A HISTOLOGICAL STUDY OF SKELETAL MUSCLE AND CONNECTIVE TISSUE IN VITAMIN C-DEFICIENT GUINEA PIGS

by

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B. S., Kansas State College
of Agriculture and Applied Science, 1941

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1943

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INTRODUCTION AND REVIEW OF LITERATURE

Although the dietary disease known as scurvy or scorbutus was known as early as the sixteenth century little extensive research was done on the subject until it was found that a deficiency of vitamin C or ascorbic acid brought about the scorbutic conditions.

Pioneers in the experimental study were Cohen and Mendel (1918) who showed that scurvy could be produced experimentally at will in guinea pigs by regulation of the diet.

Much of the first research that followed their findings dealt with the effects of the deficiency on the blood vessels. Aschoff and Koch, as reported by Eddy and Daldorf (1937), proposed that the hemorrhages in scorbutic individuals were the result of altered cement substances in the walls of the blood vessels. Later Hess (1920) attributed the hemorrhages to lesions of the endothelial cells; and Findley (1921) also reported that vitamin C-deficiency led to swelling and degeneration of the capillary endothelium resulting in hemorrhage.

Later experiments dealt with the effects of the deficiency on the bones and teeth. Howe (1922) and Harman, Kramer, and Kirgis (1938) worked on bones and teeth in scorbutic guinea pigs and found that the scorbutic condition

is characterized by a resorption of bone and dentine substance. Although the odontoblasts and osteoblasts are not destroyed they become incapable of laying down dentine and calcium. Oftentimes, however, in extreme cases of the deficiency a collagen-poor connective tissue is formed which takes the place of dentine and calcium.

Bessey, Menton, and King (1934) found that the most marked effect of deficiency on the histological structure of various organs and glands in guinea pigs was on the connective tissue.

Extensive studies of collagen formation in relation to the scorbutic state were carried on by Wolbach and Howe (1925) and Wolbach (1933, 1936). They concluded that the formation of collagen is the result of secretory activity of the fibroblasts and that in the scorbutic condition these cells are incapable of producing collagen.

Harman and White[1], who studied the comparative strength of muscle fascia in vitamin C-deficient and normal guinea pigs found that, "the connection between muscle and periosteum and between periosteum and bone was 25 to 50 percent weaker in animals on a diet deficient in vitamin C than in similar animals on a diet containing sufficient vitamin C to maintain health and growth".

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[1] Harman, Mary T. and White, Roger F.
The effect of a vitamin C-deficient diet upon the tensile strength and muscle fascia of guinea pigs (unpublished).

Of the comparatively small amount of literature to be found concerning the histological structure of skeletal muscle in vitamin C-deficient individuals the studies of Dalldorf (1929) are probably the most extensive. He found that the scorbutic state in guinea pigs was characterized by a degeneration of fibers of the intercostal, masseter, and diaphragmatic muscles. Degeneration in other muscles was absent unless produced by enforced exercise of those parts of the scorbutic animal.

In summarizing and accounting for the general scorbutic effect Wolbach (1937) stated that, "all the pathological features of scurvy are understandable only on the basis of the characterization as a condition of cessation of formation and maintenance of intercellular substances".

The purpose of the present study was to continue the work of Harman and White by making a histological study of the connective tissues involved and to enlarge upon the effects of vitamin C-deficiency on body tissue by examining histologically the skeletal muscles in scorbutic guinea pigs.

MATERIALS AND METHODS

Number and Treatment of Experimental Animals

Four different lots of animals were used for the experiment. The first lot consisted of 24 mature guinea pigs,

most of which were well over 400 g. All of the animals were fed for several days on a normal diet containing fresh green alfalfa until they became accustomed to their new environment. Then they were divided into two groups of 12 each. The animals of the first group were designated as positive controls and were given vitamin C daily in the form of "Cebione" at the rate of .66 mg per 100 g of body weight. This has been calculated to be a sufficient amount for proper maintenance of the guinea pig body. The 12 aminals of the second group were designated as negative or deficient animals. They were given approximately .14 mg of Cebione per 100 g of body weight. This is about one-fifth the required amount. The animals were not completely deprived of the vitamin in order to insure a more gradual decline of tissues. Both the deficient and the normal animals received, in addition to the vitamin C, the regular Sherman, LaMer, and Campbell (1922) vitamin C-free diet.

The second lot of animals consisted of 16 comparatively immature guinea pigs. They were placed on a normal diet with greens for a week to accustom them to their new environment, after which time they were divided into two groups of eight each. The normal and the deficient animals were given Cebione in amounts determined by body weight as were the first lot. At the time the animals were placed on the control diet most of them weighed slightly over 300 g.

The deficient animals of both the first and the second

lots were watched closely after ten days on the deficient diet and were killed shortly before they were about to die from the effects of scurvy. A normal animal of the same sex and of approximately the same weight was killed at the same time as a deficient one.

The third lot of animals consisted of 30 guinea pigs which weighed approximately 300 g each at the beginning of the experiment. These were divided into four groups. The number of animals in each group varied somewhat due to the fact that during the experiment blood was being taken from them for other experimental purposes. This process killed five of the animals within the first four days of the experiment. After this time, however, the animals showed no apparent effects of the treatment. Since none of the animals that were killed were on a deficient diet for more than four days their tissues were fixed and used for comparison as normal tissues. Of the remaining 25 guinea pigs the first two groups each consisted of three deficient animals and one normal, the third group of six deficients and two normals, and the fourth group of seven deficients and two normals. The deficient animals of the first group were placed on a partially deficient diet, determined by weight as in lots one and two, for one week, after which time they were killed. Those of the second group were partially deficient for two weeks before they were sacrificed. The third group, after two weeks of partial deficiency, were placed on a completely deficient

diet one week before they were killed; while the fourth group, after two weeks of partial deficiency, were completely deprived of the vitamin C for two weeks.

The normal animals of each group were sacrificed at the same time as the deficient ones.

The fourth lot of animals consisted of 27 guinea pigs, all of which were under 250 g at the beginning of the experiment. These animals were conditioned for the experiment by receiving the basic diet supplemented by 10 mg of vitamin C per day for 27 days. This amount is approximately 8 mg in excess of that found to be necessary for normal maintenance of the guinea pig body.

After the 27 days of conditioning a group of five animals was killed. Nineteen of the remaining 22 animals were completely deprived of Cebione and three were kept as normals. At this time their weights ranged from 300 to 350 g. The deficient animals were divided into smaller lots of four or five each. The plan was to kill one group each week after they were completely deprived of the vitamin; thus four animals were killed after seven days of deficiency, four after 14 days, and five after 22 days. Six of the deprived animals succumbed to the deficiency before their alloted time and it was necessary to kill them on various dates between the seven day and 22 day periods. The three normals were killed on the seventh, 14th., and 22nd. days, respectively. They were fed the excess amount of vitamin daily until they were sacrificed.

Kinds and Treatment of Tissues

When the animals of the first lot were killed and dissected, tissues were taken from the muscles of the foreleg. Later, when the second lot was killed, sections were taken from the masseter and hindleg as well as the foreleg muscles in an effort to detect any differences in the effects of the deficiency on different muscles. Sections of those muscles taken from the second lot showed such variations in the effects that still other muscles were included in the study of the third and fourth lots. In these animals muscles were chosen which might vary greatly in the degree to which they were used, thus tissues taken from the third and fourth lots included the occipital, intercostal, and diaphragm muscles as well as the foreleg, hindleg, and masseter.

