seem science-based. More often than not the Internet site for the magical herbal ingredient will tell you that scientists have shown it kills cells (if the cells are bad) or saves them from free radical damage (if the cells are good). Sometimes they even give a reference. Of course, the studies they refer to are usually done in a dish, with whopping doses of whatever natural product they are plugging, and the bioavailability of the stuff taken orally is usually dismal, but you wouldn't know that from reading the sales pitch.

And there are so many studies to cite, because every year the number of published papers that examine natural product effects goes up. And up. And up and up.

For instance, papers on Medline citing curcumin went from a mere 161 in 2000 to a whopping 1,320 in 2006. 'Garlic' AND 'cell' generated 43 hits in 2000, but 480 last year. The black pepper constituent piperine was barely on the map with 17 papers in 2000, but scored 194 in 2006.

So we scientists love our natural products. The only trick we have missed is COMBINING two or more of these agents in a single paper. For instance, searching 'curcumin AND garlic AND cell' together generated only a measly two hits – which would strike anyone who likes a good curry as clearly daft.

Anyway, the take home message is that, no matter what natural product someone wishes to tout as a treatment for something, there will be a good few kosher scientific papers they can cite – or rather mis-cite – as support.

Which is where we came in. Because what I say is: if you can't beat 'em… join 'em. Or rather, we should cut out the middle man. After all, it's our work these Snake Oilers are using to make money flogging this stuff to the punters. Why shouldn't we scientists be the ones raking in the profits?

Ergo, step one in the Cain masterplan: publish a paper – any journal will do – combining several spice or food ingredients in a study on cells in a dish that suggests a possible Alternative Health use. For instance, making cells release more of something your body needs, like insulin or some other critical hormone.

Step Two: start selling the same mixture online, plugging the research paper at every opportunity as the proof that it is 'scientifically designed'.

So if you spot me creeping off to the local cash 'n' carry to stock up on 5 kg bags of turmeric, ground red and black pepper, and garlic powder, you will know why. Coming soon to an Internet site near you:

'NEW InsulinoSpice™ – the carefully formulated herbal treatment for type II diabetes. Scientific research by Dr Mark Cain and his colleagues at Popplechester University has shown that the spices in InsulinoSpice™ act together to help your cells produce the insulin your body needs. InsulinoSpice™ is formulated in accordance with the latest advanced research. It brings you the perfect balance of high-purity ingredients, designed to work in harmony with the body's natural mechanisms to ensure optimum health.'

That should ensure a nice steady income in retirement.

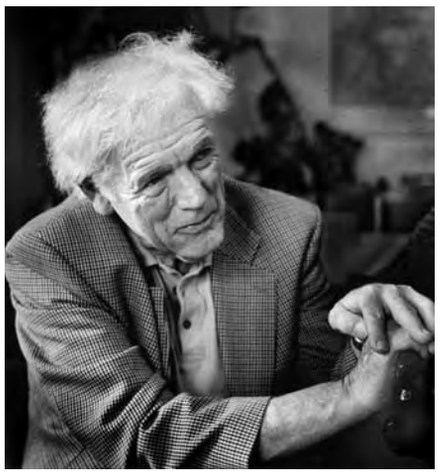
Of course, I will have to stay in my current job long enough to get the research done for step one, and to use the lab balance to weigh out the spice mixture into those nice little plastic vials we use for scintillation counting. And maybe I could get the undergraduate students to build the web site and design the labels, all as part of their 'Communication Skills' module, of course.

So – today the culture dish – tomorrow the world. You heard it here first.

Mark Cain

Eric J Denton

1923–2007



Professor Sir Eric Denton was one of the most distinguished marine biologists of the past century. He had the rare ability to see fascinating and important problems manifested in common animals such as herrings and mackerel that others had neither tackled nor even noticed. He worked chiefly with fish, squid and cuttlefish, and the scope of his work was notable: from buoyancy, to eyes and vision, photophores, camouflage and intraspecific signalling.

