5-1-1938

Myopathies

William Osheroff
University of Nebraska Medical Center

THE MYOPATHIES.

William Osheroff

Senior Thesis Presented to the

College of Medicine,

University of Nebraska,

Omaha.

1938

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INTRODUCTION

Introduction.

Since the era of Greek medicine, muscle diseases have been noted and mentioned innumerable times, and were characterized generally as varieties of metaphysical ailments; little of significance was known until about 1850 when the French school first attempted to accurately describe and classify them. From that time on, interest in these conditions has constantly increased. It was necessary for a proper understanding of the various muscle disorders that metabolic processes of muscle be known; and this information has been gradually accumulating in the past few decades to the point where accurate conceptions of the pathological physiology involved are being predicated. Neurologists and orthopedists have quarreled for many years as to which field properly contained the muscle diseases. It is in the last few years under the guidance of the experimental physiologists and clinicians that understanding of these conditions has reached the stage where therapeusis is possible.

Since the muscle diseases were first described there has been a great amount of worthless literature published concerning them. For quite a number of years it was the fashion for everyone who

had a single case to report on it and to either borrow the ideas if not the words of preceding investigators in his discussion or make thoroughly groundless assumptions on the basis of much too small an amount of material. If he noted one insignificant new finding he described it in great detail. Thus, there has been accumulated a wealth of material which is valueless to any basis of understanding of the muscle diseases. This paper, therefore, will not be an attempt at complete coverage but rather, in as much as is possible at the present time, a significant coverage.

The production or transfer of a disease to laboratory animals is a step which has preceded proper understanding of many disease conditions. Since 1931 it has been possible to produce, by dietary means, a condition in guinea pigs and rabbits which both pathologically and chemically closely resembles progressive muscular dystrophy in man. With this knowledge, the outlook for the myopathies becomes more encouraging.

Dr. Sergius Morgulis and his associates in the Biological Chemistry laboratory have been studying nutritional muscular dystrophy quite intensively for several years. We are grateful to have had the opportunity to work under him in the investigation of one

phase of this problem, the mineral metabolism.

In view of the close similarity of nutritional muscular dystrophy and human dystrophy, we are including in this paper a fairly comprehensive discussion of the chemical and pathological findings in this condition, and also material (in a much abbreviated form) from a discussion of the mineral metabolism from a thesis leading toward the degree of Master of Arts in Biological Chemistry.

THE MUSCLE ATROPHIES

The Muscle Atrophies

Muscular atrophy may occur in any condition effecting the ventral horn cell, its axone, or the muscle itself. It may be a primary condition or follow secondarily to disuse, trauma, various neuritides, poliomyelitis, syphilis, plumbism, etc. The secondary atrophies properly belong to the study of the various disease entities which produce them or to orthopedic surgery which offers the chief hope of amelioration at the present time.

The first clear division of the primary muscle atrophies is on the basis of the causal pathology; that is, whether the muscle affection follows neural pathology (the myelopathic type), or is in the muscle itself (the myopathic type). Of these, the myelopathic type is much less common and much less understood than the myopathic.

In this paper "muscular atrophy" will be used to indicate any of the wasting diseases of muscle; in a more restricted sense it has been used to describe the myelopathies. "Muscular dystrophy" will be used in referring to muscular wasting accompanied by degeneration.

The general subject of muscular atrophies is too wide in scope for a paper of this nature, and since much more is known of the myopathies it is with

this group that we propose to deal. The myopathies are classified as follows (3):

The Myopathies

Sporadic

(1) Amyotonia congenita (Oppenheim, 1900).

Familial

(1) Myasthenia gravis (Wilks, 1877 and Erb, 1878).

(2) Family periodic paralysis (Cavare, 1853).

(3) Progressive muscular dystrophy (Erb, 1882 and Landouzy, 1884)

a. True hypertrophic type (Spiller, 1913).

b. Pseudo-hypertrophic type (Duchenne, 1860).

c. Fascio-scapulo-humeral type (Landouzy, Dejerine, 1884)

d. Distal type (Gowers, 1902).

(4) Myotonia (Thomsen, 1876).

(5) Dystrophia myotonica (Deleage, 1890).

A number of characteristic features are found in the myopathies, which include:

(1) Widespread distribution, which is rather commonly in patterns.

(2) Onset is generally before puberty (except Myasthenia gravis and dystrophia myotonica)

(3) More common in males (except myasthenia gravis and family periodic paralysis).

(4) Tend to be familial (except amyotonia congenita)

(5) Tendon reflexes are diminished or abolished.

(6) Quantitative electrical changes, but no reaction of degeneration.

(7) Frequent accompaniment of endocrinopathies.

(8) Lack of disturbance of the nervous system.

a. No fibrillations.

b. No sensory changes.

c. No qualitative electrical alterations.

(9) Decreased preformed creatinine excretion and lowered creatinine coefficient, or

(10) Increased creatine coefficient, giving practically normal total creatinine coefficient.

In the following pages are descriptions of each of the myopathies.

THE MYOPATHIES

Progressive Muscular Dystrophy

Progressive muscular dystrophy is a chronic wasting disease of the skeletal musculature, accompanied by muscular degeneration, pseudo-hypertrophy, and sometimes true hypertrophy. It was first identified as a clinical entity by Erb in 1883 and Landouzy in 1884, although a fairly adequate description of two cases in brothers was made by Coste and Gioja as early as 1838 (61). The incidence according to Hough (29) is six per hundred thousand; which makes it the second most common of the myopathies. As mentioned before, the principal types are the true hypertrophic type of Spiller (65), the pseudo-hypertrophic type of Duchenne, the fascio-scapulo-humeral type of Landouzy and Dejerine, and the distal type of Gowers. A number of other name syndromes have been described which are variations of those mentioned, and are uncommon.

This disease practically never appears sporadically, and the hereditary factors are being established on the basis of the large amount of literature accumulated. In general, there seem to be three main types of inheritance, which are: Mendelian dominance, parental cosanguinity, sex bound dominance (30).

Duchenne in 1862 and 1869 presented two family histories in which the fascio-scapulo-humeral

type was evidently a Mendelian dominant. Barker (4) agreed, but held that the more frequent pseudo-hypertrophic type is usually recessive. In regard to the parental cosanguinity factor, the most interesting family history is that mentioned by Minkowsky and Sidler (5). They found a large number of cases in an isolated Swiss village of a population of 1700, in which all the afflicted individuals were from two very much intermarried families. In each case the development of dystrophy occurred only in an individual whose father and mother both were descendents of both families.

The third type mentioned is the common situation of an unaffected daughter transmitting the disease to her sons. Dittrich found six cases of this in three generations and Voshell ten cases among seventy-five individuals in four generations. Kostakow found the characteristic a purely sex bound recessive in his series of fifteen cases among forty-nine individuals in four generations. Riese, Davidenkow, and Barnes, however, have described the factor as a sex bound dominant.

There is enough work being done on the subject of inheritance that there is little doubt, Barker has stated, that eventually close studies of the hereditory factors will lead to a classification based on

Mendelian inheritance.

Endocrinopathies have been commonly associated with progressive muscular dystrophy, and the significance of the findings have been much debated. Following is a list of endocrine (or possibly endocrine) changes with the names of the investigators who found them, as taken from Günther(27):

Myxedema. Schlesinger, Marco.

Thyroid struma. Prager, Werdt, Michelsen.

Basedow disease. Moebius, Sattler, du Cazal, Miesowicz, Chvostek, Cardarelli, Ditischeim.

Dystrophia adiposogenitalis. Spiller, Fränkel, Schaefer, Funsten, Landé, Lewin, Minkowsky, Gareiso, Duken.

Hypogenitalism. Marco, de Nicolo.

Infantilism. Friedreich, Falta.

Cryptorchism. Collins.

Acromegaly. Eulenburg, Bregman, Friedman, Janney.

Pineal shadows. Timme.

Parathyroid dysfunction. Pevitz.

Hypopituitarism. Bramwell and Wright.

Hypersuprarenalism. Marinesco.

Macroglossia. Janney, With, Bing.

Hypoglycemia. Mc Crudden, Janney.

It is obvious, as Günther states, that merely a glance at the list demonstrates how difficult it is

to attempt to ascribe to any one endocrine change an etiological significance. Certainly progressive muscular dystrophy cannot be produced by both hypo and hyper thyroidism. Günther lists many errors in the diagnosis of endocrine disease in the presence of dystrophy, for example: atrophy of the orbicularis palpebrae may produce typical "pop eyes", atrophy of the soft tissues of the fingers causes the joints to appear abnormally large.

Gibson, Martin and Buell (22) describe the following as indications of endocrine disorder in muscular dystrophy: the development of the disease in polyglandular disturbances, improvement sometimes following endocrine therapy, and occasional spontaneous recovery at puberty when alterations in glandular balances take place.

Although there is evidently some association between progressive muscular dystrophy and glandular dysfunction, the number of types of endocrinopathies described indicates that the relationship is not of any etiological significance.

Bony changes have been very much discussed by the European investigators, although we fail to find as widespread mention by Americans. Among the changes described are included: deformities of the posture, lordosis, skoliosis; deformities of the

skull, hydrocephalus, plagiocephalus, microcephalus, and others; prognathus; and bony atrophy. This last was described by Friedreich, Lloyd, Schultze, Spiller, Marie-Crouzon, Noiça, Schlippe, Dreyer, Landouzy, Merle, Timme, Janney and Zabriskie. Bony changes are so constant that Günther states, "Muscular dystrophy and bony dystrophy are concommitant characteristics of the same disease." The bony dystrophy, the degree of which does not necessarily correspond to the degree of muscular dystrophy, affects the shafts of long bones, sparing the epiphyses, and progresses swiftly to produce great deformities.

The disease is, generally speaking, a combination of atrophy of one muscle group with pseudohypertrophy of other muscles, which may follow a period of true hypertrophy (3). Both sides are involved and usually, but not always, symmetrically. The proximal muscles are most affected, with the distal portions including especially the hands and fingers spared until late, except in the distal type. Atrophy takes place in the sternal portion of the pectoralis major (sparing the clavicular portion), trapezius, serratus magnus, latissimus dorsi, biceps, brachialis, quadriceps femoris, and the adductors of the thighs. Pseudohypertrophy takes place in the infraspinatus, deltoid,

triceps, sartorius, glutei, and especially the calf muscles. Changes in the heart muscle have been described and also paroxysmal tachycardia. Iraus, Boas, and Lowenburg believe that the tachycardia is due to diminished venous return from the lost pumping action of the skeletal muscles.

Loss of sphincter control is rare. Occasionally involvement of the diaphragm and intercostal muscles takes place, often resulting in lung infections.

When the pelvic, thigh, and spinal muscles are involved there is a waddling gait and lordosis, with protrusion of the abdomen and backward slant of the trunk. Due to the shoulder girdle involvement the scapulae are prominent. Gowers' sign is usually present; this consists of a peculiar difficulty in rising. The hands and knees are employed--the legs extend--the hamstrings come into play; one shin is grasped by one hand--the weight is thrown backward over the hips--the opposite leg is caught below the knee; then the standing posture is attained by advancing the hands up the legs. This is described as "climbing up his own legs."

In a fair proportion of cases the orbicularis oris is involved, and stuttering is common.

The age of onset is generally quite young, as opposed to the myelopathic homologues. Hough stated that 12.5 per cent began before two years, 50 per cent

from two to four, and 30 per cent from five to seven. The earlier the onset, the more severe is the condition; Gowers found that his worst cases began before six. It is found in all races. The sex distribution is rather striking: in three series of cases described by Gowers, Mettel and Slocum, and Hurwitz (31), the incidence in males was 86.4, 97.8 and 75 per cent respectively.

The prognosis is rather discouraging at best, even with treatment. Hough (30) found that 35 per cent were unable to walk at five, 50 per cent were disabled at eight. After puberty the condition advances more slowly, if the case survives the upper respiratory infections common to that age.

The pathological findings of all the types of progressive muscular dystrophy are characteristic. Grossly the fibers are pale or yellow from increased fat content. Erb gave an adequate description of the microscopic pathology in 1883, which follows: ".....true hypertrophy, subsequent atrophy, splitting of muscle fibers with proliferation of nuclei, connective tissue proliferation, vascular hyperplasia and fat between the muscle bundles." In the same field may be seen the increased number of nuclei inside the muscle fibers, the increased size of the intrafascicular septa, and hypertrophied and atrophied

fibers side by side. Later the muscle fibers break down and are replaced by connective tissue and fat.

A fairly large amount of laboratory investigation has been done on this condition.

The basal metabolic rate has been found to lie anywhere between - 36 and 35.5 (31), probably due to associated endocrinopathies.

The blood sugar is frequently low; the blood NPN, urea, CO_2 combining power, Ca, P, K, lactic acid, cholesterol, creatine, creatinine are usually normal. McCrudden and Sargent (44) found a hypocholesterolemia, but this has not been confirmed.

A low creatinine and high creatine output which is characteristic of this disease, was first found by Levene and Kristeller in 1909, and has been generally confirmed by later investigators. They found that orally increased protein increased the creatinuria. Other workers have convirmed this finding, but showed that after a few weeks the creatinurea diminishes. Burger, in 1919, found a low total creatine coefficient in cases of definite dystrophy, and a normal total creatine coefficient in other atrophies. He claimed this difference to be constant enough to be used in differential diagnosis. A simple guanidine derivative was isolated from the urine of

dystrophic patients by Sullivan (66), who claimed it to be of universal frequency.

Collazo, Barbudo and Torres (15) found the following chemical changes in muscle:

	Normal		Dystrophy	
	average	range	average	range
Phosphagen	54.6	40.8 to 68.5	36.2	227- 44.4
Glycogen	690		490	586-186
Lactic acid	107.5	81.1 - 122	106	150- 78.8
Dry substance	20.7 %	25.8 to 16.6	28.8	36.3-23.8

Although the glycogen, phosphagen were decreased about a third, the lactic acid was about normal. The increased solid content of the dystrophic muscles is interesting in view of the same finding in the muscles of our animals with artificially induced dystrophy.

