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Biochemistry of muscle contraction and its aberrations in nutritional muscular dystrophy

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THE BIOCHEMISTRY OF MUSCLE CONTRACTION AND ITS ABERRATIONS IN NUTRITIONAL MUSCULAR DYSTROPHY

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Table of Contents

Introduction

One of the most rapidly advancing fields of biological research in the past decade and a half has been that of the biochemistry and biophysics of muscular contraction. The intense interest of physiologists in this subject has been stimulated in part by the fact that the energy-supplying processes which take place in muscle are apparently fundamental in the metabolism of almost all other tissues of the body (6). Another inviting aspect of the study lies in the mechanical properties of muscle tissue. Energy expended may be measured either mechanically, as work, or thermodynamically, as heat. A gain of knowleuge has taken place which would have been considered incredible twenty years ago. Skeletal muscle has been extensively and fruitfully analyzed in its chemistry, gross metabolism, thermodynamics, and molecular structure. The result has been a correlation of structural, chemical, and mechanical properties which far surpasses that for any other tissue. It has even been possible to construct, using purified tissue extractives, a synthetic muscle fiber which exhibits all of the mechano-chemical properties of God's original product (17,19,20). Nearly all of the enzymes hypothecated for the various reactions underlying muscle

contraction have been isolated in pure, many in crystalline, form.

The study of non-infectious myopathies has benefited greatly from the knowledge gained in research on the biochemistry of contraction. Clinically, the muscular dystrophies remain at present one of the most baffling groups of diseases, but chemical and metabolic analysis has done much to clarify the basic pathology and to suggest possible routes of therapy. Such analysis has, in turn, contributed considerably to our knowledge of normal muscle physiology, if only by way of verification through aberration. Of the myopathies, nutritional muscular dystrophy in animals has received the greatest amount of attention in the research laboratory. From a clinical standpoint, it is very similar to progressive muscular dystrophy in humans, and the two terms have come to be used almost interchangeably. Whether this is justified awaits confirmation.

The purpose of this paper will be to discuss the biochemistry of normal muscle so far as it is known and then to correlate it with recent studies of muscular dystrophies in an attempt to identify the mechanisms of nutritional dystrophy and to determine whether human progressive dystrophy has a nutritional etiology.

History

The present most widely held concept of the chemistry of muscle contraction dates from the classical work of Fletcher and Hopkins in 1907 (1). They disproved the theory, proposed by Hermann and Pflüger many years previously, that CO_2 and lactic acid which appeared in muscle contracting in an atmosphere of nitrogen were products of the breakdown of hypothetical "inogen". They showed that such contraction was truely anaerobic, for the CO_2 was produced by the action of the lactic acid on bicarbonate present in the muscle fluids. Further, their experiments demonstrated that oxidation does not occur in muscle in an atmosphere of oxygen until after contraction is over, and that lactic acid is the substance oxidized to produce CO_2. A few years later, Parnas and Meyerhof identified the source of lactic acid as glycogen. It was also shown by Meyerhof that only about one-fifth of the lactic acid disappearing in aerobic recovery of muscle is oxidized to CO_2 and that glycogen increases at this time.

Thus, the groundwork for the "carbohydrate cycle" in muscle was laid. Glycogen breaks down into lactic acid in the anaerobic phase of contraction, and one-fifth of the lactic acid is oxidized to CO_2 and water

in the aerobic phase of recovery, while the remaining four-fifths are resynthesized to glycogen. The nature of the intermediate steps in the breakdown and resynthesis of glycogen remained unknown until Embden, who had previously shown the importance of inorganic phosphate in muscle metabolism, proposed a hexose-phosphate as the precursor to lactic acid (2). From this point, the many complicated steps known today to occur in glycolysis were elucidated by a number of workers. Their researches, although dating from 1933, comprise too great a volume of material to be recorded here. References may be found in the excellent reviews by Needham (3), Parnas (4), Meyerhof (5), and Potter (6).

At the time of Embden's early work, it was thought that the immediate source of energy for muscle contraction lay in glycogen breakdown. Oxidation of lactic acid was thought to supply energy for the resynthesis of glycogen. In 1927 creatinephosphosphate was discovered simultaneously by Eggleton and Eggleton, who named it "phosphagen", and by Fiske and Subbarow (7,8). Two years later, Lundsgaard (9) revealed the importance of this compound as a source of energy by noting its decomposition into creatine and phosphoric acid in contracting muscle whose carbohydrate cycle had been poisoned by sodium iodoacetate. He pro-

ceeded to show that such a decomposition occurs in normally contracting muscle before the breakdown of glycogen takes place, and that if the latter process is prevented, the muscle ceases to contract only when creatinephosphate stores are depleted. Following this discovery, another revision of thought, analogous to that which took place with the discovery of the carbohydrate cycle, occurred. Credit for the immediate energy for contraction had been shifted from oxidation to glycolysis; now it was shifted from glycolysis to phosphocreatine breakdown, and glycolysis, in turn, was thought to supply energy for phosphocreatine resynthesis. However, the picture was as yet incomplete, for at this time (1929) adenosinetriphosphate was discovered in muscle extracts by Lohmann (10). He found that its concentration in normally contracting muscle is nearly constant but that in severe or prolonged contraction it breaks down to yield adenylic acid (adenosine monophosphate) and two molecules of phosphoric acid. This splitting is accompanied by the liberation of large amounts of energy. It was not until 1934 that the significance of this process in normally contracting muscle was seen by the same investigator. Using dialyzed muscle extract, he proved that phosphocreatine cannot break down on contraction until adenosinetriphosphate

has broken down. It soon became apparent that the immediate energy for contraction is supplied by the breakdown of adenosinetriphosphate, but that the latter is resynthesized simultaneously by phosphocreatine breakdown (11). Meyerhof and Lohmann later found that adenosinetriphosphate is both the source of phosphorus and the coenzyme for the phosphorolysis of glycogen, which initiates the carbohydrate cycle. The role of adenosinetriphosphate in muscle metabolism has since been found to be even more complex, but again, the literature is much too voluminous to be followed in detail in this paper. However, a simple scheme for the chemical reactions of muscle contraction can be presented at this point and will be expanded on later, as the more recently known intermediate steps are discussed.