The tissues were fixed in Zenker's or Bouin's fixative and stained in alum haematoxylin and triosin. Most of the embedding was done in paraffin, but due to difficulty in properly infiltrating tendon some of the tissues were doubly embedded in pareffin and nitrocellulose. Sections were made from seven to ten microns thick. A special effort was made to obtain longitudinal sections through tendon and muscle in order to show any effects the deficiency might have on the tissue in the region of the muscle-tendon connection. Although all of the tissues were sectioned longitudinally most of those of the first lot were also sectioned transversely

to allow closer comparison. In such cases the same tissue that was sectioned longitudinally was reblocked and sectioned transversely in order to study different views of the same part of the tissue. In addition to the sections that were made a few of the deficient and of the normal tissues were teased apart and stained with Azan triple stain.

OBSERVATIONS

Muscle Degeneration

A breaking down or disintegration of the muscle fibers was one of the most obvious effects of complete deficiency. This seemed to vary greatly in degree in different animals and in different muscles of the same animal.

In the most extreme cases of degeneration much of the muscle tissue resembled connective tissue containing only a few scattered fragments of muscle fibers (Plate III, Fig. 2). In other muscles not so completely broken down more of the fibers were remaining, but these fibers were very irregular in width and often contained small centrally located loculi (Plate II, Fig. 2). Along the length of the fiber some parts were greatly reduced while other parts of the same fiber appeared swollen or degenerated. In many of the swollen areas the substance of the muscle fiber appeared as a granular mass with no striations (Plate I, Fig. 2). It was only when the

fiber substance had completely broken down, however, that the striations disappeared. They were usually distinct even in the small fiber fragments left surrounded by "connective tissue" (Plate III, Fig. 2). Often clefts were found along the sides of the fibers which usually occurred opposite each other and alternately, giving the fibers a wrinkled appearance (Plate IV, Fig. 1).

Numbers of Nuclei. In some tissues there was an apparent increase in the number of nuclei. In contrast to the normal nuclei, which usually were located along the sides of the fibers within the sarcolemma, the nuclei of the deficient muscles were clustered in groups in the center of the fibers and sometimes apparently between the fibers (Plate III, Fig. 1).

No mitotic division was observed in the sections and neither was there any evidence of amitotic division.

Thinning of the Muscle Fibers. In some of the more compact tissues of the deficient animals in which degeneration was not extreme the muscle appeared much thinner than in normal tissue. This condition often paralleled the apparent increase in the number of nuclei (Plate IV, Fig. 2).

Classification of Muscle Degeneration

It was noted while studying the deficient tissues that the degree of degeneration of the muscle fibers tended to increase

with an increase in the number of days the animal was on a deficient diet. To simplify the study of the successive phases of the degenerating process and to bring out more distinctly the tissue differences in each animal a system of classification was devised. According to this system four major classes of tissues were recognized. Each of these classes was again divided into two minor divisions. The classes were more or less distinctly separated from each other by the presence or absence of some degenerating character, but the divisions within the classes were determined for the most part by the degree to which a character occurred. Those tissues which were the most degenerated were placed in the first class, while those which appeared normal were placed in the fourth class. Classes two and three represented intermediate stages of degeneration. The characteristics of each of these classes and their subdivisions are given in Table 1.

Differences Observed Between Lots of Tissues

By classifying all of the tissues it was found that the muscles from animals that were partially deprived of the vitamin, as a whole, did not show as extreme degeneration as those which were completely deprived at the beginning of the experiment.

In comparing the tissues from animals of lots three and four it was found that although some of the animals of lot three were completely deprived of the vitamin after two weeks

Table 1. The classification of muscles.

Class	Description
I Muscle fibers completely degenerate	a. Those tissues in which degeneration had occurred to such a degree that the greater part of the section appeared as connective tissue containing many scattered nuclei
	b. The tissues in which extreme degeneration was universal, but small sections of muscle fiber could still be distinguished among the scattered nuclei
II Fibers partially degenerate	a. Tissues whose extreme degeneration was quite evident, but not universal in the section. The greater part of the tissue was broken down, but in some parts the fibers were more compact, although usually wavy and irregular in width
	b. Those tissues in which the extreme degeneration seemed confined to small "strips" surrounded by comparatively compact tissue
III Fibers thin and irregular	a. The tissues in which short sections of some of the fibers had begun to break down. This was usually accompanied by extreme waviness, narrowness, or irregularity of the muscle fiber and also by the appearance of increased numbers and clusters of nuclei
	b. Tissues with no observable breaking down of the fibers. Abnormality was evidenced only by waviness and thinning of the fibers, or by seemingly increased numbers and groupings of nuclei
IV Fibers normal or nearly normal	a. Those tissues which had the general appearance of normal tissues, but had some slight feature which might indicate the effect of deficiency thus distinguishing them from characteristically normal sections
	b. The tissues which appear normal in every way; the standard for normality being based upon the usual appearance of normal animals studied

of partial deficiency, none of the tissues from that lot showed as extreme degeneration as did those from the preconditioned animals of lot four which were completely deprived (Plate VI, Figs. 1 and 2).

There was also a distinct contrast between the normal muscles of the animals which were fed a sufficient amount of vitamin C and those which were fed an excess of the vitamin throughout the experiment. In classifying the normal tissues of lots one, two, and three it was necessary to place some of them in the class III b. due to their wrinkled appearance. When the muscles of the animals of lot four were examined none of them could be placed in the third class and the majority were classified as IV b., that is, normal in every way (Plate VII, Figs. 1 and 2).

Differences Between Individual Muscles As Shown by Statistical Analysis

To determine the effects of the vitamin deficiency on any one muscle each muscle was given a score determined by its degree of deficiency, that is, its place in the classification (Table 1). The scores ranged from one, corresponding to the classification I a., through eight, corresponding to the class IV b. The scores for each muscle for all of the deficient animals killed on the same date in each lot were averaged and were represented at the proper location on a

graph. The graphs of the muscles of animals in lots three and four are shown in Plates VIII and IX. Regression lines were calculated for each muscle in these two lots of animals. The lines of progressive degeneration of the muscles of animals of lot four (Plate IX) showed two very erratic peaks at the points where animals were necessarily killed because of their dying condition. In order to ascertain whether these animals in any way affected the statistical analysis separate calculations were made for this lot excluding the seven erratic animals.

The regression lines were determined by the regression formula obtained by the Method of Least Squares (Snedecor, 1940):

$$E(\text{estimate of } Y) = \bar{y} + \frac{Sxy}{Sx^2}(X - \bar{x})$$

In this formula Y refers to the score and X to the date.

Analysis of Muscles from Animals of Lot Three. The regression coefficients (Table 2) show that the masseter and occipital muscles responded only slightly to the deficiency. There was also only a slight correlation between the number of days deficient and the scores of the two muscles, as shown by their correlation coefficients. In the other muscles of this lot the correlation coefficients were found to exceed the 0.1 percent level of significance.

