Green College Lectures

Discoveries on muscle: observation, theory, and experiment

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The understanding of muscular contraction is a good topic to use for illustrating the various ways in which discoveries in medical science are made. During the past 60 years there has been a series of revolutions, each entailing the overthrow of some generally accepted doctrine about the way in which a muscle uses energy from a chemical reaction to do mechanical work.

The first use of the word "revolution" that I know of in connection with muscle is in the title of an article by A V Hill (1886-1977) "The revolution in muscle physiology," published in 1932 in *Physiological Reviews*.[1] The revolution in question was the end of the "lactic acid era" and the start of the "phosphagen era." The production of lactic acid by a muscle when it is active was known to Berzelius early in the nineteenth century, and during the first quarter of the twentieth century it was almost universally supposed that the primary biochemical reaction in muscular contraction was the release of lactic acid from a hypothetical large precursor molecule referred to as "lactacidogen"; the hydrogen ions liberated were supposed to neutralise negative charges on the contractile protein filaments, allowing them to fold and shorten.

Hill dates the outbreak of the revolution to the last day of 1926, when Philip and Grace Eggleton submitted to the *Biochemical Journal* a paper reporting that the amount of an unidentified organic phosphate compound present in muscle decreased during a contraction, with a corresponding increase in the amount of inorganic phosphate.[2] Later in 1927 Fiske and Subbarow published their independent discovery of this phenomenon,[3] and identified the organic phosphate compound as phosphoryl creatine. Supporters of the lactic acid theory did not, however, give in immediately, and the revolution was not over until 1930, when Lundsgaard published his observation that a muscle poisoned with iodoacetic acid will perform a considerable number of normal contractions but produces no lactic acid.[4]

This revolution followed the pattern described in T S Kuhn's *The Structure of Scientific Revolutions*,[5] in that the actual overthrow of the old theory was preceded by a period during which it encountered one serious difficulty after another, including the following:

(1) A mainstay of the theory had been the existence of a well defined maximum to the amount of lactic acid produced in one contraction, suggesting the complete conversion of a precursor present in limited amount. Kondo found, however, that adding a buffer greatly increased the amount of lactic acid that could be formed by muscle press juice, implying that the limit was set not by the exhaustion of a precursor but by the fall of pH that the enzymes would tolerate.[6]

(2) Embden reported that much of the lactic acid was produced after the contraction itself was over[7]; this corresponded to the delayed heat production found a few years earlier by Hartree and Hill.

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(3) The lactic acid was shown to come from glycogen, not a plausible "lactacidogen."

(4) Tiegs showed that creatine escape is accelerated when a muscle is fatigued,[8] a forerunner of the discovery of phosphoryl creatine breakdown.

I have been told by Professor Hermann Blaschko that the crucial observation of Lundsgaard in 1930 was an accidental byproduct of an unrelated investigation into the "specific dynamic action" of proteins. He was testing the effects of individual amino acids on the basal metabolic rate, and also tried acetic acid and some of its derivatives, including iodoacetic acid, finding that a muscle poisoned with this substance would contract without liberating lactic acid.

Discovery of ATP

Even before this revolution was over the seeds of the next one were being sown: in 1929 Lohmann discovered adenosine triphosphate (ATP) in muscle extracts.[9] In 1934 he claimed that the only pathway for the utilisation of phosphoryl creatine was to rephosphorylate adenosine diphosphate (ADP) to ATP, and it was soon accepted that the primary reaction providing the energy for contraction was the conversion of ATP to ADP plus inorganic phosphate. The splitting of phosphoryl creatine, as well as the production of lactic acid, was thus relegated to the role of a recovery process. Conclusive proof of this theory was, however, long delayed. No decrease in the content of ATP could be detected; this was attributed (correctly, though without direct evidence) to rapid rephosphorylation of ADP from phosphoryl creatine. The experiment equivalent to Lundsgaard's "alactacid contraction" was not achieved until 1962, when Cain, Infante, and Davies showed that a muscle poisoned with fluorodinitrobenzene could perform several normal contractions with the utilisation of ATP but not of phosphoryl creatine.[10]