As the stimulus for contraction reaches the muscle fiber, adenosinetriphosphate (ATP) breaks down, liberating energy for contraction and phosphoric acid for the phosphorolysis of glycogen. Immediately, phosphocreatine breaks down to supply energy and phosphate for the resynthesis of ATP. Glycolysis, having been initiated, proceeds with the production of lactic acid for oxidation and of energy and reliberated phosphate for the resynthesis of phosphocreatine from creatine. Up to this

point, contraction or the anaerobic phase has occurred. The aerobic phase of recovery begins with the oxidation of one-fifth of the lactic acid to produce energy for the resynthesis of the remaining four-fifths to glycogen.

Discussion

In reviewing the modern concept of muscle biochemistry, it will be expedient to present first some diagrams showing all of the known reactions and then to correlate these reactions with the simple scheme outlined above. Figure I shows the Meyerhof scheme for glycolysis, modified to include recent findings as suggested by Potter (6). Figure II shows the Krebs citric acid cycle, modified to what may be called the tricarboxylic acid cycle, as suggested by Krebs himself (12).

Considering the glycolysis scheme, the first step is the breakdown of ATP by adenosinetriphosphatase in the presence of calcium ions to yield adenosinediphosphate (ADP) and phosphoric acid. The latter is used for the phosphorolysis of glycogen, which is catalyzed by phosphorylase in the presence of adenylic acid. The result of this reaction is glucose 1-phosphate (d(+) 1-phospho-d-glucopyranose). In the presence of phosphoglucomutase and magnesium or manganese ions, the phosphate group undergoes an intramolecular shift to produce glucose 6-phosphate (d(+) 6-phosphoglucopyranose; this compound is then changed to fructose 6-phosphate by the enzyme phosphohexose isomerase. The fructose 6-phosphate (d(+)-

6-phosphofructofuranose) then calls upon ATP for more phosphoric acid, which is supplied through the mediation of an enzyme not yet isolated. The products of this phosphorylation are fructose 1,6-diphosphate (d(+) 1,6-diphosphofructofuranose) and adenosinediphosphate. Under the influence of zymohexase the former compound is split into an equilibrium mixture of 3-phosphoglyceraldehyde and phosphodihydroxyacetone. The equilibrium is established by the action of phosphotriose isomerase, and phosphodihydroxyacetone predominates until the reaction is shifted to the left by the removal of 3-phosphoglyceraldehyde in the following step. 3-phosphoglyceraldehyde is oxidized by dehydrogenation and takes on another molecule of phosphoric acid, from the ATP first broken down, to form an acyl-phosphate bond. This is catalyzed by phosphoglyceraldehyde dehydrogenase and coenzyme I, the latter being reduced by accepting one atom of hydrogen from the 3-phosphoglyceraldehyde and one from the phosphoric acid molecule. The products of this important reaction are 1,3-diphosphoglyceric acid and dihydrocozymase.

The next step is the first of two in which energy is given off by the carbohydrate cycle. 1,3-diphosphoglyceric acid is broken at the acyl bond by a suitable

enzyme to yield phosphoric acid to adenosinediphosphate. The products are ATP and 3-phosphoglyceric acid. Phosphoglyceromutase causes an intramolecular shift of the phosphate group of the latter compound to form 2-phosphoglyceric acid. This, under the influence of enolase is converted to (enol)-phosphopyruvic acid. Here, the second energy-releasing step occurs: (enol)-phosphopyruvic acid donates more phosphoric acid to ADP in the presence of a suitable enzyme and magnesium and potassium ions. The products, of course, are ATP and pyruvic acid. The latter is possibly in part (4/5) reduced to lactic acid by hydrogen from the dihydrocozymase formed above and in part (1/5) used directly in the Krebs cycle of oxidation. The Krebs cycle will be discussed later.

The assumption of a "suitable enzyme" occurs three times in the glycolysis scheme shown. Such an assumption is necessitated in step V (see Figure I) by the fact that phosphorylase will not catalyze the phosphorylation of a monosaccharide. In steps IX and XII ADP is required as coenzyme; the enzymes simply have not been isolated as yet. The presence of ADP in these two steps demonstrates the important point that the glycolysis scheme does not supply energy and phosphoric acid directly to creatine for the resynthesis of phosphocreatine, as sug-

gested in the previous section of this paper, but rather through the intermediary of ATP.

Thus, it is evident that ATP does not have the sole function of supplying energy for muscle contraction. The accumulation of information about its activities in muscle contraction and many other metabolic processes in other tissues has shown that it is an ubiquitous carrier or transport mechanism, as well as temporary storehouse, for energy and phosphoric acid. Now, in the case of muscle, at least, phosphocreatine may be thought of as a stationary or permanent reservoir of these staples.* But the plural should not be used, for Lipmann (13) has emphasized that the energy and the phosphate of ATP always go together, the energy lying in the "energy-rich phosphate bond". An energy-rich phosphate bond, designated as ~P by Lipmann, is one containing from 9,000 to 11,000 cal-

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*As has been mentioned, the phosphocreatine store may be depleted or resynthesized according to the needs of the tissue, but the concentration of ATP remains relatively constant. This relationship is expressed by the equilibrium, 2 phosphocreatine + adenylic acid $\rightleftharpoons$ 2 creatine + ATP, as suggested by Lohmann in 1934 (11), or,

ATP + creatine $\rightleftharpoons$ ADP + phosphocreatine.

ories of energy per mole of phosphate. Compounds containing such bonds are ATP, phosphocreatine, 1,3-diphosphoglyceric acid, and (enol)-phosphopyruvic acid. Phosphate ester bonds, as found in glucose 1-phosphate, 3-phosphoglyceraldehyde, phosphodihydroxyacetone, etc., contain only 2,000-4,000 calories of energy per mole. The phosphorylation of an alcoholic hydroxyl group to form a phosphate ester linkage requires energy, which is readily supplied by the energy-rich bond residing in ATP. The formation of energy-rich phosphate bonds, however, requires all of the energy present in another such bond (as in the case of synthesis of ATP from phosphocreatine) or energy from some outside mechanism. Two mechanisms are used in the glycolysis scheme: in the first, a high-energy acyl-phosphate bond is formed from mineral phosphoric acid by the energy inherent in oxidation (step VIII); in the second, ~P is formed from a pre-existing phosphate ester bond by the removal of a molecule of water by enolase (step XI). It will be seen that in the whole course of glycolysis four energy-rich phosphate bonds are formed, but since one is expended in the phosphorylation of glucose 6-phosphate, the resulting number is three, per mole of glucose 1-phosphate.

