BIOGRAPHICAL MEMOIRS

Alec Douglas Bangham. 10 November 1921 — 9 March 2010

Sir Brian Heap and Gregory Gregoriadis

Biogr. Mems Fell. R. Soc. 2011 **57**, 25-43, published 31 August 2011
originally published online August 31, 2011

ALEC DOUGLAS BANGHAM

10 November 1921 — 9 March 2010

Biogr. Mems Fell. R. Soc. **57**, 25–43 (2011)

ALEC DOUGLAS BANGHAM

10 November 1921 — 9 March 2010

Elected FRS 1977

By Sir Brian Heap[1] CBE FRS and Gregory Gregoriadis[2]

[1]*St Edmund's College, Cambridge CB3 0BN, UK*
[2]*School of Pharmacy, 29–39 Brunswick Square, London WC1N 1AX, UK*

A first meeting with Alec Bangham would leave an impression of a larger-than-life scientist with a penetrating curiosity and formidable features characteristically portrayed in the dramatic portrait painted by his nephew, Humphrey Bangham, which currently hangs in one of the Royal Society's rooms. Or, as Jeffrey Watkins (FRS) experienced on reaching the Institute of Animal Physiology at Babraham in September 1963, 'at the moment of my arrival he was avidly peering down a microscope and excitedly proclaiming the wonders of myelin figures as viewed through crossed polaroids … [and that] they might prove useful as model systems for the study of structure and function in biological membranes. It was decided that I should work immediately along these lines.' So what were the formative events that led to these first impressions? What had contributed to the formation of a scientist who became known worldwide as the father of liposomes, the inventor of Artificial Lung Expanding Compound (ALEC)—and someone who never stopped thinking about membranes, surfactant, anaesthetics, cricket, vegetables, family and friends?

Early days

Alec was the eldest of three children of Donald Hugh Bangham MC MA DSc and Edith Bangham (*née* Kerby), born on 10 November 1921. His father was a surface chemist who died prematurely at the age of 55 years while Director of Research at the British Coal Utilisation Research Association. He recruited Rosalind Franklin shortly after she received her doctorate and together they published a couple of articles on the properties of coal. Previously he was

The electronic supplementary material is available at http://dx.doi.org/rsbm.2011.0004 or via http://rsbm.royalsocietypublishing.org.

http://dx.doi.org/10.1098/rsbm.2011.0004

Dean of the Faculty of Science in the newly founded Egyptian University, Cairo (1926–36). Edith was born in St Petersburg, Russia, in 1892, daughter of the Managing Director of the City of St Petersburg New Waterworks, Co. Ltd, a later item for Alec's stupendous photographic collection, albeit photographed covertly in 1972. As Edith Kerby she acted as interpreter for Mrs Pankhurst on the occasion of her meeting with General Bochkareva, First Women's Battalion of Death. Harvey Pitcher, a well-known writer on Soviet and Russian matters, included a chapter of Alec's mother's personal recollections in his book entitled 'When Miss Emmie was in Russia' published by John Murray in 1977.

School

Bangham's early days were spent in temporary homes in England, on summer holidays in the Gower, South Wales, and on commutes to Egypt preceded at the age of 4 years by tonsillectomy at St Mary's Hospital, Manchester, from where a strong recollection of chloroform was to return at a later date. He suspected that his father, like himself, was an atheist, yet Alec was placed at The Downs, Quaker Preparatory School, Colwall (1929–35), where Alan Hodgkin (FRS 1948; PRS 1970–75) was an Old Boy and where he overlapped with Frederick Sanger (FRS 1954). He was to find himself embarrassed by ignorance of the Lord's Prayer, and he left with a mark of less than 5% in religious education for the Common Entrance Examination. However, the memories of this remarkable school, which was heavily subsidized by the Cadbury daughter married to the headmaster, Geoffrey Hoyland, remained with him for a lifetime. He recalled how W. H. Auden taught him English in his final two years and his delight in this maverick teacher who paid five cigarettes for Alec's photograph of him and his 'wife of the day', Erica Mann, daughter of Thomas Mann. The glass quarter-plate negative remains in the Bangham archives to this day. The school permitted Alec to visit his parents in Egypt when his father was Professor of Physical Chemistry in the University of Cairo. There he photographed Professor Jack (later Sir John) Gaddum (FRS 1945), who had been appointed by Alec's father to the Chair of Pharmacology, only to meet him again later at Babraham, where Alec was one of the burgeoning scientific staff when Gaddum became Director.

Both Sanger and Bangham went on to Bryanston, Dorset (1935–39), where most of his reports ended with a discouraging sentence, 'could do better'. One source of inspiration was the biology teacher, Dick Harthan, who led Alec to the grave of Benjamin Jesty, the farmer who vaccinated his children with cowpox (another negative in the photographic collection). He read Paul de Kruif's *Microbe hunters* (de Kruif 1926) and decided to study medicine, his parents finally leaving Egypt for London, where Alec no longer needed to spend his life in a boarding school.

Bombs and demobilization

After Alec left what he confessed was a remarkable Quaker school, though with barely the Common Entrance marks to get into Bryanston, the story of his early academic career did not portend the highly original and insightful research scientist who was to emerge. When war was declared he was not eligible for call-up and was without Latin so he was ineligible for Oxbridge. He needed French and 1st MB to get into University College London, so he

enrolled at Chelsea Polytechnic (now University of the Arts London, Chelsea) in September 1939. It was at University College that he met a medical colleague, Rosalind Barbara Reiss, daughter of Captain Richard and Mrs Reiss who was Chairman of the Garden Cities and Town Planning Association of Great Britain and a co-founder of Welwyn Garden City. It led to a loving union that lasted well beyond their diamond anniversary in 2003.

In late summer of 1940 the college was bombed and the Anatomy and Physiology departments were moved to the precincts of a minor boys' preparatory school at Leatherhead, Surrey. Air raids were frequent and there were no formal air-raid shelters, so protection was provided under steel tables supporting the corpses that were otherwise the subjects of dissection. In hot summers a special dispensation allowed dissections to be performed on the lawns, and work was hard and pressurized, part of the prevailing pressures of warfare. Contemporaries included Douglas Wilkie (FRS 1971) and Naomi Datta (FRS 1985).

Second MB was reached in 1942, followed by the Clinical School, University College Hospital, based at its evacuated premises at North Watford with dormitories, improvised lecture rooms and clinical cases in the large Victorian mental asylum at Langley. He revelled in the teaching of the physician, scientist and naturalist Sir Thomas Lewis FRS and the support of his remarkable personal assistant, John Honor, who helped Alec build an automatic blood sedimentation rate recorder, ideal for ward rounds. He was not very good at examinations and had to take 'medicine' papers three times before qualifying MRCS LRCP and MB BS (London) in 1945. Yet soon he was a Casualty Officer at Addenbrooke's Hospital, Cambridge, where penicillin was available to military personnel only. Such was its scarcity that Alec collected urine from fortunate cases to send across the road to the Department of Biochemistry, where it was re-extracted and made available to civilians. He recalled a case of infected tendon sheath that responded in a matter of hours.