Nevin (53) investigated muscle phosphorus fractionation in this and other types of myopathies. The following chart summarizes his findings:

	Mg %					% of total			
	Inorg P	Phos phoCr P	ATP P	undet erm. P	TOT. P	inorg P	Phos pho-Cr P	ATP P	undet erm P
Myasthenia gravis	15	56.6	53.8	14.1	139.5	10.7	40.6	38.6	10.1
Myasthenia+Graves'	10.4	46.0	50.8	4.4	111.6	9.3	41.2	45.5	3.9
Periodic weakness	11.8	45.9	39.4	4.1	101.8	11.6	45.1	38.7	4.6
Progressive dyst. (pseudohypertr)	6.7	13.7	17.8	3.6	41.8	16.0	32.8	42.6	8.6
Progressive dyst. (pseudohypertr)	2.1	1.5	2.8	1.5	7.9	26.6	19.0	35.4	19.0
Dystrophia myotonica.	11.3	18.5	21.6	7.4	58.2	19.4	31.8	36.1	12.7
Normal	19.1	51.7	51.9	1.8	124.5	15.3	41.5	41.7	1.4

It is evident that the undetermined P fraction in all types of myopathies studied is increased. The phosphagen-P is much decreased in pseudo-hypertrophic muscular dystrophy and in dystrophia myotonica, while in the other myopathies it is about normal. Also evident is the increased proportion of inorganic phosphorus in progressive muscular dystrophy and in dystrophia myotonica, and the decreased proportion of inorganic P in the other myopathies studied.

Wang, Kaucher and Wing (71) investigated the mineral metabolism by balance experiments and found that although the mineral intake in two dystrophic boys was 30 to 60 per cent higher than in their normal adolescents, the retention of calcium and phosphorus was definitely lower.

Retentions in mg. per kilo per 24 hours.

	Calcium	phosphorus
Normal adolescents	11	5
Dystrophic boys	4	1

The adolescents they worked with in their investigations were girls, and therefore we would expect a somewhat higher retention in their case than in the boys. However, this difference is too great to be accounted for merely on the basis of a sex difference. The ratio of Ca/P retention for the control group was 2.2 whereas in the dystrophic group it was 4. This is interesting in view of the fact, as we shall demonstrate, that rabbits made dystrophic by nutritional means retained much more phosphorus in relation to the calcium retained than the normal, the opposite of that which occurred here.

The pathogenesis, except for the hereditary and possibly the endocrine factors, remains rather vague. Kure and Okinaka's hypothesis of a double innervation for striated muscles has been discredited, and therefore their theory of etiology based upon this assumption is also untenable. Among other theories of the etiology is that of Erb--a disturbance of a trophic center in the mid brain. All the ideas advanced for the causation of progressive muscular dystrophy have

proven inadequate, but possibly physiological researches will give sometime in the future a working hypothesis of the basic disturbance involved.

Dystrophia Myotonica (Deleage, 1890)

This condition, which is also called myotonia atrophica, is a combination of dystrophy and myotonia. As is generally the case with the myopathies, it is hereditary and is accompanied commonly by endocrine dysfunctions.

The most characteristic findings in the condition, as summarized by Morgulis and Young (52) are as follows:

(1) The onset is generally in the third decade.

(2) Myotonia is limited to the hands, tongue and legs.

(3) Atrophy is found in the face, sternocleidomastoid, hand, forearm, muscles of the leg.

(4) General emaciation.

(5) Alopecia.

(6) Testicular atrophy.

(7) Cataract.

(8) Speech defect.

(9) Ataxia and Westphal's sign.

Although the disease is hereditary it may not show as a myopathy for several generations, presenting as the only evidences: cataract, frequent celibacy, many childless marriages, and the disappearance of

several branches of the family. If the disease is well developed it cannot be inherited since the gonads are destroyed.

Myotonia is often present, and is much the same as found in myotonia congenita, as shown by Lindsley and Curnen (11) by the use of electromyographic studies. The after-contraction which persists following voluntary movement is accompanied by action currents, and "is due to persistent discharge of hyperexcitable sensory end-organs in the muscle." They found that injections of calcium gluconate into the blood, although the serum calcium was entirely normal, served to decrease the after-contraction action currents.

Atrophy of the muscles of the face and neck give the characteristic "hatchet face" and "polelike neck" facies. When the peronei are involved there is a broad based and steppage gait, resembling closely that of tabes dorsalis. Due to the atrophy of the oral and lingual muscles the voice is low pitched, monotonous and nasal. Paralysis of the vocal cords has been reported.

Although atrophy is found chiefly in the regions mentioned, it may spread to adjacent or any other muscles. The most constant atrophy is that of the orbicularis oris and the sternocleidomastoidius. In about half the cases atrophy of the dorsiflexors

of the feet is present.

Cataract is present in ten to thirty per cent of the cases; frequently stellate opacities in the posterior lammelae of the lens in a young individual serves as a helpful diagnostic feature.

The alopecia mentioned is usually frontal in distribution, although it is sometimes found over the entire body. The atrophy of the gonads is usually complete in a comparatively short time.

There is a very great loss of weight, much more than can be accounted for by atrophy of muscles. This is evidently due to decalcification of the bones, since there are always bony deformities. This last is yet another point wherein this condition resembles progressive muscular dystrophy.

Certain neuro-muscular features are present, which include: loss or diminution of the tendon reflexes, severe pain in the legs, myotonic electrical reaction, lessened response to faradic and galvanic currents, and rarely fibrillary twitchings.

The course is slow but progressive, and most cases do not live over forty five. Death is from asthenia, or its accompanying intercurrent infections.

Certain mental alterations have been noted. Adie and Greenfield, Bramwell and Addis, Curschman, Baake and Voss, Naegeli, and Hauptmann have all des-

cribed poor intelligence in many of their cases; but both Curschmann and Hauptmann have maintained that this is accidental. Most cases are dull, selfish, unsociable, and all this is possibly due to psychic reaction to their condition. Maas and Paterson (42) found four congenital mental defectives and seven cases somewhat retarded in twenty five cases. In their particular series they found several psychotic types: schizophrenia, paranoia, hypomania, hysteria and status epilepticus.

The condition is a heredo-degenerative disease and as such is frequently accompanied by endocrine pathology. Atrophy of the testes has been mentioned previously. Bramwell (6), Adie and Greenfield (2), and Keschner and Davison (34) have described suprarenal dysfunction. Pathological changes in the pars intermedia of the hypophysis have been described by Adie and Greenfield, Keschner and Davison, and Guillain, Bertrand and Rocques. Curschmann (17) does not believe the glandular dysfunctions have any etiological significance, and indicts "dysplastic defects in the vegetative centers of the mid-brain."

Metabolism studies (52) have demonstrated a marked persistent creatinuria which is not effected by gelatine feeding, normal blood contents of sugar, uric acid, lactic acid, non protein nitrogen and

creatine-creatinine, and apparently normal excretion of uric acid and organic acids. The muscles demonstrated an inability to retain creatine and synthesize phosphagen.

The muscular pathology is very similar to that found in progressive muscular dystrophy (23). There is an extreme variation in size of the muscular fibers, of which some are atrophied and some hypertrophied. In some fibers there is a great increase in the number of nuclei throughout the fibers. Some of the nuclei are shrunken and pyknotic. On longitudinal sections there is noticed increased markings of the striations of the functional fibers, and lighter areas of degeneration between the fibers.

Nevin (53), as previously shown, found in a muscle biopsy specimen a very low total phosphorus, and a decreased proportion of phosphagen, and an increased proportion of inorganic phosphorus.

Myotonia Congenita

This condition, first described by Thomsen in 1876, is characterized by the presence of clinically sustained contraction after voluntary movement. This after-contraction, as shown by Lindsley, is accompanied by action currents, and is much the same as that found in dystrophia myotonica (41). Indeed, the closest relationship exists between the two diseases.

The onset is in the earliest childhood, although sometimes it is first noticed at puberty or even later. There is little or no progression, but rarely improvement. It occurs most frequently in males, and appears to be a dominant Mendelian factor. (3).

The spasmodic contractions mentioned are aggravated by fear, strong emotion, fatigue, cold, feverish illnesses, and forced effort. Warmth benefits as do moderate quantities of alcohol and frequent movement. The patient usually finds it advantageous to "limber up" before attempting his work.

The muscles are large, but their size belies their lack of power. All the muscles are involved usually, although there may be a variation in degree. The ocular, tongue, throat, respiratory muscles are not usually involved but may be.

The mechanical excitability of the muscles

is markedly exaggerated. Percussion produces a deep dimple at the point of impact and a persistent contraction which may last as long as half a minute. The "myotonic reaction" (prolonged contraction) is given on stimulation with moderate faradic or galvanic current.

Sensations and reflexes are normal.

Paramyotonia of Eulenburg is a similar condition, in which there is a rigidity of the face and neck and the muscles of deglutition and of the extremities just at the onset of cold weather. This lasts 15 minutes to several hours, and is followed by generalized weakness which lasts sometimes for days.

Myotonia is associated with other glandular, muscular or neural dystrophies, which may be scattered through other members of the family.(3). The close association to dystrophia myotonica has been mentioned.

Rosenbloom and Cohoe (60) described in a case a negative nitrogen balance, no creatinuria except after high creatine feeding, a negative sulfur balance with normal partitioning, a negative calcium and a negative magnesium balance, with a good retention of phosphorus.

The pathological findings are as follows: macroscopically the muscles are slightly paler than normal, with no apparent fatty change. Microscopically,

the muscle fibers are seen to be enlarged, rounder than normal, and to possess poorly marked transverse striations. The sarcolemma nuclei are increased in number. The nerves and cord are normal.

Amyotonia Congenita

This rather rare condition was first described by Oppenheim in 1900. Following is Oppenheim's description of the disease, as quoted by Collier and Wilson (16): "....a condition of extreme flaccidity of the muscles associated with an entire loss of the deep reflexes, most marked at the time of birth and always showing a tendency to slow and progressive amelioration. There is a great weakness but no absolute paralysis of any muscle. The limbs are most affected, the face is almost always exempt. The muscles are soft and small, but there is no local muscular wasting. Contractures are prone to occur in the course of time. The faradic excitability in the muscles is lowered and strong faradic stimuli are borne without complaint. No other symptoms indicative of lesions of the nervous system occur." The first case noted in the United States was described in 1907.

The disease is congenital and most usually is noted at birth or within a few hours; that is, hypotonus is usually present. The parents frequently do not notice anything wrong until they attempt to get the child to walk, when the extreme weakness of the muscles is evident. Certain cases begin following acute infections and develope very rapidly. There is

no demonstrable familial aspect.

The etiology is unknown. Although a number of cases have followed infections and toxemias, these factors are not conspicuous. The children are of good size and weight and are apparently healthy at birth, except for hypotonus.

Clinically it is characterized by an extreme limpness of muscles; there is always some voluntary power present, although this may be so slight that the child may not be able to raise his limbs against gravity. Relaxation of the ligaments produces flail joints, easily capable of extreme hyperextension. In decreasing order of degree of affection are: the legs, the arms, the trunk, and leastly the face. Although the muscles are small, there is no true atrophy and no fibrillations. The tendon reflexes are abolished; but if the condition improves they later return. Sensations, speech, and mentality are normal while the superficial reflexes, as opposed to the tendon reflexes, are always normal. The response to faradic current is greatly lessened or completely lost and the ability to bear strong faradic currents is greatly increased. Sometimes with galvanic current the response is normal, although usually it is decreased; and polar reactions are normal.

The diagnosis may be made on the age, the

symmetrical flaccidity, the weakness without complete paralysis, and the loss of deep reflexes. It must be differentiated from poliomyelitis, post-diphtheritic palsy (which is rare during the first year), and the Werdnig-Hoffmann type of infantile muscular atrophy. In later years this last differentiation has become more and more difficult, so that at the present time a clear cut differentiation can hardly be made except by the course and pathology (26).

The course is generally that of slow but progressive improvement. Twenty to thirty per cent die during the first year of some intercurrent malady; the survivors recover in five to ten years but they are always weak. The progressive improvement is the principal basis upon which differentiation from infantile muscular atrophy may be made, since with the latter condition death usually occurs in a few months.

Laboratory findings in a case studied by Zeigler and Pearce (75) were: a creatinuria and low creatinine output on a low protein diet, normal uric acid excretion (indicating no breakdown of muscle cells), increased nitrogen and neutral sulfur excretion. The phosphorus excretion was normal, while the chloride excretion was diminished.

The pathology has been a moot subject since few cases have been adequately studied. Grinker (26)

in 1927 was able to collect pathological reports on only twenty two cases. Some investigators have linked the condition to the myopathies, others to infantile muscular atrophy. Spiller (26) in 1907 published the first comprehensive pathological report. He noted that the hypotonicity is so great that rigor mortis does not set in. He described the muscles as pale, with some hyalinization, some connective tissue and nuclear increase, and the presence of small fibers which show indistinct longitudinal striations. Others have reported the muscles as normal save for the miniature size of the fibers. Spiller reported the peripheral nerves as normal, which finding has been generally confirmed. Baudouin (26) first noted changes in the anterior horn cells and roots. In general there seem to be two types of anterior horn cell pathology: in one there is only a paucity and a deformation of ganglion cells; neuronophagia, and changes in the glial cells, and actual degeneration of the ganglion cells are found in the other. Grinker attempts to classify the latter type as the Werdnig-Hoffmann condition, and regards the former as being true amyotonia congenita. He believes that the site of the changes is the same and that, since they sometimes merge, the fundamental difference is only a matter of the time at which the

ganglion cells are affected.

The smooth muscle is apparently normal, and the lateral horn cells are well developed. The heart muscle shows thin fibers of the fetal type, sometimes producing death from myocardial insufficiency.

Krabbe holds the condition as benign, and perhaps due to retarded development of the muscles (38). The laboratory and electrical findings, the muscle pathology, and the inconsistant neural pathology are the bases upon which this disease is included with the myopathic group. In many neurology texts it is included with the myelopathic group--we do not regard it as such.

Family Periodic Paralysis

Family periodic paralysis is a primary myopathy, hereditary in nature. It was first described by Cavare in 1853, although probably the best description was that of Holzapple, a general practitioner of New England(28).

The familial nature of the disease has been from the first well defined. The family studied by Holzapple serves as a good illustration of the hereditary nature of the disease. He found in four generations of the family 22 cases of the paralysis, of which 6 cases had an affected father and 4 cases had an affected mother.

The condition is characterized by irregular attacks of flaccid complete or partial paralysis in an otherwise apparently healthy individual. The onset is at puberty or earlier, and the condition remains throughout life although after middle age the attacks come on at longer and more irregular intervals. The arms and legs are most often involved, although occasionally all the muscles below the neck are involved. The face, eyes, tongue, speech, deglutition and sphincter muscles are usually spared.

The attacks usually begin at night, and last a few hours to three days. When the attacks begin during the day the legs are first affected, then the

arms, trunk and neck in order, progressing from proximal to distal. The paralysis usually passes off in a reverse order, and recovery may be very rapid or require half a day (3).

During the attacks the patient is more or less prostrated, sometimes completely, but the mind is always clear. The tendon reflexes are completely lost, and the muscles exhibit the "cadaveric reaction" which consists of a loss of all excitability. The pulse is weak and irregular and frequently the heart shows signs of dilatation. Death has occurred during an attack.