The Krebs cycle, illustrated in Figure II, has been widely accepted as the most satisfactory scheme for the oxidation of the end products of glycolysis to carbon dioxide and water. An early theory which led to the present one by Krebs supposed that lactic acid is first oxidized to pyruvic acid by the removal of two atoms of hydrogen and that two molecules of pyruvic acid then combine to form a hypothetical six-carbon compound. This compound was thought to go through a series of reactions whereby one-half of the molecule is oxidized to carbon dioxide and water and the other half is set free as pyruvic acid to combine with another molecule of the same in starting the cycle again. Krebs (14), in the formulation of his early cycle, suggested that the hypothetical 6-carbon compound is citric acid and that it is formed not from two molecules of pyruvic acid but from one molecule of oxalacetic acid (the end-product of the cycle) and one molecule of a triose derivative, probably pyruvic acid. Later experiments proved, however, that citric acid is not in the direct path of the cycle but is formed by a collateral equilibrium with cis-aconitic acid. Hence, the modern "tricarboxylic acid" cycle of Krebs.*

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*It should be noted that there is no necessity for assum-

Though the compounds in this cycle have not been isolated in phosphorylated form, it is probable that phosphorylation is an integral part of the process, for inorganic phosphate is necessary for its functioning. More specifically, Ochoa (15) has demonstrated that the oxidation of α-ketoglutaric acid fails in the absence of phosphate.

The energy of oxidation is used in the resynthesis of glycogen. As the glycolysis scheme shows, all of the reactions from glycogen to lactic acid are reversible; up to a short time ago, this reversibility had been verified experimentally in all but one----the splitting of (enol)-phosphopyruvic acid. It was thought that this terminal reaction could not reverse through a simple equilibrium reaction, and so one or two theories were proposed to explain its reversal in a detour of several steps. Very recently, however, Lardy and Ziegler (16) have proven by the use of radioactive phosphorus that the resynthesis of phosphopyruvate is a direct enzymatic reaction, taking place in the presence of ATP.

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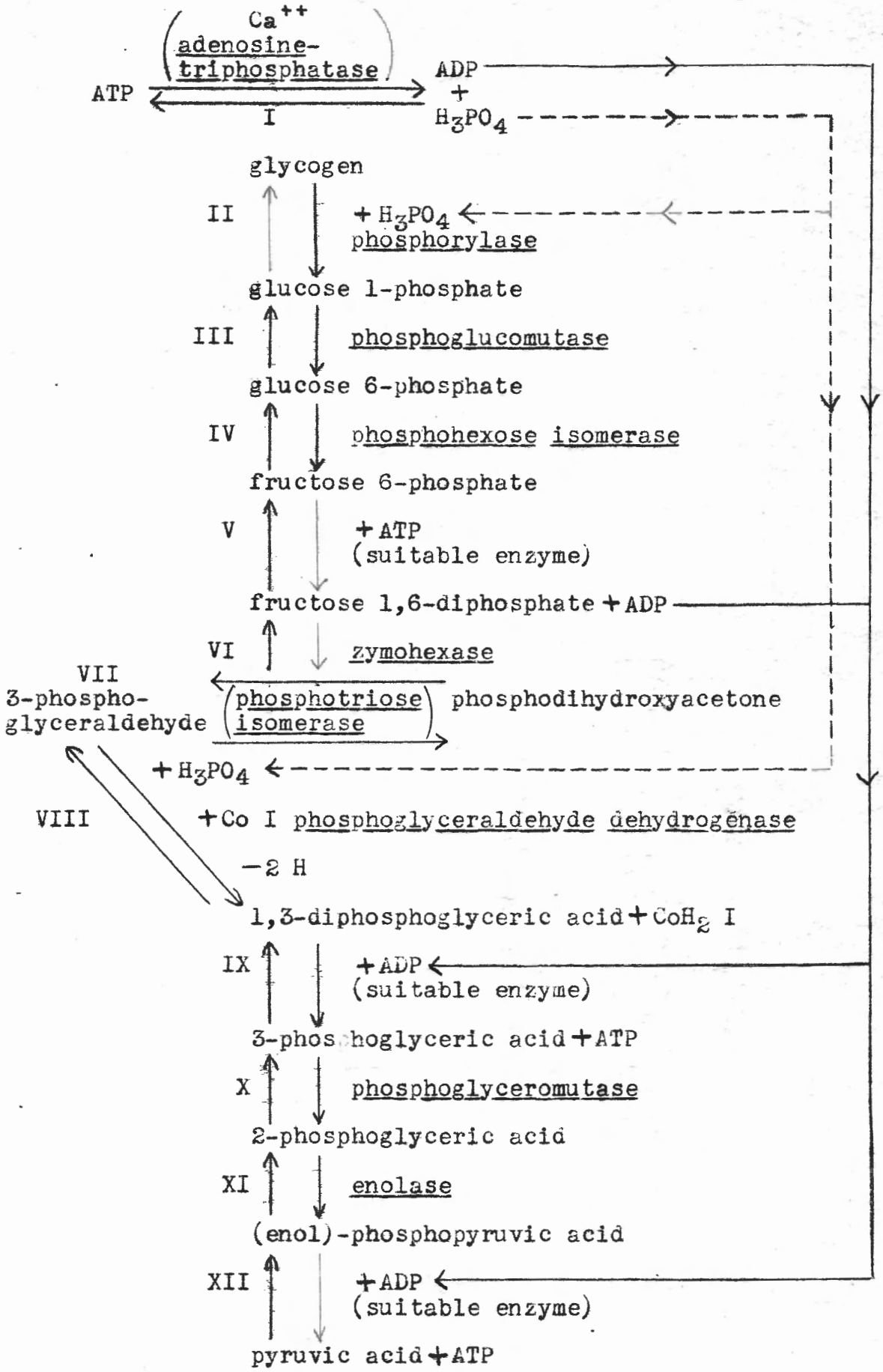
ing that the portion of pyruvic acid destined for oxidation ever gets to the lactic acid stage. It may be used directly as pyruvate.