Denton began his scientific career at TRE, the radar research establishment at Malvern, towards the end of the war. He took a degree at St John's College, Cambridge, and then joined the biophysics research unit at University College London, which was a valuable nursery for successful biophysicists. He then took up a lectureship in physiology at Aberdeen where he stayed until 1956, when he began his long and illustrious career in Plymouth at the Marine Biological Laboratory (MBA) on Plymouth Hoe.

He was first a staff physiologist, then a Royal Society research professor seconded from Bristol until 1974, and director of the laboratory from 1974 to 1987. After retiring as director, he continued to work at the laboratory as a research fellow until 2005.

As director he led the mixed (and sometimes awkward) team of individualists on the laboratory staff with much success, walking around regularly asking: 'What did you discover today?' With a lesser man this might have been irritating, but Denton's obvious interest and pleasure in talking about others' work made it encouraging and helpful.

Under his direction, with the support of an eminent president and council, the MBA flourished and attracted many visiting workers. Living squid from the laboratory trawler were available, and attracted many visitors such as Sir Alan Hodgkin, Sir Andrew Huxley and Professor Richard Keynes. They came to work on the astonishing giant nerve fibres of the squid mantle, and began their day when the squid arrived, retiring in the small hours, to reappear for a mid-morning coffee. Denton himself began work on bound water within squid axons, but soon turned to the way physiology underpinned the whole lives of marine animals.

This first main interest was signalled by a long paper on the buoyancy of fish and cephalopods, where he reviewed what was then known about the way in which the swimbladder of bony fish was filled with gas, and showed how many squid floated on tanks of ammonium chloride. He later found that the giant squid *architeuthis* used this mechanism.

His work on cuttlefish showed that the cuttlebone was an ingenious and adjustable buoyancy device, osmotically driven, and this result (extended to the shells of the little spirula and much larger nautilus) enabled him to explain the buoyancy of the long-extinct ammonites. Later work on buoyancy showed that many deep-sea dogfish sharks achieved neutral buoyancy by storing especially light oil in their enormous livers, as do basking sharks.

The next topic that attracted him was the silveriness of many fish scales. Like many of Denton's discoveries, his work on silver camouflage seemed obvious, but it needed his insight to see the answer and how to tackle it with simple home-built apparatus. He had realised that the symmetrical polar distribution of light in the sea made it possible for fish to camouflage their sides with mirrors. This they did by silvering their scales with guanine, but this left the problem of how fish camouflaged themselves against predators looking upwards, when the potential prey would be silhouetted against the light. He showed that fish such as herring and sprat do this by making their ventral surfaces knife-like.

Other fish, such as the bizarre sternoptychid hatchetfish *argyropelecus*, which Denton studied during Atlantic cruises on the large research vessel *RRS Discovery,* use a much more sophisticated method. They shine light downwards from their ventral surfaces, exactly matched to the natural light. Denton's investigation of this complex system, involving colour filters, half-silvered mirrors and intensity matching, was a real tour de force. It was on a *Discovery* cruise too, that he found a dragonfish (*malacosteus*) with a red headlight and discovered that the visual pigment was red-sensitive, so that it could illuminate and see the fish it preyed upon with light that they could not see (their eyes being tuned to blue-green only).

Denton's last main field was the perception of vibrations, including sound by herring. Working with the electrophysiologist Sir John Gray, he showed that the complex and particular link of the herring ear to the swimbladder together with the lateral line enabled herring to be vibration specialists, unlike most fish in the sea.

Such wide-ranging studies, allied with a friendly, considerate and generous nature, brought him many scientific friends worldwide. He received many honours, from the Royal Society's Royal Medal to the International Prize for Biology by the Japanese Society for the Promotion of Science.

Denton is survived by his wife Nancy, a daughter and two sons.

(*Eric Denton became a Member of The Physiological Society in 1952 and an Honorary Member in 1988.*)

Alan G Brown

1939–2006

Alan Brown was a distinguished Edinburgh neurophysiologist who will be remembered for his originality, enthusiasm and humanity. He died on 6 December 2006 at the age of 67. His enduring interest was in the physiology and anatomy of the mammalian central nervous system, especially those components concerned with somaesthetic mechanisms in the spinal cord. His 1982 book *Organisation of the spinal cord* contains numerous neuronal reconstructions reminiscent of Cajal's illustrations, but accompanied by precise information as to their functional characteristics, the nature of their afferent inputs and the potential role of descending pathways from higher centres in the dynamic regulation of somaesthesia. He contributed prominently to our understanding of the diversity of sensory mechanisms in the dorsal horn and his unique contribution was the correlation of these physiological mechanisms with detailed morphology and ultrastructure.