Among the suggestions for the etiology which have been offered are those of Head--anaphylactic reaction to foods, and of Gardner--toxins from metabolic defects (19).

The blood and urine have been extensively studied chemically with apparently normal findings; potassium, calcium, urea, uric acid, and non protein nitrogen are all apparently within normal range (19).

General metabolic studies have shown little of significance. The fluid intake and output are quite variable and may be high (3). The creatinuria found in other myopathies is not evident here (19), while the creatinine coefficient is normal or low, reaching the low level of 4.8 mg. per kilo in a case studied by Diller and Rosenbloom. Singer and Goodbody (64)

found normal excretion of nitrogen, urea, uric acid, chlorides, total sulfur, alkaline sulfur, ethereal sulfur and phosphorus, and this has been confirmed by Crafts and Irwin and Diller and Rosenbloom. Mendel, working with Mailhouse (43), found a low excretion of calcium and magnesium during the interval between the attacks, but in view of the mineral balance studies of Diller and Rosenbloom this is to be discounted.

Studies of the paralyzed muscles show some very definite changes. The oxygen consumption of the paralyzed muscles is about 50 % of normal. The phosphorus fractions and creatine are very definitely decreased, as evidenced by the following table from Brand and Harris (8):

	Acid soluble P in mg. per 100 g. of muscle				
	Total	Organic	Total inorg.	Phosphagen-P	Creatine
Case	76	13	63	28	278
Normal	143	53	90	34	401

The histopathology may be slight. Occasionally the microscopic appearance of the muscles is normal, but usually there is vacuolization of some fibers with granular material in the vacuoles.

Myasthenia Gravis

Myasthenia gravis is a primary type of muscular disease, characterized by abnormal fatigability. It was described by Wilks in 1877 and by Erb in 1878.

The age of onset is generally between thirty and forty years, although a case as young as two and one-half years and one as old as 69 have been reported (35). The disease is more common in females. No direct familial aspect has been demonstrated with certainty but the existance of a number of cases in the same family has been described frequently, and there is certainly a relationship to thyroid and thymus pathology which have familial aspects. Although the etiological significance is somewhat hazy, in a large number of cases described, the onset of the condition followed various acute infections, pregnancy, and toxemias (those of plumbism especially).

The symptomatology at the onset is frequently vague, but weakness, general or localized, is usually the first sign. Occasionally pain in the involved muscles, headaches and paresthesias precede the weakness. The ocular muscles are usually the first to be involved, and are almost invariably involved at some time during the course of the disease; of these, the

levator palpebrae superioris (sometimes unilaterally) then the internal, superior, and external recti are involved in the order mentioned, producing external ophthalmoplegia. The mimic musculature of the face is next affected, then the muscles of speech, of mastication and deglutition, of the accessory respiratory group, the trunk, and finally of the arms and legs. In general, the most used muscles are most involved.

The characteristic facies of the condition are: a sleepy stare, a nasal snarl, very slight emotional expression of the face, loose hanging head (the head usually falls forward, unless the patient holds it erect with his hand). Hypotonus is present only during exhaustion, and no atrophy is present except in cases of long standing. Although diminution of vision and hearing are not uncommon, sensation is usually normal. The sphincter and detrusor muscles of the bladder are rarely involved, so there is neither bladder weakness nor incontinence.

Later in the course of the disease, paroxysms of dyspnea appear which last a few minutes to several hours, at first only after exertion but later spontaneously, and sometimes produce death. The ergograph (74) shows that the work output of the muscle rapidly decreases but recovers after a few moments of rest. The myasthenic reaction of Jolly appears--rapid decrease

of contractions to complete cessation when the muscles are stimulated by a faradic tetanizing current, with recovery established in about two seconds. This reaction has been shown to follow intellectual fatigue (Mosso), family periodic paralysis (Markeloff), post-diphtheritic paralysis (Kramer), angioneurotic edema (Salmon), hysteria, traumatic neurosis and exophthalmic goiter (Meyer-Gottlieb) and veratrine poisoning (Keschner and Strauss). Therefore this reaction cannot be considered specific. Neither the reaction of degeneration nor fibrillations are present. The tendon reflexes are normal but are completely fatigued after about twenty taps. Psychic disturbances due to mental reaction and difficulty of social adjustment sometimes occur.

There is almost always a relative, and sometimes an absolute lymphocytosis. Williams and Dyke (73) demonstrated a marked creatinuria; Bookman and Epstein (14), Diller and Rosenbloom, and also Pemberton (14) have demonstrated a diminished creatinine excretion. Muscle creatine content is low (73). The glucose tolerance may be markedly diminished. Fat and protein metabolism are normal. Neutral sulfur excretion in relation to total sulfur excretion is below normal (73). The basal metabolic rate is not characteristic, when hyperthyroidism accompanies the myasthenia the rate is

high, otherwise it is normal or even low.

Without treatment the patient gradually becomes dyspneic, cyanotic, and subject to insomnia. Remissions usually occur, but ultimately invalidism results. After several months to ten or more years, the patient succumbs to exhaustion or pneumonia. With treatment the prognosis is better; this will be discussed in the section devoted to therapeusis.

Wolff, Keutmann and Cobb, using electromyograms, decided the defect was probably in the muscle cell (74), but the etiology is unknown. As previously stated, acute infections and various toxemias frequently precede the disease. There is a definite relationship between myasthenia gravis and exophthalmic goiter, as shown by Cohen and King (14). They mention that although relatively few cases of myasthenia gravis show evidences of exophthalmic goiter, the majority of exophthalmic goiter cases show some evidences of myasthenia. In both are found: 1. hyperplasia of lymphatic tissues, 2. lymphorrhages in muscles and in the suprarenal cortex, 3. lymphocytosis, 4. lowered glucose tolerance, and 5. creatinuria on a creatine-free diet.

The pathology is rather characteristic. Lymphorrhages in ill defined clumps are found between the muscle fibers, generally in the vicinity of

capillaries. These lymphorrhages may be absent in the muscles affected most, but may always be found somewhere in the body (35); for instance, in other muscles, in the liver, adrenals, kidneys, lungs, pancreas, and even in the ganglia, the gray matter and the pyramidal tracts. Occasionally some muscle fibers are degenerated and some are irregularly atrophied; there is a tendency to narrowing of the fibers, increase in nuclei, and increase in striations. There are no consistent central nervous system findings. In about half of the cases the thymus is involved. It is large through failure of regression or hypertrophy, with degenerative and proliferative changes, and sometimes from small benign growths, "benign thymomas," which Ewing (35) believes are manifestations of infectious granuloma. The thyroid shows lymphorrhages, interstitial fibrosis, colloid degeneration of the fibrous stroma, and proliferation of the epithelium with formation of new follicles. Hyperplasia of the lymphatic tissues is found. A number of other pathological findings, especially in the parathyroids and other endocrine glands, have been reported.

THERAPEUSIS IN THE MYOPATHIES

Therapeusis

In the past electricity, arsenicals, strychnine, calcium, thorium, thyroid extract, parathyroid extract, x-ray therapy of the thymus have been tried and have all proven valueless. To date the treatment of only one of the myopathies, myasthenia gravis, is satisfactory. Treatment of progressive muscular dystrophy has not proven generally successful.

Milhorat, Techner, and Thomas demonstrated in 1932 that the creatinuria which accompanies glycine feeding in myopathies diminishes in from two to eight weeks, with a coincident rise in the preformed creatinine, and definite clinical improvement. Clinical and chemical improvement in myasthenia gravis has been attested by Adams (1), Chanutin, Butt and Royster (12), Tripoli and Beard (68), Cuthbertson and Maclachlan (18), Kesner, West and Key (36), Mettel and Slocum (46), Brand and Harris (7), Boothby (9,10,11), Schmitt (63), Reinhold and his associates (57), and by Reese, Burns and Rice (56).

Occasionally the first evidence of clinical improvement is the presence of paresthesias in the involved muscles. This is followed by the typical decrease in fatigability, increase in muscular strength, and increase in weight. Reinhold, et al, have reported

not only chemical, but also histological evidences of improvement. Boothby (10) stated that over 90 per cent of patients are benefitted by glycine plus ephedrine.

The glycine is given in divided doses of from 5 to 20 g. daily, and improvement is generally noted (if there is to be any) in two to eight weeks. The benefits from this treatment are not permanent, but slowly recede after the glycine feeding is stopped. Reese, et al, suggest that following the initial improvement, the glycine feeding should be discontinued until the creatine excretion again begins to go up.

The manner in which glycine benefits is unknown. That there is no shortage of glycine in the body of myopathic patients was demonstrated by Freiberg and West (21), by the normal ability to eliminate hippuric acid on the feeding of benzoic acid. Apparently glycine stimulates the creatine metabolism in such a manner as to restore the capacity of the body to utilize creatine.

Glutamic acid has been substituted for glycine, but the unpleasant taste discourages the patient.

The epinephrine-pilocarpine combination advocated by Kure and Okinaka has been as thoroughly discredited as their double innervation theory of skeletal muscle. Apparently when it has some benefit the power

of suggestion is more useful than the drug. About the only results in most cases on administration of this regime are palpitation and other untoward symptoms.

Chanutin, Bütt and Royster (12) have reported improvement following the feeding of creatine, but this finding needs confirmation.

Ephedrine, 3/4 gr. per day was advocated by Edgeworth, who was herself a victim of myasthenia gravis. Although some have reported good results with this therapy, ~~while~~ others have emphasized the frequency of lack of tolerance. Nevertheless it is worth trying, as Boothby has reported benefit in 93 per cent of a series of 47 cases at the Mayo Clinic.

The latest treatment advocated is prostigmin. Jolly in 1895 attempted to use physostigmine, but found too many side reactions accompanied, such as diarrhea, excessive perspiration, etc. Walker (67), noting the resemblance of the condition to curare poisoning, attempted to give physostigmine and later the homologue prostigmin, and reported good results. Pritchard (55) demonstrated that after administration of prostigmin to a myasthenic patient, a normal myogram was obtained on stimulation of the ulnar nerve. Benefit with prostigmin has been repeatedly confirmed (67).

Understanding of the manner of action of prostigmin dates back to the time when Loewi and his

Coworkers identified acetyl choline as "vagus-Stoffe," and demonstrated that eserine inhibited its destruction by some substance found in serum and tissue extracts. Dale and his associates (67) demonstrated in 1936 that striated muscles are activated by acetyl choline, which is liberated at the myoneural junction except during exhaustion. The substance in the tissue extracts and serum that destroys acetyl choline has been called choline esterase, and methods are extant for its quantitative determination. McGeorge (45) has shown that while the concentration of the choline esterase is normal in the serum of myasthenic patients, it is decreased for four or five hours to one-half or one-third of the resting level on the administration of prostigmin. This period of inhibition of the esterase corresponded to the period of clinical improvement.

Prostigmin is given in doses of 15 mg., five to nine times evenly distributed throughout the waking day. The occasional diarrhea that is noticed is easily controlled by a hundred fiftieth of atropine sulfate. Such constantly satisfactory results that its use has been suggested as a diagnostic test, have been obtained from its use (67).

Other therapeutic agents have been given in conjunction with prostigmin, especially KCl (since

K has been demonstrated to stimulate production of acetyl choline) and benzedrine, the neurological panacea; results, however, have proven no better than with prostigmin alone.

Suprarenal cortex has been used in the treatment of myasthenia gravis, but the reported good results require confirmation.

Treatment of progressive muscular dystrophy is yet in its infancy, and still unproven. Some investigators have stated that improvement results from glycine feeding, while Brand and Harris, and Boothby, and Schmitt, and Reinhold state definitely that it is of no value. Hurwitz (31) after reviewing the literature, states that the chance of symptomatic improvement after glycine therapy in progressive muscular dystrophy is good enough that it should be tried.

Tripoli and Beard (68) have suggested the use of glutamic acid.

Use of creatine in the treatment of both progressive dystrophy and myasthenia gravis has been advocated by Chanutin, Butt and Royster, who reported improvement in both conditions. This, however, lacks the required confirmation.

Suprarenal extract and cortin have been suggested, not only for myasthenia but also for pro-

gressive muscular dystrophy, but the results are not conclusive, as is true of anterior pituitary extract.

Calcium is still being used on the continent, but no remarkable improvement has been reported.

Physiotherapy and braces and other orthopedic appliances aid somewhat at least in maintaining posture and stability; and therefore they will probably continue to be used until some really worthwhile therapeutic agent appears.

NUTRITIONAL MUSCULAR DYSTROPHY

Nutritional Muscular Dystrophy

In 1931 Goettsch and Pappenheimer (25), studying the effect of a diet of which the vitamin E content was purposefully reduced to a minimum, observed the occurrence of a characteristic and specific disease affecting the skeletal muscles of rabbits and guinea pigs. Since little can be added to their original description of the pathological findings this is followed very closely. The muscle of the thigh and abdomen were particularly abnormal, although the muscles of the back and extremities, of the diaphragm, and the intercostal muscles were also involved. The myopathy, then, is fairly generalized.

Grossly the muscles appear atrophied, pale and definitely less translucent than the normal muscles. The lungs may show pneumonic consolidations but the heart, kidneys, adrenals, gastrointestinal tract are normal in appearance. Microscopically the myocardium and the smooth muscle fibers of the intestine, bronchi, blood vessels, and the uterus are entirely unaffected. The microscopic lesions are restricted to the striated skeletal muscles of the trunk and the extremities, in which the lesions are not only characteristic but also profound and widespread. In guinea pigs practically every muscle, including the

diaphragm, is affected to a varying degree with the exception of the masseter and tongue muscles, which preserve their integrity to the end.

"The essential and primary lesion is a coagulative necrosis of the muscle fiber presenting the familiar picture of waxy, hyaline, or Zenker's degeneration. This is followed by reactive cellular multiplication with attempts at regeneration. Many of the necrotic fibers become resorbed and their place taken by fat and connective tissue. The late stages resemble closely the changes seen in progressive muscular dystrophy of man.

"The earliest change in the fiber is shown in the appearance of transverse ridges or contraction bands, in which the striations are brought more closely together. The discs become disarranged and lose their individual identity, fusing into a swollen hyaline mass. Fibers cut in their long axis may show a succession of more or less globular hyaline masses, between which the sarcolemma is collapsed, containing only granular detritus. The sarcolemma nuclei are displaced and distorted, and tend to become oriented at right angles to the long axis of the fiber; subsequently they undergo karyorrhexis and may disappear completely.