The importance of adenosinetriphosphate breakdown, in supplying the immediate energy for contraction and in initiating glycolysis and respiration, has been emphasized several times. This breakdown is dependent upon the action of adenosinetriphosphatase. In 1941, Englehardt (17) published the results of some remarkable work concerning the nature of this enzyme. Through a series of experiments in purification and inactivation of ATP-ase preparations, he proved that the enzyme is either identical with or inseparable from myosin, a protein comprising 40 per cent of all muscle protein and known to harbor the contractile properties of the muscle fiber. Furthermore, he showed that artificial myosin threads, formed after the method of Weber (18), exhibit a lengthening or increase in extensibility when placed in a solution of adenosinetriphosphate. No other substances tried would replace the latter in producing this effect. Englehardt concluded that myosin is not only the enzyme catalyzing the reaction producing energy for contraction but is also the transformer which converts that energy to mechanical work. His results were confirmed by Needham (19) and Szent-Györgyi (20), and it was acknowledged that the unification of the mechanical and chemical events in muscle contraction had at last begun. Further observation has led to

the finding that myosin threads set up in the fashion of Englehardt will contract when calcium ions are added to the solution of adenosinetriphosphate (21,22). Calcium having thus been identified as the activator of ATP-ase, the earlier knowledge that calcium ions are liberated at the time of stimulation of muscle tissue becomes significant (23,24). This is in accordance with the disclosure of Heilbrunn and his students that stimulation releases calcium from an organic complex in a number of different tissues (including muscle) (25). That the production of acetylcholine at the motor end-plate, or the introduction of this compound into muscle and ganglion extracts results in an increase in calcium ion concentration has been known for some time (26). Consequently, another unification of knowledge has occurred, in that the chemistry of the nerve impulse and the chemistry of muscle contraction have joined into a continuous process through the intercession of calcium.

In an attempt to conciliate the mechanical and chemical changes in the myosin molecule during contraction, Binkley (27) has recently proposed a theory based upon certain known characteristics of the structure of the molecule. The myosin molecule has been accepted for

some time to be elongated or micellar in shape (18,28). Binkley visualizes the contracted state as one wherein there is a condensation between sulfhydryl and hydroxyamino acid side chains, resulting in a thio-ether linkage. The relaxed state is presumed to be brought about by the phosphorylation of such a linkage with the production of the original sulfhydryl group and the hydroxyamino acid group in phosphorylated form. Adenosinetriphosphate would be the phosphorylating agent which cleaves the thio-ether linkage. Binkley's diagram, reproduced in Figure III, shows that considerable shortening of the myosin molecule would result from such a reaction if the sulfhydryl and phosphorylated hydroxyamino acid groups were suitably situated in the polypeptide chain. The energy for contraction is liberated from these groups when the thio-ether pattern is formed.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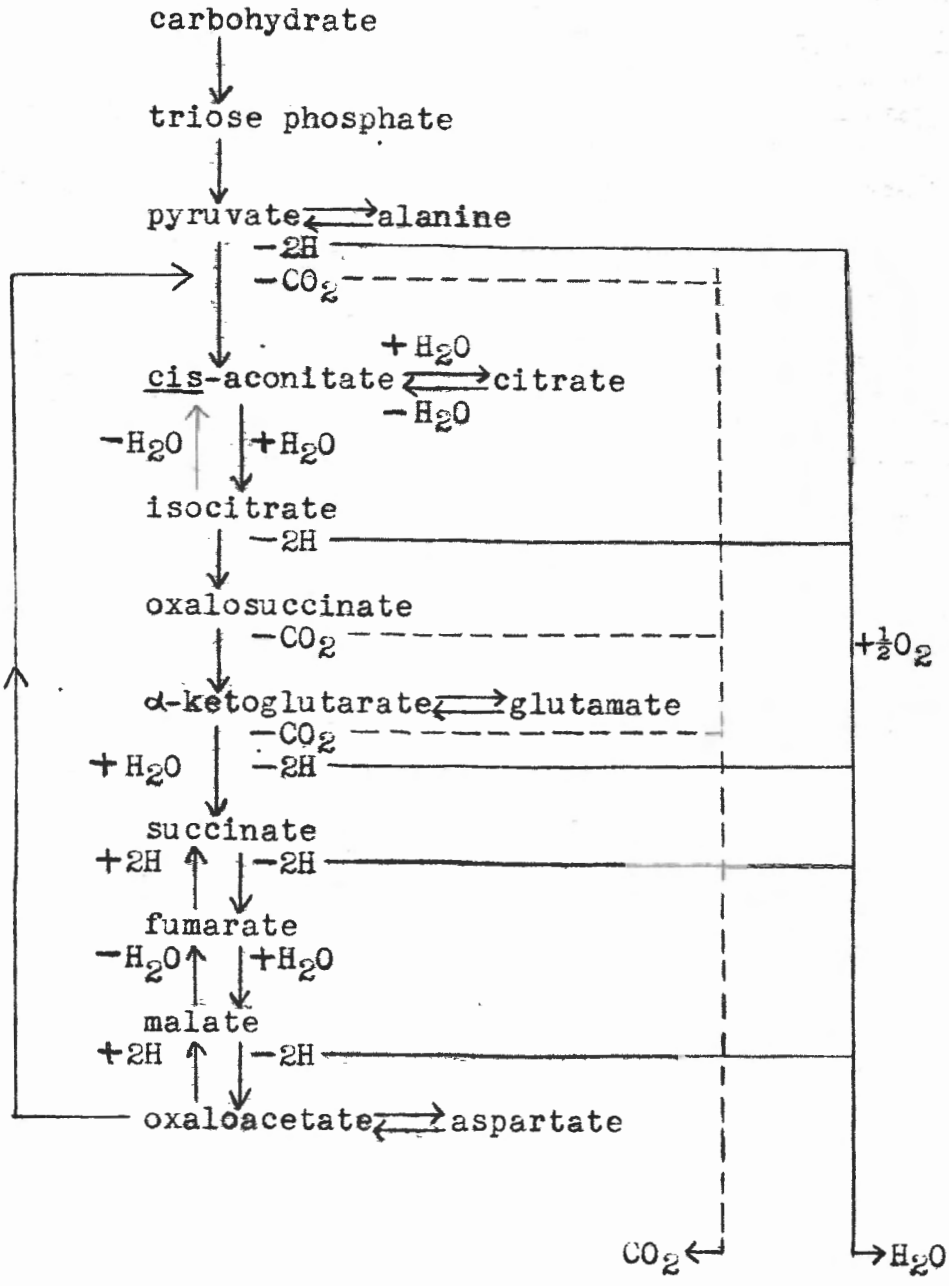
adenosine-triphosphatase
ATP
I
ADP + H3PO4
glycogen
II
+ H3PO4
phosphorylase
glucose 1-phosphate
III
phosphoglucomutase
glucose 6-phosphate
IV
phosphohexose isomerase
fructose 6-phosphate
V
+ ATP
(suitable enzyme)
fructose 1,6-diphosphate + ADP
VI
zymohexase
VII
3-phospho-glyceraldehyde
phosphotriose isomerase
phosphodihydroxyacetone
+ H3PO4
VIII
+ Co I phosphoglyceraldehyde dehydrogenase
− 2 H
1,3-diphosphoglyceric acid + CoH2 I
IX
+ ADP
(suitable enzyme)
3-phosphoglyceric acid + ATP
X
phosphoglyceromutase
2-phosphoglyceric acid
XI
enolase
(enol)-phosphopyruvic acid
XII
+ ADP
(suitable enzyme)
pyruvic acid + ATP