Table 2. Regression coefficients, correlation, and standard error of individual muscles.

Muscle	n	b	r	P
Masseter	30	-.0169	.02	> .100
Occipital	29	-.0189	.03	> .100
Foreleg	30	-.0495	.59	< .001
Diaphragm	30	-.0637	.72	< .001
Intercostal	30	-.0703	.65	< .001
Abdominal	30	-.0717	.77	< .001
Hindleg	30	-.0946	.75	< .001

In the table above:

b = regression coefficient = $\frac{Sxy}{Sx^2}$

r = correlation coefficient = $\frac{Sxy}{(Sx^2)\ (Sy^2)}$

P = probability that this large a"b" or "r" could have arisen from sampling variation alone

n = number of animals

In order to determine any differences in the rate of degeneration of the muscles an analysis of covariance was applied to the individual scores and the regression lines were tested for homogeneity. The test for homogeneity follows (Tables 3, 4, and 5).

Table 3. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, and foreleg muscles (Occipital, masseter, and hindleg omitted).

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Mean square	P
Deviation of adjusted muscle means from average regression	115	74.16		
Deviations from individual muscle regressions	112	72.96	.65	
Differences among muscle regressions	3	1.20	.40	>.500

Table 4. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, foreleg, and hindleg muscles (Occipital and masseter omitted).

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Mean square	P
Deviation of adjusted muscle means from average regression	144	104.05		
Deviations from individual muscle regressions	140	99.87	.71	
Differences among muscle regressions	4	4.28	1.07	.200

Table 5. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, foreleg, hindleg, masseter and occipital muscles.

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Mean square	P
Deviation of adjusted muscle means from average regression	202	159.75		
Deviations from individual muscle regressions	196	140.31	.72	
Differences among muscle regressions	6	19.44	3.24	$<<.001$

In Table 3 the intercostal, abdominal, diaphragm and foreleg muscles were tested as a group. As the variance of their individual regression lines, when adjusted to a group mean, is less than the variance of the points determining each line these four muscles may be thought of as being very homogeneous in their degenerating rate. Table 4 shows that when the hindleg was tested with the above muscles the group became slightly heterogeneous, while Table 5 indicates the extreme heterogeneity of the group when all of the muscles were included in the test.

Thus it is seen that in this lot of animals the intercostal, abdominal, diaphragm, foreleg, and hindleg muscles were homogeneous in their reaction to the deficiency, while, when the masseter and occipital were added to this group, it became very heterogeneous. This is in agreement with Table 2

in which the regression rates of these two muscles were shown to be insignificant.

Analysis of Muscles from Animals of Lot Four. The regression coefficients (Table 6) show that the masseter muscle responded only slightly to the deficiency and that the correlation between the number of days deficient and the scores of the muscle was also very slight. In the other six muscles of this lot the correlation coefficients were found to be very highly significant.

Table 6. Regression coefficients, correlation, and standard error of individual muscles.

Muscle	n	b	r	P
Masseter	27	-.0331	.03	> .100
Occipital	27	-.1331	.73	< .001
Diaphragm	27	-.1584	.76	< .001
Abdominal	27	-.1827	.80	< .001
Foreleg	27	-.1933	.69	< .001
Intercostal	27	-.2129	.75	< .001
Hindleg	27	-.2745	.86	< .001

The tests for homogeneity for this lot of muscles are given in Tables 7, 8, 9, and 10.

Table 7. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, foreleg, and occipital muscles (Masseter and hindleg omitted).

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Errors of estimate: Mean square	P
Deviation of adjusted muscle means from average regression	129	242.80		
Deviations from individual muscle regressions	125	236.03	1.89	
Differences among muscle regressions	4	6.77	1.69	>.500

Table 8. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, foreleg, occipital, and hindleg muscles (Masseter omitted).

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Errors of estimate: Mean square	P
Deviation of adjusted muscle means from average regression	155	304.26		
Deviations from individual muscle regressions	150	282.83	1.88	
Differences among muscle regressions	5	21.53	4.31	.049

Table 9. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, foreleg, occipital, and masseter muscles (Hindleg omitted).

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Errors of estimate: Mean square	P
Deviation of adjusted muscle means from average regression	155	292.85		
Deviations from individual muscle regressions	150	254.73	1.70	
Differences among muscle regressions	5	38.12	7.62	$< .001$

Table 10. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, foreleg, occipital, hindleg, and masseter muscles.

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Errors of estimate: Mean square	P
Deviation of adjusted muscle means from average regression	181	428.54		
Deviations from individual muscle regressions	175	301.43	1.72	
Differences among muscle regressions	6	127.11	21.18	$<< .001$

Table 7 shows that when the group of muscles from animals of lot four were tested for their similarity of rate of degeneration the group was found to be homogeneous when the masseter and hindleg muscles were excluded. When the masseter was included in the test the group became heterogeneous (Table 8) and when the hindleg was included the heterogeneity was very highly significant (Table 9). Also all seven of the muscles, when tested together, showed even more extreme heterogeneity (Table 10).

Thus the intercostal, abdominal, diaphragmatic, foreleg, and occipital muscles from the animals of lot four were found to have degenerated at the same rate while on a deficient diet. The hind leg, if added to this group, causes it to become heterogeneous due to the extreme degeneration shown by this muscle.

Analysis of Muscles from 20 Animals of Lot Four. The regression coefficients in Table 11 indicate that, regardless of the omission of seven erratic animals from lot four, the masseter muscle still showed very little response to the deficiency and that the correlation between the number of days deficient and the scores of the muscle was also very slight. The other six muscles were found to react similarly to those shown in Table 6.

Table 11. Regression coefficients, correlation, and standard error of individual muscles.

Muscle	n	b	r	P
Masseter	20	-.0105	.13	> .100
Occipital	20	-.1180	.76	< .001
Diaphragm	20	-.1349	.83	< .001
Foreleg	20	-.1545	.73	< .001
Abdominal	20	-.1630	.85	< .001
Intercostal	20	-.1714	.85	< .001
Hindleg	20	-.2570	.91	< .001

Table 12. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, foreleg, and occipital muscles (Masseter and hindleg omitted).

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Errors of estimate: Mean square	P
Deviation of adjusted muscle means from average regression	94	95.91		
Deviations from individual muscle regressions	90	88.99	.99	
Differences among muscle regressions	4	6.92	1.73	.170

Table 13. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, foreleg, occipital, and hindleg muscles (Masseter omitted).

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Errors of estimate: Mean square	P
Deviation of adjusted muscle means from average regression	113	131.34		
Deviations from individual muscle regressions	108	109.09	1.01	
Differences among muscle regressions	5	22.25	4.45	.0015

Table 14. Test for homogeneity of intercostal, abdominal, diaphragmatic, foreleg, occipital, and masseter muscles (Hindleg omitted).

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Errors of estimate: Mean square	P
Deviation of adjusted muscle means from average regression	113	140.47		
Deviations from individual muscle regressions	108	99.37	.92	
Differences among muscle regressions	5	41.10	8.22	$<<$ .001

Table 15. Test for homogeneity of rate of degeneration of intercostal, abdominal, diaphragmatic, foreleg, occipital, hindleg, and masseter muscles.