"Preparations from many of the animals were obtained in this stage, before any marked cellular

reaction had occurred. In most instances, however, side by side with the necrobiotic changes, there was found an active multiplication of cellular elements with numerous mitoses. The cells have deeply staining oval nuclei, and the purplish cytoplasm is rather small in amount. They lie in clefts in the coagulated and necrotic muscle substance, and may completely fill the original sarcolemma sheath. The cylindrical columns of closely packed cells constitute the "Muskelzellenschläuche" of Waldeyer. The origin of these cells, whether from invading histiocytes or from the division of the uninjured muscle nuclei with their surrounding sarcoplasm, has been often discussed. Reference may be made to the excellent studies of Forbus who by means of preliminary vital staining, was able to distinguish the invading phagocytic histiocytes, which aid presumably in the removal of the necrotic material, from the proliferating muscle nuclei, which are concerned in the regeneration of new fibers.

"It has seemed to us that the majority of the mononuclear cells making up the "Muskelzellenschläuche" were derivatives of the muscle cells themselves, although clear leucocytes. Many of them, as Forbus and others have pointed out, undergo subsequent degeneration, but others assume a spindle

shape, align themselves in rows and, by the development of myofibrils on their surface, give origin to regenerating muscle fibers. .....In many of our preparations, there is a conspicuous formation of large multinucleated plasmatic masses, lying against the necrotic remains of the muscle substance.

"It has been stated that in the more chronic cases, only occasional necrotic fibers were to be seen, but the remaining fibers were often atrophic and widely separated by strands of cellular connective tissue or by fat. <u>These muscles with replacement fibrosis and lipomatosis closely resemble those of progressive muscular dystrophy in man</u>."

No significant changes have been found in the central nervous system or peripheral nerves either by Goettsch and Pappenheimer or by Dr. W. A. Willard who kindly cooperated with the Department of Biological Chemistry in the study of this condition. Goettsch and Pappenheimer were certain that the disease of the muscles was not associated with degeneration of ganglion cells of the nervous system or of the peripheral nerve trunks. In a special investigation by Rogers, Pappenheimer and Goettsch (59) on the condition of the intramuscular innervation of the degenerated muscles, no visible alterations could be found either in the finer nerve branches or in their motor terminals.

This is especially significant because marked changes in the terminal branches and of the end-net of the axis cylinder have been described in the muscles from animals suffering from experimental polyneuritis. It corroborates the statement that the muscle affections observed in beri-beri are secondary to nerve degeneration, whereas the dietary myopathy is a genuine primary muscle lesion which bears very close resemblance pathologically to the lesions in human muscular dystrophy.

Quite a large amount of chemical study has been done on nutritional muscular dystrophy, and the findings are especially significant because the studies that have been made on progressive muscular dystrophy of man have given essentially the same findings. For a more detailed discussion the reader is referred to the published work of Morgulis and Spencer (49, 50, 51) and of Spencer, Morgulis and Wilder (648).

Muscle glycogen.

The following table illustrates the findings from muscle glycogen studies:

Muscle glycogen, mg. per cent.

	Average	Range
Normal rabbit	556	434-716
Normal rabbit fasting 3 days	358	
" " " 6 "	273	
" " " 14 "	149	
Earliest signs of dystrophy	172	209-141
More advanced "	133.5	143-113
Severe "	91.5	126-69
Normal human deltoid (15)	690	
Dystrophic " " "	490	586-186

It is evident that there is a progressive decrease in muscle glycogen, which parallels roughly the histological degree of dystrophy. On two animals which had recovered, the muscle glycogen approached the normal. That this decrease in glycogen is the result of rather than the cause of dystrophy is evidenced by the fact that in animals fed magnesium gluconate dystrophy developed while the muscle glycogen was still relatively high. The values on deltoid biopsy specimens from humans are included to illustrate yet another point of similarity of the two conditions.

Muscle acid-soluble phosphorus.

The total acid soluble-P is usually noticeably

reduced, and the decrease generally is proportional to the histological extent of degeneration. The relative distribution of the different P fractions is hardly altered from normal. The per cent of phosphagen is the same in both, the inorganic phosphate and the undetermined acid soluble-P is somewhat greater, and the hexosemonophosphate is smaller in the degenerated muscles. These differences, however, are still within the normal range. It can be seen that here again nutritional dystrophy resembles progressive muscular dystrophy, as the changes correspond well with quoted results on human biopsy material (53).

Muscle creatine.

Goettsch and Brown (24) found that the creatine content of both white and red muscles was decreased roughly in proportion to the histological degree of dystrophy, although the brain and heart muscle showed normal amounts of creatine. Victor (69) found that the creatine content of the adductor muscles of ducklings made dystrophic by nutritional means had decreased from a normal of 458 to 186 mg. per cent.

Muscle creatine changes

Control creatine	387 mgm %
Control, fasting 6 days	398
Dystrophy 1 plus	382
Dystrophy 2 to 4 plus	268 to 75

The decrease is not due to fasting, since in the initial phase of fasting the creatine increases. As the total creatine decreases the percentage of esterified creatine increases, when it has decreased to 150 mg. the esterified form is about 80 % of the total, while in the normal it is about 65. In progressive muscular dystrophy in humans, as has been shown, the muscle creatine is markedly diminished.

Muscle nitrogen.

Goettsch and Brown (24) reported a very great decrease in the total nitrogen of dystrophic muscles. This finding has been confirmed by Spencer, Morgulis and Wilder although they never found the degree of decrease Goettsch and Brown reported (6%). Since there is a great increase in fibrous tissue histologically, it seemed worthwhile to attempt to measure this chemically. Accordingly, a method for measuring the gelatin nitrogen was devised (6%) and the following table illustrates the findings:

Gelatin nitrogen, per cent of total N

	Normal	Dystrophic
Gastrocnemius	14.3	32.3
Biceps femoris	11	18.6
Triceps	13.7	23.4

It is obvious then that there is two to two and one-half times as much fibrous tissue in the dystrophic muscles as in the normal. On a progress experiment it was found that fibrosis (chemically) developed with marked suddenness at about the time the weight curve began to waver and before outward evidences of dystrophy had appeared. They found that an individual determination might give a fallacious picture, since the same muscles are not always affected to the same degree. It is interesting to note that the characteristic lymphorrhages in myasthenia gravis are not always found in the expected locations.

Muscle cholesterol.

The cholesterol content of the degenerating muscles is two to three times as great as that of the normal muscles. This is true of all of the skeletal muscles examined, although certain of the muscles (the abdominal and intercostal) have a less pronounced increase than the more active muscles of the extrimities. The heart, stomach, kidney and brain exhibit practically no variation so far as the cholesterol is concerned from that found in the normal rabbits. The abdominal organs had an actually decreased cholesterol content. The normal cholesterol value in the heart fits in well with the normal histological finding of Goettsch and Pappenheimer.

Blood studies.

Blood sugar values were found to be the same for both normal and dystrophic animals. The dystrophic animals had peculiar glucose tolerance curves, and this peculiarity consisted of a less steep initial rise and decline than normal. This conceivably is the result of endocrine involvement, since the adrenals and the gonads are considerably reduced in size in the dystrophic animals (50).

The lactic acid content of the blood is apparently unchanged, the value (average) being 16.3 mg. per cent as compared with the normal of 16.6.

The total blood phosphorus is increased about twenty per cent, but the proportions of the various fractions of all save the lipid-P which is increased as much as one-half are practically normal.

The blood cholesterol of dystrophic animals is increased, this rise being parallel to the development of the muscular degeneration. This is evidenced by the following data:

Rabbit no.	Biopsy findings	Cholesterol
274	+	205
275	++	233
260	+++	260
286	++++	293

Metabolism experiments.

The results of the metabolism experiments as quoted from their summary are as follows:

"During the period of approaching dystrophy: The weight curve wavers or drops slowly, while the consumption of food and water becomes less. The urine excretion also decreases and it becomes acid in reaction. The urinary chlorides decrease gradually while the phosphorus elimination increases. The total nitrogen excretion decreases somewhat, but the creatinine excretion remains practically constant while that of creatine increases by leaps and bounds.

"At the 'critical point': The body weight declines sharply. The ratio of urine output to the water intake approaches the value of 1:1 as the acidotic metabolism develops. There is marked diuresis accompanied by a relatively slight increase in creatinine, total nitrogen, phosphorus and chloride excretion and an enormous increase in the creatine elimination. At this point the creatine N, calculated as per cent of total N, doubles or trebles itself and the nitrogen balance becomes definitely negative.

"During the period of progressive dystrophy, i.e., from the 'critical point' to the time of death: The weight continues to fall rapidly. The chloride and phosphorus excretion gradually decrease. The

creatinine and total nitrogen decrease from the high level reached at the "critical point," while the creatine excretion continues to rise till the time of death. The urine volume decreases following the brief strong diuresis at the critical point, but just before death a marked diuresis occurs again.

"During the period of recovery, following a change of diet at the "critical point": The weight increases at first slowly, then very rapidly. The food and water intake increases gradually. The ratio of the urine output to water intake soon returns to the normal value of 1:2. The urine almost immediately becomes alkaline. The excretion of phosphorus and chloride approaches the values normal for that diet. The nitrogen balance becomes positive and the creatine elimination approaches the normal level. Meanwhile, the rabbit gradually loses the symptoms of dystrophy and behaves perfectly normal."

EXPERIMENTAL METHODS

Procedure of the Experiment

We selected Diet 313 (50) for inducing dystrophy because it has a rather constant mineral composition, which is not true for Diet 13 with its high ferric chloride concentration. Ralston Purina Chow was selected for the control diet in as much as it contains a liberal quantity of all known food requirements. For the rabbits used only for analysis of muscles, Diet 313 supplemented with 10 or 20 parts of whole wheat germ, respectively were used also as control diets. Cures were accomplished by adding whole wheat germ (in one case after a preliminary administration of lettuce and cold press wheat germ oil) and in one case with Purina Chow.

At the beginning of the metabolism experiment, enough of the basic ingredients of Diet 313 to last the entire period were weighed out in the following proportions: Rolled oats, 355 parts; wheat bran, 180 parts; and casein, 75 parts. These were thoroughly mixed in a mechanical mixer and kept in a large tin can with a tight lid. For each batch of the diet, 10 g. of sodium chloride and calcium carbonate, 15 g. weighed to one hundredth of a g. were added to 610 g. of the above mixture and thoroughly mixed before 80 g. of melted lard and 10 g. of cod liver oil were added.

The material was then thoroughly mixed and treated with an ethereal solution of superoxol made up as follows: 20 cc. superoxol (30%), 30 cc. distilled water and 100 cc. redistilled ether. The material was allowed to stand with occasional shaking for at least two hours. It was then spread in a wide shallow Pyrex dish and allowed to dry overnight. Then 275 g. of dried skimmed milk powder were added, and the Diet again thoroughly mixed.

The curative and control diets were prepared in portions of not over 500 g. at one time and stored in the refrigerator to prevent spoilage.

Enough Purina Chow for the entire experiment was thoroughly mixed and stored in a tightly covered can. To guard against the possibility that the rabbits might eat only certain portions of the Purina, this was pulverized in a mechanical grinder, enough of the diet being prepared to last about a week, and stored in a well stoppered bottle.

The metabolism experiments were carried out in specially constructed cages. The cage proper is a wire screen cylinder twelve inches in diameter and ten inches high, with no bottom and a detachable screen top, and rests upon a chromium plated truncated cone about three inches deep and provided with a fine mesh

screen at the bottom. The cone in turn sets into a glass funnel holding a small amount of Pyrex glass wool to filter the urine. A graduated cylinder was attached to the funnel by means of soft rubber tubing. A few drops of a solution of thymol in chloroform and of toluol were used as preservatives for the urine. Food was placed in a small jar with a metal top so cut as to prevent the animal from scattering the food. The rabbits were supplied with distilled water.

At the same time each day the rabbit was weighed, the food consumption determined by difference, the feces weighed, and the urine measured. The weighed feces were dried in an oven at 105°C. The weight of the fresh feces and also its dry weight were determined for each day. The water consumption was also measured by difference. The funnel was rinsed with enough water to dilute the urine to 150 cc. Each day a 50 cc. aliquot was taken to prepare composite samples for each period. The reaction of the urine to litmus was noted daily. The entire apparatus was scrupulously cleaned every day.

Some of the dystrophied rabbits were allowed to die, but the controls and the cured animals were killed by bleeding. As soon as possible the rabbit was dissected and the following muscles removed: 1. two heads

of the quadriceps femoris, 2. biceps femoris, 3. gastrocnemius, and 4. anterior tibialis. The muscles were freed as much as possible of fascia, tendons, nerves and blood vessels, and the blood was soaked up on filter paper. They were then finely cut with surgical scissors into tared weighing bottles. After weighing, the material was covered with pure acetone. This was allowed to evaporate spontaneously, and then the weighing bottles were placed in a vacuum desiccator until the material had reached constant weight. The dried muscle was powdered, and stored in small vials.

Analytical methods

The mineral composition of Diet 313 was determined by analysing separately the basal portion, the dried skimmed milk powder, the cod liver oil and lard, and calculating from the relative proportions in the diet. The Purina chow and the wheat germ, and also the basal portion of Diet 313 were finely pulverized in a drug mill before analysis.

For all the analyses, except for the chloride, the material was ashed with a nitric-perchloric acid mixture (71).

Reagent:

100 cc. of yellow fuming nitric acid (Sp. G. 1.49) and 50 cc. of perchloric acid (Sp. G. 1.615) are added to 100 cc. of distilled water.

Procedure:

About two grams of the solid materials were weighed in a small filter paper (Whatman No.44) and transferred to a Kjeldahl flask. The liquid materials were placed in tared Kjeldahls, and the weight of the substance being measured calculated by difference.

After adding 40 cc. of the digestion reagent, the flasks were heated slowly over electric heaters, with frequent agitation to prevent bumping. When the flask filled with white fumes and the material darkened

10 cc. more of the reagent were added. The heating was continued until the solution became nearly colorless. Sometimes water had to be added repeatedly and boiled down to clear up the solution, occasionally a few drops of superoxol were also added. After evaporating to dryness and heating over the electric heater for ten minutes, the flask was cooled and the ash was dissolved in a few cc. of water acidified with nitric acid. The dissolved ash was poured through a small ashless paper into a 100 cc. volumetric flask, washed repeatedly and the solution finally made up to volume with water.

A slight modification was introduced in the analysis of the lard. It was placed into a tared flask and weighed by difference. Before adding the digestion mixture, however, it was first partially charred by moderate heating over the electric heater and then cooled. From this point on, the procedure was the same as before described.

The cod liver oil was digested in the same manner, but the mixture was much more explosive and had to be handled with utmost care. It was not found necessary to char the oil as was done with the lard.

For the analysis of some of the minerals in the urine trichloracetic acid extracts were used.

The extracts were shaken with charcoal and filtered. The charcoal used in these preparations was specially purified by boiling Norit A with dilute hydrochloric acid and washing with distilled water until the wash water failed to give a test for the specific mineral to be determined.