Figure I.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triose phosphate
pyruvate
alanine
−2H
−CO2
+H2O
cis-aconitate
citrate
−H2O
−H2O
+H2O
isocitrate
−2H
oxalosuccinate
−CO2
+½O2
α-ketoglutarate
glutamate
−CO2
+H2O
−2H
succinate
+2H
−2H
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oxaloacetate
aspartate
CO2
H2O

Figure II

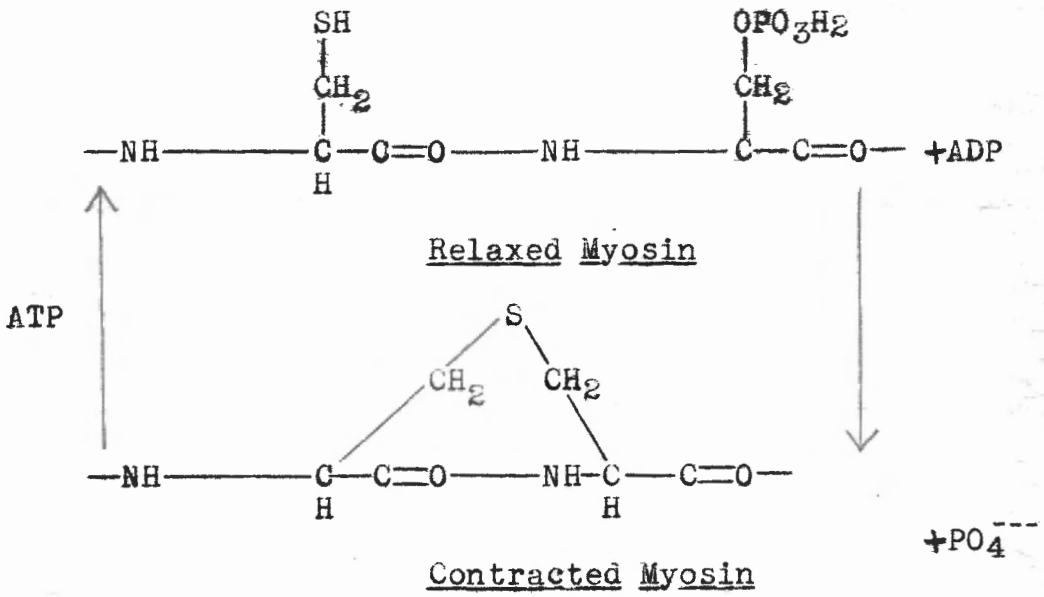
SH
OPO3H2
CH2
CH2
—NH—C—C=O—NH—C—C=O— +ADP
H
Relaxed Myosin
ATP
S
CH2
CH2
—NH—C—C=O—NH—C—C=O—
H
H
+PO4---
Contracted Myosin

Figure III

The Biochemistry of Common Muscular Dystrophies

In discussing the subject of nutritional muscular dystrophies, one naturally is expected first to define the term. As the remainder of this paper will reveal, a definition of the category requires consideration of recent facts which actually tend to disprove the existence of such disease entities in humans and to limit the category to one specific pathology in animals. The one myopathy in animals which is known to have a nutritional etiology is identical in all respects, except response to treatment, to progressive muscular dystrophy in humans. Therefore, this disease shall receive most of the attention given to the problem in the following pages.

The great body of work done on metabolism of non-enfectious muscle diseases in general shows that almost all have a common characteristic: an upset in creatine and creatinine metabolism. Hence, it will be meet to consider each of these diseases separately so that common etiological factors may be recognized if they exist. The diseases are listed, along with their characteristic changes in creatine-creatinine metabolism, in the chart of Figure IV.

Myasthenia Gravis

It is now commonly accepted that the cause of myasthenia gravis is a disturbance in acetylcholine metabolism, whereby this substance is prevented from acting at the myoneural junction by a curare-like substance (29). Creatinuria, low creatinine output, and low creatine tolerance, when they occur in this disease, are believed due secondarily to destruction of muscle tissue or marked alteration of metabolism in very severe cases. It has been emphasized that uncomplicated myasthenia gravis is not associated with any change in the fundamental chemistry of muscle contraction.

Myotonia Congenita

Myotonia congenita (Thomsen's disease) is apparently the converse of myasthenia gravis. It is supposedly caused by an increase of acetylcholine at the motor endplate. However, there are two definite changes in creatine-creatinine metabolism in this myopathy. High retention of administered creatine and complete absence of creatine excretion, even in children (in whom it is physiological), are characteristic. Quinine is the most effective therapeutic agent in reducing the hyperactivity of muscle in this condition.

Myotonia Atrophica

Endocrine dysfunction is of great importance in myotonia atrophica, which is typified by cataracts, alopecia, and testicular atrophy as well as muscular atrophy. Quinine is also of value in the treatment of this kind of myotonus, but no increase in muscular strength follows its use. Histological studies show that there are two distinct pathological processes in this disease: increased muscle tone without significant change in structure, and muscular atrophy with definite destruction of tissue. Creatinuria, decreased creatinine excretion, and decreased creatine tolerance are probably due to the latter of these processes.

Familial Periodic Paralysis

Recurring flaccid paralysis distinguishes this disease. The changes in creatine-creatinine metabolism are the same as in progressive muscular dystrophy, except that creatinine excretion, normally constant, is signally variable. Both abnormally high and low values are found. During attacks, the serum potassium level drops, and administration of potassium salts orally or intravenously is highly successful in treatment. Potassium absorbed from the intestinal tract is excreted in the urine at an excessive rate, and water diuresis may provoke an attack.

Calcium and magnesium balances are normal in this disorder.