Source of variation	Degrees of freedom	Errors of estimate: Sum of squares	Errors of estimate: Mean square	P
Deviation of adjusted muscle means from average regression	132	183.64		
Deviations from individual muscle regressions	126	119.47	.95	
Differences among muscle regressions	6	64.17	10.70	$<<.001$

The homogeneity tests (Tables 13, 14, and 15) of this group of 20 animals showed that the differences brought about by the omission of the erratic animals of this lot were very slight. The intercostal, diaphragmatic, occipital, abdominal, and foreleg muscles formed a homogeneous group, as in the previous test. It was noted, however, that the group of muscles tested without the hindleg and masseter was slightly less homogeneous in this analysis (Table 12) than in the preceding one (Table 7), ($P = .170$ as compared to .005). Also the group of muscles excluding only the masseter (Table 13) shows a more distinct heterogeneity ($P = .0015$) when compared with the previous test ($P = .049$).

Comparison of Analysis Groups

It has been shown that when the seven erratic animals were included in the analysis of the muscles from lot four the rate of degeneration for each muscle was increased and the correlation coefficients were slightly decreased. However, it has also been shown that excluding these seven animals from the test did not in any way change the conclusions that might be drawn from this part of the experiment. Therefore, the results from the analysis of the muscles from all 27 animals of lot four were used for further comparison with lot three.

In contrasting the rate of degeneration shown by these two lots of animals the regression coefficient, "b",(Tables 2 and 6) showed that the animals of lot four, which were preconditioned with an excessive amount of the vitamin and then completely deprived, exhibited more rapid and more complete degeneration than those of lot three which were not preconditioned. However, the significance of this regression was slightly less in all muscles from the preconditioned animals.

The tests of homogeneity have shown that the individual muscles of these two lots of animals behaved similarly in their relation to one another, with the exception of the occipital and hindleg. The occipital, which, in lot four, had the lowest rate of degeneration of its homogeneous group,

was found to be similar to the masseter in lot three in that there was no significant correlation between the number of days deficient and the score. The hindleg, in lot four, showed a significantly greater rate of degeneration than any of the other six muscles tested, but, in lot three, although it still showed the highest rate of degeneration, was found to be homogeneous with the intercostal, abdominal, diaphragm, foreleg, and occipital muscles. The masseter muscles in both lots showed no significant rate of degeneration.

Effects of Deficiency on Connective Tissue

The effects of scorbutus on such connective tissues as fascia and tendon seemed minor in contrast to the extreme effects on the muscles. In some of the more advanced cases of scurvy it appeared that the amount of connective tissue was increased due to the fact that the muscle fibers had, for the most part, disappeared and their place had been taken by collagen-poor connective tissue. As a rule the external and internal perimysium of the deficient muscles could not be distinctly differentiated from that of the normals.

In the tendon, also, little change was noted except that occasionally the tendon fibers of the deficient muscles appeared more narrow and had a tendency to be more "wrinkled" or wavy than in normal tissues.

In some of the deficient muscles it appeared that the

connection between tendon and muscle had become weakened, for the muscle fibers seemed to be pulled away from the tendon, which at that point appeared as loosely arranged connective tissue. This weakness at the connection point was also evident when the muscles were teased apart. In normal sections the muscle fibers were always closely applied to the tendon and in some cases the fibrils of the muscle appeared to continue into it. When normal fibers were teased apart muscle and tendon could not be separated.

DISCUSSION

Comparison of Present Study with Previous Work on Muscles

The occurrence of muscular degeneration in scorbutic guinea pigs as found in the present study was similar to that reported by Dalldorf (1929). However, the two studies differ in several respects.

Muscle Degeneration. Dalldorf's study showed that degeneration of muscle fibers occurred only in the masseter, diaphragmatic, and intercostal muscles. He stated that, "a search for similar changes in the extensor of the thighs and fore legs and in the psoas muscle disclosed no comparable changes". The reason given for this was that "the

weakened scorbutic guinea pig spares the muscles of the skeleton except those necessary to vital functions such as the respiratory muscles". To test this theory Dalldorf exercised scorbutic guinea pigs in rotated barrels. Microscopic examination of the leg muscles afterwards revealed muscular degeneration similar to that found in the intercostal muscles.

In the present study muscles were chosen which would probably vary in degree of use. It was found that, although the muscles tended to differ somewhat in degree of degeneration, all of those studied showed evidences of breaking down at some time.

In contrast to the comparative lack of degeneration in the leg muscles, as found by Dalldorf, the present study has shown muscles from the foreleg and hindleg to be similar to the abdominal, intercostal, and diaphragm muscles in their reactions to the deficiency. It has also been shown that, under the extreme conditions of preconditioning with an excessive amount of the vitamin followed by complete deprivation, the hindleg muscles showed greatly increased degeneration. Under all conditions studied the masseter muscles responded only slightly to the deficiency.

Correlation Between Exercise and Degeneration. According to Dalldorf the masseter is a muscle which is necessarily used by even a scorbutic animal. However, it has been noticed in caring for the animals in this experiment that the scor-

butic guinea pig has very little tendency to eat after a few weeks on a deficient diet. If, as Dalldorf has proposed, degeneration and exercise are correlated this might account for the comparative normality of masseter muscles in deficient animals. The occipital muscle also is little used in the sick animal and its low response to deficiency might be accounted for in the same way as the masseter.

Possibilities of any excessive exercise which might have produced degeneration in the leg muscles were excluded due to the fact that a scorbutic guinea pig usually, after ten days on a deficient diet, has almost no tendency to move of its own accord. However, the animal was usually disturbed when the cages were cleaned and it is possible that the exertion of movement of the body at this time was great enough to parallel the constant movement of the intercostal, diaphragm and abdominal muscles which are used in breathing. Thus it was suggested that the intensity of use of the muscle is an important controlling factor in the degree of degeneration.

Striations. Dalldorf (1929) stated that the process of degeneration was accompanied by a disappearance of striations. Whether or not he referred to a completely degenerated fiber in making this statement is not made clear. The present work has shown that striations were evident during the process of degeneration until the muscle fiber was completely broken down and had become granular in appearance.

Numbers of Nuclei. In many of the slides of deficient muscle studied there was apparently a great increase in the numbers of nuclei. This was difficult to account for in view of the fact that no mitosis was found, although some sections were specifically stained to show it if present. Dalldorf made no mention of nuclear proliferation although he did mention the occasional occurrence of mitotic figures. In the present study it was concluded that the proliferation was merely apparent as it was observed only when the muscle fibers were very thin, crowding the nuclei closer together (Plate IV, Fig. 2) or when the fibers were completely degenerated leaving scattered collagen fibers and nuclei (Plate III, Fig. 2).

Discussion of Differences in Lots of Tissue Studied

As a whole the muscles from partially deficient animals did not show as extreme effects from the deficiency as those which were completely deprived of vitamin C. It seemed that the animals were able to survive for from five to nine weeks on a very low level of vitamin C, while with complete deprivation degeneration was in some cases extreme within ten days.