One of Alec's key career changes was his decision to renege on clinical medicine and become an apprenticed clinical pathologist. It was a momentous decision with long-term repercussions that he must have reflected on as he sought to emulate his mentor, Dr Francis Camps, the famous forensic pathologist who would drive frantically from Black Notley (Essex) hospital to conduct the large number of weekend coroner's cases in East London. Later, Alec was called up and posted to Palestine, where, as Captain in the Royal Army Medical Corps, he had charge of the Pathology Department, Bir Jacob British Military Hospital. This was a depressing experience with a modern ring—autopsies on regular and conscripted soldiers—but it was followed by a more interesting time after the British Mandate ended in 1948, with a posting to the Central Middle East Pathology Laboratory at Fayid, Egypt. The facilities were good, the spectrum of diseases was informative and he published a joint paper on typhoid carrier rates among Egyptian food handlers on account of regular summer epidemics. Chloramphenicol only became available after 1949 and overcame some of the losses of the past. 'Some of us in the laboratory inoculate ourselves with the "old" formaldehyde vaccine, the British Army having been inoculated by an alcoholic vaccine just pre WWII', wrote Alec in his notes.

True to form, Alec took the opportunity while in Egypt to show Rosalind the Marine Biological Station set up by his father at Hurghada. He and Rosalind joined Colonel (later Lord) John Hunt of Everest fame and Mary, his wife, for a crazy week driving 300 miles down the edge of the Gulf of Suez. The Hunts had wanted to go by taxi, but Rosalind's hilarious account in her letter to her mother says that 'this was impossible for us as it would cost 25 pounds EACH at least!' After further setbacks, the situation was eventually resolved: Alec travelled on a motor bike with Rosalind in the sidecar, and the Hunts drove a dilapidated

prewar Standard 9. The Hunts had set their hearts on reaching a point from which to climb the 5000 ft Gebel Shayib, the highest peak in western Egypt, and both parties wanted to visit the Roman mining town Mons Claudianus, which they did with immense fascination. John Hunt's account of the expedition speaks of 'the Bangham's (who) were a delightfully unconventional pair. We took an immediate liking to them'. Incidentally, Rosalind recalls that 'Alec's chain and the mechanism for arranging the two wheel drive broke, but that did not seem to matter, he just removed it!!'

The early scientist

Alec must have always been a scientist. He was not very good at examinations, but his penetrating curiosity, which went straight through you, and his questioning mind were just right for the next phase of life. Demobilization was a devastating time for some as they adjusted to a civilian life, but Alec was awarded a grant (and lectureship) by Sir Roy Cameron FRS at University College Hospital to study the effects of cortisone on wound healing alongside the routine job of autopsies on geriatric patients at St Pancras Hospital. Within two years he had published a couple of papers and was 'particularly chuffed to have made and used radioactive mini glass spheres resembling bacterial cocci to follow lymph drainage'(1, 2)*. By 1952 he had been recruited by Dr I. de Burgh Daly FRS to the founding staff of the newly established Agricultural Research Council (ARC) Institute of Animal Physiology at Babraham, near Cambridge, where he worked until statutory retirement in 1982. He became the first Principal Scientific Officer of the Pathology Department, later led by Sir Alan Drury FRS. As a concession to his new employers he decided to scan the haemoglobins of various breeds of cattle, as the electrophoresis of proteins was in vogue at the time. Original findings tumbled into his lap and into *Nature* (3–5). First he found a new allele carried exclusively in Jersey, Guernsey and South Devon cattle (16), and then two non-allelic haemoglobins in horses (7). His research on species distribution of bovine haemoglobins introduced him to Arthur Mourant (FRS 1966) from Jersey and to Hermann Lehmann (FRS 1972), and that on equine haemoglobins to Max Perutz FRS, who was presenting his early X-ray pictures of horse haemoglobin that Alec felt 'obliged' to tell him were 'composite'.

During early years at Babraham, Victor (Lord) Rothschild FRS was an authority on spermatozoa and was Chairman of the ARC. Alec rose to his challenge about whether sex determining spermatozoa could be separated by electrophoresis (11). He was also invited to test a gravitational method of separation described by a young veterinarian, Mr B. C. Bhattacharya. This second test, which involved the then Director, Richard Keynes FRS, required a considerable amount of work, including a clinical trial that proved entirely negative (22). But this was not the end of the story because Alec naturally accepted the open challenge from his Lordship to explain how spermatozoa manage to swim (apparently) against a flow of liquid (positive rheotaxis), as claimed from Rothchild's movie shown to the Royal Society. After numerous experiments and consultations with colleagues, notably A. E. Walton, Bangham concluded that rheotaxis was not strictly taking place. In his own words, and having in mind his fascination with sailing, living as he did within easy access of the East Anglian coastline, sperm, on account of their uniquely slippery head and long tail, 'behave like a boat lightly moored over

* Numbers in this form refer to the bibliography at the end of the text.

a tidal current'. Despite his reservations about Rothchild's ideas, 'over a period of years', he said, 'I was unable to achieve acquiescence from his Lordship or for that matter any reply.'

By this time Bangham's curiosity about the biophysics of cell surfaces was bubbling. He spoke with his father about why red cells or white cells behave differently with regard to their inherent 'stickiness' to each other. What is it about cell surfaces that allows erythrocytes and spermatozoa to remain discrete cells? This led to the Department of Colloid Science in Cambridge and the laboratory of Jack Schulman, Brian Pethica, Geoffrey Seaman and others, and to the skills and devices required for measuring surface tension. In 1958 they published a paper which suggested that the differential behaviour of blood cells depended on the species and density of anionic groups on the surface of the cells (5). They used phospholipid dispersions (now called liposomes) as model surfaces presenting carboxyl, phosphate, choline and/or amine discrete sources of charge. Together with his increasingly important collaborations with the lipid biochemist Rex Dawson (FRS 1981) at Babraham, these studies led to new insights into the coagulation of blood, the many properties of liposomes, and why lecithinases failed to attack films of pure lecithin (6, 8–10, 12). This was the phase in his life when the physics and chemistry of cell surfaces relating to adhesion and/or fusion (13–15, 17), and the interactions of phospholipases with phospholipids, provided a perfect prelude as it started to overlap into the next era.

