THE DEVELOPMENT OF MUSCLE-CHEMISTRY, A LESSON IN NEUROPHYSIOLOGY

by

ALEXANDER VON MURALT

Hallerianum, Bern (Switzerland)

In the development of muscle-chemistry four different periods can be distinguished: the pre-lactic acid era, the lactic acid era, the period of phosphorylations and the myosin period. The name of OTTO MEYERHOF is intimately connected with three of them. In no field of physiology has knowledge advanced so far towards the fundamental and elementary processes of function as in muscle chemistry. This advancement is mainly due to OTTO MEYERHOF's brilliant conception of chemical and physical aspects and to the unparalleled cooperation of two masterminds in different fields, OTTO MEYERHOF AND A. V. HILL.

In the prelactic acid era, although it starts paradoxically with BERZELIUS, who discovered in 1841 that muscles of exhausted deer contained more lactic acid than muscles of animals with partially paralysed extremities[1], the rôle of lactic acid was quite unrecognized. There was even a very temperamental discussion as to what might be the fuel for muscular work. FICK AND WISLICENUS[2], who climbed the Faulhorn (1956 m), between the lake of Brienz and the valley of Grindelwald, collected their urine and showed conclusively in a famous paper in 1865 that the excreted nitrogen corresponded only to 37 g of protein, which by no means accounted for the work done. This statement caused the long-held belief of LIEBIG, that protein is the source of muscular activity, to be discarded and attention to be drawn to carbohydrates. Six years later WEISS[3] showed that the glycogen content of muscle decreases with the work done, and it seems that LUCHSINGER[4] in Zürich was the first to recognize the importance of nutrition for the maintenance of a sufficient glycogen content of the muscles, and to point out that glycogen is the intermediate energy carrier between ingested foodstuffs and activity. The next step was only reached in 1893 when PANORMOFF[5] showed that glycogen in muscle is hydrolysed to glycose. Among the many original observations which DU BOIS-REYMOND made, it seems that he was the first to recognize that a muscle becomes acid with activity and to relate this finding to BERZELIUS's observation of the formation of lactic acid[6]. It is quite amazing to see how, as early as 1859, a very clear conception existed and how it's development was delayed by the following accumulation of a great mass of very unimportant evidence up to the end of the century. This is even more surprising when we see that HEIDENHAIN[7] had found that the amount of lactic acid increased with the amount of work done. NASSE[8] who seems to have had great influence at this time however believed that lactic acid was only formed in rigour and death, and did not recognize the importance of HELMHOLTZ's[9] fundamental finding that the alcoholic extract of muscle decreased with activity, whereas the aqueous extract increased,

References p. 129.

thus giving the first well founded evidence for chemical events, and suggesting that glucose and lactic acid increase at the expense of glycogen. It seems almost unbelievable that M. v. FREY wrote even in 1909 about chemical changes in muscular activity ... "which acid is responsible cannot be stated to-day, since lactic acid seems not to account for it" (referring to the acidification of active muscle!)

The importance of phosphates seems to have been recognized for the first time by SALKOWSKI[10], who described the liberation of inorganic phosphate from an organic compound during activiry, a finding which was rejected by v. FÜRTH, another of those most unfortunate cases (which occur so often!) where the authority of one man has delayed development.

It was MACLEOD[11] who took up the point and found that inorganic phosphate increased and organic phosphate decreased, and MONARI[12] first seems to have observed that the creatine-content of muscle increases with activity (phosphagen not being determined in his experiments). These—in our present point of view—most important findings could not be corroborated at that time to give a clear conception and were almost burried by a great deal of other chemical evidence which we consider to-day as entirely uninteresting and which filled the periodicals of the time.

The lactic acid era started in 1907 with the classical paper of FLETCHER AND HOPKINS[13], in which they definitely established that fact that lactic acid is formed during activity and that it is absent (or practically absent) in resting muscles. This opened up a vast field and led to MEYERHOF's great work, which is summarized in a hypothesis, which was called the lactic acid theory of HILL AND MEYERHOF. The milestones of this development were the discoveries of the PASTEUR-MEYERHOF reaction, of the independence of initial heat of oxygen, the very accurate measurements of muscle heat by A. V. HILL and his colleagues, and their relation to chemical and calorimetric values obtained by MEYERHOF, the extensive study of lactic acid metabolism in muscle in all conditions of work, rigour and death, and finally the brilliant adaptation of this theory to muscular work in man by A. V. HILL[14] and his conception of oxygen-debt. It was a one-sided picture—as we all know to-day—and yet it is one of the golden pages of scientific discovery, because every new finding fitted into the theory and led to a very clear conception of what is taking place in a working muscle. It was very fortunate, that MEYERHOF published in 1930 his famous book on chemical events during muscle contraction, in which he gave an admirable account of the lactic-acid hypothesis[15].