The more sudden and extreme reaction of the deficient animals of lot four is difficult to explain. It is a question as to whether the extreme response of these animals was due to

their complete deprivation, to their preconditioning, or to both factors. It has been pointed out that in lot three those animals which were partially deficient for two weeks and then completely deprived for two weeks did not show as extreme or as sudden degeneration as those animals of lot four which were preconditioned. Thus it would seem that the preconditioning is perhaps, in this case, the more important factor. In an attempt to explain this it has been suggested that the animals of this lot had become so accustomed to the high level of vitamin during the 27 days of conditioning previous to the beginning of the experiment that they were unable to adjust themselves again to such an extreme change as a higher requirement level had been induced. Thus it is possible that those animals which were placed on a partially deficient diet could adjust themselves somewhat to the low level of vitamin C and therefore were able to withstand the deficiency for a longer period of time.

The appearance of the "super normals" of lot four is also difficult to account for. It is possible that the condition of wrinkled fibers observed in any of the muscles studied was the result of the fixing process and that there was a variation in the resiliency and "toughness" of the muscle fibers causing the weaker ones to "wrinkle" with treatment. This was more apparent in deficient than in normal muscles and was often accompanied by other more definite degenerating

characters. It is possible that the normal muscles of lot four under the influence of an excessive amount of vitamin C had become strong enough and resilient enough to withstand the fixing technique.

Theories Concerning Muscle Degeneration

The Role of Vitamin C in Maintenance of the Muscle Fiber. The exact cause or causes of the breaking down of the muscle fibers are not known, but it is known that deficiency of vitamin C has a distinct effect on the connective tissue of a body. As the exact nature of a muscle fiber or muscle cell is a point of much controversy it is not easy to discern which part of the muscle fiber is affected by the deficiency. If the muscle fibril is thought of as a connective tissue element it might be assumed that these elements suffered the same effects of deficiency as other connective tissues and that the breaking down of these fine supporting fibrils was responsible for the breaking down of the whole structure of the muscle fiber.

It is also possible that the role of maintaining sarcoplasm belongs as much to vitamin C as that of maintaining intercellular materials such as collagen.

Indirect Causes of Muscle Degeneration. For some time it was thought that the breaking down of the muscle fiber was caused by deficient oxygination of the tissues resulting

from hemorrhages. However, it has been found that the greatest degree of degeneracy is not always found in the areas where hemorrhages occurred.

As the scorbutic animals ate very little after the first week on a deficient diet a question arose concerning the possibility of producing the same muscular degeneration in scorbutic guinea pigs by merely starving them. This experiment was not carried out in the present work, but results have been reported on it before. In 1939 Chor and Dolkart starved guinea pigs and studied their muscular tissues. They reported that, "microscopically many fibers showed fading of the cross striations. These fibers were narrow and pale but showed no definite necrosis or degeneration". Thus the effects of starvation could not be confused with the effects of scorbutus.

Disuse atrophy has also been studied in some animals. Chor and Dolkart (1936) in reporting on the disuse of muscles in the monkey stated that, "disuse atrophy consists primarily of a uniform reduction of the bulk of each muscle cell, especially of the sarcoplasm. It is not attended by any evidence of degeneration or attempts at regeneration". Thus the possibility that the mere disuse of the parts studied could produce the scorbutic effect was also discarded.

It was found by Chor, Dolkart, and Davenport (1936) that the changes occurring in denervated skeletal muscle of the monkey and cat consisted of narrowing of the individual fibers

followed by granular and vacuolar degeneration with replacement by fibrous tissue. This description of degeneration is strikingly similar to that found in scorbutic guinea pigs as given in this discussion. Meyer and McCormick (1928) reported that a degeneration occurred in the nervous tissue of scorbutic animals but there has been no study made concerning the correlation of nervous and muscular degeneration in vitamin C-deficient animals. The fact that these two types of degeneration are so similar in their progressive nature and in the rapidity of their progression suggests a new and possibly important aspect of the study of muscular degeneration in scorbutus.

EXPLANATION OF PLATE I

Fig. 1. Longitudinal section from the hindleg of a normal animal. This section shows the compactness of normal tissue, the evident striations, and the usual distribution of nuclei. This tissue was classified as IV b.

Fig. 2. Longitudinal section from the foreleg of an animal partially deficient for seven weeks. In this section it is seen that some of the muscle fibers are very irregular in width and in some of the swollen areas the sarcoplasm has become granular in appearance. This tissue was classified as II b.

PLATE I

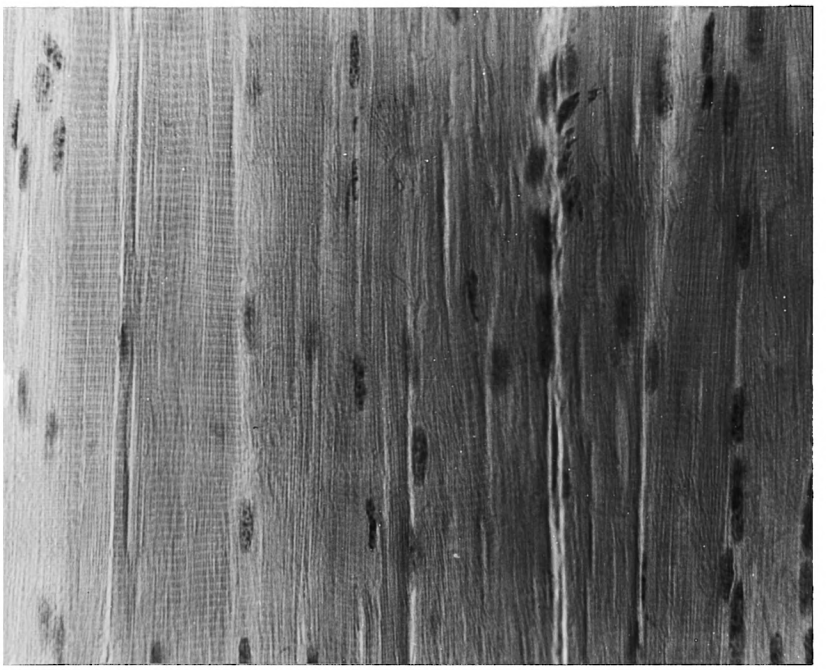

Fig. 1

Fig. 2.

EXPLANATION OF PLATE II

Fig. 1. Transverse section of foreleg muscle of normal guinea pig. This figure shows the regularity in width of the normal muscle fibers and the compactness of the fibers. The longitudinal section of the tissue was classified as IV b.

Fig. 2. Transverse section of foreleg from guinea pig partially deficient for four weeks. The irregularity in width of the deficient muscle fibers can be noted here. In some of the fibers the sarcoplasm has begun to break down and small, centrally located loculi are seen. The fibers are loosely held together by connective tissue. The longitudinal section of this tissue was classified as II a.