From bangasomes to liposomes

By the early 1960s, Alec was fired by his interactions with colloid science and by the book *Electrophoresis of proteins and the chemistry of cell surfaces* (Abramson *et al*. 1942), which he claimed was almost as influential in his life as the early reading of *Microbe hunters*. He had a persistent fascination with platelets and polymorphonuclear leucocytes that were able to aggregate towards the edge of a blood smear, unlike erythrocytes, which seemed not to absorb proteins or to adhere to glass; they resisted being filtered off by reticuloendothelial cells and seemed not to accelerate the clotting reaction even though they represented such a huge area. Highly purified phospholipids or smectic mesophases, as liposomes were called at the time, had been used in his seminal 1958 paper (5) (smectic, or liquid crystals with molecular layers oriented in parallel layers; mesophase, phospholipids in water where the liquid crystalline phospholipids form multilayered parallel plate structures, each layer being a bilayer, the layers separated by aqueous medium). Ultra-pure lecithins (and starch particles) had zero electrophoretic mobility, which led Bangham to wonder whether the absence of charge at the hydrodynamic plane of shear had anything to do with the stickiness or invisibility of a particle *in vivo* and whether there were other surfaces to which proteins failed to absorb. 'Anonymity is difficult to sustain if one enters the blood stream, or for that matter, the tissues of an animal', he wrote in 1995 (37);

> Almost all surfaces are tagged within milliseconds of being exposed to plasma, which after all, is an abundant source of animal glue. … But suppose the surface of the material introduced into the plasma already looks like the surface of a slice of (bulk) water. Then the polyionic proteins will not find anything to tag, there is no entropy gain, no attractive interactions and the interloper is invisible.

Bangham was convinced that there was a family of small molecules, yet to be identified and with a molecular mass of less than 300 Da, whose job was to shield ionic and possibly

complex antigenic sites in much the same way as a soap molecule shields a glob of hydrocarbon. In the absence of suitable bouquet shielding molecules the macrophage sees a foreign surface that has entered the circulation. This provocative idea lived with him until the end (38, 39) because he gained further reasons to believe it: the purer the lecithin, the less likely it was to be hydrolysed by phospholipases (5), and ultra-pure lecithin was neutral in blood clotting experiments unless mixed with amphipathic anions (9). These early experiments reveal the extent of Bangham's hands-on experience of phospholipids long before they became known as liposomes, and also what they told him about invisible hydrodynamic interfaces, a long-sought-after property for surrogate cells such as those that might, someday, carry haemoglobin.

When Babraham acquired its first electron microscope in 1961, Bangham had the privilege of working with Robert Horne to look at 'dispersions of phospholipids (alias liposomes) in water solutions of negative stains. … We argued', Bangham went on to say, 'that the integrity of a biomolecular leaflet of phospholipids depends as much on the presence of water as it does on the precise composition and structure of the participating lipid molecules.' From among the very first electron microscope photos Horne and Bangham proudly selected pictures that now adorn book covers, advertisement and walls all over the world: concentric rings of material in the glorious, unambiguous black and white of a negative stain identifying the oil and water phases (figure 1). Here were phospholipids that had spontaneously formed into closed membrane systems when offered an unlimited water phase (18). Horne and Bangham's excitement that this was a valid model of a real biological membrane was dented when Richard Keynes presented them with a preprint of a paper by Mueller *et al*. (1962) showing how to make a black lipid membrane, which was as irresistible to play with as Alec's favourite soap bubbles, enabling one to study real-time electric parameters across a membrane. Alec was convinced that he could see swelling and shrinking in his liposomes as a consequence of osmotic challenges, and sought to convince Jeff Watkins (FRS 1988), who joined him from the laboratory of David Curtis (FRS 1974) in Canberra, Australia, for a sabbatical, that this was the case. Watkins, who had a deep interest in neuronal cell membranes, had heard rumours that Alec was the 'brightest scientist at Babraham' and that he was beginning to investigate black films as a general model for cell membranes. 'As it happened', writes Watkins, 'we did not work on black films together but on a new model membrane system arising from a "eureka moment" of illumination that Alec had had in the meantime, and which has since given the world the word "liposome" and a whole new field of biophysical and medical research.'

The introduction to the substantial paper by Watkins, Standish and Bangham, 'Diffusion of univalent ions across the lamellae of swollen phospholipids' (19), published in 1965 in *Journal of Molecular Biology* remains the most succinct description of a liposome (figure 1). This was the year when Bangham was awarded a doctorate of medicine by the University of London. The paper was later to be awarded a Citation Classic. Jeff Watkins presented their early results at a meeting of the Biochemical Society in Birmingham in the spring of 1964 in which he reported that liposomes were not only permeable to water but also (unexpectedly) to Cl^- , while being relatively impermeable to Na^+ and K^+, results that were confirmed and much extended after Jeff returned to Australia. Alec's letter to Jeff reflects his excitement as he recounts presenting the data to a Tea Club run by the Pharmacology Department in Cambridge 'to which Hodgkin, Rushton, Glynn and Haydon came. Still no objections, well, not quite! Haydon took exception to describing his membranes as being fundamentally "anaesthetized".' David Deamer recalls that by 1965 the crucial experiments had been published: lipid bilayers

