PART I

MUSCLE

A CHALLENGE TO BIOCHEMISTS

by

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OTTO MEYERHOF has always been betwixt and between: a physiological chemist or a chemical physiologist, perhaps we should call him a "chemiologist". On my shelves are about two hundred of his reprints, his and his colleagues'. The first of these, with its accompanying letter addressing me as "Sehr geehrter Herr Kollege" dated 1911 from Naples, dealt with the heat production of the vital oxidation process in the eggs of marine animals. Next follow papers on the energy exchanges of bacteria, the heat accompanying chemical processes in living cells, the inhibition of enzyme reactions by narcotics (1914). Some time in those apparently peaceful years, before the explosion of 1914, he visited us at Cambridge. Then comes a gap, so far at least as my collection of OTTO MEYERHOF's reprints is concerned. By 1919 he had moved to HÖBER's laboratory at Kiel and the long succession of papers began on the respiration, energetics, and chemistry of muscle. And when I say muscle, I mean muscle: living muscle, resting, contracting and recovering from contraction, developing tension and doing work, producing lactic acid and removing it again, using oxygen and glycogen, giving out CO_2 and heat, all things which living muscles are accustomed to do. And since I too was working on living muscle, we were in frequent communication again, after the five years' gap. In the summer of 1922, following a suggestion to HOPKINS, he visited Cambridge and gave lectures there. I remember "Hoppy" expressing concern lest some anti-German demonstration might take place, but appearing to be satisfied by the comment that if so I should be proud to remove the demonstrator: nothing of course happened. Later, he stayed with me at Manchester and I recall, as an example of his scientific perspicacity, the complete disbelief which he, first of anyone, expressed in experiments he witnessed which six months later were proved to be fraudulent. That was our first reunion after the War, there were many others, in London, Plymouth, Barcelona, Heidelberg, Berlin, Rome and elsewhere. The photograph shows us driving together to Stockholm for the Physiological Congress in 1926.

The results of his researches, and those of his colleagues, are a part of scientific history. They are linked with most that is known of the chemistry of muscle and with much that is established of changes involving phosphate and carbohydrate in the cell. For some years his investigations were concerned mainly with muscle — living muscle: more recently they followed the trend in biochemistry, perhaps even they helped to establish the fashion, of dealing *in vitro* with the enzyme systems of muscle. As late, however, as 1935, he was working on the volume changes of living muscle during contraction and relaxation and relating them to the underlying chemical cause. I read these papers again recently, very carefully, having come to the conclusion that the

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reversible part of the volume change is attributable mainly or wholly to pressure set up by contraction. The elegance and clarity of MEYERHOF's work and its description impressed itself again as it had done in earlier days. One might criticize some of the conclusions, but not the methods or results. To read these papers once more was a sudden pleasure, after so many in which one could not be sure what an author had really done!

My last reprint from Heidelberg is dated 1938. Perhaps if Hitler had not driven him from the beautiful Institute and the excellent colleagues and facilities he had there, the succession of papers on muscle—living muscle—might have continued. Alas that they could not! This paper, however, is to challenge him and his disciples to make a few more chemical investigations on living muscle, to see how far the chemistry *in vitro* of muscle extracts can be fitted to the physical facts of muscular contraction.

It is customary for biochemists, (*e.g.*, BALDWIN[1], p. 341) to describe "The probable course of events in normal muscular contraction" is some such terms as these:

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"On the arrival of a nerve impulse, ATP is broken down, giving rise to ADP and inorganic phosphate, furnishing at the same time the contraction energy. The ADP is promptly converted again into ATP at the expense of phosphagen and no change in the ATP content of the muscle can be detected . . ." Others suppose that contraction is associated with the formation of myosin — ATP and that ATP is broken down in relaxation. By SANDOW[2] a slight initial lengthening (in a muscle under tension) after a stimulus ("latency relaxation") is attributed to the formation of a complex between activated myosin and ATP. Most of this is pure speculation, without direct experimental evidence. Unlike Mr. Stalin (HISTORICUS[3]) I have no general theory of revolutions, but I did once write an article (1932), which I think is still worth reading, on "The Revolution in Muscle Physiology"[4]. That was after phosphagen had deposed lactic acid from pride of place as the chief chemical agent in contraction. At that date one could write: "On stimulation, phosphagen breaks down . . .: this is the primary change by which energy is set free". Only four years earlier RITCHIE[5] wrote: "On stimulation of a muscle fibre the wave of excitation passes down it; by increasing the permeability of a membrane or by some other means it causes the liberation of lactic acid from a carbohydrate source. The liberated hydrogen ions neutralize the negative charge on a surface of protein, MEYERHOF's *Verkürzungsort* . . . and thereby alter the type of structure, the area of surface, and the mechanical constants. This will be the fundamental change." In the lactic acid era the evidence that the formation of lactic acid was the cause and provided the energy for contraction seemed pretty good. In the phosphagen era a similar attribution to phosphagen appeared even better justified. Now, in the adenosinetriphosphate era lactic acid and phosphagen have been relegated to recovery and ATP takes their place. Those of us who have lived through two revolutions are wondering whether and when the third is coming.