A native of Nottingham, Alan Brown read Medicine at the University of Edinburgh, qualifying with an honours BSc in Physiology in 1961 and MBChB in 1964. After graduation he joined the newly formed Department of Veterinary Physiology at the Royal (Dick) Veterinary College, University of Edinburgh. He received his PhD in 1968, progressed to a Readership in 1976 and to a full Professorship in 1984. He received some prestigious research awards including a Beit Memorial Research Fellowship and MRC Research Fellowships during this period. A member of The Physiological Society since 1968, he was elected Fellow of the Royal Society of Edinburgh in 1984 and Fellow of the Institute of Biology in 1988. He served as a member of the MRC Neurosciences Grants Committee and held editorial positions on *The Journal of Physiology*, the *Quarterly Journal of Experimental Physiology*, the *Journal of Neurophysiology*, *Neuroscience* and *Brain Research*/*Brain Research Reviews*. He was Head of Department of Preclinical Veterinary Sciences for much of the 1990s.

Alan mentored a number of distinguished neuroscientists who achieved high positions in their own right in North America, Australia, UK and elsewhere. They have written uniformly of the warmth of the welcome they received when they visited his laboratory, and of their happy memories of time spent in Edinburgh and the high productivity at that time in their careers. His personality and character were those of the true academic, with dedication and commitment to his discipline, integrity in the conduct and presentation of his research, and a love of the broader intellectual and cultural life that enriches and enlivens the human spirit. He approached life with a characteristic meticulous and thoughtful approach, lacking hubris and self-promotion, and his enthusiasm for scientific debate in the best tradition of The Physiological Society will be remembered by many. Other visitors to his laboratory felt they learned through their visits that the destructive behaviors that existed in their home institutions were not inevitable. Many have commented on how they and their families were welcomed into his home, in the best of Edinburgh traditions, during their visits. Other senior visitors during the period of his headship have commented that despite successive rounds of cuts, his department was a settled one, due not least to his own personal qualities and support for his staff.

My own links with Alan and his family date back to his undergraduate days in Edinburgh and I fully concur the feelings of his distinguished students and colleagues from the 1980s and 1990s. I too enjoyed some time in his laboratory, but my main contact was with him and his family in our home environments over a span of more than 40 years, when the he would often talk about science, music, politics, art, literature and education, not to mention everyday matters of family and work, and one of his favorite hobbies, gardening. Our families visited each other regularly, and our shared enthusiasm for music originated from participation in chamber music of various genres. Alan was a skilled violinist and in adulthood also learned to play the cello; in recent years he was an active member of the New Edinburgh Orchestra.

Alan took enormous pride in his two children, Jeremy and Jessica, who are both in academia. He is survived also by his mother, his first wife, Judith, and his second wife, Patricia, whom he married just weeks before his death, and who looked after him lovingly throughout his final illness.

John F B Morrison

W E Balfour

1927–2007

Bill Balfour died on 1 February after a very long illness. He was educated at Harrow County Grammar School, but because his father had died when he was 11, Bill left immediately after passing School Certificate in 1942 and went to work in the local Kodak factory. While there he took evening and weekend courses at Birkbeck

College. After gaining Higher School Certificate he was awarded a Kitchener Scholarship that enabled him to go to King's College London to take a BSc. He then went to Edinburgh on a scholarship from the Agricultural Research Council (ARC) to do a PhD with Catherine Hebb. Though offered a junior lectureship in Edinburgh, he opted to move to The Physiological Laboratory in Cambridge where he remained for the rest of his working life, first as an ARC Fellow and then as a member of the academic staff.