Another very important, though less widely recognised, revolution concerning the metabolism of active muscle took place in 1938 with the publication of the well known paper of A V Hill, which gave a quantitative account of the increase of heat production (and therefore of the amount of chemical change) which takes place when a stimulated muscle is allowed to shorten and thus to do mechanical work.[1] This refuted the widespread assumption that a fixed amount of energy was made available on stimulation so that the energy appearing as heat ought to decrease when the muscle is allowed to do work; it also gave an interpretation of the relation between the speed of shortening and load in terms of an influence of the mechanical conditions on the rates of chemical reactions, as against the previous assumption that this relation was governed by the "visco-elastic" properties of the muscle. The increase of heat production had in fact been shown qualitatively by Fenn, working in Hill's laboratory[11]; the importance of the result was recognised by Hill in 1923 in his Nobel lecture, but he admitted much later (1965) that he had continued for many years to think in terms of the viscoelastic theory.[1] Even now, many biochemists do not pay sufficient attention to the influence of mechanical conditions on chemical rates.

An astonishing fact, to which A V Hill drew attention (reference 1, p 9), is that the increase of heat liberation when an active muscle is allowed to shorten had been seen by Rudolf Heidenhain in 1864 and by Fick a few years later.

Major steps in "normal science"

Two discoveries made around the start of the second world war were tremendously important but cannot be counted as "revolutions" since they did not overthrow previously accepted ideas; they were major steps in "normal science," to use Kuhn's phrase for advances that take place in the intervals between revolutions. They were, firstly, the discovery in Moscow by Engelhardt and his wife Ljubimova that myosin is itself an ATPase and that its physical properties are changed while this enzymic activity is in progress; the second was the discovery by Straub in the laboratory of Albert Szent-Györgyi at Szeged that the material previously known as "myosin," and recognised as being the contractile substance, was itself a complex of two proteins, "actin" and what is now known as "myosin." These two discoveries were built into the theory that on excitation the two proteins combined to form continuous "actomyosin" filaments, which hydrolysed ATP and were thereby caused to fold and thus to make the muscle shorten.

Two of the earliest publications containing useful electron micrographs of muscle claimed to substantiate this theory by demonstrating the existence of these filaments running continuously through the A and I bands of the striation pattern (reference 12, p 34). In both cases the objects examined were whole myofibrils (bundles of many thousands of filaments), and with hindsight clearly there was no possibility of establishing whether the filaments were continuous or not.

The next major advance, the discovery (1953-4) that a striated muscle fibre changes its length by relative sliding motion of two sets of interdigitating filaments within each unit of the striation pattern, was indeed a revolution since it contradicted several features of the theory outlined in the last paragraphs, which was universally believed at the time though with variants as to the nature and cause of the folding which made the muscle shorten.

The three key observations, in the order in which they were published, were as follows. Firstly, myosin is present only in the "A bands" of the striation pattern and is the material which gives those bands the high refractive index by which they are visible in ordinary light, phase, or interference microscopy; this contradicted the belief that both actin and myosin were distributed uniformly along the filaments. Secondly, transverse sections under the electron microscope showed the presence of two (thick and thin) sets of interdigitating filaments, contradicting the idea of continuous filaments.[13] Thirdly, features of the band pattern in whole muscle fibres or in separated myofibrils, corresponding to the lengths of these two sets of filaments, were found not to change during either stretch or active contraction, contradicting the idea that contraction took place by shortening of the filaments.

Relationship to Popper's views

The fact that these features of the new theory were contradictions of what was currently believed could be taken as supporting the view of Karl Popper that scientific advance takes place only by a sequence of "conjectures and refutations."[14] Nevertheless, I regard this aspect of these discoveries as less important than their positive features—the presence of two sets of overlapping filaments, neither of which changes length appreciably (except in extreme shortening), and with totally different composition.