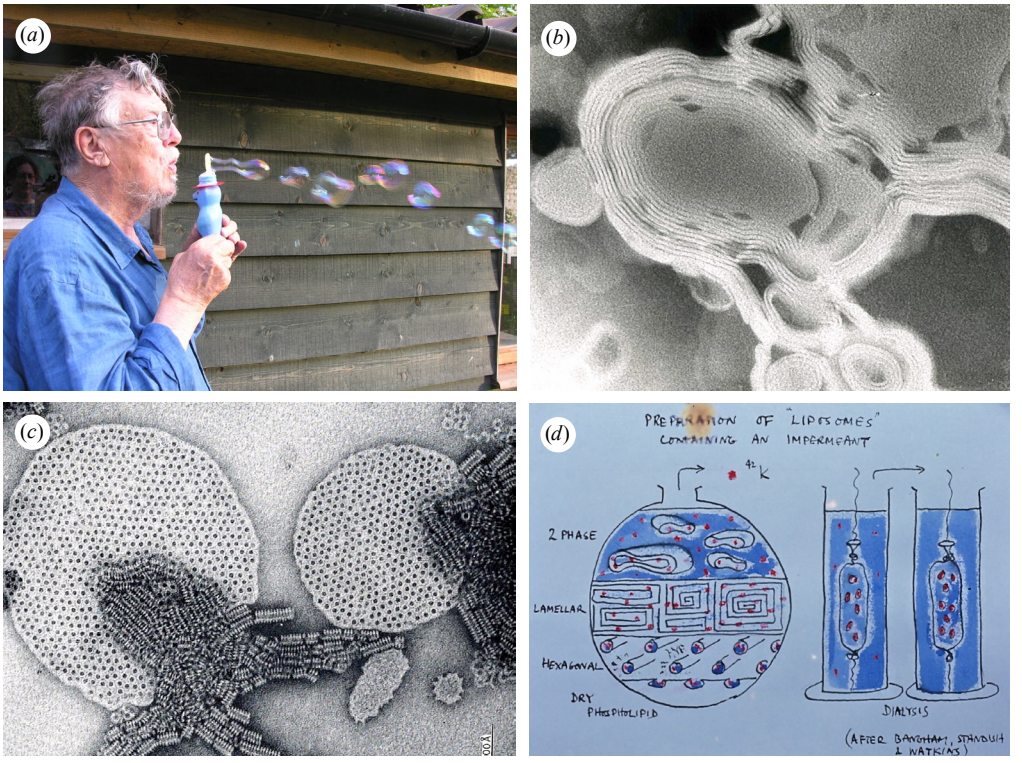

Figure 1. Lipid membranes and liposomes. (*a*) Alec Bangham and his fascination with lipid membranes; at home. (*b*, *c*) The first sight of liposomes; electron micrographs of multilamellar phospholipid vesicles: (*b*) ovolecithin liposomes revealed by negative staining with 2% potassium phosphotungstate; (*c*) dispersion of lecithin and cholesterol in water with a molar ratio of 1:1, treated with 0.2% (w/v) saponin, and finally mixed with 2% potassium phosphotungstate. (*d*) Alec Bangham's portrayal of the production and function of 'liposomes'—lipid bilayers of vesicles that could maintain concentration gradients of ions such as potassium and sodium (from (19)). (Panels (*b*) and (*c*) reprinted from *Journal of Molecular Biology*, vol. 8, A. D. Bangham and R. W. Horne, Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope, pp. 660–668, copyright 2011, with permission from Elsevier.) (Online version in colour.)

of the vesicles could maintain concentration gradients of cations such as K^+ and Na^+ and that when the bilayer was perturbed the gradient was disrupted (20, 21, 23). 'This evidence,' he wrote, 'along with the planar bilayer models being developed at the same time, established that lipid bilayers are the primary permeability barrier of all cell membranes. It was the membrane equivalent of finding the double helix structure of DNA, another Cambridge discovery in the life sciences' (Deamer 2010).

The origin of the name 'liposome' is of some interest. According to the immaculate laboratory notebooks of Guy Greville, Head of Biochemistry at Babraham at the time, 'Bangasome' was Greville's word for 'liposome' (2 November 1965). David Deamer of the University of California at Santa Cruz, one of Alec's early visitors, recalls that it was Gerald Weissmann who was one of the first visitors to the Bangham laboratory who coined the word 'liposomes' by analogy with the terminology of lysosomes. The name stuck, although 'Banghasome' emerged occasionally, albeit lightheartedly. Over the next 40-odd years liposomes grew into a minor industry

and the word was incorporated into book titles, a scientific journal, an international society, three successful companies, a drug delivery agent, cheese making and fish food preparation, a treatment for infantile respiratory distress syndrome, and even cosmetic formulations of Dior and Lancôme. PubMed has listed more than 35 000 articles on liposomes (Deamer 2010).

Gerald Weissmann returned to New York and set about proselytizing the liposome model vigorously. He had worked with Alec on the effect of a range of steroids on liposomes (20) and the relation between lytic activity on biological single-membrane structures and increased lytic leakage of cations, which, in Alec's own words, was phenomenal. There was easy access to colleagues at Babraham; the claims of Bangham and his team were beginning to offer liposomes as a versatile model (24), and interest grew apace (26) (Heap *et al*. 1970, 1971). The laboratory received a stream of visiting scientists including Demetri Papahadjopoulos, Hans de Gier, Maurice Wilkins FRS, Arthur Kornberg ForMemRS, Peter Mitchell FRS, George (later Lord) Porter FRS (PRS 1985–1990), Colonel Carl Alving (Washington DC) and representatives from L'Oreal (Niosomes) and Christian Dior.

Volume 1 of *Methods in Membrane Biology* (28) contained an account of the techniques at that time available in their laboratory, including facilities for self-diffusion of isotopic solutes, rapid osmotic shape changes that follow the permeation of water and very permeable solutes, X-ray diffraction of concentrated dispersions, microelectrophoresis and Langmuir trough for surface tension studies—probably the apogee of liposomes at Babraham when Alec was elected FRS (1977), the first native Babraham scientist. At the celebratory laboratory party with champagne and food, Alec regaled us with stories of his dismal start in academic life, his failed Latin and repeated resits, his skills on the cricket field on behalf of the institute and the village of Babraham, and his prowess at boules in The George before the local hostelry replaced straw on the floor with something more auspicious. There was no 'side' to the man, and he boldly followed his ideas wherever they took him. Few realized that his own laboratory was unpretentious and consisted of a set of connected barrack structures left over from the 1940s and never exceeded two staff members—a technician (Nigel Miller) and a physicist (Sheena Johnson and then Martin Hill). Yet he was deeply aware that scientists in research institutes were envied by their peers in the university because many had permanent appointments, technical assistants and a freedom to follow their curiosities. However, he recognized the deprivations such as the feedback generated by teaching.

Liposomes at large

The history of the liposome technologies and their application took a dramatic leap forwards on the occasion of the 1st International Conference on the topic, held in New York in September 1977. The conference was convened by close colleague, Dimitri Papahadjopoulos, held under the auspices of the New York Academy of Sciences and sponsored by 16 multinational pharmaceutical companies. The leap was made by Brenda Ryman, who brought a gasp of incredulity from the hall concerning the prospect of injecting vesicles into patients. Her riposte was that 'when liposomal administration of therapeutic agents is commonplace, the liposome, the biophysicist's membrane model, will truly come of age.' Representatives of pharmaceutical companies were seen to leave the conference persuaded that the time was right to exploit liposomes to smuggle insulin into the body by mouth, to wrap up vaccines in biodegradable adjuvants, and to address the delivery of anti-cancer drugs (29).

Meanwhile Gerald Weissmann, back in New York, had been writing on the basis of Alec's work:

> in the beginning there must have been a membrane! … were lipids of whatever origin to find themselves in the vicinity of primordial [synthetic] reactions it would not be too difficult to imagine them forming self-assembled bubbles within which to segregate the new thing—life, as it were—from the hostile sea.

At that time it was pure theory that phospholipid assemblies could form closed bilayer structures, just like red cells, and they could capture molecules such as enzymes and nucleic acids. It became a mantra of the National Institute of General Medical Sciences of the National Institutes of Health, Washington DC. Enter David Deamer, who had been seeking to reduce theory to experiment since the spring of 1975, when he and Alec had tossed astrobiology around in an Austin Mini after Alec had ventured the title of his lecture in Bristol as 'Membranes came first'. Deamer pursued this idea at his home campus and found that phospholipids could be synthesized in prebiotic conditions, that membrane-forming amphiphilic molecules were present in the Murchison meteorite (which had been found to be loaded with some of the important and complex molecules needed for life), and that simple cell-like structures could be formed by encapsulating functional nucleic acid polymerases in lipid vesicles (Hargreaves *et al*. 1977; Deamer 1985).