PLATE II

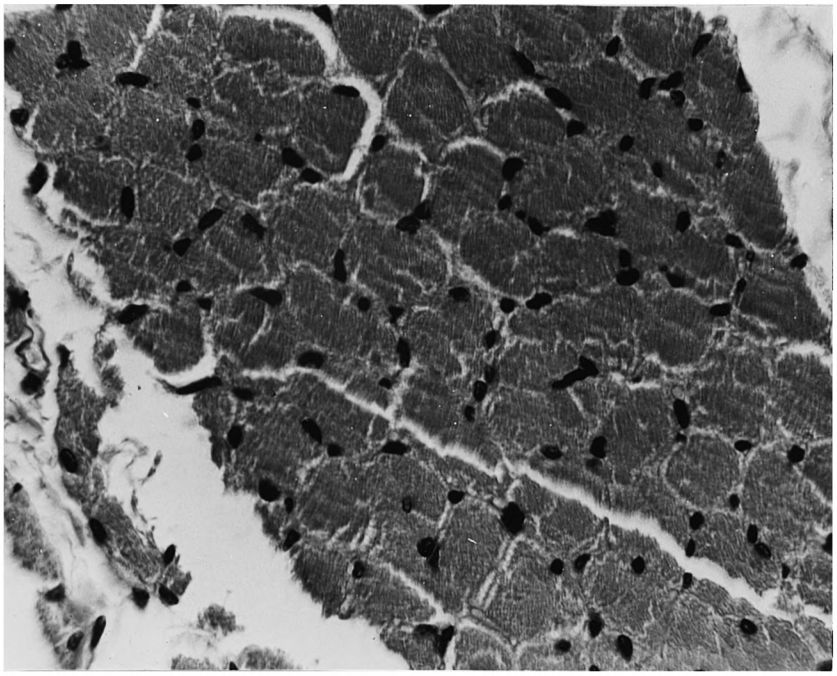

Fig. 1

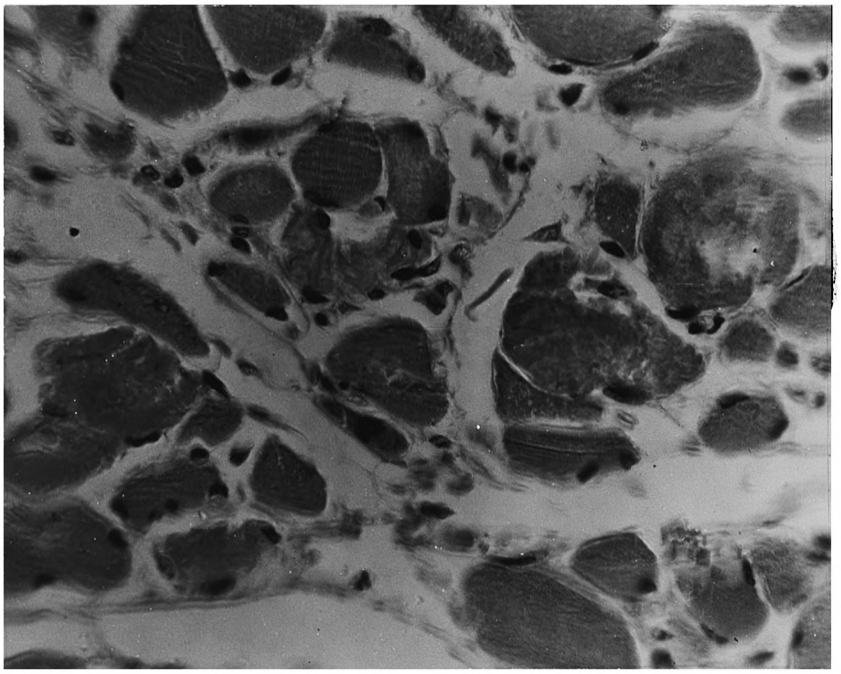

Fig. 2

EXPLANATION OF PLATE III

Fig. 1. Longitudinal section from hindleg of an animal partially deficient for ten weeks. In this tissue parts of some of the vibers are completely degenerated and in their place only loose connective tissue remains. The tissue was classified as II a.

Fig. 2. Longitudinal section of foreleg muscle from an animal partially deficient for nine weeks. Here only a few sections of muscle fibers are remaining; for the most part the tissue appears as loose connective tissue with many scattered nuclei. It is noted that in the few fibers seen the striations are still evident. This tissue was classified as I a.

PLATE III

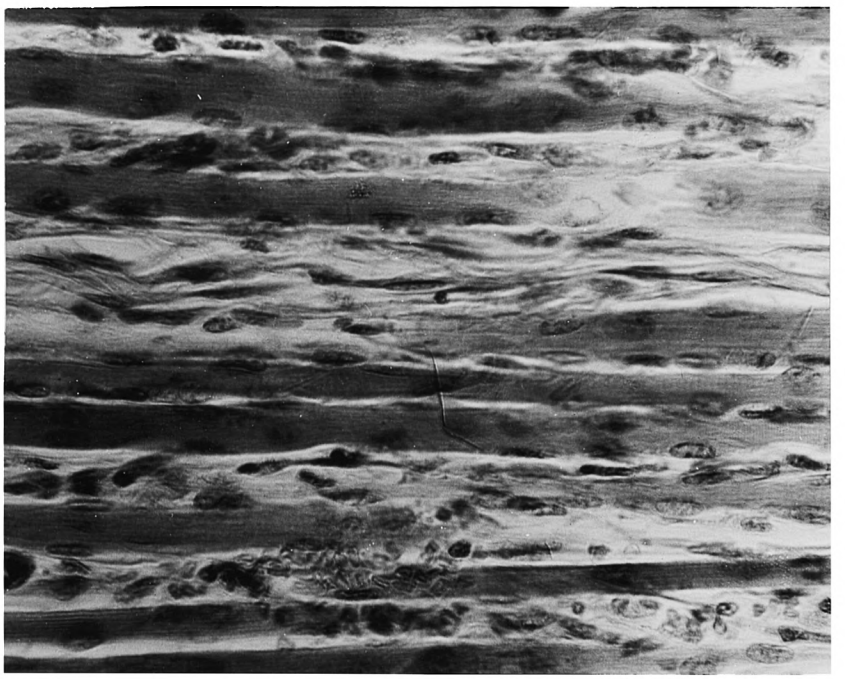

Fig. 1

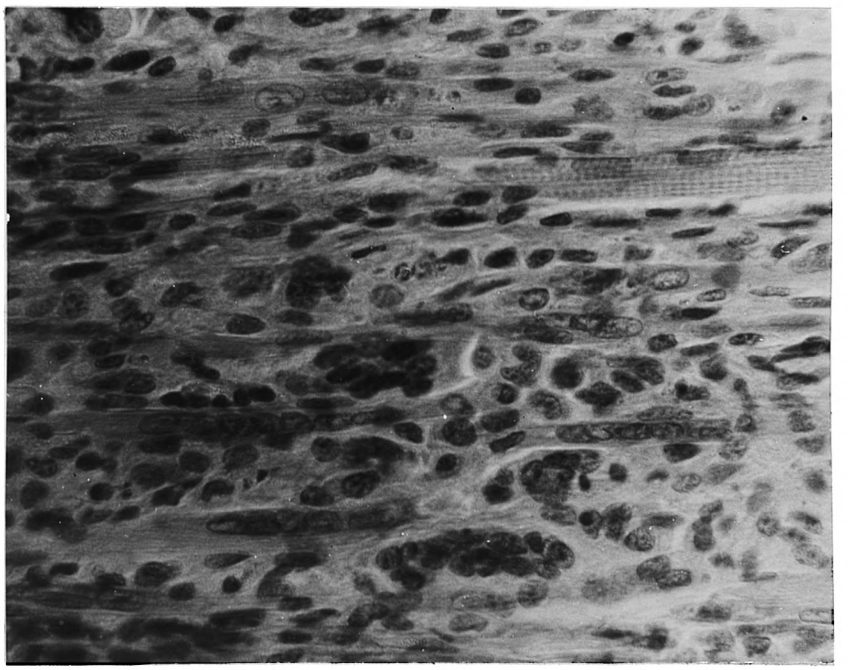

Fig. 2

EXPLANATION OF PLATE IV

Fig. 1. Longitudinal section of diaphragm muscle from an animal partially deficient for two weeks followed by one week of complete deficiency. Here no degeneration can be seen in the fibers, but they show abnormality by their extremely wrinkled and narrowed fibers. This tissue was classified as III b.