Bill was elected a Member of The Physiological Society in 1955 and served on the Committee from 1980 to 1984. News of his death prompted a flow of emails from his Cambridge colleagues and ex-students. Woven together here, these highlight his standing as a physiologist, a teacher and a good and wise friend.

Bill's lab was a solitary affair, untidy, full of antique equipment with reprints all over the place. Although constantly at the bench his publication list is short. He was always reluctant to publish unless he felt he had something worth writing about. His preference, when he did publish, was for a letter to *Nature*. He enjoyed discovering novel, important, and frequently counter-intuitive phenomena that would act as a stimulus to others to head off in new directions. He preferred, in his own quiet way, to lead the pack rather than to follow, and a letter to *Nature* is a time-honoured way of doing just that. Having cracked a problem, or demonstrated something important, he was content to let others sort out the boring bits while he moved on to new but always fertile ground.

Bill worked largely alone though early collaborative work included studies on the synthesis of acetylcholine in brain, carrier proteins for thyroxine and triiodothyronine, the role of the carotid body in the regulation of erythropoiesis, the secretion of progesterone by the adrenal gland, and absorption of colostrum in the newborn calf. Later, his main interest was in the regulation of blood volume. As a willing source of updated and critical wisdom on all aspects of kidney function and endocrinology he was invaluable to his colleagues who appreciated what one described as his high standards and sceptical integrity about science.

A trademark of his experiments was their simplicity. This is exemplified by an experiment in the late 1970s. It had been established that when a muscle-derived preparation of ATP was used in measurements of Na^+-K^+-ATPase the enzyme activity was less than that obtained with ATP prepared from yeast. The suspected inhibitory contaminant was eventually identified as vanadate, then attracting interest as a possible mediator of the diuretic effect of atrial distension. However, when sodium orthovanadate was injected into rats its diuretic effect was small and transient. On being consulted by the colleagues involved in this experiment, Bill's reaction, to the surprise of all concerned, was 'What a huge effect!' He set out to prove that the apparently disappointing result was attributable to dehydration. An anaesthetized rat fitted with an intravenous cannula was put on an old-fashioned balance and a primitive but ingenious feedback system set up to keep the rat's weight constant by intravenous infusion. Vanadate induced a diuresis comparable to the animal's weight in a few hours. A beautiful experiment, with the simplicity only profound expertise can deliver.

Bill was a dedicated teacher and an excellent and caring Director of Studies in King's College. His lectures on endocrinology are remembered for their clarity, logical progression, and the vast amount of fascinating information conveyed at a speed that allowed copious note taking (although, according to one student, this was at the expense of an aching hand).

Progressive ill health meant that in his later years Bill became increasingly reclusive but as a young man he had a passion for exotic cars. These included a red Gordon-Keeble – a British car of which only 100 were built, two of them being owned by fellow physiologists in the Cambridge Lab. Another enterprise was making his own trousers and shirts which he did with some success.

Our sympathy goes to Bill's wife Margaret, his three daughters and his grandchildren.

Ann Silver

(using contributions from Hal Dixon, Alan Findlay, James Fitzsimons, James Hickson, Arieh Lew, Miranda Potter (née Harrison) and David Tolhurst).

Paul Lauterbur

1929–2007

Paul Lauterbur, who has died aged 77 from kidney disease, published the first magnetic resonance image in a short letter to *Nature* in 1973 (**242**, 190-191). Though the name he coined for the technique – zeugmatography - never caught on, the method has revolutionised medical imaging, particularly of soft tissues. An estimated 60 million or more magnetic resonance imaging (MRI) scans are now carried out each year. The invention of MRI won Lauterbur many prizes and awards, culminating in the 2003 Nobel Prize for Physiology or Medicine jointly with the British physicist Peter Mansfield.

Paul Lauterbur enjoyed being something of a scientific maverick. As a teenager he built his own basement chemistry laboratory, and would recall as an early inspiration a chemistry teacher who let him get on with doing self-designed experiments 'while the rest of the class got a lecture'. The lifelong independence of mind led him to give up MRI research in his 70s, as he joked, 'just in time for the Nobel', to work on the possible pregenomic chemical origins of life.