One of Alec's most enjoyable events arising from liposomes was in 1986 when his wife, Rosalind, received a mailshot from Joshua Taylor, a well-loved department store in Cambridge, now defunct. Part of the text read: 'CAPTURE is being launched next month and is the result of research undertaken over several years in the Christian Dior laboratories in conjunction with the Pasteur Institute. CAPTURE a gel which deals with the very cause of skin ageing thanks to an exclusive process: CHRISTIAN DIOR LIPOSOMES.' Rosalind went by moped to pick up her free sample, only to be told they had not arrived from London and the salesperson had not received her training. Always ready to help, Bangham sent the salesperson a set of reprints. Ten days later he received a phone call from London expressing delight that the inventor of liposomes was alive and lived in Cambridge and 'could Lola McAlister, National Consultant UK, take them out for dinner'. Alec seized the opportunity and asked to see liposomes being made in kilogram quantities. He and Rosalind flew, by air taxi, to Paris for dinner with Monsieur Roget, President of Christian Dior. Nowadays almost every cosmetic formulation uses liposomes 'to deliver some virtue or other to that remarkable organ, the skin', although not without some criticism over excessive claims. This cosmetic appeal of liposomes could be seen in a recent exhibition of Colipa, the European Cosmetics Association, at the European Parliament in Brussels, 7–8 December 2010, entitled 'Science, beauty and care: innovation in cosmetics'. The attendees greatly outnumbered those at the exhibitions of other lobbyists for armies, photography, tourism, and so on, and the organizers were pleased to hear of the inventor, whom they did not know, but were sad to learn of his recent demise. No doubt Alec's riposte should have been recalled—'Drugs and cosmetics appeal, ultimately, to their users and a good bedside manner hurts no one.'

Lung surfactant

Bangham was forced to retire at the age of 61 years as a result of quaint Civil Service rules that gave greater importance to age than to creativity. As to be expected, he found ways around

this and with Colin Morley at Addenbrooke's Hospital pursued an earlier discovery that solid, rather than liquid, lung surfactant prevented lung tissue from filling up with fluid. This dramatic development had come from an accidental meeting between the two in 1977.

Morley, a trainee paediatrician and Research Fellow in the Nuffield Institute of Medical Research in Oxford, was trying to understand how pulmonary surfactant worked and whether it could ever be used as a treatment for babies who were born so prematurely that their lungs did not aerate properly at birth. Colin has described (personal communication) how he had recovered 'surfactant' from sheep's lungs, by saline lavage, centrifugation and Folch extraction. The result was a sticky brown mess at the bottom of a tube, looking like earwax. He had no idea whether this was surfactant or how to analyse it. Pulmonary surfactant is mainly phospholipids with the unusual ability to lower the surface tension of water to very low levels.

> My mentors suggested I should approach Dr Rex Dawson at the ARC Institute of Animal Physiology, Babraham, Cambridge, and ask his advice because he was an expert on analysing phospholipids. I keenly drove to Cambridge early one morning to meet Rex Dawson, only to find that he was away. His chief technician suggested that, having travelled all that way, I should talk to Alec Bangham, who was an expert on surface tension measurements of phospholipids through his famous studies on model membranes, and had a laboratory in the same 'temporary' barrack-type building (now demolished).
>
> Alec was not very welcoming to an uninvited visitor because he was busy with his experiments. He almost dismissed me, saying he had thought about pulmonary surfactant before, with Dr Douglas Gairdner, a paediatrician in Cambridge, and did not understand how it worked and did not want to get involved in that again. However, when I explained the situation and showed him my tube with brown wax he generously agreed to test its surface tension properties while I was there. He scraped a little of the wax from the tube, shook it up with some saline, and pipetted it onto the surface of the surface tension balance trough. The surface tension dropped instantly. Suddenly, the atmosphere changed. 'Wow, where did you get this?' he said. 'This is some of the most surface active stuff I have seen … come and have a coffee; let's hear more about this.' Like all good scientists we went back to repeat the experiment but it did not work so well. Alec then realized that the surfactant wax had been sitting in the saline for a while and had probably become hydrated and changed its physical properties to a smectic mesophase. We repeated the experiment by placing a small particle of pure wax on the surface tension trough and the surface tension fell rapidly again. Alec was elated: he realized that the important physical properties of surfactant that had previously eluded him were present when it was in a 'dry state' and the phospholipid bilayers were all tightly packed. Interestingly, surfactant is stored in the alveolar lamellar bodies as tightly packed anhydrous layers. This led to 'Physical and physiological properties of dry lung surfactant', published in *Nature* (30). This letter was initially rejected, but Alec lambasted the editor about how important the concept was, and then it was published with no revisions!

Morley and Bangham studied the effect of placing the surfactant extract into the trachea of preterm rabbits and showed it had a profound effect on their lung aeration. Eventually they realized that the main concept that pulmonary surfactant reduced the surface tension to zero was erroneous and an artefact of the technique of measuring surface tension. Alec showed that under lateral compression a pulmonary surfactant monolayer changed its state from liquid to solid so that the surface solidified. In this state it would splint the alveoli open at end expiration, like an archway of bricks, and although Morley was convinced by this explanation it was an idea that challenged orthodoxy and was never accepted by people who had been teaching zero surface tension for a long time.

The question was this: Could 'dry surfactant' treat very preterm infants and help their immature lungs aerate at birth? However, to obtain carefully extracted, sterilized, surfactant

from animal lungs was an overwhelming task and probably beyond serious consideration by pharmaceutical licensing authorities. Alec was undaunted and decided that he could design an artificial surfactant with the same properties. 'I remember him sitting in the coffee room of his lab', writes Morley, 'writing out the calculations on the back of an envelope. It needed to be predominantly dipalmitoylphospatidylcholine, a saturated 16-carbon phospholipid which was the main constituent of pulmonary surfactant.' In its pure form this phospholipid is 'solid' at body temperature and so would not be effective. Morley goes on to note:

> Alec calculated that to lower its transition temperature to below 37 °C about 30% of other appropriate substances should be added. As unsaturated phosphatidylglycerol was another major component of pulmonary surfactant, Alec thought we should try 30% of that. When used as a dry powder this mixture lowered the surface tension perfectly. We spent many weeks trying other mixtures, but none were any better than Alec's original calculations.

Once Morley and Bangham had a synthetic pulmonary surfactant they tested it on the lung function of premature rabbit pups and showed beneficial effects (31, 32). The surfactant was made by Alec's chief technician, Nigel Miller, and led to the possibility of clinical trials in babies.