It may very well be the case, and none will be happier than I to be quit of revolutions, that the breakdown of ATP really is responsible for contraction or relaxation: but in fact there is no direct evidence that it is. Indeed, no change in the ATP has ever been found in living muscle except in extreme exhaustion, verging on rigor. This is explained by supposing that as soon as ATP is broken down into ADP and phosphate it is promptly restored in the so-called "LOHMANN reaction" at the expense of creatine phosphate.

$$\mathrm{ADP} + \mathrm{CP} \rightarrow \mathrm{ATP} + \mathrm{C}$$

If this happens after each stimulus, then the smallness of the changes involved and their quickness make it extremely difficult to gain any direct evidence on the subject. In a single twitch, for example, the heat set free is about 3 millicalories per gram, which would correspond to the liberation from ATP of $2.5 \cdot 10^{-7}$ g molecule of phosphate per gram of muscle. To measure so small a change, reversed within the duration of a single twitch, might well seem an impossible task.

We should not, however, be so satisfied with the explanation of why no change in ATP is ever found in living muscle that we cease to look for it: for another possibility exists. The total energy available from all sources (lactic acid, phosphagen and ATP) for the anaerobic phase of contraction is about 1 cal/g, corresponding to about 400 twitches. The total energy similarly available after poisoning with iodoacetate (from phosphagen and ATP) is about 0.25 cal/g corresponding to about 100 twitches. From the known amount of ATP present is muscle, the total energy it could provide by breaking

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off one phosphate is about 0.05 cal/g, corresponding to about 20 twitches. Is it not possible that as stimulation proceeds a balance is reached at some intermediate level between breakdown and restoration? That is the case with phosphagen and lactic acid; in a muscle steadily stimulated (in the presence of oxygen) a certain amount of phosphagen is broken down, a certain amount of lactic is formed, and a steady level is reached between breakdown and recovery. At a still earlier stage one might expect steady stimulation to provide at least a temporary balance between ATP breakdown and restoration.

In frogs' muscles at 20° C, if ATP were the only source of energy a maximal tetanus would lead to its complete breakdown in about 0.5 sec. The suggested balance, if it occurred, would presumably be reached within that time, and when the stimulus ended restoration of the ATP might be completed within another 0.5 sec. The times involved are far too short for chemical manipulation: but biochemists need not be disheartened, frogs' and rabbits' muscles are singularly ill-suited to the enquiry, they are much too quick, why not use muscles which contract more slowly? The muscles of the Mediterranean land tortoise, *Testudo graeca*, commonly imported before the War into England and sold on barrows for 1/– in London streets, take about fifteen times as long to contract as those of a frog and their speed can be further reduced about nine times by lowering the temperature from 20° C to 0° C, or about five times by lowering it to 5° C. This means that the time available for chemical manipulation can be reckoned in large fractions of a minute instead of fractions of a second. Provided, therefore, that the chemical technique is capable of determining a substantial part of the total ATP with reasonable accuracy, the time involved can be made so long that sufficient resolution ought easily to be obtained.

The experiment ought certainly to be made and nobody could make it better than OTTO MEYERHOF — for he knows how to handle living muscles. The result may not be unequivocal — but it very well may. If no change in ATP is found, but only a change in phosphagen, the *status quo* remains and we can all believe what we like, provided it is consistent with the physical facts described below. But suppose it is found that ATP is broken down at a rate decreasing from the start, reaching a steady concentration after half a minute's stimulation (corresponding to half a second in a frog's muscle at 20° C) and is restored to its original level after (say) a further half minute of rest and recovery. Then at least we can be assured that ATP is really concerned either with the contractile process itself, or with the very early stages of recovery. There are other possibilities and, without trying, it is useless to speculate too much. A German clinician is said to have remarked: "Der Versuch muss gemacht werden und sollte er hundert Bauern kosten". A decision on this important matter is certainly worth a hundred tortoises.

But whatever may be the outcome of this challenge to biochemists, I would invite them also, in their speculations about muscle, to take note of the following facts, all referring to contraction and relaxation, as distinguished from recovery.

1. There is no sign of an endothermic process at any stage of contraction or relaxation. If endothermic processes occur they are balanced, or overbalanced, by exothermic ones.

2. No heat at all is produced during relaxation, apart from that derived from the degradation of work previously performed during contraction (in raising a load, or in stretching elastic material in series with the muscle). When a muscle relaxes without load or tension, no heat is produced after the contractile phase is over.