Paul Lauterbur was born in Ohio in the American Midwest, and got his Bachelor degree from Case Institute of Technology (now part of Case Western University) before being drafted into the US army. As he described in a 2003 interview with *Physiology News* (**55**, 12-15), it was in the Army that he first got to use an NMR spectrometer, publishing several papers.

Following return to civilian life and completing graduate school, Lauterbur became an Associate Professor at the State University of New York (SUNY) in Stony Brook. Here he carried on pioneering work with multinuclear and particularly 13C NMR, including the first 13C NMR studies of proteins. In the summer of 1971 he got involved with an NMR instrument company in Pennsylvania, and the seed that led to the idea of MRI was planted when he watched living tissue experiments there. He often recounted the story of how he made the first notes of the basic concept of MRI on a napkin in a hamburger restaurant later that night. Back at Stony Brook that autumn he did the pioneering work single-handed, often using the NMR machine at night, and making early images of simple 'phantoms' (capillary tubes of water and heavy water) and of seashells collected by his elder daughter Sharyn. Lauterbur liked to point out, in discussions on the beginnings of MRI, that *Nature* only published the seminal 1973 paper after he had argued long and hard with their original rejection of it, and also that SUNY's patent department thought the whole idea of MRI too far-fetched to patent.

Through the 1970s Lauterbur's lab at Stony Brook became a focus for others interested in the idea of imaging with NMR, and once MRI machines became a reality in the early 1980s he worked tirelessly to convince radiologists that the technique would provide new data not available from CT (computed tomography) scans. He took great satisfaction from the way that new applications of MRI in science and medicine continued to emerge, stating in 2003 that among developments of MRI that had most gratified him were its use in 'functional imaging' of things like brain activity and heart movements.

In 1984 Lauterbur married for the second time, to the American physiologist Joan Dawson (then at UCL), and they moved to the University of Illinois at Urbana. He is survived by Joan, their daughter Elise, and his son and daughter from his first marriage.

Austin Elliott

Paul Christian Lauterbur, pioneer of 13C NMR and co-inventor of MRI. Nobel Laureate, member of the US National Academy of Sciences (1985) and Honorary Member of The Physiological Society (2004).

A century ago in *J Physiol*

As many readers will already know, the full archive of *J Physiol* is now available on Highwire, and can be reached from The Society web site. This allows one to see what was being published 25, 50 or even 100 years ago.

The four papers in *J Physiol* (**35.4**) (http://jp.physoc.org/content/vol35/issue4/) come from just two universities, Cambridge and UCL (March 1907). The authors include a future Nobel Laureate, Frederick Gowland Hopkins (Nobel 1929), a brilliant experimental physiologist killed in a WWI flying accident (Keith Lucas), and the Cambridge ur-pharmacologist W E Dixon. Two authors are among the UK founding fathers of sciences that 'spun off' from physiology – biochemistry (Gowland Hopkins) and pharmacology (Dixon) - or perhaps even three if one views Keith Lucas as the father of UK biophysics The papers run the full gamut of the experimental systems of the day, ranging from amphibian and crustacean nerves in Lucas' work, through frog muscle, to rabbits, cats, dogs and humans.

Papers 100 years ago came in all sizes. When I started in physiology *J Physiol* had a reputation for printing very long papers. This may well be an ancient tradition, as volume 35.4 opens with a paper by Fletcher and Gowland Hopkins that runs to a hefty 62 pages, albeit the smaller pages of the pre-1994 *Journal.* Fletcher and Hopkins' paper on *Lactic acid in amphibian muscle* is heavy on methodology, with an Appendix even giving the (heroic?) numbers of frogs used in each experiment! Their painstaking experimentation does, however, give a picture of the conditions under which muscle lactate is produced that holds good to this day.

Keith Lucas' paper includes some delightful diagrams of the self-designed and built equipment he used to deliver brief stimuli to nerves (e.g. his Fig. 2). Lucas worked in a special vibrationally-shielded basement room in the Cambridge Physiological Laboratory, inherited after his tragic early death by his former student E D (later Baron) Adrian. Lucas' paper employs the amphibian sciatic nerve – gastrocnemius muscle prep still in use (one hopes) in undergraduate physiology labs. It is one paper of a staggering 25 Lucas published in *The Journal* between 1904 and 1914.