> It was a time when other surfactants were being developed and tested by other groups and we needed a name for our surfactant. While at a surfactant meeting in Canada I named it Artificial Lung Expanding Compound (ALEC) in honour of the inventor. ALEC was tested in three clinical trials in very preterm infants. The results (33) showed that their mortality was almost halved by this surfactant treatment.

To continue with the development of ALEC, a pharmaceutical partner was required that could manufacture, license and distribute the product. After many discussions, with an industry that knew little or nothing about treating very preterm babies and naturally were cautious, Britannia Pharmaceuticals successfully licensed and distributed ALEC (Pumactant) for several years until 2000. During the years that ALEC was developed and tested it became clear that natural pulmonary surfactant contained apoproteins that affected its clinical properties, in particular the speed of action. Despite this, Bangham was adamant from his experience that proteins would not be important for surfactant function, and would not countenance the consideration that they should be used with ALEC (35, 36).

History and liposome-based medical applications today

The seminal discovery of liposomes by Bangham in the 1960s, and their immediate use as a model for the study of cell membrane biophysics, was followed in the early 1970s by the demonstration that liposomes could serve as a carrier system for the delivery of drugs and vaccines in therapeutic and preventive medicine. This initial work on animals and humans (Gregoriadis 1976) attracted worldwide attention from both academia and industry, in turn leading to the general adoption of liposomes as the 'gold standard' of drug delivery systems. Applications included the treatment of lysosomal storage diseases, anti-cancer and antimicrobial chemotherapy, the treatment of diabetes, conventional and genetic vaccines, and therapies involving short interfering RNA (siRNA).

Such applications were promoted by two parallel developments. In the first technological developments, methods were invented for the efficient entrapment of a wide range of

pharmacologically active agents (such as conventional drugs, proteins and peptides, enzymes, nucleic acids and viruses) into liposomes, which could be produced cheaply on an industrial scale in a stable, non-toxic form. The second development was the optimization of the behaviour of liposomes *in vivo* in terms of their stability in the blood circulation so as to avoid the leakage of entrapped agents, and control of their pharmacokinetics and pharmacodynamics. This was achieved by two approaches: appropriate manipulation of the liposomal size and lipid composition, and the anchoring of poly(ethylene glycol) (PEG) on the vesicle surface.

By the early 1980s, three liposome-based companies were founded, namely the Liposome Company, Liposome Technology, and Vestar (vesicle targeting), all in the USA. After extensive multi-centre clinical trials, several injectable liposome-based products were marketed. Prominent among these were Doxil (pegylated liposomes containing doxorubicin, for the treatment of certain cancers) and AmBisome (a liposomal formulation incorporating amphotericin B, for the treatment of systemic fungal infections). Both products substantially reduce the toxicity of the drugs they contain, thus allowing their aggressive use. It is generally accepted that Doxil and AmBisome have extended the lives of countless patients or led to their cure. All three companies were bought in the 1990s by larger pharmaceutical concerns for US$500–700 million each. More recent developments in several newer companies include the design of liposomal formulations for siRNA therapy, gene therapy and genetic vaccines.

Forty years have elapsed since the introduction of liposomes in therapeutics, yet they remain the premier drug delivery system in the continuing battle against disease. Alec Bangham was fortunate to live long enough to witness the fruits of his original observation made nearly half a century ago in his modest laboratory at Babraham.

The man himself

Bangham was a man of firm views. His research was driven by curiosity, and he followed wherever his ideas led, even if it meant disputation or disregard. Anaesthesia action was his first fascination with liposomes as a model membrane system to test a hypothesis that anaesthetics partitioned into the lipid bilayer moiety and in some way inhibited nervous function. With co-workers Keith Miller and Sheena Johnson, he found that liposomes exposed to anaesthetics became more permeable to ionic solutes and that this effect was reversed by pressure, helping to explain why anaesthesia could be reversed by hyperbaric pressure (25, 27). The action of anaesthetics by their presence in both the aqueous and lipid membrane phases could be understood in thermodynamic terms involving pressure, temperature and ionic permeability. Neurotransmitter vesicles that trapped either weak acids or weak bases would be vulnerable to changes in their permeability to cations (such as K^+): pH would decrease, catecholamine concentration would fall, and weak-acid concentrations would rise—here he saw a potential way to orchestrate neurotransmitter release and a mechanism responsible for varieties of unconsciousness.

In a letter to *New Scientist* in 1980 he said that his ‘proton pump-leak’ theory would be resisted strongly by traditionalists in the field (34), a comment of some prescience. Amusingly, he could see his theory epitomized by consideration of how brandy carried by the St Bernard dog might be life saving. A climber caught by an avalanche is about to die because deep body temperature causes the failure of proton pumps and synaptic pathways. He receives

> an unlimited supply of 11 molar ethanol … a bad anaesthetic because its partition coefficient, membrane/water is near unity. … You drink the brandy and it diffuses into membrane and aqueous

> space indiscriminately, thereby changing the Gibbs free energy of both the aqueous and membrane phase. The effect is equivalent to adding antifreeze to a radiator of water at 0 °C!

As he speculated, 'your pumps would be released into activity by the best thermodynamic equivalent of "instant heat" and you retire to the safety of the monastery for further warming.'

There were yet other 'critical beliefs and experimental conclusions' that he felt he still needed to 'brief someone or write myself', in this case about lung dynamics. His world of liposome research had advanced from conventional vesicles (first generation) to long-circulating liposomes (second generation) to stealth liposomes modified by the inclusion of the synthetic polymer PEG in liposome composition. This extended blood circulation time while decreasing the uptake of mononuclear phagocytes by the system. Other formulations encapsulated active molecules with high target efficiency and activity, including monoclonal antibodies and ligands such as asialoglycoproteins targeting the hepatic parenchymal cells, vitamins and specific antigens. The ability of suitably designed liposomes to target cell types in the body was compared to the Trojan-horse (Gregoriadis 1973) delivery of drugs that would otherwise be systemically delivered, with the aim of reducing natural toxicity if delivered only to diseased tissues. Reflecting on these latter-day applications and the mass of nostalgic correspondence at the 30th Liposome Birthday Conference held in 1995 at Babraham and St Catharine's College, Cambridge, Bangham was pleased to note that although his roots could no longer support the massive foliage, 'the blossom is glorious'. Not only had liposomes fuelled work on new ways of delivering drugs to target tissues, they had also inspired letters, poems and anecdotes. Gregoriadis wrote:

> little fatty vesicles of bilayer fame
> protean and elusive, fragile all the same,
> aloof and enigmatic beneath your many skins,
> unyielding to the vigour of thousands of spins,
> descended from the pastures of Babraham we are told,
> you never ceased to wrinkle, expand and then to fold
> embracing sodium ions and such electrolytes.
> Twinkling guide stars to throngs of acolytes
> desirous of your membranous semi-barriers.
> Precursors of bion, potential drug-carriers.