Chlorides in the Solid Materials.

At first a modified Carius digestion, employing silver nitrate, red fuming nitric acid, and superoxol was used.

Reagents:

1. 0.1 N $AgNO_3$, made by dissolving 16.99 g. and diluting to a liter, and standardized against NaCl by the gravimetric procedure.
2. 0.01 N NH_4CNS, made by dissolving 0.761 g. and diluting to a liter. This was standardized against the $AgNO_3$.
3. Saturated ferric alum.
4. Red fuming nitric acid.
5. Superoxol.

Procedure:

Transfer 0.25 to 1.0 g. samples of the material to 150 cc. Kjeldahl flasks calibrated at 125 cc, wash down any particles adhering to the neck, then add 3 cc. 0.1 N $AgNO_3$ and 10 cc. fuming nitric acid. Bring carefully to boiling, and if much foaming occurs heat the foam in the flask. Boil slowly, adding 5 cc. of

fuming nitric acid as needed, until the reaction ceases. Then cool somewhat, add 5 cc. superoxol, and continue heating gently.

When digestion is completed add 6 cc. saturated ferric alum, dilute to the mark with distilled water, and filter through a dry ashless paper. Titrate 25 cc. aliquots, equal to 0.2 of the surplus $AgNO_3$, with 0.01 N NH_4CNS, according to the Volhard method.

$$3.546 \times \left[\text{cc. 0.1 N } AgNO_3 - \frac{5\ (\text{cc. 0.01 N } NH_4CNS)}{10}\right] =$$

mgm of Cl in the sample analyzed.

It was later found to be more convenient to ash the solid materials. The material (250 mg.) was placed in quartz crucibles, moistened with a few drops of Bloor's reagent (alcohol-ether, 3:1), and then 1 cc. of saturated $Ba(OH)_2$ or of $NaHCO_3$ (13,54) was added. The material was ashed overnight in an electric furnace at about 600 C. The next morning the residue was dissolved in 5 cc. HNO_3 and transferred with repeated washing to a 100 cc. volumetric flask, containing 3 cc. 0.1 N $AgNO_3$ and 5 cc. saturated ferric alum. After diluting to the mark and mixing, filter. Twenty cc. aliquots were titrated with 0.01 N NH_4CNS.

$$3.546 \times 4 \times \left[\text{ccs. 0.1 N } AgNO_3 - \frac{5(\text{ccs. 0.01 N } NH_4CNS)}{10}\right] =$$

mgm Cl per g. of sample.

Urinary chlorides were determined with the same reagents, using a modified Folin procedure.

Five cc. of the composite urine were pipeted into a 100 cc. volumetric flask, then 50 cc. of water, 5 cc. of saturated ferric alum, and 10 cc. 0.1 N $AgNO_3$. The mixture was made up to volume, mixed, and filtered through a dry ashless paper. Five cc. of the filtrate were titrated with 0.01 N NH_4CNS.

$$\left[\text{cc. 0.1 N } AgNO_3 - \frac{(\text{cc. 0.01 N } NH_4CNS \times 20)}{10}\right] \times 3.546 \times 30 = \text{mg. Cl excreted per day.}$$

Phosphorus

Total urinary phosphorus was determined by the method of Fiske and Subbarow (20).

Reagents:

1. Molybdic acid solution, made by dissolving 2.5 g. ammonium molybdate in 100 cc. of 5 N H_2SO_4.
2. 0.25 % aminonaphtholsulfonic acid. Dissolve 14.25 g. of sodium bisulfite, 0.25 g. aminonaphtholsulfonic acid in 80 cc. of water, add 10 g. 5 % sodium sulfite and make up to 100 cc.
3. Standard phosphate solution, made by dissolving 0.4394 g. of dry KH_2PO_4 in water and diluting to a liter. This contains 0.1 mg. of P per cc.

Procedure:

Depending upon the phosphorus content of the composite urine, 1 to 4 cc. were measured into a 100 cc.

volumetric flask and diluted with 65 cc. of H_2O. After mixing 10 cc. of the molybdic acid were added and the solution again mixed. A standard was prepared by measuring 5 cc. of the standard solution into a 100 cc. volumetric flask, followed by 10 cc. of molybdic acid as before. The color was developed in each flask by adding 5 cc. of the aminonaphtholsulfonic acid and, after diluting to the mark with water, leaving to stand five minutes. The results were calculated by the familiar equation:

$$\frac{\text{Reading S}}{\text{Reading U}} \times \frac{\text{value of S}}{\text{vol. of urine}} \times \text{daily vol. of urine} = \text{mg. of P per day.}$$

For phosphorus determination of solid materials, the HNO_3-$HClO_4$ digest was analyzed by the method of Kuttner (40).

Reagents:

1. 10 N H_2SO_4. Add 282 cc. of concentrated H_2SO_4 to 600 cc. of water and, after cooling, make to a liter.
2. 7.5 % sodium molybdate.
3. Stannous chloride. The stock solution is made by dissolving 10 g. in 25 cc. of concentrated HCl. This keeps four to six weeks. For immediate use dilute 1 cc. to 200 cc. with water.
4. Phosphorus standard. Use the same standard as mentioned in urinary P determination as the stock. This is diluted to give 0.003 and 0.006 mg. per cc. respectively.

Procedure:

Measure enough of the ash solution to contain 0.012 to 0.04 mg. P into a small test tube calibrated at 10 cc. Standards are prepared by adding to similar tubes 5 cc. of each standard, equivalent to 0.015 and 0.03 mg. P. Add to each tube 4 cc. of molybdic-sulfuric acid reagent (1 cc. 10 N H_2SO_4, 2 cc. H_2O and 1 cc. Na molybdate) and mix by inversion. Add 1 cc. diluted stannous chloride, immediately invert and mix, then make up to the mark. Compare in the colorimeter, and calculate as follows:

$$\frac{\text{Reading S}}{\text{Reading U}} \times \text{value of S} \times \frac{\text{Volume U}}{\text{vol. U used}} = \text{mg. P in the solution.}$$

Calcium

All calcium determinations were made by the method of Wang (71).

Reagents:

1. 20 % sodium acetate.
2. Bromcresol green, 0.016 %.
3. 0.1 M ammonium oxalate, made by dissolving 1.42 g. and diluting to 100 cc. Add a small amount of thymol and store in the refrigerator.
4. 1.5 N acetic acid. This contains 42 cc. of glacial acetic (99.5%) per 500 cc.
5. Ammonia, diluted 1:1 and 1:3.
6. Wash solution. Add 20 cc NH_4OH to 980 cc.

of a mixture of 1 part redistilled alcohol, 1 part redistilled ether and 1 part distilled water.

7. 1 N H_2SO_4. This contains 27 cc. of H_2SO_4 per liter.

8. 0.005 N $KMnO_4$. Standardize this against the oxalate standard.

9. 0.01 N oxalate standard. Dissolve 0.670 g. of $C_2O_4Na_2$ and dilute to a liter. This solution keeps for a month in the refrigerator.

Procedure:

The sample to be analyzed should contain 0.06 to 0.22 mg. Ca. Using either the HNO_3-$HClO_4$ digest or in the case of urine the trichloracetic acid filtrate, to the proper amount measured into a 15 cc. graduated centrifuge tube, add enough water to make the volume to 5 cc. Add 1 cc. of 20 % sodium acetate and 6 drops of Bromcresol Green. Then add drop by drop, taking care not to leave any on the wall of the tube, 1 cc. 0.1 M ammonium oxalate and stir with a fine glass rod.

Using the dilute ammonia solutions and the 1.5 N acetic acid, adjust to a pH 5 by comparing with a phosphate buffer containing the same quantity of indicator. Remove the rod and rinse with a few drops of water. Make up the volume to 10 cc. with H_2O, mix thoroughly, stopper and allow to stand at least an hour (preferrably overnight).

After centrifuging for 8 minutes, aliquots of

the supernatant fluid are removed for the Mg determination. Carefully decant the remainder of the supernatant fluid and let the tube drain on filter paper for five minutes. After removing traces of the supernatant fluid clinging to the mouth and wall of the tube by wiping with dry ashless filter paper, wash down the tube wall with 3 cc. of the wash solution. Add water in a fine jet just above the level of the liquid while the tube is held at an angle of 60 and rotated, to a total volume of 7 cc. Break up the precipitate with a fine Pyrex rod and mix thoroughly. Then remove and rinse the glass rod, making up the volume to 8 cc.

Again centrifuge for 5 minutes, decant, drain, and wash twice as before. The tube is placed in the oven at 80°-100°C for an hour to remove the organic solvents.

Introduce 2 cc. of 1 N H_2SO_4 down the side, stir with a fine glass rod and place the tube in a boiling water bath for one minute. Keeping the tube in a water bath at 70°- 75°C, titrate with 0.005 N $KMnO_4$. The results are calculated as follows:

cc. 0.005 N $KMnO_4$ X 0.1 = Mg. Ca in sample.

Magnesium

Magnesium was determined by precipitating as ammonium magnesium phosphate and then determining

P by the method of Samson (33) or of Kuttner (40). Kallinikowa's method of precipitation (33) is as follows:

Reagents:

1. 5 % $(NH_4)_2HPO_4$.
2. 10 % HNO_3.
3. NH_4OH, concentrated and diluted 1:1.
4. 30 % NH_4NO_3.
5. 3 % $(NH_4)_6 Mo_7O_{24}$.
6. 1 % KNO_3.
7. 0.04 N NaOH. Dilute a 0.1 N solution and standardize before using.
8. 0.04 N HCl. Dilute a 0.1 N solution and standardize before using.

Procedure:

Measure enough of the Ca-free supernatant fluid to contain 0.019 to 0.056 mg. Mg into a centrifuge tube with a fine point. Make up the volume to 6 cc. with H_2O, add 1 cc. 5 % $(NH_4)_2HPO_4$ and 2 cc. of strong NH_4OH, mix thoroughly, cover with tinfoil and leave stand overnight.

Centrifuge sharply for 10 minutes, carefully decant the supernatant fluid from the precipitate, wash down the wall of the tube with 3 cc. of dilute NH_4OH and stir the precipitate into the solution. Repeat the washing in this manner twice more, and then

dissolve the precipitate in 2 cc. of 10 % HNO_3. The P is determined by Samson's method. Add 1 cc. 30 % NH_4NO_3 and 1 cc. 3 % $(NH_4)_6Mo_7O_{24}$, mix, and heat in a water bath at 85°- 90°C for three minutes. Allow the solution to cool and the precipitate of ammonium phosphomolybdate to settle out. Layer 0.5 cc. 95 % ethanol over the fluid and centrifuge 5 minutes.

Decant the supernatant fluid and wash with 5 cc. of 1 % KNO_3 layered with ethanol. Repeat the washing until the fluid is no longer acid to litmus. Dissolve the residue in 1 to 5 cc. 0.04 N NaOH and titrate the excess alkali with 0.04 N HCl, using phenolphthalein as an indicator. The results are calculated as follows:

(cc. 0.04 N NaOH - cc. 0.04 N HCl) X 0.03745 = mg. Mg in sample.

An alternate and somewhat simpler procedure is to determine the P by the Method of Kuttner. In this case, however, it is necessary to use graduated centrifuge tubes. Add a drop of 10 N H_2SO_4 to the washed precipitate of ammonium magnesium phosphate, dissolve and dilute to 10 cc. with water. Aliquots of this solution are analyzed by the Kuttner micro-colorimetric method. The results are calculated as follows:

$$\frac{\text{Reading S}}{\text{Reading U}} \times \frac{\text{Value of S}}{0.7835} \times \frac{10}{\text{cc. used}} = \text{mg. Mg in sample.}$$

Potassium

Potassium was determined by the cobalt nitrite method of Kramer and Tisdall (39) with certain modifications(48).

Reagents:

1. Potassium stock standard. This contains 1.9069 g. of KCl per liter, and is equivalent to 1 mg. K per cc. This stock is so diluted that 1 cc. of the dilute solutions contain 0.1 and 0.2 mg. respectively.

2. Sodium cobaltic nitrite solution.
   Solution A. 50 g. of cobalt nitrate crystals are dissolved in 100 cc. of H O, and to this solution 25 cc. of glacial acetic acid are added.
   Solution B. 50 g. of K-free sodium nitrite are dissolved in 100 cc. of water.
   Mix six volumes of solution A and ten volumes of solution B. Air is drawn through the solution until all the nitric oxide has passed off. After allowing the reagent to stand in the ice box at least 24 hours, it is filtered through ashless paper.

3. 0.02 N $KMnO_4$, prepared by diluting 0.1 N.

4. 0.01 N sodium oxalate. Dissolve 0.670 g. per liter. Stored in the ice box, it keeps about a month.

5. 4 N H_2SO_4.

6. Sodium nitrite, containing 50 g. per 100 cc. of solution.

Procedure.

To a series of centrifuge tubes with fine tips containing 0.1, 0.2, 0.25, 0.3, 0.35 and 0.4 mg. of K all made up to 2.5 cc. volume, add 0.5 cc. sodium nitrite. Add drop by drop 2 cc. of sodium

cobaltic nitrite reagent, and mix thoroughly. After allowing to stand one half hour, centrifuge for 7 minutes, and syphon off all but the last 0.3 to 0.4 cc. of the supernatant fluid.

Introduce 5 cc. of water being careful not to disturb the precipitate, mix with the remainder of the supernatant fluid by rotating gently. Centrifuge for 5 minutes and syphon off as before. The washing must be repeated (three or four times) until the wash water becomes colorless.

Add from a microburet an excess of 0.02 N $KMnO_4$ and 1 cc. of 4 N H_2SO_4. Mix with a glass rod and heat in a boiling bath until the solution becomes clear and pink, which generally requires about one minute. Decolorize with 2 cc. of 0.01 N sodium oxalate and titrate back to a faint pink color with the standard permanganate. A graph is constructed from these titration values, using amounts of K as absissae and the volumes of $KMnO_4$ as ordinates.

A volume of the filtrate containing 0.1 to 0.4 mg. of K is measured into a graduated centrifuge tube with a fine taper, and the determination carried out as described above.

The K value corresponding to the titration value was read off directly from the prepared graph.

Sulfur

Total sulfur was determined iodometrically by the method of Morgulis and Hemphill (47).

Reagents:

1. 0.02 N $BaCrO_4$ in 0.2 N HCl. Dissolve 2.53 g. of the chromate in 100 cc. of 2 N HCl and dilute to a liter.
2. NH_4OH diluted 1:5.
3. 0.005 N sodium thiosulfate.
4. 1 % starch solution in saturated NaCl.
5. Superoxol, Merck's c.p.
6. $Ca(OH)_2$, dry powder.
7. 5 % Potassium iodide.
8. 10 N H_2SO_4.