The year 1930 brought, what A. V. HILL called the revolution in muscle physiology. LUNDSGAARD's[16] paper on mono-iodoacetic acid poisoned muscles and the absence of lactic acid formation in these muscles was—as it seemed at first—a heavy blow to the lactic acid hypothesis. It is very interesting to read to-day the conclusions BETHE[17] drew at that time and it is equally astonishing to see, how quickly MEYERHOF reacted and how he and LUNDSGAARD kept the lead. The conception of energetic coupling between different reactions was worked out and proved to be a new and extremely useful aspect in the classification and understanding of the chemical events including adenylpyrophosphate, creatinphosphate and fructosediphosphate breakdown. RITCHIE[18] introduced the idea that all chemical events might be recovery processes and therefore furnish the energy for the next contraction. This led to the conception that energetically coupled reactions furnish in steps the necessary free energy to restore the energyloss which occurs in an explosive way during contraction. This conception has been recently summarized by MEYERHOF[19] in an article which contains all the classical points of view

References p. 129.

of the era. This era might be called the period of phosphorylations and it is characterized by the discovery of the PARNAS-reaction, the LOHMANN-reaction and the complete series of steps in glycolysis in muscle, with the isolation of the corresponding enzymes.

In 1939 the myosin period started with the paper of ENGELHARDT AND LJUBIMOVA[20], which was followed by SZENT-GYÖRGYI AND BANGA's[21], NEEDHAM's[22], BAILEY's[23] and KLEINZELLER's[24] papers. Myosin, the "muscle machine" or what A. V. HILL has always called the fundamental process, became the center of attention. Myosin had been known, of course, for quite a long time. In 1930 my friend JOHN EDSALL and I published experiments, which showed that myosin must be the contractile element of muscle. The important point about ENGELHARDT AND LJUBIMOVA's paper is, however, that they found that the enzyme associated with the breakdown of ATP was associated with myosin. With this it became evident at once that there is a close relation between the "muscle machine" and the whole set of coupled chemical reactions. SZENT-GYÖRGYI and his coworkers[25] have added a great deal of very interesting new information about the nature of the muscle machine and thus we are just now in the midst of a "myosin era". MEYERHOF has attached his name to this period by the almost simultaneous isolation of ATP-ase from myosin, first described by PRICE AND CORI[26].

What is the lesson neurophysiology can learn from this development?

1. A rather long period of widespread chemical research has to precede the definite identification of those chemical reactions which are really essential. I am afraid that the smallness of nerve and the impossibility to accumulate break-down products connected with the absence of fatigue in peripheral nerve has prevented any extensive chemical work. Such work preceded the lactic acid era in muscle chemistry. The ground for neurophysiology therefore is not as well prepared as it was for muscle-physiology in 1907.

2. Once the importance of lactic acid was established, an intensive attack was made from all sides, yielding an astounding amount of information. Looking back it can well be said, that the prejudiced concentration on lactic acid was very much worthwhile! Is acetylcholine in neurophysiology a problem which will prove to be as fruitful as lactic acid was in muscle physiology? I doubt it and I realize that in this respect I disagree with my colleague NACHMANSOHN[27] who has published an admirable amount of work on the subject.

3. In muscle the energy expenditure is the main function. In nerve, nature gives us an opposite example of maximal economy in energy expenditure connected with function. The energy changes are so small that it took even A. V. HILL 15 years to measure them. This renders the task of corroboration between physical and chemical events in nervous excitation extremely difficult and tedious.

4. In muscle physiology it was possible to study the interesting reactions *in vitro*, to measure the various steps of glycolysis and to isolate the important enzyme-systems. Sodium fluoride and isoacetic acid have been powerful tools in this work. In nerve-physiology the material is complex and there is, as far as I can see, no definite clue to any chemical reaction of primary importance. GERARD[28] has contributed most valuable studies on nerve-chemistry by working along lines similar to those used by muscle physiologists, but I think he will agree with me in saying, that our knowledge of what is going on chemically in order to restore the energy expenditure of the ionic changes (potassium going "out", sodium going "in" and vice versa, cf. HODGKIN[29]) is very far from being satisfactory. I think it is well to emphasize that brain-brei is in no way a

References p. 129.

model for peripheral nerve chemistry and that the application of results obtained with brain-brei must be regarded with caution.