Deamer (2010) observed that Alec was never ambitious in the usual sense and never fitted well into the British scientific establishment. His creative instincts were directed towards science and experiments rather than administration, although, as we have seen, they were by no means limited to liposomes. He was addicted to cricket, especially against Australia (he had been a notable batsman in his day), to picture-making in dark rooms with photographic paraphernalia, to boats, cars and scooters, and in earlier days to restoring Caucasian rugs and making facsimiles of classical clarinet mouthpieces. He claimed to have processed 70 000 negatives (black and white, colour and digital) and it is on record that he is the only scientist invited to speak at the Friday Evening Discourse at the Royal Institution in London to have taken a wide-angle photograph of the audience with a blinding flash. The purpose was to obtain a head count of somnolent individuals in the wonderful, but then poorly ventilated, eighteenth-century lecture theatre. Such was the fascination of his lecture that the count must surely have been surprisingly low.

In similar vein, when invited to give a seminar at the University of California at Davis he borrowed Deamer's Teflon Langmuir trough, some water saturated with diethyl ether, a

sample of phospholipid, a graduate student and a fire extinguisher to repeat an experiment in public that he had performed in many famous venues. To a packed audience he spoke of the barrier properties of a bilayer membrane and demonstrated how a lipid monolayer could inhibit molecular diffusion across an interface. 'He poured the ether-water into the trough,' writes Deamer, 'stationed the graduate student and fire extinguisher nearby, and held a match to the trough, which erupted in flaming ether! He then dipped a glass rod in to the phospholipid sample and touched it to the water surface. In a few seconds, as the monolayer spread over the trough, the flames were magically extinguished.' To show how any surface-active substance works, he repeated the experiment, this time with earwax from his ear, promptly extinguishing the fire.

A SURREAL MOMENT

Bangham was devoted to his family, and visitors who came to work with him were soon drawn into a scientific family with Alec and Rosalind at its centre. Rosalind, a wonderful match, was sharp-witted and perceptively sympathetic, and had her own practice as a physician. In later life she and Alec rejoiced in grandchildren numbering in double figures, and gatherings at their house vibrated with conversation, cooking with fresh produce from the large garden at Great Shelford, Cambridge, and a stream of visitors from the laboratory and around. It was a family that reached around the world and over many generations. Needless to say, many of the extended family followed in the footsteps of medicine, invention and engineering. Ros predeceased Alec by a few months, and this was a painful time for Alec who felt bereft. However, he was surrounded and supported by his family of four, who by now had distinguished themselves: James Andrew, Professor of Computer Science, University of East Anglia; Janet, artist and wife of the Cambridge biological scientist Paul Edwards; Oliver Butts, Management Consultant at Eastman Kodak plc; and Daniel Hugh, clarinet maker and proprietor of Wood, Wind & Reed Music Shop, Cambridge.

Towards the end, he was still bursting with ideas and still publishing. Colin Morley (personal communication), writing of his time with Alec, tells how

> he never stopped thinking, night and day. … he was confident that his ideas were correct. Right up to his dying days he was developing ideas and publishing, about how a body's cells tolerate each other, why pregnant women do not reject their foetus and how in the future transplants might not be rejected.

This last hypothesis proposed that any one person's bouquet of weak-acid volatile organic compounds (VOCs) can either collectively identify an individual if they lack charge, or neutralize cell surfaces to make them free and invisible when charged, ideal for overcoming rejection. A visitor to the house would find diagrams on the kitchen table to show how VOCs would fit, like edge pieces in jigsaws, the unique puzzle for which they were made (38, 39). Perhaps these were the compounds that would reduce immune responses to transplanted organs. It was perplexing and not a little irritating that transplant surgeons bombarded with his idea failed to rise to the challenge and never replied to his letters. His sole-author paper on the subject (39) was published a few months before he died at the age of 88 years, and he was distraught after the first week that no one had asked for reprints, as had happened in the past and which

he had found so encouraging! To wait for citation figures was a step too exasperating, and so it proved a surreal moment when he gathered a small group of friends and colleagues to tell them all about it on the evening before he died.

But the last word must go to Rev. Dr Martyn Hill, a loyal and insightful physicist who worked with Alec for many years; at Alec's funeral he reflected on why an Anglican clergyman was taking it. Alec was quite emphatic about his atheism but had told Martin shortly after he was ordained that if any clergyman was to take his funeral it had to be him. Martyn spoke of a kind, generous companion who was also extremely competitive. 'He loved gardening and was generous with his produce, but he did grow the largest cabbages, longest runner beans, heaviest marrow, tallest sunflower, in the whole area! Well if not this year, next!!' As we watched as he was lowered into the ground, we blew bubbles from small party bottles provided for the occasion. They floated high into a wintry sky close to his home of many years in celebration of a man who brought artificial membranes to life, and who lived life to the full.

Acknowledgements

We are deeply indebted to the Bangham family, Jeff Watkins FRS, Colin Morley, David Deamer and Ed Munn for their very generous contributions to this memoir.

Dr Ed Munn, a former colleague of Alec Bangham at Babraham, Cambridge, and creator of *OneLinerImages*, has kindly provided a personal addition to the memoir of his hand-drawn liposome illustration. The illustration and description are available in the electronic supplementary material online.

The frontispiece portrait, 'A. D. Bangham and the liposome', by Humphrey Bangham (oil on canvas, 1985), is from the collection of the Royal Society. (Online version in colour.)

References to other authors

Abramson, H. A., Moyer, L. S. & Gorin, M. H. 1942 *Electrophoresis of proteins and the chemistry of cell surfaces*. New York: Reinhold.

Deamer, D. W. 1985 Boundary structures are formed by organic components of the Murchison meteorite. *Nature* **317**, 792–794.

Deamer, D. W. 2010 From 'Banghasomes' to liposomes: a memoir of Alec Bangham, 1921–2010. *FASEB J.* **24**, 1308–1310.

De Kruif, P. 1926 *Microbe hunters.* New York: Harcourt Brace & Jovanovich.

Gregoriadis, G. 1973 Molecular Trojan horses. *New Scient.* **60**, 890–893.

Gregoriadis, G. 1976 The carrier potential of liposomes in biology and medicine. *N. Engl. J. Med*. **295**, 704–710 and 765–770.

Hargreaves, W. R., Mulvihill, S. J. & Deamer, D. W. 1977 Synthesis of phospholipids and membranes in prebiotic conditions. *Nature* **266**, 78–80.

Heap, R. R., Symonds, A. M. & Watkins, J. C. 1970 Steroids and their interactions with phospholipids: solubility, distribution coefficient and effect on potassium permeability of liposomes. *Biochim. Biophys. Acta* **218**, 482–495.

Heap, R. B., Symonds, A. M. & Watkins, J. C. 1971 An interaction between oestradiol and progesterone in aqueous solutions and in a model membrane system. *Biochim. Biophys. Acta* **233**, 307–314.