Procedure:

Urine was shaken with dry $Ca(OH)_2$, and after standing an hour, centrifuged. One cc. of the P-free supernatant fluid is measured into a large Pyrex tube and digested over an electric heater with 0.5 cc. of fuming HNO_3, to which 15 to 20 drops of superoxol are added at intervals. The digestion is carried out to complete dryness; however, if the ash is not perfectly white it is to be taken up with a small quantity of water and the digestion continued to completion. The white ash is dissolved in a few cc. of water and evaporated, this procedure being repeated twice in order to drive off completely the excess nitric acid

and peroxide. After cooling, the residue is dissolved in 5 cc. of water acidified with HCl. Then 5 cc. of $BaCrO_4$ reagent are added, and after 15 minutes, 5 cc. of dilute ammonia. The mixture is then centrifuged for 2 minutes and an aliquot of the supernatant fluid (3 or 5 cc.) measured into a small Erlenmeyer flask, treated with 1cc. of 5 % KI, then with 2 cc. of 10 N H_2SO_4, and titrated immediately with the 0.005 N $Na2S_2O_3$, using a few drops of the starch solution as an indicator.

The same procedure was followed with the wet ashed solutions, except that no further digestion with HNO_3 and H_2O was necessary.

The results are calculated as follows:

cc. $Na_2S_2O_3$ X Factor = mg. of S in sample.

This Factor, representing the quantity of S equivalent to 1 cc. of $Na_2S_2O_3$, is determined by analyzing known quantities of S (using a standard $(NH_4)_2SO_4$ solution) with each batch of reagents used.

Sodium

Sodium was determined by the triple acetate method of Salit (62).

Reagents:

1. Uranyl zinc acetate reagent (72).
   Solution A. 77 g. of uranyl acetate and 14 cc. of glacial acetic acid are dissolved by gentle heating and stirring in 400 cc. of H O and made to 500 cc. in a volumetric flask.
   Solution B. 231 g. zinc acetate and 7 cc. of glacial acetic acid are dissolved by gentle heating and stirring in 400 cc. of water and diluted to 500 cc.
   Mix the two solutions while hot, let stand 24 hours and filter.

2. Absolute or 95 % alcohol.

3. Wash reagent. 1 g. of the triple acetate is placed in a bottle and moistened with a minimum quantity of distilled H_2O. To this is added 1 liter of glacial acetic acid and the bottle well shaken. It is filtered before use.

4. 20 % aqueous solution of K_4FeCN_6.

5. Standard NaCl. 5 g. of NaCl are dissolved and made up to a liter with distilled water. This stock solution is diluted to give standards containing 0.39345 and 0.19673 mg. of Na per cc.

Procedure:

For urinary Na determination, the interfering phosphates are first removed by shaking for an hour with dry $Ca(OH)_2$ and centrifuging. Protein when present was removed by adding 1 cc. of 50 % trichloracetic acid to 5 cc. P-free urine. After centrifuging off the

protein precipitate the solution was neutralized with NH OH and made up to such volume that 2 cc. were equal to 1 cc. of the original urine. For Na determination on the ash solutions, the material was first neutralized and shaken with dry calcium hydroxide to remove the phosphates. The standard NaCl was similarly treated.

Two cc. of the sample, containing 0.05 to 0.5 mg. Na and the same quantity of a standard NaCl solution containing approximately the same amount of sodium were measured into similar 15 cc. centrifuge tubes. To each tube 6 cc. of freshly filtered uranyl zinc acetate reagent were added. Then 0.3 cc. of absolute alcohol are added and the contents well stirred. When the greater part of the precipitate has settled, another 0.3 cc. of alcohol were added and stirred, in such a manner as not to disturb the precipitate. Additional 0.3 cc. portions of alcohol were added at intervals of a few minutes until a total of 2.1 cc. have been added.

Centrifuge for 10 minutes, decant, and allow the tube to drain. Introduce 5 cc. of freshly filtered wash reagent and stir the precipitate. Again centrifuge for 10 minutes, drain the inverted tube on filter paper, remove traces of the reagent from the mouth of the tube. Add a drop of glacial acetic acid, wash down the side

with water, and transfer the dissolved precipitate quantitatively to a 50 cc. volumetric flask and make up to the mark.

If the total sodium content is 0.1 to 0.2 mg. measure 20 cc. into a test tube, but if the sodium content is 0.2 to 0.2 mg. use 10 cc. and dilute to 20 cc. with water, treating the unknown and the standard in like manner. Add 0.5 cc. $K_4Fe(CN)_6$, mix, wait 5 minutes for full development of the color, and compare in the colorimeter. The results are calculated according to the equations:

$$\frac{\text{Reading S}}{\text{Reading U}} \times \frac{\text{Value S}}{\text{vol. U}} = \text{mg. of Na per cc. of solution.}$$

EXPERIMENTAL RESULTS AND DISCUSSION

PERIOD	Ave. wt. per period	SODIUM				POTASSIUM				CALCIUM				MAGNESIUM				CHLORIDE				PHOSPHORUS				SULFUR			
		INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol
		in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day
4	590	644	2	390	2.74	1152	12	834	1.95	1128	44	140	6.02	212	7	181	0.25	984	4	572	3.30	868	31	89	6.04	156	6	72	0.61
4	670	624	3	356	2.88	1116	12	960	0.92	1096	22	50	6.40	208	6	140	0.65	952	4	496	3.18	840	30	88	5.83	152	6	60	0.69
4	790	904	16	416	5.13	1620	39	1302	1.78	1588	36	232	8.89	300	48	176	0.80	1380	10	744	4.41	1220	96	146	7.86	220	10	144	0.52
2	900	386	14	156	4.70	692	17	537	1.77	678	44	50	7.30	128	6	72	1.04	590	4	278	4.34	520	34	52	7.01	94	4	72	0.28
2	960	322	14	172	2.96	576	16	360	2.56	566	44	10	6.40	106	6	52	1.00	492	3	187	4.26	434	34	60	5.48	78	4	24	0.78
2	1020	300	14	156	2.83	534	18	588	-0.92	524	44	17	5.80	98	6	48	0.92	456	2	228	3.18	402	33	71	4.81	72	2	30	0.62
2	1010	108	15	75	0.40	254	16	654	-5.32	206	44	6	1.95	44	6	36	0.04	168	2	134	0.45	206	32	98	1.22	50	2	32	0.25
4	1070	788	32	548	2.26	2304	52	1518	4.70	1336	176	28	7.08	348	24	92	2.42	1216	8	1016	1.35	1052	120	178	6.08	240	10	126	0.81
4	1190	964	36	740	2.04	2824	80	1650	7.00	1644	360	40	7.78	424	72	182	1.77	1484	12	1252	1.55	1284	252	246	6.34	296	26	114	1.22
4	1290	880	20	728	1.43	2588	56	1656	5.60	1508	276	24	7.55	388	36	158	2.00	1364	10	1340	0.10	1180	232	214	5.92	268	16	128	0.97
2	1390	428	10	376	0.46	1252	29	969	3.25	736	138	14	7.30	188	16	76	2.00	658	4	626	0.39	570	116	130	5.23	130	8	76	0.72

Rabbit 13
Diet 313 above
" " +10% Wheat germ below

PERIOD days	Ave. wt. per period	SODIUM				POTASSIUM				CALCIUM				MAGNESIUM				CHLORIDE				PHOSPHORUS				SULFUR			
		INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in mMol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in m-Mol
		in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day
4	615	284	35	84	1.80	2032	147	702	7.12	872	96	20	4.73	277	23	172	0.85	484	46	368	0.49	520	46	-	3.83	356	56	176	0.97
4	715	360	35	192	1.44	2600	147	1704	4.76	1116	96	89	5.83	354	22	240	0.96	620	48	292	1.97	664	46	-	4.98	452	58	268	0.98
4	840	428	89	84	2.78	3084	458	732	12.08	1324	232	220	5.45	420	60	284	0.80	736	86	344	2.15	788	190	-	4.78	536	104	364	0.53
4	940	540	88	220	2.52	3884	457	2244	7.57	1669	232	1084	2.18	529	61	376	0.96	928	86	436	2.86	996	188	-	5.52	676	104	364	1.62
4	1050	556	126	328	1.11	4016	557	714	17.52	1720	298	1028	2.46	550	90	360	1.04	960	158	540	1.84	1028	322	-	5.70	700	102	380	1.62
4	1160	624	126	256	2.63	4500	556	2644	8.32	1932	298	944	4.31	614	90	340	1.92	1076	158	508	2.88	1152	322	-	6.69	784	100	408	2.15
4	1270	608	140	464	0.04	4380	516	3544	2.04	1880	348	992	3.38	598	96	372	1.35	1048	134	560	2.42	1124	304	-	6.61	764	102	452	1.58
4	1390	628	140	456	0.35	4520	514	3544	2.95	1952	346	1000	3.79	616	94	364	1.65	1080	132	536	2.90	1156	304	-	6.83	788	102	428	2.01
4	660	1044	39	660	3.75	1868	76	630	7.42	1832	186	196	9.06	344	32	252	0.62	1596	8	1312	1.94	1408	97	49	10.17	252	33	89	1.01
4	810	1392	39	812	5.88	2492	76	996	9.08	2452	188	86	13.61	460	34	248	1.85	2124	6	1854	1.86	1878	96	154	13.10	336	34	144	1.23
4	960	1376	32	798	5.93	2460	63	488	12.20	2420	203	112	13.16	456	38	224	2.02	2096	10	924	8.18	1852	171	133	12.45	332	32	176	0.97
2	1065	596	16	302	6.05	1062	32	438	7.58	1050	204	60	8.58	194	19	125	1.04	910	6	774	1.83	804	84	58	10.67	144	16	54	1.15
2	1075	468	16	299	3.33	836	32	495	3.97	822	203	29	7.04	154	19	84	1.05	712	4	678	0.42	630	84	120	6.88	112	16	66	0.47
2	1040	238	24	80	2.91	428	46	264	1.51	416	142	18	3.02	78	16	68	-0.12	362	16	298	0.68	320	92	140	1.42	58	13	85	-0.62
2	965	84	24	120	-1.30	150	46	148	-0.56	148	142	9	1.20	28	16	50	-0.86	128	16	220	-1.52	114	90	128	-0.97	20	12	62	-0.84
2	900	--			-0.78	-	44	150	-2.47	-	142	3	-1.81	-	16	7	-0.48	-	16	55	-0.78	-	90	78	-2.71	-	11	35	-0.72

Rabbit 11 above - Diet Purina Chow

" 12 below - " 313

PERIOD	SODIUM				POTASSIUM				CALCIUM				MAGNESIUM				CHLORIDE				PHOSPHORUS				average weight during period	ratio water intake to urine volume
	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in M-mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE In M-mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in M mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in M-mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in M-mol	INTAKE	OUTPUT FECES	OUTPUT URINE	BALANCE in M-mol		
days	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day	in mgm per period			per day		
4	964	38	620	3.33	1720	34	1400	1.62	1696	83	103	9.44	320	12	256	0.54	1468	16	1324	0.90	1296	66	50	9.50	830	2.34
4	1068	38	772	2.80	1908	34	760	7.77	1880	85	208	10.00	352	14	240	1.02	1628	16	1740	-0.90	1440	65	21	10.88	970	1.99
4	1188	54	648	5.29	2124	223	2436	-3.35	2092	337	152	10.00	392	50	268	0.77	1812	20	1476	2.32	1704	370	68	9.40	1135	2.41
4	1244	54	808	4.15	2224	223	1632	2.36	2192	337	220	10.22	412	50	284	0.81	1900	20	1860	0.14	1680	368	68	10.05	1285	1.59
4	1292	64	1104	1.35	2304	346	1648	1.98	2272	508	276	9.30	428	84	64	2.92	1968	20	1068	6.20	1740	540	116	8.75	1410	1.76
4	1436	64	884	5.30	2568	346	1572	4.54	2528	508	256	9.02	476	84	302	0.94	2192	20	1128	7.35	1936	541	89	10.50	1545	1.66
4	1344	133	1144	0.73	2408	609	576	7.82	2368	662	144	9.76	444	110	308	0.27	2552	22	1432	7.82	1812	716	160	7.56	1615	1.55
4	1472	133	1148	1.00	2632	609	1080	6.02	2588	660	312	10.10	488	108	268	1.17	2244	20	1904	2.38	1984	715	149	9.05	1715	1.58
4	1412	82	1112	2.37	2524	687	784	6.73	2484	530	352	10.00	468	78	288	1.05	2152	26	1920	1.45	1900	578	130	9.62	1810	1.45
4	1240	83	860	3.24	2216	687	1280	1.59	2180	530	228	8.89	410	78	252	0.83	1888	26	1840	0.15	1668	576	170	7.44	1880	1.66
4	904	80	480	3.74	1616	424	928	1.68	1592	374	84	7.09	300	38	170	0.95	1376	18	1208	1.06	1216	388	104	5.84	1930	1.96
4	980	80	722	1.96	1740	426	1264	0.32	924	374	212	2.11	324	38	162	1.33	1496	18	1394	0.59	1320	388	132	6.46	1945	1.73
4	1124	132	746	2.67	2008	414	1784	-1.22	1580	658	404	3.86	372	92	188	0.96	1716	30	1624	0.44	1516	668	180	5.39	1970	1.42
4	1180	132	680	4.00	2112	414	1248	2.88	2080	658	586	5.23	392	92	272	0.30	1800	30	1612	1.11	1592	668	384	4.35	1975	1.35
2	522	66	412	0.96	932	222	680	0.38	916	354	244	3.98	172	84	77	0.23	796	21	833	-0.82	704	339	225	2.25	1955	1.16
2	480	66	242	3.74	860	220	520	1.52	842	354	226	3.28	158	84	100	-0.54	734	20	814	-0.84	648	338	140	2.73	1900	1.09
2	382	66	274	0.91	682	220	508	-0.59	670	354	154	2.02	126	84	48	-0.13	784	22	482	3.94	514	338	122	0.71	1825	1.12
2	20	16	98	-2.04	22	90	600	-8.55	34	232	64	-3.28	6	46	22	-1.29	29	7	154	-1.86	25	181	146	-4.87	1685	0.64
2	-	16	120	-2.96	-	90	486	-7.37	-	232	24	-3.20	-	45	15	-1.25	-	6	74	-1.13	-	180	192	-6.00	1515	0.31
3	-	16	94	-2.39	-	90	368	-5.86	-	232	14	-3.08	-	46	14	-1.25	-	6	124	-1.83	-	180	130	-5.00	1420	

Rabbit 1
Diet 313

Mineral Metabolism Studies.