Another failure to fit in with Popper's ideas is that none of these observations was made in an experiment designed to test the point that was contradicted. Nor was the possibility of sliding filaments in mind when any of these experiments was being planned: Hasselbach told me that his observation of myosin being the A substance was a byproduct of a re-investigation of the amounts of myosin and actin present in muscle; H E Huxley and Jean Hanson saw the A bands of myofibrils being dissolved away when a pyrophosphate solution intended as a plasticiser was applied[15]; H E Huxley found the double array of filaments in a straightforward electron microscopic investigation of the structure of muscle[13]; and A F Huxley and Niedergerke noticed the constancy of the width of the A band during experiments aimed at investigating the formation of "contraction bands."[16]

With hindsight, it is difficult not to echo the sentiment of my grandfather T H Huxley when he took in the central point of *The Origin of Species*: "How stupid not to have thought of that before." So far as I have been able to discover, the only person to have thought of sliding filaments before 1953 was Dorothy Hodgkin.[17]

This revolution was not preceded by a period in which there was difficulty in fitting fresh observations into the current theory, such as preceded the end of the lactic acid era. There were, however, a few observations which might have led a perceptive individual to suggest sliding filaments. Thus, *x* ray diffraction work in the 1930s, although generally regarded as supporting the idea of specific folding of protein chains, in fact showed little change in the wide angle pattern and would have been better interpreted as suggesting that the filaments themselves did not shorten. Further, Ernst Fischer found (in smooth muscle) that the strength of birefringence was not much increased when the muscle was stretched[18]; he interpreted this as implying that the birefringence was due to oriented structures in the muscle that changed their relative positions, but were not individually stretched, when the muscle as a whole was lengthened. Then the existence of the separated proteins actin and myosin in the contractile material might have suggested that they were distributed in relation to the striation pattern.[19] Finally, Dorothy Needham had pointed out that the form of A V Hill's relationship between speed of shortening and rate of liberation of energy (heat plus work) suggested a cyclic action of the contractile elements, an idea that is difficult to incorporate in a theory based on progressive folding of protein filaments but is natural if one thinks in terms of sliding filaments.[20] None of these points received much attention, however, and almost the only feature of folding theories that was generally regarded as unsatisfactory was the longstanding one that they gave no explanation for the existence of the striations.

Forgotten observations

In fact, many observations hidden in the nineteenth century literature might have suggested a sliding filament system—for instance (references in reference 12), the constancy of the A band width (Krause, 1869; Engelmann, 1873); myosin in the A bands (Krause, 1869; Schipiloff and Danilevsky, 1881); and birefringence not increased on stretch (von Brücke, 1858).

Unfortunately, these observations, although common knowledge in the late nineteenth century, were to all intents and purposes forgotten by the mid-twentieth century, and were rediscovered only after the sliding filament theory had been established. The belief that most of the length change during passive stretch took place in the I bands was reversed early in the twentieth century, mainly on the basis of the papers by Meigs and by Hürthle. I have discussed the causes of these mistakes elsewhere.[12]

The other aspect of muscular activity that I wish to discuss is the turning on of contraction when a muscle fibre is stimulated. It has long been recognised that excitation is an event occurring primarily in the surface membrane, which raises the question of how this event influences the contractile material in the interior of the fibre. It was generally supposed that an activating substance liberated at the surface membrane diffused into the interior, though in a well known paper Tiegs had proposed that the Z line was a spiral structure and that excitation was conducted along it, not specifically affecting the surface membrane.[21] It is now known that the Z line is usually spiral, but electrophysiology gives no support to the idea that the longitudinal conduction of excitation takes place along it.

The problem was raised in an acute form after the second world war by measurements made by A V Hill which showed that after excitation the whole of the cross section of each fibre became fully

active in a time which was too short to be accounted for by simple diffusion. Hill's suggestion was that diffusion was accelerated by an autocatalytic reaction.

My interests in structure and in microscopy led me to think about the possibility that inward spread took place along some specialised structure which formed part of the striation pattern. The obvious structure to consider first was the Z line, or "Krause's membrane," both because of Tiegs's suggestion and because there was much old evidence from light microscopy that the Z lines in adjacent myofibrils were joined together so as to form a continuous structure across the fibre.[22] Taylor and I devised an experiment to test this possibility: we depolarised a very small patch of the surface membrane of an isolated muscle fibre (about 1 μm diameter) by placing the end of a micropipette in contact with the surface and applying a negative electric potential to the fluid contained within the pipette.[23] We saw immediately the result that we had hoped for: a highly localised contraction, entailing shortening of only a single I band, took place but only when the pipette was placed opposite an I band, the Z line being at the centre of I. No contraction occurred when the pipette was placed opposite an A band.