Academics in those days were clearly particular about their affiliations. Lucas does not list any degrees but gives his personal affiliation as 'Fellow of Trinity College', while Fletcher and Gowland Hopkins also give their college details.

The volume also includes a paper from the Department of Physiology at UCL (then run by Ernest Starling) by David Henriques de Silva. Silva later worked at King's College, collaborating with W D Halilburton among others, and seems to have had biochemical inclinations; his later papers cover many subjects, often with a focus on digestive juices and blood.

The final paper in volume 35.4 is the only one not to come from a physiological laboratory. This is W E Dixon's *The action of alcohol on the circulation*, detailing experiments on humans, dogs, cats and rabbits (!) to try and determine absolutely whether alcohol is a cardiac stimulant or depressant. The following excerpt particularly caught my eye:

'*If moderate doses of alcohol well diluted with water be administered to animals or men the pulse rate does not alter. I have tried these experiments over and over again and always with the same results. The popular fallacy that alcohol quickens the pulse is clearly derived from the conditions under which alcohol is usually taken. It is well known that excitement of any kind quickens the heart and alcohol is generallv taken under exciting circumstances*.'

There is a highly entertaining account of Dixon's career written by Alan Cuthbert in his 2001 W D M Paton Memorial Lecture (Cuthbert AW (2001). *Brit J Pharmacol* **133,** 945–950). Cuthbert wryly points out that though Dixon was the founding father of pharmacology in Cambridge, Cambridge never got round to making him a Professor. Dixon *was* a professor, however, as for many years he held a lectureship in Cambridge simultaneously with a Professorship at King's! Cuthbert quotes Dixon's motto as 'Dire n'est rien , faire est tout', and tells a fascinating story of how Dixon almost discovered neurotransmission almost a decade before Otto Loewi.

Austin Elliott

Laboratory skills for science and medicine

By Maxine Lintern
2007, Radcliffe Publishing, Oxford. 118 pp, £19.95
ISBN 1 84619 016 9

This handy little paperback is a mine of information for the wannabe research scientist. Aimed at final year undergraduates embarking on their first research project and postgraduates at the start of their PhD, it is full of useful advice and practical tips on how to get going and, just as important, how to keep going on your first research project right up to the last page of the report or thesis. The book is divided into two sections. The first deals with basic concepts such as health and safety, designing and managing experiments, solutions and dilutions, keeping track of data and the knack behind scientific writing, reinforced with illustrations and top tips for good practice. The second, much shorter section gives a glimpse of some of the more common techniques utilised in biomedical research laboratories at the present time and ends with a consideration of the use of animals in biomedical research. This section is very limited and deals only with protein and histological techniques, cell culture and a bit of molecular biology, but is OK as far as it goes.

What I liked about the book was its mixture of common and practical advice sense applied to good laboratory practice and scientific writing, all delivered in a very accessible style. It is not like reading a textbook. It is more like a chat with a friendly but knowledgeable supervisor or experienced post doc who has not only been there and seen it all but is also prepared to take the time to tell you all about it. You learn what to do and why you should be doing it in a certain way and, in the case of the author's painful experience with a stolen laptop, what can happen if you don't take your own advice to obsessively back up computer files.

The book hands out good advice that many of us hope we already impart to our own students. However, Maxine Lintern's advice gains authority by virtue of being transcribed into the printed word and placed between two shiny covers. The copy I bought for my lab is already much thumbed. Those who take its messages to heart and follow the guidelines will not regret it.

Thelma Lovick

Adaptation to life in the desert

The special physiology and history of the black Bedouin goat. By Amiram Shkolnik & Itzhak Choshniak. 2006. ARG Gantner Verlag KG, 142 pp.
ISBN 3 906166 54 6

Black Bedouin goats live on both sides of the Gulf of Eilat, in a sun-scorched desert environment with large distances between water sources. In this fascinating book, Amiram Shkolnik - with the last chapter written by Itzhak Choshniak - presents the results of years of physiological studies on the many ways in which these animals have adapted to live in such an environment.