Two rabbits were allowed to remain on Diet 313 until they died of dystrophy, and one animal was allowed to recover after reaching the "critical period" by supplementing the Diet 313 with whole wheat germ. One animal on Purina Rabbit Chow served as a control.

The study of mineral metabolism is complicated by the fact that we are dealing with actively growing animals. If we compare the growth curves of the four animals we find that they all grow well, and at first the ones on the dystrophy producing diet may even grow at a higher rate than the control. After a time, the animals on dystrophy producing diets show irregular growth curves and soon the weight drops off rather precipitously. Although during the last four or five days the animals actually starve, the loss of weight really begins before they have ceased eating. In these animals the urine becomes neutral or even acid.

The intake and excretions per period and the balance per day, calculated in millimoles by difference between the intake and output, are charted on the accompanying tables.

It will be observed that the rabbits on the Diet 313 all show a very marked retention of sodium.

Although this Diet 313 is richer in Na than the Purina Rabbit Chow, this can hardly be the cause of the greater retention (100 to 200 per cent increase) because the extent of retention among the rabbits on the Diet 313 is not in any way correlated to the amount of sodium consumed, and, furthermore, on the recovery diet with an increase of nearly 35 per cent in Na intake the retention actually decreases to nearly one half. In rabbit 12 a negative Na balance occurs only during the last four days, when the animal, because it no longer could take food, was actually in a state of inanition. The curve of the Na retention for rabbit 13 resembles very closely that for rabbit 12, but as soon as its diet has been supplemented with whole wheat germ the Na retention was restored to the same level as prevailed in the control rabbit 11.

The situation with regard to the potassium is different. Only rabbit 12 showed a retention which paralleled closely that found in the control rabbit during the first twelve days of vigorous growth, the other two rabbits on the dystrophy producing diet showing a markedly smaller retention of potassium. Rabbit 13, which was cured by the addition of wheat germ to its diet, showed a great negative K balance even greater than that shown by rabbit 12 at the time just

preceding death. However, as soon as it was placed on the curative diet the potassium balance immediately became positive and varied within the same range as that of the control animal. It is significant that this great loss of K takes place even before there is an appreciable loss in body weight and while the animal is still eating fairly well. It must be correlated, therefore, with the loss of potassium from the muscles which we have shown to occur in the dystrophic process, and the sudden loss of K by the animal coincides with this event in the development of the disease.

The study of the calcium metabolism also reveals that on the dystrophy producing diet all the rabbits retain much more than does the control animal, the difference being four to five fold. This greatly increased positive Ca balance which manifests itself from the very start of the experiment, cannot be attributed to the difference in the calcium intake because we found that even doubling the Ca intake may leave the balance unchanged or in fact yield only half as large a retention. We are led to conclude that, as in the case of sodium, the rabbits on the dystrophy producing diet retain relatively very great amounts of calcium. Rabbit 13, whose diet had been supplemented with wheat germ at the time it became dystrophic on Diet 313, shows an immediate large rise in the calcium balance.

The sodium and potassium balance on the recovery diet, as we have seen before, falls within the range of variation observed in the control rabbit, but during the recovery the calcium balance again becomes much greater than in the control. A similar behavior marks the phosphorus metabolism as will be shown presently.

The magnesium balance is not definitely affected, and only in one of the three rabbits on the dystrophy producing diet was there evidence of a marked increase in Mg retention over a limited period (4th to 12th day) on this diet. Otherwise there was no immediate tendency to greater retention and the retention curves of the dystrophic and control rabbits run a fairly parallel course. Rabbit 13, which was in a negative Mg balance at the time the cure with wheat germ began, immediately showed a great increase in Mg retention attaining even a somewhat higher level than in the control animal at about the same age. These results on the Mg metabolism tally very well with our findings that the magnesium content of the muscles is very little affected by the process of dystrophy.

A study of the chloride metabolism shows generally a rather close resemblance to that of the sodium. It is retained more in the rabbits on the dystrophy producing diet than in the control, the

higher balance level occurring from the start of the diet, and, as in the case of the sodium, the curve of the chloride balance likewise manifests a peak of greater or less extent and duration coinciding in a general way with the former. On placing rabbit 13 on the recovery diet there is temporarily an increased retention but the chloride balance is far below that of the control. That these changes in balance are not to be attributed to changes in chloride intake but to intrinsic metabolic processes is again corroborated by the fact that these variations in intake and retention are frequently in opposite directions. Thus rabbit 13 when placed on the recovery diet consumed, on the average, about 30 per cent more chloride per day but the chloride balance during that period was only a little more than one fourth as large as on the dystrophy producing diet.

The study of the total sulfur metabolism reveals little of any significance. In rabbit 12 on the dystrophy diet the S balance before the "critical point" hardly differs from that of the control. In rabbit 13 the retention is definitely below that in the control and increases more or less when it is put on the recovery diet.

The total phosphorus metabolism follows a course resembling very much that of the calcium. Here

likewise the rabbits on the dystrophy producing diet show a positive P balance which at times is two to three times as large as in the control animal. Furthermore, the P and Ca balance curves are almost parallel to each other so as to suggest that the retention of Ca dnd P are correlated, just as the retention of Na and Cl seem to be. But whereas on the recovery diet (rabbit 13) the Ca balance immediately rises far above that of the control, the same does not hold true for the P, although both curves (the Ca and P retention curves) still remain strictly parallel.

To test whether the mineral composition of the Diet 313, being higher in Na and lower in K than the Purina diet, could account for its dystrophy producing quality, we so adjusted its salt content that it was practically the same as that for Purina. The rabbits developed dystrophy in record time even when the Na content of Diet 313 was lowered and its K content raised. That the mineral composition of the diet is not a factor in causing dystrophy is further corroborated by the fact that Diet 313 plus 20 per cent wheat germ is both preventive and curative, although the mineral composition is not very different from that of Diet 313.

Although averaging of the results in the case of the dystrophic animals is hardly justifiable,

because of the diphasic nature of the experimental period, a spell of vigorous growth alternating with a regression, nevertheless we shall present a series of average ratios of the retained elements because they do show the general trend of the mineral metabolism.

Ratios (m-Mol.) of retention of mineral

Average	Na/Cl	K/Na	Ca/P	Ca/Mg
Control (11)	0.75	6.2	3.3	1.6
Rabbit 1	1.47	0.7	1.2	8.5
Rabbit 12	1.47	1.5	1.0	7.5
Rabbit 13	0.96	0.2	1.1	10.0
	1.95	4.1	1.1	3.6

This table shows that, whereas the normal rabbit retains less Na than Cl, on the dystrophy producing diet almost the relative retention of Na is twice as great. The K retention which is about six times as large as that of the Na in the control diminishes to from 1/4 to 1/30, but on feeding a recovery diet (Rabbit 13) the K/Na tends to return to that of the control. The Ca/P ratio is rather interesting because while in the control animal 3.3 times as much Ca is retained as P, in the dystrophic animals, where the absolute retention is very markededly increased, the ratio is about one third of this, i.e. much more P is retained in proportion to the retained Ca. The

Ca/Mg ratio also manifests the great increase in Ca retention which, on the basis of the Mg balance, is nearly five times as large. On feeding the curative diet this ratio also tends quickly to return to that of the control animal.

Another point of interest is the change in fecal excretion, of the various mineral elements. This decreases enormously on the dystrophy producing diet. Calculating the fecal elimination per 100 mg. consumed, a few comparisons will reveal the extent of this decrease. The normal rabbit's feces contain (on the average for the entire experiment) 19.4 mg. Na and 12.4 mg. Cl per 100 mg. Na and Cl consumed. In the dystrophic rabbits these values fall to 7.1-2.4 and 1.2-0.6 respectively. Similarly the fecal K, per loo mg. K consumed, falls from 11.6 mg. to values as low as 2.2 mg.; the fecal P also generally decreases from 23.2 mg. in the control to as low as 6.5 mg. The fecal Ca, on the basis of 100 mg. Ca consumed, was greatly reduced in only one animal, but in the other three it was unchanged in one and definitely increased in the other two.

Summing up the results of the study of the mineral metabolism of rabbits on a dystrophy producing diet, there is a marked tendency for retention of Na, Ca, Cl, and P, the first three of which also accumulate in the dystrophic muscles. The loss of potassium from

the affected muscles is reflected in a smaller tendency of the animals on the dystrophy producing diet to retain this element. However, the lower K balance is probably not due to the availability of K from the degenerating muscles. At the time when the dystrophic process in the muscles gains great momentum there is so great a loss of K that the animals are actually in a negative balance even before the inanition stage has been reached. It is also worth observing that, when the recuperative processes are initiated by supplementing the diet with wheat germ, the balance for the different minerals becomes very rapidly restored to the normal level.

Muscle Minerals.

Fenn and Goettsch (1938) reported a number of determinations on the electrolytes of normal and dystrophic rabbit muscles. We also determined the electrolyte content of muscles in connection with our study of the mineral metabolism of rabbits made dystrophic and of rabbits which subsequently recovered from the myopathy. In some respects the results of these independent investigations have many points in common, but also some striking discrepancies.

Four control animals were used, two of which were on our diet 313 supplemented with 10 per cent wheat germ, and two were on Purina Rabbit Chow. Five animals were kept on the dystrophy producing diet until death. Three other animals were also on Diet 313 (18 to 20 days) until the dystrophy was well developed, when they were allowed to recover either by adding whole wheat germ (10-20 per cent) to Diet 313 or by changing to the Purina Chow. Their muscles were analyzed after the animals had been on the recovery diet 43 to 70 days. For our analyses, as has been stated, we chose a mixed sample consisting of gastrocnemius, biceps femoris, quadriceps femoris and anterior tibialis.

The analytical results are summarized in the table in which, besides the composition of the ash

in mg. per cent of the dry muscle substance, the average concentrations in millëequivalents per kilogram of fresh muscle are also recorded. The phosphorus was regarded as monovalent in the calculation.

(See following page for table)

Our results on the water content of both normal and dystrophic muscles are much more consistent than those of Fenn and Goettsch, the dystrophic muscles containing about one per cent less water than the normal. In the muscles from recovered rabbits the water content was again normal. Comparing the data on the electrolyte concentration we note the following changes.

The sodium content of the dystrophic muscles is markedly increased from an average of 17.9 to 46.3 mE, or about 2.6 times. The potassium, on the other hand, is greatly decreased, by one-third, the changes in Na and K practically compensating each other. In the recovered animals the sodium concentration is still 50 per cent too high (on the average) but it is noteworthy that in two out of the three animals it is actually restored to the normal level. The potassium, however, is still low which probably indicates that the regeneration of new muscle fibers is not yet complete. We found practically no changes in the magnesium concentration, but the calcium was increased 500 per cent on the average, although in individual cases the increase

No.	Days, Diet, etc	g.1% in fresh muscle Solids	g. % in fresh Muscle Water	Na	K	Ca	Mg	Cl	P
2	72 - Diet 313+10% W.G.	23.68	76.32	190.3	1400	27	120	301	906
3	38- " " " "	22.06	77.94	197	1600	24	98	252	855
4	30 - Purina Chow	22.55	77.45	191	1830	51	94	284	1086
11	36- " "	22.37	77.63	147	1890	35	94	192	1086
	Average.	22.66	77.34	17.87	97.16	3.88	19.24	16.47	146.97
13	Dys. 19 - 10% Wg. - Killed 43	22.01	77.99	205	1240	26	118	288	963
14	" 18 - " " 52	22.74	77.26	198	1400	31	108	206	1071
65	" 20 - Purina - " 70	22.53	77.47	393	1310	43	97	435	927
	Average.	22.43	77.57	25.90	75.67	3.74	20.11	19.56	128.50
5	Dystrophic - Dedd 21	23.65	76.35	439	890	320	94	425	1060
6	" " 24	23.43	76.57	450	1300	107	128	385	978
8	" " 28	23.33	76.67	592	825	148	96	564	901
10	" " 20	23.91	76.09	504	1080	252	100	512	950
12	" " 22	23.84	76.16	273	1280	46	106	316	856
	Average.	23.64	76.36	46.34	65.05	20.65	20.67	29.40	130.18

was almost twice as great. In rabbits recovering from the dystrophy the calcium was entirely normal again. This would suggest that the calcium must be held in some easily mobilizable form. Of the anions we determined only the phosphorus and the chlorides. In view of the fact that the acid-soluble-P is reduced to about one-half in severely dystrophic muscles it is surprising that there is no alteration in the total phosphorus. Our normal P values are in good agreement with those recorded and also with those of Fenn and Goettsch (73.1 mE. as compared to our 71.5 mE.). The only way we can account for this is by assuming that the accumulation of Ca in the dystrophic muscle is associated with a retention also of P, and that the constancy of the total P is really only apparent, the decrease in the acid-soluble-P being compensated by the coincident retention. It is rather significant, on glancing over the Ca and P values of the dystrophic muscles, that the two are varying together. The findings with regard to chlorides are very striking, showing a great increase in the dystrophied muscles, where even the lowest value is somewhat larger than the highest value in the control muscles. On the average, the concentration of the chlorides is 1.8 times greater in the dystrophic than in the normal muscles. In the muscles of rabbits recovering from dystrophy the chlorides in two out of

the three animals were entirely normal, as was also the case with the sodium.

As mentioned previously, in spite of the general agreement between Fenn and Goettsch's and our own findings, there are certain discrepancies which require some explanatory comment. To begin with, the average results show certain quantitative differences, our Na, K and Mg being lower and the Ca and Cl higher than those given by Fenn and Goettsch. It is therefore, interesting to compare our results with the data for rabbit muscles gathered from the literature by Riesser (58) in his monograph on comparative muscle physiology.

Mg. per cent in fresh muscle	Na	K	Mg	Ca	Cl
Riesser	45	380.5	25.5	(8.6)	51
Our results	41	380.7	23.0	7.8	58.3
Fenn-Goettsch	52.7	464	30.2		39.8

In none of the muscles studied by us have we ever found calcification of the magnitude described by Fenn and Goettsch, and this is also true of the many muscles which were examined histologically. We have never found any evidence of recognizable calcification in our muscle preparations. Although we found a very large increase in Ca (up to about 1000 per cent), Fenn and Goettsch report increases even up

to 30,000 per cent! The only way we can explain this is by the fact that these authors have used Diet 11 which brings on dystrophy very slowly (rabbits have been on this diet up to two years!) so that the lesions developing under these conditions must be chronic, whereas on our Diet 313 the dystrophy developes rapidly and is undoubtedly an acute condition.