This appeared to confirm our hypothesis, and we published the result as a letter to *Nature* entitled "Function of Krause's Membrane." However, when we showed our film at a meeting of the Physiological Society an electron microscopist in the audience produced from his pocket a slide showing transverse tubules in a striated muscle fibre.[24] These were obviously promising structures for conveying excitation inwards from the surface membrane, but they were in the wrong place: in each repeat of the striation pattern there were two sets of tubules, one either side of the Z line. I have set out elsewhere the sequence of observations that led finally to the clear result that is now universally accepted—namely, that excitation spreads not along Krause's membrane itself but up tubules which are continuous with the surface membrane and are open to the extracellular space, but the course to this simple conclusion was tortuous on account of very unexpected differences between the muscles of different animals and also because of fixation artifacts in electron micrographs obtained before the use of glutaraldehyde as a fixative became general.[25]

This revolution had aspects that fit with Kuhn's ideas (widespread dissatisfaction with the diffusion theory before it was displaced). It also had aspects that fit with Popper's propositions, in that the key experiment was designed to test a conjecture. But progress was made not by refutation but by finding the expected result. Nevertheless, the outcome emphasises an important feature of Popper's ideas: finding an expected result does not necessarily mean that the hypothesis on which the expectation was based is correct; it is always possible—as in this case—that the result is due to some other circumstance that one had not thought of.

A parallel with the situation regarding sliding filaments is that highly relevant and suggestive observations had been made with the light microscope about the end of the nineteenth century but had been totally forgotten. I have set these out elsewhere,[25] but I must mention the paper published by Veratti in 1902 with beautiful pictures of the transverse networks formed by the tubules and shown by the Golgi method. This paper was rediscovered by H S Bennett after he had seen indications of continuous transverse structures with the electron microscope and was republished in English translation.[26]

Manner of discoveries

These examples illustrate several features of the way in which discoveries have been made in one branch of medical science. Firstly, there are many different ways in which a scientist may be led to his discovery. Secondly, a discovery is apt to be forgotten unless it is not only established firmly but also built into a theoretical structure which is accepted. Thirdly, finding an expected result does not prove the correctness of the hypothesis underlying the expectation. Fourthly, progress is often held up by the assumption that each function is carried out in the same way in every kind of living organism—evolution leads to diversity as well as providing resemblances due to common ancestry.

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What is erythromelalgia and how should it be treated?

Erythromelalgia, or erythermalgia, is a condition characterised by painful, burning extremities when exposed to heat, either local or general. On examination the feet are red and hot. There is a primary form where there seems to be no underlying disorder, and a secondary form where there is an underlying disease such as a myeloproliferative disorder, hypertension, venous disease, diabetes, systemic lupus erythromatosus, or rheumatoid disease. It needs to be distinguished from the painful, burning but cold feet seen in patients with chronic ischaemia. It is rare. In the many thousands of patients I have seen in the peripheral vascular clinic of the Royal Infirmary of Edinburgh over the past 16 years I have not found a convincing case. Patients with burning, painful feet from various causes are seen but do not have the so called characteristics of erythromelalgia of hot feet with the symptom brought on by exposure to heat. The condition must be differentiated from painful peripheral neuropathy, diabetic neuropathy, and local causes of pain in the feet. There does not seem to be any satisfactory treatment. Aspirin has been said to be effective, raising the possibility of some abnormality of prostaglandins, but it does not appear to be uniformly effective. Methysergide, vasodilators, naproxen, and dipyridamole have all been tried with doubtful results. Propranolol has been reported to be effective in one case and sodium nitroprusside infusions have been reported in isolated cases and are perhaps worthy of a trial.—E HOUSLEY, consultant vascular physician, Edinburgh.

Boda JL. Treatment of erythromelalgia with propranolol. *Lancet* 1977;ii:412.