5. Physical phenomena, accompanying the chemical changes have been a great help in establishing the sequence of events in muscle. Volume change, change of p_H, variation of birefringence, of light scattering and change of electrical resistance have been studied with great success, and it is one of the outstanding characteristics of MEYERHOF's work that he always was able to make a fruitful correlation between these phenomena and the chemical aspect. In nerve, all these effects—if they exist at all—are probably extremely small. DAVID HILL (personal communication) has been able to detect changes of light scattering and volume changes in certain nerves. This may be the beginning of a new development. But on the whole,—except for action potentials— the nerve does not offer many good points for attack from the physical side.

The problem of the function of nerve remains, as A. V. HILL[30] has stated 17 years ago, intellectually quite a respectable one. For all those who are attracted by it the study of the development of muscle chemistry is a lesson of how to proceed. OTTO MEYERHOF's lifework with its unique combination of physical and chemical aspects furnishes the pattern which must be followed, if we want to understand what "excitation" really means.

REFERENCES

1 C. G. LEHMANN, *Lehrbuch d. physiol. Chem. I*, 103 , Leipzig 1850.
2 A. FICK AND J. WISLICENUS, *Vierteljahresschr. naturforsch. Ges. Zürich*, 10 (1865) 317.
3 S. WEISS, *Sitzber. Akad. Wiss., Wien*, 64 (1871) 1.
4 L. LUCHSINGER, *Vierteljahresschr. naturforsch. Ges. Zürich*, 20 (1875) 47.
5 C. PANORMOFF, *Z. physiol. Chem.*, 17 (1893) 596.
6 E. DU BOIS-REYMOND, *Monatsber. Berl. Akad.*, 288 (1859).
7 R. HEIDENHAIN, *Mechan. Leistung bei der Muskeltätigkeit*, Leipzig 1864.
8 O. NASSE, *Hdb. d. Physiol.*, 1 (1879) 288.
9 H. HELMHOLTZ, *Arch. Anat. u. Physiol.*, 72 (1845).
10 T. SALKOWSKI, *Z. klin. Med.*, 17 (1890) Suppl. 21.
11 I. I. R. MACLEOD, *Z. physiol. Chem.*, 28 (1899) 535.
12 A. MONARI, *Jahresber. Tierchem.*, 296 (1889).
13 W. M. FLETCHER AND F. G. HOPKINS, *J. Physiol. (London)* 35 (1907) 247.
14 A. V. HILL, *Muscular activity*, Baltimore 1926.
15 O. MEYERHOF, *Die chemischen Vorgänge im Muskel*, Berlin 1930.
16 E. LUNDSGAARD, *Biochem. Z.*, 217 (1930) 162.
17 A. BETHE, *Naturwissenschaften*, 18 (1930) 678.
18 A. D. RITCHIE, *Nature* (1932) 165.
19 O. MEYERHOF, *Ann. N. Y. Acad. Sci.*, 47 (1947) 815.
20 V. A. ENGELHARDT AND M. N. LJUBIMOVA, *Nature*, 144 (1939) 668.
21 A. SZENT-GYÖRGYI AND I. BANGA, *Science*, 93 (1941) 158.
22 D. M. NEEDHAM, *Biochem. J.*, 36 (1942) 113.
23 K. BAILEY, *Biochem. J.*, 36 (1942) 121.
24 A. KLEINZELLER, *Biochem. J.*, 36 (1942) 729.
25 A. SZENT-GYÖRGYI, *Studies Inst. Med. Chem. Univ. Szeged.*, Basel 1941–43.
26a W. H. PRICE AND C. F. CORI, *J. Biol. Chem.*, 162 (1946) 393.
26b B. D. POLIS AND O. MEYERHOF, *J. Biol. Chem.*, 163 (1946) 339.
27 D. NACHMANSOHN, *Ann. N. Y. Acad. Sci.*, 47 (1946) 395.
28 R. W. GERARD, *Physiol. Revs.*, 12 (1932) 469.
29 A. HODGKIN, *J. Physiol.*, 108 (1949) 37.
30 A. V. HILL, *Chemical wave transmission in nerve*, Cambridge 1932.

An account of some aspects of our present knowledge in neurophysiology has been given by the author in his book *Die Signalübermittlung im Nerven*, Basel 1946.

Received April 16th, 1949