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TITLE

NUTRITIONAL MUSCULAR DYSTROPHY

A BIOCHEMICAL AND METABOLIC STUDY

BY

Howard C. Spencer

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NUTRITIONAL MUSCULAR DYSTROPHY
A BIOCHEMICAL AND METABOLIC STUDY

by

Howard C. Spencer

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THE BIOCHEMISTRY OF MUSCLE

Investigators early realized that muscle is the tissue in which the process of energy transformation can best be studied since the external work can be measured and correlated with phases of chemical activity and heat production. Because of this, muscle tissue has been the subject of many metabolic, functional, chemical and physical investigations.

EARLY CONCEPTS OF THE BIOCHEMISTRY OF MUSCLE

In sketching the history of muscle biochemistry, the work of Fletcher[32] (1898), may be cited as a starting point. This work is important since it provided the first wedge for the overthrow of the inogen theory.

Ever since the discovery by Lavoisier (1774), that life is dependent upon the availability of oxygen, oxidation has been looked upon as a means of energy liberation and distribution.

The inogen theory held that muscle energy depended upon the sudden splitting of an unstable compound, inogen to yield oxygen for the combustion of the remainder of the molecule. Both lactic acid and carbon dioxide were produced by this breakdown, and as oxygen was provided within the molecule, energy was liberated as well under anaerobic as aerobic conditions.

Evidence accumulated from the work of Fletcher, Hopkins, Hill and others, enabled Fletcher in 1913[33] and Meyerhof in 1922[78] to definitely disprove the inogen theory.

The "modern period" of knowledge concerning the biochemistry of muscle can be said to have begun with the work of Fletcher and Hopkins,[34] in 1907, on the lactic acid changes in amphibian muscle. Following this came the myothermic investigations of Hill[43] and his co-workers and the chemical investigations of Meyerhof.[77]

During this first period in the modern development of muscle chemistry, from 1906 until 1926, the investigators developed the hypothesis that the primary change in muscular contraction is the anaerobic breakdown of glycogen to lactic acid followed by oxidative recovery.

According to this hypothesis, under anaerobic conditions the reaction which furnishes the initial energy for contraction is:

$$4n(C_6H_{10}O_5)n + 4\ H_2O = 3\ C_6H_{12}O_6 + 2\ C_3H_6O_3$$

Glycogen — Glucose — Lactic Acid

During the recovery phase about four-fifths of the lactic acid is resynthesized to carbohydrate and the equivalent of about one-fifth is burned. The equation for aerobic recovery may be written:

$$5(C_3H_6O_3) + 3\ O_2 \rightarrow 3\ CO_2 + 3\ H_2O + 2\ C_6H_{12}O_6 \rightarrow 2/n(C_6H_{10}O_5)n + 2\ H_2O$$

Lactic Acid — Glucose — Glycogen

From a historical point of view, it would be most interesting to sketch the work of this period in detail. However, the features of this "lactic acid theory" which find a place in the more modern concepts will be given consideration in the following discussions. It should be remembered that even if much of the older theory is discarded, any new hypothesis must be compatible with the experimental findings of these earlier workers.

THE REVOLUTION IN THE BIOCHEMISTRY OF MUSCLE

Since 1926, significant discoveries have been made which require a complete revision of the hypothesis of the chemical transformations accompanying muscular activity. Chief among these discoveries are the following:

(1) The finding in 1927 of a labile phosphoric acid ester, phosphagen, in muscle tissue by the Eggletons[13] and the demonstration by Fiske and Subbarow[29,30,31] that this compound was phosphocreatine.

(2) The discovery of adenylic acid in muscle by Embden and Zimmermann[26,27], 1927, and its ortho- and pyro-phosphoric acid compounds by Lohmann[63], 1920.

(3) The work of Lundsgaard[68] showing that muscles poisoned with iodoacetic acid could perform a certain amount of work without any lactic acid production, and that the phosphocreatine in these muscles was completely broken down during activity.

Along with these major findings much data has accumulated favoring the formulation of a new hypothesis. Among these are the following:

(1) Lactic acid formation does not begin until after contraction is well under way, and continues to be formed after relaxation (Embden[15,22,23,24]).

(2) During contraction ammonia is liberated. (Embden[15,22,23,24], Parnas and Mozolowski[96]).

(3) The pH of the intact muscle, upon stimulation of that muscle, shifts towards the alkaline side. (Meyerhof and Lohmann[79]).

(4) The non-appearance of lactic acid in muscles poisoned with iodoacetic acid is not due to a prompt reconversion to carbohydrate. (Smith and Visscher[107]).

(5) The chronaxie of different muscles is inversely proportional to the concentration of phosphagen. (Nachmannsohn[88]).

These discoveries, made during the brief period from 1926 to 1930, obviously invalidated the hypothesis that the formation of lactic acid was the primary reaction in muscular activity, and placed the emphasis on the breakdown of phosphocreatine as the energy-yielding reaction. In 1931 Lundsgaard proposed the hypothesis, accepted by Meyerhof (1931) and also by Hill (1932) that '' the primary chemical change in muscle contraction is the (hydrolytic) breakdown of phosphocreatine; the recovery process, which may be oxidative or anaerobic, is the resynthesis of this phosphocreatine; the energy for this anaerobic resynthesis being furnished by the formation of lactic acid from glycogen.''