Fig. 2. Longitudinal section of foreleg muscle from an animal partially deficient six weeks. This section appears normal except for the extreme narrowness of the muscle fibers and numerous nuclei. The tissue was classified as IV a.

PLATE IV

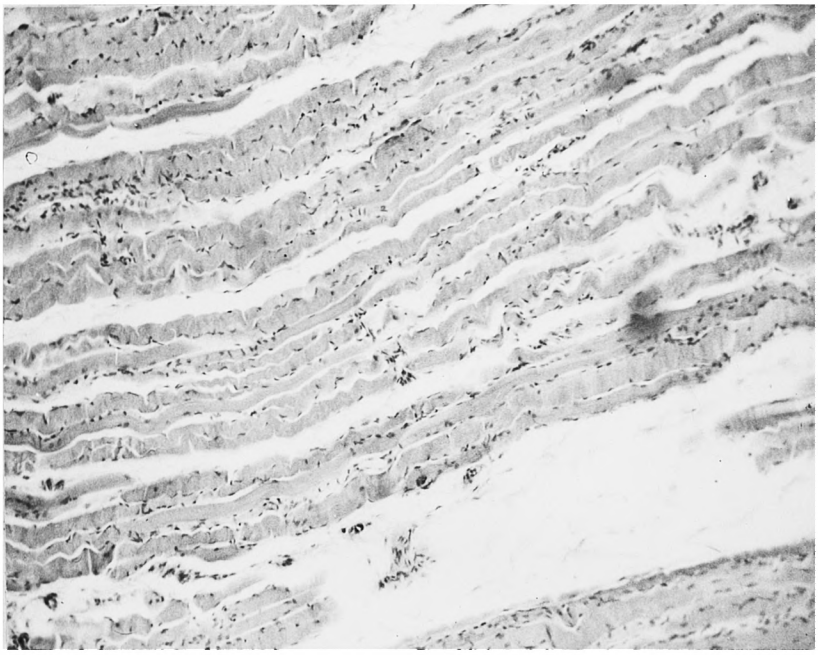

Fig. 1

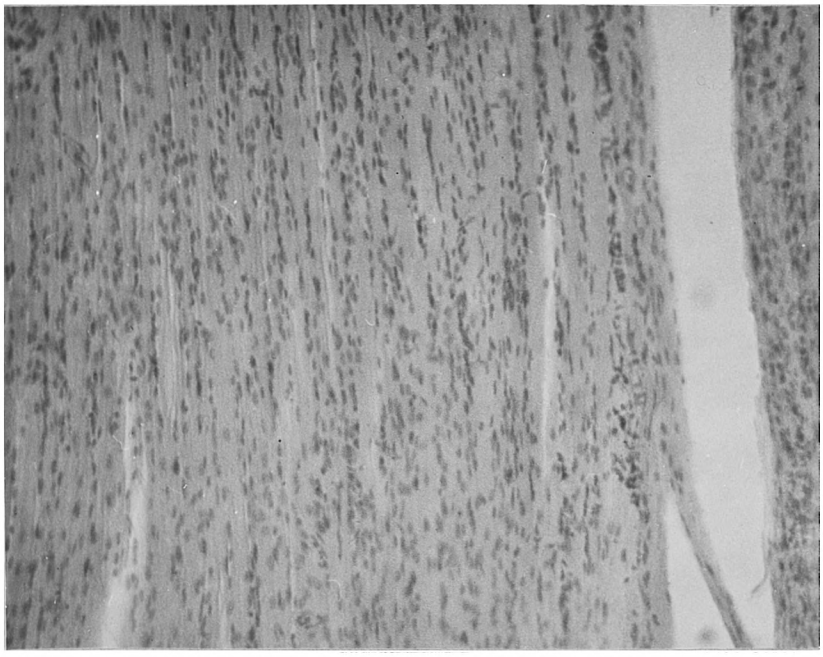

Fig. 2

EXPLANATION OF PLATE V

Fig. 1. A longitudinal view of teased muscle from the foreleg of an animal on a partially deficient diet for two weeks and then a completely deficient diet for two weeks. In this figure only one degenerating fiber is seen among many others which appear normal. A longitudinal section of this muscle was classified as III b.

PLATE V

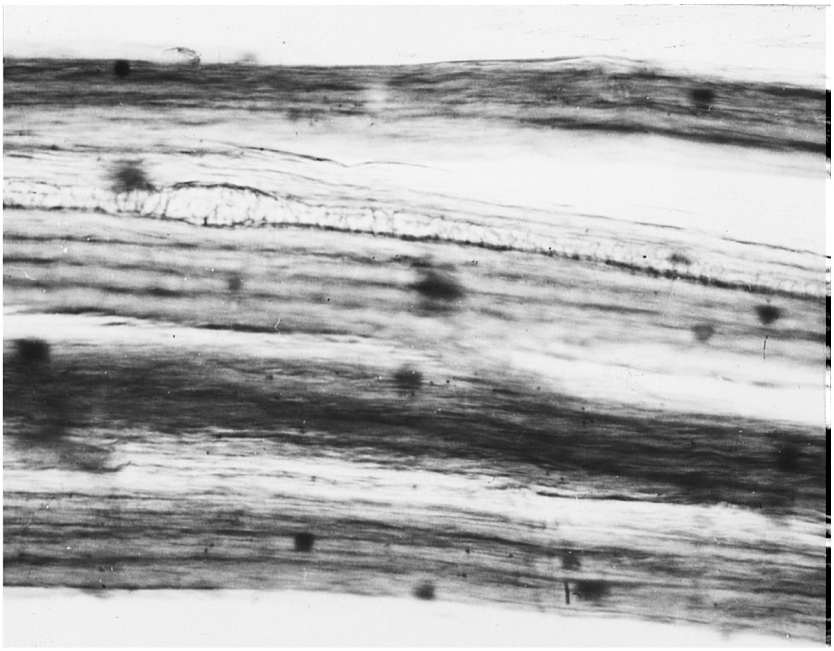

Fig. 1

EXPLANATION OF PLATE VI

Fig. 1. This block diagram rêpresents the classification of the seven muscles studied from the animals of lot three. The animals which were killed after four to 14 days had been partially deficient for that length of time. The first group of animals killed after that thme had been partially deprived of the vitamin for 14 days and had then been completely deprived of it for one week. The second group had been partially deprived for 14 days and then completely deprived for two weeks. The diagram shows the tendency for a gradual increase in the degree of muscle degeneration as the days of vitamin C-deprivation are increased.