One example is the development of a rumen with such a large capacity that a goat weighing 23 kg can drink up to 5 litres of water , thereby allowing it to graze in the desert for up to 4 days before it must drink again. After foraging in the desert for several days, even the sight of water causes an instantaneous drop in the level of antidiuretic hormone in the blood, thus enabling the kidneys to rapidly change the rate of urea production in anticipation of drinking the water. Since the plant foliage that they eat is generally of very poor nutritional quality and resistant to digestive processes, these goats have developed a very efficient chewing ability, thus enabling them to digest the roughage that they eat. Because of the low protein content of their food they also have developed the ability to recycle the urea from their urine in order to conserve the nitrogen required for protein synthesis. One of the many other adaptations that have evolved in these animals is their ability to maintain a high rate of sweating, to prevent body temperature from rising in the extremely hot environment. To achieve this, their sweat glands have as much as a 10-fold higher secretion rate than those of other bovine species. To gain insight into the adaptations required to ensure the survival of new born kids, measurements were made of milk yield and composition, water requirement, and mechanisms of water conservation during lactation.

The results of these and many other physiological studies are presented in this very well written book, and I recommend it not only to students and academics in all areas of biology, but also to anyone interested in how evolution has worked to allow these animals to survive and reproduce in such an extreme environment.

Jacob Joseph Blum

23 problems in systems neuroscience

Edited by J Leo van Hemmen and Terrence J Sejnowski. 2006. Oxford University Press, 514 pp, £49 (hardback).
ISBN 0 19 514822 3

Why 23 problems you might reasonably ask? According to the preface, it seems that, in 1900, the mathematician David Hibbert posed 23 mathematical problems as challenges to twentieth century mathematicians and this book mirrors that with challenges to twenty first century neuroscientists. Hibbert's challenges were apparently idiosyncratic and quirky and the chapters of the present edited volume are equally so, but taken together they make fascinating reading.

The book is divided into five sections, each made up of an eclectic collection of papers and the section titles give a flavour of the questions being posed:

- How have brains evolved?
- How is the cerebral cortex organized?
- How do neurons interact?
- What can brains compute?
- Organization of cognitive systems.

The Editors have done well to allow each of the authors to express themselves, without forcing every chapter into a common format. The chapters read well, are well illustrated, and have useful reference sections. Even if your favourite area in neuroscience is not covered, you will find plenty to interest you in this volume.

Bill Winlow

Lavish limited edition hardback out soon

Burroughs Wellcome & Co.

Knowledge, trust and profit, and the transformation of the British pharmaceutical industry, 1880-1940

Roy Church and E M Tansey

Over the last half century the pharmaceutical industry has been one of the great success stories of British industry, achieving world leadership through innovation and exports. Yet, when the partnership of two young American pharmacists, Silas Burroughs and Henry Wellcome, was established in London in 1880 the British industry had been lagging well behind its German and American competitors. In the early years the company traded in imported products, including the highly profitable Kepler malt extract, and compressed medicines marketed under the 'Tabloid' trademark.Rarely in modern British history has a medium-sized company exercised such a dominant influence on an individual industry as did Burroughs Wellcome & Co. Within 30 years it became the largest manufacturer of pharmaceuticals in Britain, and the most significant company in this sector before the Second World War. Part of its commercial success was based upon its deployment of innovative marketing methods, especially product development, branding, advertising, salesmanship, market research, and resale price maintenance (of which Burroughs Wellcome was a pioneer as early as the 1880s). Perhaps more importantly the pre-eminence and influence of the company lay in its extensive promotion of scientific research. Through a series of research laboratories established by the company in the 1890s – unprecedented in the industry – remarkable scientific advances were made which won the company unique prestige and established an unrivalled reputation and level of trust, especially among the medical community which used and recommended its products. Of particular note was the success of Henry Wellcome in achieving registration for the laboratories under the Cruelty to Animals Act, which allowed staff to perform animal experiments from the beginning of the 20th century onwards. This was the single most important factor in promoting innovative physiological and bacteriological research within the company and was to serve, to a great extent, as the pattern for all future pharmaceutical research in Britain.