Peroneal Muscular Atrophy

Little is known about this disease. It is accompanied by pathological changes in the spinal cord and nerve roots. These changes may be primary to the muscular atrophy, in which case the malady could be included in the next category. Creatine-creatinine metabolism is essentially normal.

Progressive Muscular Atrophy and Secondary Muscular Atrophy

This category encompasses almost all myopathies which are secondary to nerve disease. Amyotrophic lateral sclerosis, poliomyelitis, and peripheral neuritis may be cited as examples. Peroneal muscular atrophy, progressive muscular atrophy, and secondary muscular atrophies all present the same general type of creatine metabolism. When the total mass of muscle involved is small, the picture is substantially normal. When destruction of muscle is extensive, typical changes take place: creatinuria becomes prominent, creatine tolerance decreases, and creatinine output as well as creatinine coefficient diminishes.

Amyotonia Congenita

As the name implies, amyotonia congenita occurs in the first year of life and is identified by small, weak muscles. However, there is no true atrophy. Lack of the normal concentration of anterior horn cells in the spinal cord may be the cause; if it is, atrophy has occurred only in the potential sense. Creatinine output is pathologically low, and creatinuria is raised proportionally above the physiological level for infants. Tolerance to exogenous creatine is lacking.

Of the diseases mentioned, the following have been proven to be hereditofamilial: myotonia congenita, myotonia atrophica, progressive muscular dystrophy, familial periodic paralysis, peroneal muscular atrophy. The use of glycine (aminoacetic acid) in therapy of the myopathies will be taken up in the discussion of progressive muscular dystrophy below. For references to the literature and further information concerning diseases of muscle, the reader is referred to Biochemistry of Disease by Bodansky and Bodansky (30).

	Creatine Tolerance	Muscle Creatine	Urine Creatine	Urine Creatinine
Myasthenia Gravis	normal	normal	normal or sl. ↑	slight ↓
Myotonia Congenita	↑	normal	none	normal
Myotonia Atrophica (Dystrophica Myotonica)	↓	slight	↑	↓
Progressive Muscular Dystrophy	↓	↓	↑	↓
Familial Periodic Paralysis	↓	↓	↑	↓ or ↑ (fluctuant)
Peroneal Muscular Atrophy (Charcot-Marie-Tooth)	normal	normal	none or normal	normal
Progressive Muscular Atrophy (& Secondary Muscular Atrophy)	normal	normal	normal	normal
Amyotonia Congenita	↓	?	↑	↓

Figure IV

Nutritional Muscular Dystrophy & Progressive Muscular Dystrophy

Late in the 1920's and in the early 1930's a number of investigators reported successes in the treatment of progressive muscular dystrophy with orally administered glycine (aminoacetic acid). They contended that this substance would cause a decrease in urinary creatine and an increase in muscle creatine (31). Glycine is probably one of the important precursors of creatine, and as such its therapeutic effects in a disease entailing loss of creatine from the tissues were explained. Apparently these conclusions were drawn from too scanty observation, for subsequent work showed that clinical improvement was temporary, lasting from three to four months. In fact, in 1929 Brand and his associates proved that there is actually an increase in urine creatine and a decrease in muscle creatine when glycine is given (32). The amino acid has been tried in most of the muscular dystrophies, but the results have been largely discouraging except in the case of myasthenia gravis, where it seems to be of some value.

The importance of vitamin E in muscular dystrophy was first suspected in 1928 by Evans and Burr (33). They deprived laboratory animals of this substance and produced a peculiar type of paralysis. A decade later,

histological studies in vitamin E deficient rats showed that the central nervous system lesions are identical to those of amyotrophic lateral sclerosis and the skeletal muscle pathology resembles that of progressive muscular dystrophy (34). Following this discovery, Bicknell (35) attempted to treat human progressive muscular dystrophy with vitamin E. He fed his patients dried fresh whole wheat germ, 14 grams twice daily, and reported that twelve out of eighteen showed clinical improvement. Improvement in amyotrophic lateral sclerosis was claimed by Wechsler a short time later. He used wheat germ orally and α-tocopherol acetate both orally and intramuscularly (36). With these findings, faith in vitamin E as a specific therapeutic agent began to rise. However, soon afterwards accounts of negative results began to enter the literature. Pohl and Baethke (37) tried four regimes of oral vitamin E therapy on a series of fifteen cases: (1) wheat germ oil, (2) mixed natural tocopherols in oil, (3) natural tocopherols in oil plus thiamine hydrochloride and pyridoxine hydrochloride, and (4) dried fresh whole wheat germ. Tests on muscular strength showed that the disease progressed unretarded in all fifteen cases. The year 1940 was one of conflicting beliefs about the value of vitamin E and

of the B complex, which had just been introduced as another possible aid in treatment. The affirmative stand was taken by Bicknell, who had success in 17 of 18 patients treated with ½ oz. of wheat germ oil given in 2 cc. daily doses; Stone, who improved five patients with the same plus vitamin B complex and suggested that the B complex increases the effect of vitamin E; and Antopol and Schotland, who had immediate improvement in two cases and gradual improvement in four cases who were given vitamin E plus 100-500 mg. of vitamin B_6 per week subcutaneously. The negative stand was taken by Pohl and Baethke, mentioned above; Shelden, Butt, and Woltman, who saw no recovery with administration of wheat germ oil and α-tocopherol both intramuscularly and orally; and McBryde and Baker, who found no favorable confirmation in six patients treated with wheat germ oil, α-tocopherol, and vitamin B_6 orally and intravenously, and combinations of the vitamin B complex orally. Very careful muscular power tests were made in the latter instance (38). In 1942, Lubin (39) reported a relatively large series of pediatric cases with various muscular dystrophies. Seven patients with amyotrophic lateral sclerosis, nine patients with progressive muscular dystrophy, five patients with muscular atrophy, and fourteen patients with miscellaneous myopathies were given

massive doses of synthetic α-tocopherol. Thirty-one became worse during treatment, and four became better; but melioration of symptoms in the latter was proven, by withdrawal of treatment, to be due to unknown factors.