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3. It has been found by quick stretches applied to a muscle shortly after a single shock that the full strength of the contraction, defined as the load which a muscle can just bear without lengthening (and equal to the force of a maximal tetanus) is developed abruptly immediately after the end of the latent period. It is maintained for a time and then declines in "relaxation". If stimulation is continued, each successive shock restores the strength of contraction to its full height.

4. Corresponding to (3) there is a "heat of activation" in a twitch, which is independent of all other factors except the fact of stimulation. The heat of activation starts at its maximum rate before any visible sign of contraction occurs, declining to zero at about the moment when the strength of contraction (see 3 above) begins to fall off, *i.e.*, at the end of the contractile phase.

5. The "heat of maintenance" in a prolonged contraction is the summated effect of the heat of activation following successive elements of the stimulus. It is greater at first corresponding to the more rapid relaxation after a short tetanus, but after a certain duration of stimulus it becomes constant. It is affected only to a minor extent by the length of the muscle. It is greatly increased by a rise of temperature, corresponding to the more rapid relaxation.

6. In twitch and tetanus alike, apart from the heat of activation or the heat of maintenance, energy is given out in two discrete forms, (a) as mechanical work and b) as heat of shortening. The heat of shortening is directly proportional to the change of length over the whole range of shortening, and (for a given change of length) is independent of the work done.

7. Apart from heat of activation or heat of maintenance, the rate at which total energy, *i.e.*, heat plus work, is given out, is a linear function of the load throughout a contraction:

$$(P + a)\, dx/dt = b(P_o - P)$$

where x is the amount of shortening up to time t, P is the load, dx is the heat of shortening, P_o is the maximum isometric tension and b is a constant related to the maximum velocity of shortening under zero load.

8. The constant a in (7) can be obtained either from thermal measurements or from the form of the characteristic relation between load and velocity of shortening. The agreement is good.

9. Relaxation is not an active process. A muscle completely without load or tension does not lengthen again after shortening in response to a stimulus. That its length has really changed and that its fibres or fibrils have not gone into folds is shown by the fact that its latent period is practically the same at a short length as it is at a greater one. If a muscle had to "take up the slack" in fibres or fibrils before its tension could be manifested externally, the latent period would be greatly prolonged.

10. Simultaneous with the earliest sign of mechanical activity after a shock is a change of opacity. This is due to an alteration of light scattering (D. K. HILL[6]). The earliest phase has certain characteristics which distinguish it from a later phase which continues into recovery.

11. If we can assume that excitation occurs at the surface membrane of a muscle fibre, the propagation inwards of the change there started cannot be due to the diffusion inwards of some substance, *e.g.*, Ca ions or acetyl choline, initiating contraction by its arrival at each point. Diffusion is far too slow. Some chain-reaction started at the surface is required.

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Nineteen years ago my colleagues and I found (HILL AND KUPALOV[7]; HILL AND PARKINSON[8]) in muscles stimulated to exhaustion in nitrogen, a lowering of vapour pressure considerably too large to be accounted for by chemical changes known to occur, if the precursors of the chemical substances produced were themselves osmotically active. In normal muscles complete exhaustion led to a decrease of vapour pressure corresponding to an increased concentration in the free water of a muscle of 0.12 M. The production of 0.35% lactic acid dissolved in the free water, (taken as 0.77 g per g) of the muscle, would lead to a concentration change of 0.050 M. The liberation of creatine and phosphate by the complete breakdown of phosphagen in amounts equivalent to 65 mg. P/100 g would give 0.054 M. The production of phosphate and adenylic acid from ATP in amounts equivalent to 30 mg P/100 g would give 0.012 M. The total, 0.116 M, is not far from that (0.12 M) calculated from the observed change of vapour pressure. We have assumed, however, that the phosphagen and the ATP were not themselves osmotically active; if they had been the increase would have been 0.031 M less, namely 0.085 M instead of 0.12 M. The vapour pressure measurements were certainly not that much wrong.

Again, in muscles poisoned with iodoacetate complete exhaustion led to a mean decrease of vapour pressure corresponding to an increased concentration of 0.050 M. If phosphagen and ATP breakdown are assumed, as above, to be the only chemical reactions involved, the corresponding change of concentration in the free water of the muscle would be 0.066 M. It is impossible, however, in muscles adequately poisoned to ensure that some preliminary breakdown of phosphagen has not occurred: and if the poisoning is not quite sufficient, there is likely to be some formation of lactic acid. Either cause would tend to make the observed change of vapour pressure smaller than that calculated from the assumed breakdowns. Even so, had the phosphagen and ATP originally been osmotically active, the change calculated from the constituents would have been only 0.035 M, considerably less than the 0.050 M observed.