Mueller, P., Rudin, D. O., Tien, H. I. & Wescott, W. C. 1962 Reconstitution of cell membrane structure *in vitro* and its transformation into an excitable system. *Nature* **194**, 979–980.

Bibliography

The following publications are those referred to directly in the text. A full bibliography is available as electronic supplementary material at http://dx.doi.org/10.1098/rsbm.2011.0004 or via http://rsbm.royalsocietypublishing.org.

(1) 1951 The effect of cortisone on wound healing. *Br. J. Exp. Pathol*. **32**, 74–84.

(2) 1953 (With P. N. Magee & S. B. Osborn) The effect of inflammation and other factors on the movement of radioactive glass particles from the peritoneal cavity. *Br. J. Exp. Pathol*. **34**, 1–11.

(3) 1957 Distribution of electrophoretically different haemoglobins among cattle breeds of Great Britain. *Nature* **179**, 467–468.

(4) 1958 (With B. S. Blumberg) Distribution of electrophoretically different haemoglobins among some cattle breeds of Europe and Africa. *Nature* **181**, 1551–1552.

(5) 1958 (With B. A. Pethica & G. V. F. Seaman) The charged groups at the interface of some blood cells. *Biochem. J*. **69**, 12–19.

(6) 1958 (With R. M. C. Dawson) Control of lecithinase activity by the electrophoretic charge on its substrate surface. *Nature* **182**, 1292–1293.

(7) 1959 (With M. F. Perutz, L. F. Steinrauf & A. Stockell) Chemical and crystallographic study of the two fractions of adult horse haemoglobin. *J. Mol. Biol*. **1**, 402–404.

(8) 1959 (With R. M. C. Dawson) The relation between the activity of a lecithinase and the electrophoretic charge of the substrate. *Biochem. J*. **72**, 486–492.

(9) 1959 (With R. M. C. Dawson) The activation of surface films of lecithin by amphipathic molecules. *Biochem. J*. **72**, 493–496.

(10) 1960 (With R. M. C. Dawson) The hydrolysis of phospholipids at an interface and its possible significance in the initiation of blood clotting. In *Proc. 7th Congress European Society of Haematology, London, 1959*, part II, pp. 661–666.

(11) 1961 Electrophoretic characteristics of ram and rabbit spermatozoa. *Proc. R. Soc. Lond.* B **155**, 292–305.

(12) 1961 A correlation between surface charge and coagulant action of phospholipids. *Nature* **192**, 1197–1198.

(13) 1962 (With D. J. Wilkins & R. H. Ottewill) On the flocculation of sheep leucocytes. I. Electrophoretic studies. *J. Theor. Biol*. **2**, 165–175.

(14) 1962 (With D. J. Wilkins & R. H. Ottewill) On the flocculation of sheep leucocytes. II. Stability studies. *J. Theor. Biol*. **2**, 176–191.

(15) 1962 (With J. C. Glover, S. Hollingshead & B. A. Pethica) The surface properties of some neoplastic cells. *Biochem. J*. **84**, 513–517.

(16) 1963 Cattle haemoglobins. A review. *J. R. Anthropol. Soc*. **18**, 34–40.

(17) 1964 The adhesiveness of leukocytes with special reference to zeta potential. *Ann. NY Acad. Sci*. **116**, 945–949.

(18) 1964 (With R. W. Horne) Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. *J. Mol. Biol*. **8**, 660–668.

(19) 1965 (With M. M. Standish & J. C. Watkins) Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol*. **13**, 238–252.

(20) 1965 (With M. M. Standish & G. Weissmann) The action of steroids and streptolysin S on the permeability of phospholipid structures to cations. *J. Mol. Biol*. **13**, 253–259.

(21) 1965 (With M. M. Standish & N. Miller) Cation permeability of phospholipid model membranes: effect of narcotics. *Nature* **208**, 1295–1297.

(22) 1966 (With B. C. Bhattacharya, R. J. Cro, R. D. Keynes & L. E. A. Rowson) An attempt to predetermine the sex of calves by artificial insemination with spermatozoa separated by sedimentation. *Nature* **211**, 863.

(23) 1967 (With M. M. Standish, J. C. Watkins & G. Weissmann) The diffusion of ions from a phospholipid model membrane system. *Protoplasma* **63**, 183–187.

(24) 1968 Membrane models with phospholipids. *Prog. Biophys. Mol. Biol.* **18**, 29–95.

(25) 1969 (With S. M. Johnson) The action of anaesthetics on phospholipid membranes. *Biochim. Biophys. Acta* **193**, 92–104.

(26) 1971 (With S. M. Johnson, M. W. Hill & E. D. Korn) Single bilayer liposomes. *Biochim. Biophys. Acta* **233**, 820–826.
(27) 1973 (With S. M. Johnson & K. W. Miller) The opposing effects of pressure and general anaesthetics on the cation permeability of liposomes of varying lipid composition. *Biochim. Biophys. Acta* **307**, 42–57.
(28) 1974 (With M. W. Hill & N. G. A. Miller) Preparation and use of liposomes as models of biological membranes. *Methods Membr. Biol*. **1**, 1–68.
(29) 1978 Properties and uses of lipid vesicles: an overview. *Ann. NY Acad. Sci*. **308**, 2–7.
(30) 1978 (With C. J. Morley, P. Johnson, G. D. Thorburn & G. Jenkin) Physical and physiological properties of dry lung surfactant. *Nature* **271**, 162–163.
(31) 1979 (With C. J. Morley & M. C. Philips) The physical properties of an effective lung surfactant. *Biochim. Biophys. Acta* **573**, 552–556.
(32) 1980 (With C. Morley, B. Robertson, B. Lachmann, R. Nilsson, G. Grossman & N. Miller) Artificial surfactant and natural surfactant. Comparative study of the effects on premature rabbit lungs. *Arch. Dis. Child.* **55**, 758–765.
(33) 1980 (With C. J. Morley, N. Miller & J. A. Davies) Dry artificial lung surfactant and its effect on very premature babies. *Lancet* **i**, 64–68.
(34) 1980 Breathing made easy. *New Scient.* **85**, 408–410.
(35) 1984 (With N. Miller, J. A. Davies, A. Greenough & C. Morley) Introductory remarks about artificial expanding compounds (ALEC). *Colloids Surf.* **10**, 337–341.
(36) 1987 Lung surfactant: how it does and does not work. *Lung* **165**, 17–25.
(37) 1995 Surrogate cells or trojan horses. The discovery of liposomes. *BioEssays* **17**, 1081–1088.
(38) 2008 Invisibility of tissues and the mother/fetus paradox: an hypothesis. *J. Liposome Res.* **81**, 91–93.
(39) 2009 The physical chemistry of self/non-self: jigsaws, transplants and fetuses. *FASEB J.* **23**, 3644–3646.