That natural sources of vitamin E and the insoluble synthetics (α-tocopherol and α-tocopherol acetate) are of no use in the treatment of human muscular dystrophies is now generally conceded. Nevertheless, in laboratory animals there has been considerable good fortune with the use of these substances. Nutritional (progressive) muscular dystrophy in rabbits may be cured or prevented by oral administration of d,l-α-tocopherol or its acetate. Parenteral or subcutaneous routes of administration are not as effective, according to Mattill (40), and do not work at all according to Eppstein and Morgulis, who employed 5-10 mg. intramuscular injections of the acetate in olive oil (41). Mackenzie and McCollum noted that oral administration of 20 mg. of α-tocopherol led to a decrease of urine creatine in dystrophic rabbits but that the same dose parenterally caused no change in creatine and decreased urine creatinine. They stated that injection of massive doses cures in some cases and moderately palliates in others (42).

In 1936 Madsen found that in vitro oxygen consumption of skeletal muscle from vitamin E deficient guinea pigs is considerably increased over that of normal muscle (43). Similar findings were recorded for rats and rabbits several years later by Friedman and Mattill (44). Using the Warburg technique on semitendinosus muscles from six months old rats which were tocopherol-deficient since weaning, they noted a 40 per cent increase in oxygen consumption. Thirteen months old rats who were in severe paralysis from this treatment showed a much smaller, but nevertheless definite increase. Semitendinosus muscles of rabbits subjected to the same protocol underwent a similar elevation of oxygen consumption rate. These findings have been confirmed and extended to hamsters by Houchin and Mattill, and to chicks by Kaunitz and Pappenheimer (45,46). The latter workers prevented increased oxygen consumption in unprotected young rats from vitamin E deficient mothers by giving 1 mg. of α-tocopherol acetate on the fifteenth day. Also, they perceived that muscle exhibiting such an increase early in the disease remains normal histologically, indicating that the respiratory anomaly represents aberrant metabolism in the muscle tissue itself rather than a result of invading connective tissue proliferation.

This conclusion tallies with the fact, mentioned above, that excessive oxidation is less in advanced stages of the disease, where paralysis and destruction of muscle have set in. Dystrophic muscle from hampsters shows the most marked increase in oxygen use----two-and-one-half times normal, on the average. This value approaches normal within 27 hours after oral administration of α-tocopherol and within 4 hours after intravenous injection of α-tocopherol phosphate, the water-soluble ester (47). The latter compound is also effective on dystrophic muscle respiring <u>in vitro</u> (48). Hence, it is not unfitting to assume, with Houchin, that vitamin E is the regulator of oxygen metabolism in normal muscle and that its lack leads to promiscuous abuse of oxidative mechanisms at the expense of other mechanisms in the physiologic scheme. According to Mason (49), oxidative mechanisms "get out of control" in the absence of tocopherols and "allow the muscle to be consumed by its own fire". Houchin, in interpretation of his own experiments, believes that α-tocopherol is utilized by the tissues only in its water-soluble phosphorylated form.

In searching for the mechanisms through which vitamin E exerts its oxidation regulating effect, further

data on the chemical composition of dystrophic muscle should be considered. Morgulis and Spencer, in 1936 (50), analyzed dystrophic muscle for a number of compounds and found significant changes in concentration of glycogen, acid-soluble phosphorus compounds, creatine, and cholesterol. Glyeogen in muscle from rabbits first showing signs of dystrophy was discovered to be 30 per cent of normal; in moderately advanced cases, it was 24 per cent of normal; and in very severe cases it was only about 16 per cent of normal. Total acid-soluble phosphorus was seen to decrease in proportion to the extent of degeneration of the muscle. In cases where the disease was far enough advanced to manifest such degeneration, inorganic-P, phosphagen-P, pyrophosphate-P, and undetermined-P were all diminished, but the relative proportions between them remained the same as in normal muscle. This fits the histological picture of partial fibrosis in nutritional muscular dystrophy; normal appearing muscle fibers are scattered throughout the mass of invading connective tissue, and acid-soluble phosphorus is intracellular phosphorus. However, in the early stage of the disease, there is an <u>increase</u> in inorganic phosphorus coincident with the decrease in organic phosphorus. Muscle creatine was found to be decreased in proportion to the degree of degeneration. Cholesterol was markedly increased.

Two years later, Morgulis and Osheroff determined the mineral composition of the muscles of dystrophic rabbits (51). Sodium concentration showed an average increase of two-and-six-tenths over the normal. Potassium concentration was decreased by one-third. Magnesium concentration was unchanged. Concentration of calcium (total) was increased by 500 per cent, on the average. In some cases, the increase was as great as 1000 per cent. In treated and recovered muscle, potassium ion concentration remained slightly depressed, sodium ion was increased by about 50 per cent, and calcium ion concentration was perfectly normal. In regard to anions, acid-soluble phosphate was decreased to one-half of normal in severely dystrophic muscle, even though the total phosphorus was unchanged. Chloride ion concentration was elevated to one and eight-tenths of the normal but was approximately normal in recovered muscle. In summarizing their results, these workers assumed that the loss of potassium is due to cell destruction and that the increase in sodium is a compensatory effect, keeping the total cation concentration normal. Also, it is known that both sodium ion and chloride ion increase is associated with connective tissue proliferation. Diseased rabbits usually returned to normal in six or seven weeks when their diet

was supplemented with wheat germ.

From the data cited in this section and the previous section, it is obvious that a brief of the chemical and metabolic anomalies of dystrophic muscle must take into account the distinction between early and late stages of the disease. In other words, some of the biochemical changes are due primarily to the cause of the disease, and others are due secondarily to the tissue destruction (replacement fibrosis) which results from the disease. But only certain of the data recognize this distinction, and for the most part, a separation of the changes which take place before and after tissue destruction has set in must be conjectural. The following changes are probably characteristic of <u>early</u> nutritional (progressive) muscular dystrophy: decrease in glycogen, phosphocreatine, adenosinetriphosphate, creatine, creatine tolerance, and urinary creatinine; and increase in oxygen consumption, inorganic phosphate, diffusable calcium, and urine creatine. These are explained by the "runaway" processes in the muscle. Glycogen is not resynthesized as rapidly as it is hydrolyzed and burned. Adenosinetriphosphate breaks down at an excessive rate and furnishes con-

centrations of adenosinediphosphate and inorganic phosphate far above the equilibrium values for resting muscle; these compounds in turn foster the immoderate rate of glycolysis and respiration. Phosphocreatine breaks down too rapidly in an attempt to resynthesize ATP and thereby contributes to the increase in inorganic phosphorus. The creatine produced, failing to be reconstituted as phosphocreatine, is excreted in the urine. Failure of phosphocreatine resynthesis also accounts for diminished creatine tolerance. Lowered muscle creatine is explained by the fact that 60 per cent of the total creatine in normal muscle is present as phosphocreatine (50). Decrease in excretion of creatinine, a product of normal creatine metabolism, results from abolishment of the latter process.