The data do not justify any attempt to gain an insight into the intimate changes in the ionic pattern of muscles in the process of dystrophy, except for the more obvious significance of the changes in mineral composition. Clearly, the loss of potassium is associated with the destruction of cellular elements, and the increase in sodium as is also pointed out by Fenn and Goettsch, may be a purely compensatory mechanism to restore the cation content of the muscle fibers. However, it is also well to remember that a greater amount of sodium may be associated with the increase in connective tissue. The reason for the enormous increase in the calcium is not so obvious, but the increase in chlorides is not difficult to explain. It is useless to try, on the basis of knowledge so far attained, to picture the changes within the muscle fiber undergoing dystrophy, unless one wishes to make thoroughly untenable assumptions, as Fenn and Goettsch have done. To assume that the Na and Cl are entirely

extracellular and then to draw conclusions as to the changes in the water volume of the muscle fibers, is absolutely without warrant. It must not be overlooked that at least some of the chloride is a component of the connective tissue and the striking increase in the chloride content of the dystrophic muscles is undoubtedly associated with the increase of fibrous tissue, as was shown by our study of the collagen content as well as by the histological examination of diseased muscles. Whalen and Shoemaker (728) have shown that tissues with a larger amount of connective tissue have a higher chloride content. When one bears in mind that the total base content of connective tissue is also greater than that of the cellular tissue, it is plain that it is premature to build hypotheses as to the electrolyte structure of the muscle until its tissue components can be resolved and analyzed separately. This, however, is a problem which with the present technique is still inaccessible to scientific analysis.

It is worth while to point out, perhaps, that if we neglect the calcium, the sum of the Na, K and Mg is practically the same for the normal muscles and for the dystrophic muscles (134 and 132 mE.). The muscles of the rabbits on curative diets show a different situation. The muscles of rabbit 63, which

was recovering on Purina Chow, still contain about twice as much Na and Cl as normal muscles, but the Ca is again practically normal while the K has increased, the sum of Na, K and Mg being 132 mE. However, in rabbits 13 and 14, which were fed whole wheat germ as the curative food, the Na and Cl of the muscles are already entirely within the normal range, but the sum of Na, K and Mg is decidedly lower than that of either normal or dystrophic muscles (111 and 121 mE.). It is, perhaps, reasonable to assume that on this curative diet the fibrosis of the muscles has already receded to normal limits, but the reason for the low cation content is obscure. Further studies along these lines would probably throw important light on the regenerative processes in the muscles.

BIBLIOGRAPHY

Bibliography

1. Adams, M.
1933. Metabolism studies following the administration of glycine.
J. Biol. Chem., 100, 111.

2. Adie, W. J., and Greenfield, J. G.
1923. Dystrophia myotonica.
Brain, 46, 73-127.

3. Aring, C. D., and Cobb, S.
1935. The muscular atrophies and allied disorders.
Medicine, 14, 77-118.

4. Barker, L. F.
1930. Discussion of five patients exhibiting certain forms of muscular wasting.
Med. Clin. North Am., 14, 109-125.

5. Barnes, S.
1932. A myopathic family with hypertrophic, pseudohypertrophic, atrophic and terminal stages.
Brain, 55, 1-46.

6. Bramwell, E.
1922. Observations on myopathy.
Proc. Roy. Soc. of Med., (Sect. Neurol) 16, 1-12.

7. Brand, E., and Harris, M. M.
1933. Further studies on the administration of glycine in muscular and neuromuscular diseases.
J. Biol. Chem., 100, xx.

8. Brand, E., and Harris, M. M.
1932. Phosphorus metabolism in muscular disease.
J. Biol. Chem., 97, lxii-lxiii.

9. Boothby, W. M.
1932. Myasthenia gravis; second report on the effect of treatment with glycine.
Proc. St. Meet. Mayo Clin., 7, 737-756.

10. Boothby, W. M.
1934. Effect of treatment with glycine and ephedrine; fifth report.
Proc. St. Meet. Mayo Clin., 9, 593-597.

11. Boothby, W. M.
1934. The clinical effect of glycine in progressive muscular dystrophy, in simple fatigability and on normal controls.
Proc. St. Meet. Mayo Clin., 9, 600-603.

12. Chanutin, A., Butt, H. R., and Royster, L. T.
1933. Study of progressive pseudohypertrophic muscular dystrophy in children after the administration of glycine and creatine.
J. Biol. Chem., 100, xxvi.

13. Clark, G. W.
Studies in the mineral metabolism of adult man.
University of California press. 1926.

14. Cohen, S. J., and King, F. H.
1931. Relation between myasthenia gravis and exophthalmic goiter.
Arch. Neurol. and Psych., 28, 1338-1345.

15. Collazo, J. A., Barbudo, J. and Torres, I.
1936. Der chemismus der muskels bei der dystrophia muscularis progressiva.
Deutch. Med. Wchschr., 62, 51-54.

16. Collier, J. and Wilson, S. A. K.
1908. Amyotonia congenita.
Brain, 31, 1-44.

17. Curschman, H.
Endocrine disorders. Oxford Press, London. 1929.
p. 171.

18. Cuthbertson, D. P., and Maclachlan, T. K.
1934. Treatment of muscular dystrophy with glycine.
Quart. J. Med., 3, 411-438.

19. Diller, T. and Rosenbloom, J.
1914. Family periodic paralysis.
Arch. Int. Med., 14, 869-880.

19 B. Fenn, W. O., and Goettsch, M.
1938. Electrolytes in nutritional muscular dystrophy in rabbits.
J. Biol. Chem., 120, 41-50.

20. Fiske, C. H., and Subbarow, Y.
1935. The colorimetric determination of phosphorus.
J. Biol. Chem., 66, 375.

21. Freiberg, I. K. and West, E. S.
1933. Glycine synthesis in pseudohypertrophic muscular dystrophy.
J. Biol. Chem., 101, 449-451.

22. Gibson, R. B., Martin, F. T., and Buell, M.
1922. A metabolic study of progressive pseudo-hypertrophic muscular dystrophy and other muscular atrophies.
Arch. Int. Med., 29, 82-96.

23. Gifford, S. R., Bennett, A. E., and Fairchild, N. M.
1929. Cataract in myotonic dystrophy.
Arch. of Ophthal., 1, 335-345.

24. Goettsch, M., and Brown, E. P.
1932. Muscle creatine in nutritional muscular dystrophy of the rabbit.
J. Biol. Chem., 97, 549-561.

25. Göettsch, M., and Pappenheimer, A. M.
1931. Nutritional muscular dystrophy in the guinea pig and rabbit.
J. Exper. Med., 54, 145-165.

26. Grinker, R. R.
1927. Pathology of amyotonia congenita. Discussion of its relation to infantile progressive muscular atrophy.
Arch. Neurol. and Psych., 18, 982-997.

27. Günther, H.
1937. Konstitutionspathologie der progressiven muskeldystrophie.
Zeitschr. f. Kinderh., 58, 757-768.

28. Holzapple, G. E.
1905. Periodic paralysis.
J.A.M.A., 45, 1224-1231.

29. Hough, Garry de N., jr.
1931. Progressive pseudohypertrophic muscular dystrophy. Report of results of treatment with adrenalin and pilocarpine with an analysis of twenty eight cases.
J. Bone and Joint. Surg., (N.S.13), 29, 825-847.

30. Hough, Garry de N., jr.
1936. The hereditary aspect of progressive muscular dystrophy.
New. Eng. J. Med., 214, 1189-1191.

31. Hurwitz, S.
1936. The primary myopathies; report of thirty six cases and review of the literature.
Arch. of Neurol. and Psych., 36, 1294-1316.

32. Janney, N. W., Goodhart, S. P., and Isaacson, V. I.
1918. The endocrine origin of muscular dystrophy.
Arch. Int. Med., 21, 188-215.

33. Kallinikowa, B.
1930. Titrimetric determination of magnesium in serum.
Biochem. Z., 220, 278.

34. Keschner, M. and Davison, C.
1933. Dystrophia myotonica.
Arch. Neurol. and Psych., 30, 1259-1275.

35. Keschner, M., and Strauss, I.
1927. Myasthenia gravis.
Arch. Neurol. and Psych., 27, 337-376.

36. Kesner, P., West, E. S., and Key, J. A.
1933. Effect of gelatin feeding upon cases of pseudohypertrophic progressive muscular dystrophy.
Proc. Soc. Exp. Biol. and Med., 32, 143-145.

37. Kirk, E., and Möller, E.
1933. Studies on a case of paroxysmal myoplegia.
Acta. Med. Scand., 80, 64-77.

38. Krabbe, K. H.
1920. Congenital familial spinal muscular atrophies and their relation to amyotonia congenita.
Brain, 43, 166-191.

39. Kramer, B., and Tisdall, F. T.
1921. The direct quantitative determination of Na, K, Ca and Mg in small amounts of blood.
J. Biol. Chem., 48, 223.

40. Kuttner, T., and Lichtenstein, L.
1930. Microcolorimetric studies. II; estimation of phosphorus. Molybdic acid-stannous chloride reagent.
J. Biol. Chem., 86, 671-676.

41. Lindsley, D. B., and Curnen, E. C.
1936. An electromyographic study of myotonia.
Arch. Neurol. and Psych., 35, 253-269.

42. Maas, O., and Paterson, A. S.
1937. Mental changes in families affected by dystrophia myotonica.
Lancet, 1, 21-23.

43. Mailhouse, M.
1910. Some observations on an isolated case of family periodic paralysis.
J. Nerv. and Ment. Dis., 37, 209-216.

44. McCrudden, F. H., and Sargent, C. S.
1918. Chemical changes in blood and urine in progressive muscular dystrophy, progressive muscular atrophy and myasthenia gravis.
Arch. Int. Med., 21, 252-255.

45. McGeorge, M.
1937. Choline esterase activity in disease.
Lancet, 1, 69-72.

46. Mettell, H. B., and Slocum, Y. K.
1933. Pseudohypertrophic muscular dystrophy. Preliminary report on the treatment of three cases with glycine.
J. Ped., 3, 352-358.

47. Morgulis, S., and Hemphill, M.
1932. An iodometric micro method for the determination of sulfates in biological material.
J. Biol. Chem., 96, 573-583.

48. Morgulis, S., and Perley, A.
1928. A note on the Kramer-Tisdall potassium method.
J. Biol. Chem., 77, 647.

49. Morgulis, S., and Spencer, H. C.
1936. Metabolism studies in nutritional muscular dystrophy.
J. Nutrition., 12, 191-204.

50. Morgulis, S., and Spencer, H. C.
1936. Studies on the blood and tissues in nutritional muscular dystrophy.
J. Nutrition, 12, 173-190.

51. Morgulis, S., and Spencer, H. C.
1936. A note on the condition of the endocrine glands in myopathy of nutritional origin.
Endocrinology, 20, 393-395.

52. Morgulis, S., and Young, A.
1931. Metabolism in myotonia atrophica.
Arch. Int. Med., 48, 564-575.

53. Nevin, S.
1934. A study of the muscle chemistry in myasthenia gravis, pseudohypertrophic muscular dystrophy and myotonia.
Brain, 57, 239-254.

54. Podolsky, F., and Malorny, G.
1935. Vergleichende untersuchungen über den natriumgehalf der saugetiermuskulatur.
Pfluger's, Arch. f. d. ges. Phys., 236, 339-347.

55. Pritchard, E. A. B.
1935. The use of prostigmin in the treatment of myasthenia gravis.
Lancet, 1, 432.

56. Reese, H. H., Burns, E. M., and Rice, C. M.
1935. Treatment for the primary myopathies.
Arch Neurol. and Psych., 33, 19-29.

57. Reinhold, J. G., Clark, J. H., and Kingsley, G. R., Custer, R. P., and McConnell, J. W.
1934. The effect of glycine in muscular dystrophy.
J. A. M. A., 102, 261-268.

58. Riesser, O.
1936. Vergleichende muskelphysiologie.
Ergebnisse Physiol., 36, 133-250.

59. Rogers, W. M., Pappenheimer, A. M., and Goettsch, M.
1931. Nerve endings in nutritional muscular dystrophy in guinea pigs.
J. Exper. Med., 54, 167-169.

60. Rosenbloom, J., and Cohoe, B. A.
1914. Metabolism studies in a case of myotonia congenita--Thomsen's disease.
Arch. Int. Med., 14, 262-274.

61. Ruhrah, J.
1935. A note on the history of pseudohypertrophic muscular dystrophy.
Am. J. Dis. Child., 50, 212-213.

62. Salit, P.
1932. A new triple acetate method for sodium determinations in biological materials.
J. Biol. Chem., 96, 659-672.

63. Schmitt, E. O. G.
1933. The use of glycine in the treatment of myasthenia gravis.
Ann. Inter. Med., 7, 948-959.

64. Singer, H. D., and Goodbody, F. W.
1901. A case of family periodic paralysis, with a critical digest of the literature.
Brain, 24, 257.

64 B. Spencer, H. C., Morgulis, S., and Wilder, V. M.
1937. A micromethod for the determination of gelatin and a study of the collagen content of muscles from normal and dystrophic rabbits.
J. Biol. Chem. 120, 257-266.

65. Spiller, W. G.
1913. The relation of the myopathies.
Brain, 36, 75-114.

66. Sullivan, M. X.
1935. Further studies on muscular dystrophies, With reference to intoxication by guanidine and simple guanidine derivatives.
J. Biol. Chem., 109, lxxxix.

67. Thorner, M. W., and Yaskin, J. C.
1937. The treatment of myasthenia gravis; report of six cases.
Am. Jour. Med. Sc., 194, 411-418.

68. Tripoli, C. J., and Beard, H. H.
1934. Muscular dystrophy and atrophy; clinical and chemical results following the oral administration of amino acids.
Arch. Int. Med., 53, 435-452.

69. Victor, J.
1934. Metabolic and irritability changes in nutritional myopathy of rabbits and ducks.
Am. J. Physiol., 108, 229-239.

70. Wang, C. C.
1935. Improvements in the methods for calcium determinations in biological material.
J. Biol. Chem., 111, 443-453.

71. Wang, C. C., Kaucher, M., and Wing, M.
1935. Mineral metabolism of twenty three adolescent girls, five nephrotic children and two boys with progressive pseudohypertrophic muscular dystrophy.
J. Biol. Chem., 109, xcv.

72. Weinbach, A. P.
1935. A micromethod for the determination of sodium.
Jour. Biol. Chem., 110, 95-99.

72 B. Whalen, M., and Shoemaker, H. A.
1936. The chloride content of human tissues.
Am. J. Physiol., 115, 476-479.

73. Williams, B. W., and Dyke, S. C.
1922. Observations on creatinuria and glycosuria in myasthenia gravis.
Quart. J. Med., 15, 269-278.

74. Wolff, H. G., Keutmann, H., and Cobb, S.
1928. The electromyogram in myasthenia gravis.
Brain, 51, 508-519.

75. Ziegler, M. R., and Pearce, N. O.
1920. A metabolic study of amyotonia congenita.
J. Biol. Chem., 42, 581-589.