Unless, therefore, some chemical reactions hitherto unknown occur in a muscle stimulated to exhaustion in nitrogen, we are forced to conclude that phosphagen and ATP are not themselves osmotically active in the normal muscle. This would be the case if they were bound to other molecules and their constituents only became free when they broke down. These older experiments are worth recalling now because they are pertinent to the question of how phosphagen and ATP exist in the living muscle. Looking back at them today I see no reason to question their results. If those are correct, ATP and phosphagen exist in a combined form in muscle, exerting no osmotic pressure on their own account until they are broken down.

The work which an isolated muscle of frog or toad can perform under optimal conditions may be as high as 40% of the total energy given out in the initial process, as distinguished from recovery (HILL[9]). This high efficiency is obtained just the same at 0° C as at higher temperatures, and there are no grounds at all for supposing that the nature of contraction is in any way altered, except in speed, by a change of temperature. The muscle twitch is rather stronger at 0° C than at 25° C, and quite as efficient. If theory predicts otherwise, so much the worse for the theory. The highest efficiency is obtained with a comparatively large load and slow shortening; under isotonic conditions, with a load about half the maximum which the muscle can lift. In such a contraction the work done is about twice the heat of shortening: two thirds of the total energy set free, in excess of the heat of activation (or maintenance), is external mechanical work.

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Under conditions, therefore, of maximum efficiency, the energy is liberated in about the following proportions:

HEAT OF ACTIVATION OR MAINTENANCE	WORK	HEAT OF SHORTENING
40	40	20

At the other extreme, with zero load and rapid shortening, the situation may be this:

HEAT OF ACTIVATION	WORK	HEAT OF SHORTENING
40	Nil	49

(The heat of activation is the same in both cases.)

The fact that the external work may be so large a fraction of the whole energy liberated in excess of the activation (or maintenance) heat naturally makes one ask whether the heat of shortening may not itself really be work degraded into heat in overcoming some internal resistance to shortening: in that case energy would be liberated in two forms only, heat of activation (or maintenance) and mechanical work. For two reasons, the supposed internal resistance cannot be of a viscous nature: (1) the heat of shortening is independent of the velocity of shortening, and (2) the heat of shortening per cm is the same over the whole range of possible shortening (if it were due to overcoming viscous resistance it would be inversely proportional to the length). The supposed resistance must be constant, and must reside in lines or filaments parallel to the axis of the muscle, it cannot be a volume effect. An obvious objection to the theory of a constant (*e.g.*, frictional) resistance a parallel to and inherent in the contractile elements is that there should then be a constant difference $2a$ between the load at which a muscle just shortened and the load at which it just lengthened: experiment showed (KATZ[10]) that no such difference exists. The objection would be valid if a muscle were a single contractile element, with a parallel constant resistance. In fact, however, a muscle fibre is very long relative to its thickness, and its diameter is by no means constant throughout its length. There is no reason to suppose that its maximum force is the same everywhere. If not, in an isometric contraction the stronger regions would tend to shorten at the expense of the weaker regions, and the constant resistance would hinder shortening at one point and lengthening at another (possibly a very convenient arrangement in a system of non-uniform strength). With a large number of such elements in series an increase of load would stretch the weaker elements, a decrease of load would allow the stronger elements to shorten: and the difference of load between observable lengthening and shortening would be small. The objection, therefore, is not really valid.

A stronger objection, raised in 1938[11], is that there are indications that the heat of shortening changes sign when shortening becomes lengthening; and the heat generated in overcoming a frictional resistance does not change sign when the direction of motion is reversed. The difficulty is to get muscles to lengthen reversibly except at very low speeds. Possibly the use of dogfish jaw muscles (LEVIN AND WYMAN[12]) which stand stretching well would allow more positive conclusions to be reached. One thing is certain, namely that the work done in making a muscle lengthen does not reappear completely as heat: Some of it is absorbed, presumably, in driving chemical reactions in the endothermic direction. The subject is being investigated afresh by improved methods.

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One final word — to continue my challenge to biochemists. OTTO MEYERHOF's first letter to me, as I wrote at the beginning, came from Naples: all his life he has been ready to vary not only his chemical technique but his biological material. The properties of animals, and of their muscular systems, vary over a very wide range. There is no need to stick to rabbits and frogs. If a problem seems insoluble on one muscle, one should try to define it more precisely to see where the difficulty lies. Discussion with a zoologist, or a visit to a Marine Laboratory, may provide material many times better suited to one's needs. I spent many years trying to measure the heat production of nerve: if I had made the experiment on crabs' nerves instead of frogs' the answer would have come in 1912 instead of 1926. In 1912 it was not possible to define the problem well enough to get a clear direction to non-medullated nerve, but at least one might have taken a chance and not persisted with the frog's sciatic. If one's instruments, or methods, are too slow, one can make them relatively quicker by using slower material — tortoises, toads or even sloths. That means, of course, that biochemists, like biophysicists, must also be biologists (as MEYERHOF has always been and as HOPKINS was) — but why not?

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