This Lundsgaard-Hill-Meyerhof hypothesis is the basis of our modern conception of muscular activity. A more detailed discussion of the work upon which this theory is based and of the later work on the coupling of the many chemical reactions taking place during contraction and

subsequent relaxation will be given in following discussions.

THE PRESENT CONCEPT OF THE BIOCHEMISTRY OF MUSCLE

The Composition of Muscle.

Eggleton[12] describes the chemical analysis of muscle as an indirect and at best a crude method for the determination of the chemical reactions taking place in that muscle in vivo. The analogy to the chemical analysis of an automobile as a means of determining the reactions responsible for its energy production is quite apropos. However, analysis of muscles in the resting and exhausted states, together with the study of the change in concentration of some of the constituents, is at present the most direct method for the study of the chemical transformations which furnish the energy of muscular activity.

The approximate composition of a resting skeletal muscle of a frog[12]:

Water	80.0%
Protein	17.0%
Fat and lipids	0.2%
Glycogen	0.7%
Hexose-monophosphoric ester	0.05%
Creatinephosphoric acid	0.45%
Lactic acid	0.015%
Carnosine	0.25%
Creatine	0.08%
Adenosine triphosphoric acid	0.25%
Urea	0.01%
Orthophosphate (as H_3PO_4)	0.045%

Chloride - 0.05%

Bicarbonate - 0.03%

"About 5 per cent of the phosphorus of the muscle is not accounted for by the substances in this list, and about 10 per cent of the non-protein nitrogen. The sodium content of muscle is approximately equivalent to the chloride and the bicarbonate, and the potassium is about equivalent to the total phosphate, free and conjugated."

Similar results are obtained with the voluntary muscles of other vertebrates, although there are characteristic quantitative variations with each species. In general, the more active the muscle the greater the creatine content, both free and combined. In the case of invertebrates a "phosphagen", (phosphoarginine, rather than phosphocreatine, is found.

The following table was prepared from published data, giving values for the concentration of the phosphorous compounds as found in resting rabbit muscle. It will be noticed that certain of the values have been recalculated from the data given, so that the results from different sources are comparable.

Distribution of P in mg. % in Resting Rabbit Muscle.

P-Fractions	Kerr and Blish	Milroy	Nevin	Sacks Range	Sacks Average
(1) Inorganic-P	29.7	26.5	21.0	13-22	18
(2) Phosphagen-P	52.6	38.2	71.4	59-94	74
(3) Adenosinetriphosphate-P	66.3	45.6	76.3	37-73	55
(4) Undetermined-P	22.0	28.4	7.9	3-38	20
(a) Hexosemono-P	13.2	19.8	-	-	-
(b) "Undetermined"-P	8.8	8.6	-	-	-
(5) Total-P	170.6	138.7	176.6	138-196	167

Per cent of Total P

(1) Inorganic-P	17.4	19.1	11.9		10.8
(2) Phosphagen-P	30.8	27.5	40.4		44.3
(3) Adenosinetriphosphate-P	38.8	32.8	43.3		32.9
(4) Undetermined-P	13.0	20.6	4.4		12.0
(a) Hexosemono-P	7.7	14.2	-		-
(b) "Undetermined"-P	5.3	6.4	-		-
(5) Total-P	100.0	100.0	100.0		100.0

Kerr and Blish[53], Milroy[83], Nevin[91], Sacks and Sacks[103].

Considering experiments in which analyses were performed on both resting and fatigued muscles, the following data may be cited:

(1) Rabbit muscle (Biceps femoris) frozen in situ. (Milroy[83]).

P-Fraction in Mg. %	Resting	After 7 Minute Tetanus	Difference
Inorganic-P	26.5	79.8	+53.3
Phosphagen-P	38.2	5.7	-32.5
Adenosinetriphosphate-P	45.6	31.0	-14.6
Undetermined-P	28.4	22.2	-6.2
(a) Hexosemono-P	19.8	23.4	+3.6
(b) "Undetermined"-P	8.6	-1.2	-9.8
Total-P	138.7	138.7	0.0

(2) Rabbit muscle (gastrocnemius) frozen in situ (Sacks and Sacks[103], recalculated).

P-Fraction in Mg. %	Resting	Stimulated 5 Seconds	Difference
Inorganic-P	18	18	0
Phosphagen-P	72	56	-16
Adenosinetriphosphate-P	60	63	+ 3
Undetermined-P	14	28	+14
Lactic Acid	19	56	+37

P-Fraction in Mg. %	Resting	Stimulated 15 Seconds	Difference
Inorganic-P	15	23	+ 8
Phosphagen-P	77	48	-29
Adenosinetriphosphate-P	57	57	0
Undetermined-P	19	40	+21
Lactic Acid	13	86	+73

P-Fraction in Mg. %	Resting	Stimulated 300 Seconds	Difference
Inorganic-P	19	28	+ 9
Phosphagen-P	79	63	-16
Adenosinetriphosphate-P	60	52	- 8
Undetermined-P	21	36	+15
Lactic Acid	16	134	+118