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Silas Burroughs (top) and Henry Wellcome

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Images of Baltimore and Edinburgh

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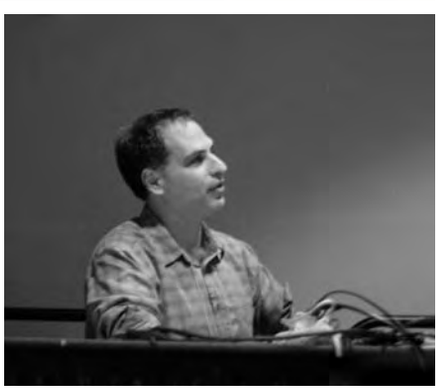
Baltimore, Maryland, USA
2 March 2007

(Report on p. 50)



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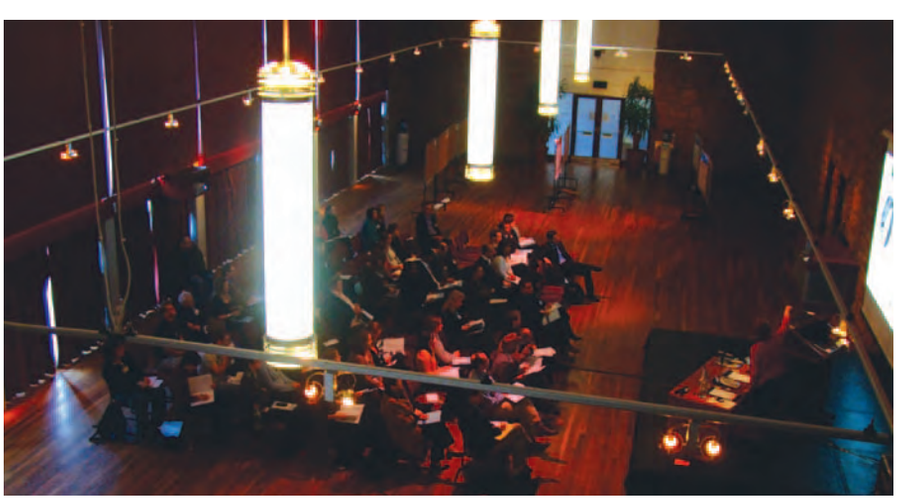

photos by David Bennett, Jonathan Goodchild and Brian Robertson

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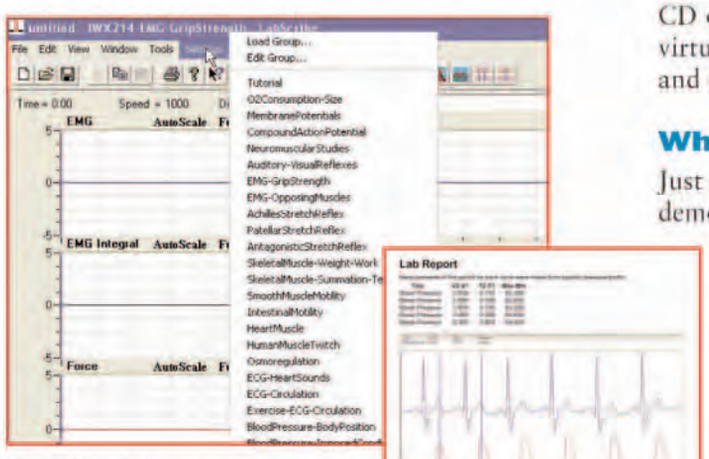

Over 150 Lab Exercises

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It's not often you get more for less, but iWorx gives you a free site license for an unlimited number of students and they don't charge for software or courseware upgrades either. They are free forever. iWorx is also expanding its courseware to complement the most popular lab manuals in publication.

More Teaching Options

iWorx has developed virtual animal and human lab experiments in cardiovascular, respiratory, and neuromuscular physiology that are presented on CD or accessible via the Web. The self-contained virtual labs are an excellent supplement to Wet labs and ideal for distance learning curricula.

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