Most of the changes of <u>late</u> nutritional muscular dystrophy (especially with the onset of paralysis) are due to the loss of functioning muscle fibers. Morgulis' statement that inorganic-P, phosphagen-P, pyrophosphate-P, and undetermined-P diminish proportionally from the norm would tend to indicate that these changes are due <u>solely</u> to cellular degeneration. However, it is probable that because of the high ratio of fibrosed

fibers to intact but abnormally functioning fibers, the changes inherent in the former far overshadow those of the latter, which are characteristic of early dystrophy. The metabolic disturbances of late dystrophy, except for inorganic-P decrease, are essentially the same as those of early dystrophy listed above, but they occur in different proportions.

We may now more directly approach the problem of how vitamin E controls the oxidative processes in muscle. The glycolysis scheme, being a chain of equilibrium reactions, shows that the rate of glycolysis, and hence of oxidation, depends upon the rate of initial breakdown of adenosinetriphosphate. Consequently, the activity of adenosinetriphosphatase is of utmost importance in determining the oxidative rate. The activation of ATP-ase by calcium ions brings to mind Morgulis' important finding of a 500 per cent increase in calcium concentration of muscle deprived of vitamin E. That this superabundance of calcium results in pathologically augmented ATP-ase activity is evident from the chemical findings in such muscle. Thus, we may justifiably postulate that vitamin E exerts its influence on oxidation through control of calcium

metabolism. This leaves only one question to be answered: what is the mechanism by which vitamin E determines the availability of ionic calcium? In vitro, the mechanism may be a very simple one. Since water-soluble α-tocopherol phosphate effects a "cure" of the dystrophic process in vitro while insoluble α-tocopherol and α-tocopherol acetate do not (48), it is possible that the former compound reduces the concentration of calcium by precipitating it as highly insoluble calcium α-tocopherol phosphate. In fact, this salt may be present in the cortex of the normal muscle cell as the metallo-organic complex which breaks down under stimulus (acetylcholine) to yield calcium ions (25). Destruction of acetylcholine by choline esterase would result in re-precipitation of calcium ions by α-tocopherol phosphate. If the latter were absent, the concentration of calcium ions in the interior of the cell would steadily increase.

In laboratory animals the insoluble tocopherols are of little or no value in treatment of nutritional dystrophy when administered parenterally. They are, nevertheless, quite effective when given orally. In humans they are valueless both orally and parenterally. The water-soluble phosphate ester, however, is extreme-

ly potent when injected intravenously in animals---- even more effectual than oral α-tocopherol itself, as discovered by Houchin and Mattill (47) and confirmed by Karrer and Bussmann (52). Yet, the latter authors found that the phosphate ester is not hydrolyzed *in vitro* by phosphatase from kidney, serum, or yeast. An explanation for this has been suggested by Jacobi (53). He points out that the ester linkage may very well embody an energy-rich phosphate bond. An enol-like linkage (-C=C-OH) is present in the vitamin E molecule. Structurally, α-tocopherol can be considered a substituted phenol, and phenols and enols in general exhibit very similar chemical properties. Thus, if enol-phosphate bonds contain high energy levels, the same must be true for phenol-phosphate bonds. Such a high-energy bond in vitamin E would not be susceptible to hydrolysis by phosphatase, but would require a more potent dephosphorylating system----the adenylic acid (AA) system. Jacobi proposes the following equilibrium reaction:

α-tocopherol phosphate + ADP (or AA) ⇌ α-tocopherol + ATP (or ADP).

Under the assumption of this reaction, α-tocopherol should be phosphorylated by adenosinetriphosphate in

the organism.

In reference to this last hypothesis by Jacobi, the failure of parenteral α-tocopherol therapy in animals may be explained. According to Potter (6), adenosinetriphosphate is an important participant in the phosphorylative mechanisms of the small intestine. If α-tocopherol is utilized in the tissues only as the phosphate ester, it may require phosphorylation in the gut before its therapeutic properties become manifest. Inability of muscle ATP to phosphorylate the parenterally administered vitamin could be due to lack in this tissue of a suitable biocatalyst, which is present in the intestine. Inefficacy of the insoluble tocopherols in humans may then be attributed to an anomalous complete lack of the biocatalyst.

Treatment of human progressive muscular dystrophy awaits an important investigation: the oral and parenteral use of α-tocopherol phosphate. If the disease responds to this substance, then it can be truly classified as a nutritional muscular dystrophy, and its identity with nutritional dystrophy in animals will be established. In the event of failure of vitamin therapy, certain other compounds might be of value. Quin-

ine, for example, inhibits phosphorylase and phosphoglucomutase in rabbit muscle (54). In thereby cutting down on the rate of glycolysis and oxidation, it would be of merit in retarding the abnormal metabolism of dystrophic muscle, providing weakness did not result from its curare-like action. These properties of quinine may be the basis of its success in the treatment of myotonia congenita and myotonia atrophica. Another candidate for trial in progressive dystrophy is atabrine, which is known to inhibit hexokinase from yeast and parasite preparations, and lactic dehydrogenase from parasite and beef heart preparations (54). Any compound which could selectively precipitate or bind intracellular calcium would, of course, be the ideal medicine for progressive muscular dystrophy.

Summary

1. A resume of established knowledge and recent findings in the biochemistry of normal muscle contraction is given, and an attempt is made to mold this material into a well integrated physiologic theory.

2. The human muscular dystrophies that are at present accepted as definite clinical entities are reviewed from a biochemical standpoint with an eye to determining which, if any, have a nutritional etiology.

3. Progressive muscular dystrophy in humans is discussed in its relation to nutritional muscular dystrophy in animals. It is concluded that glycine and vitamin E in water-insoluble forms are of no value in the treatment of human progressive dystrophy and that since this disease has not responded to such treatment, it can not yet be conclusively classified as "nutritional". It is further pointed out that although insoluble tocopherols can cure animal nutritional dystrophy when administered orally, they are of little or no use parenterally and *in vitro*; and because the soluble phosphate ester of vitamin E cures the disease parenterally and *in vitro*, even more effectively than insoluble oral tocopherols, it is

suggested that this compound be tried in human progressive muscular dystrophy. Hypotheses for the possible mechanism of therapeutic action of vitamin E are presented.

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