Fig. 2. This diagram represents the classification of the seven muscles studied from the animals of lot four. These animals were fed an excess of vitamin C for 27 days before the experiment was started and then were completely deprived until they were killed. Here the correlation between days deficient and degree of deficiency is even more evident than in Fig. 1. Also it is noted that a greater number of muscles show extreme degeneration in these animals which were completely deprived of the vitamin.

PLATE VI

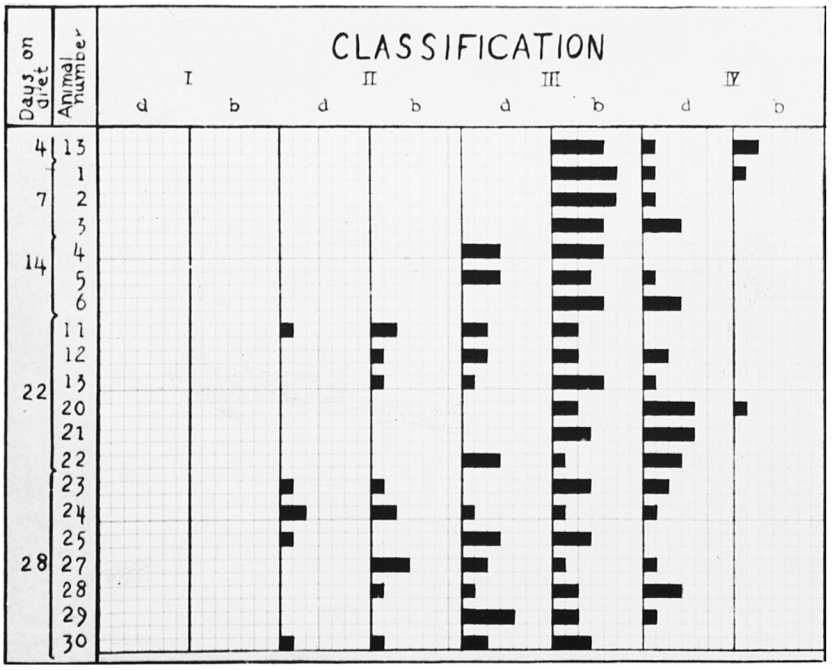

Fig. 1

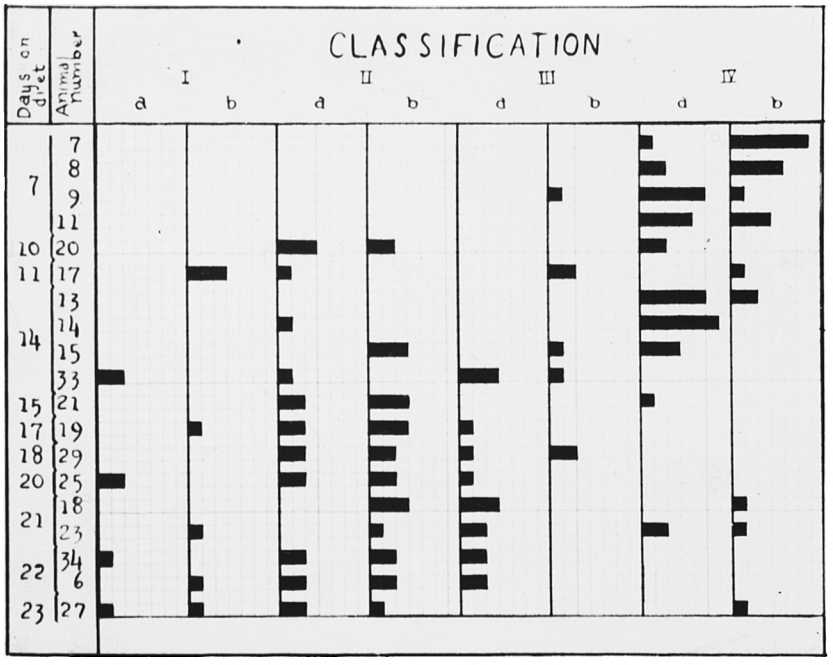

Fig. 2

EXPLANATION OF PLATE VII

Fig. 1. This diagram represents the muscles from the normal animals of lot three. It is noted, however, that they are not confined to the normal classification of class IV.

Fig. 2. This diagram represents the muscles from the normal animals of lot four. Here all of the muscles were confined to class IV a and the majority to IV b.

PLATE VII

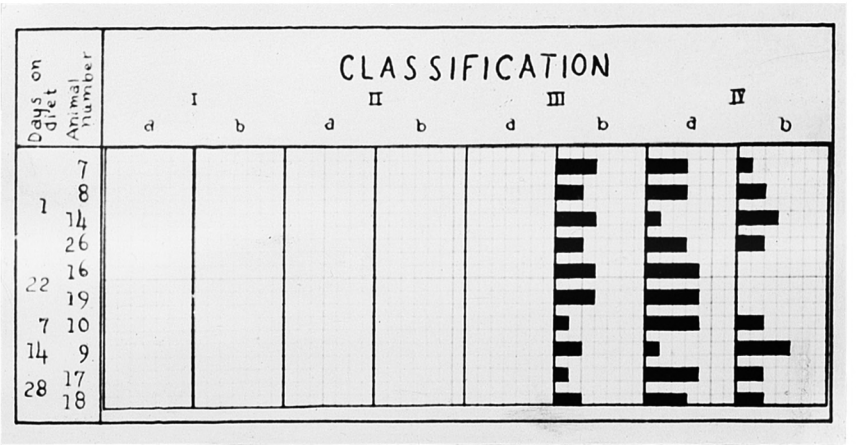

Fig. 1

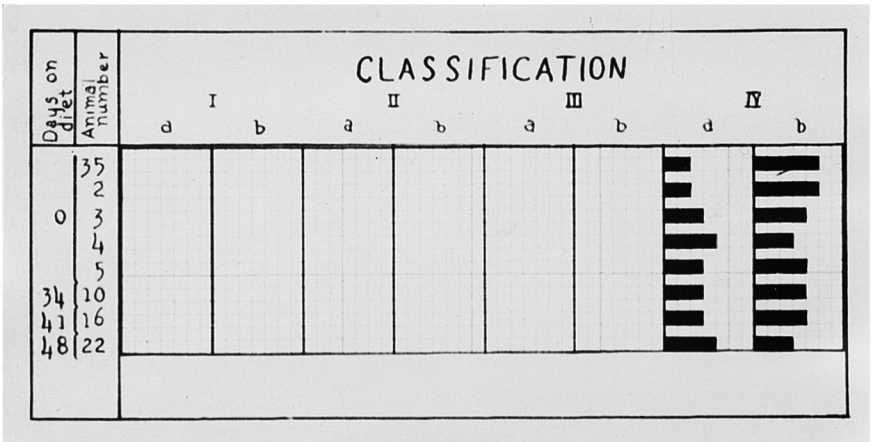

Fig. 2

EXPLANATION OF PLATE VIII

Fig. 1. On this graph the scores, i.e., the classifications of each kind of muscle studied from animals of lot three have been averaged for each group of animals killed. Thus the trend toward degeneration for any one muscle can be traced from the beginning to the end of the experiment.

PLATE VIII

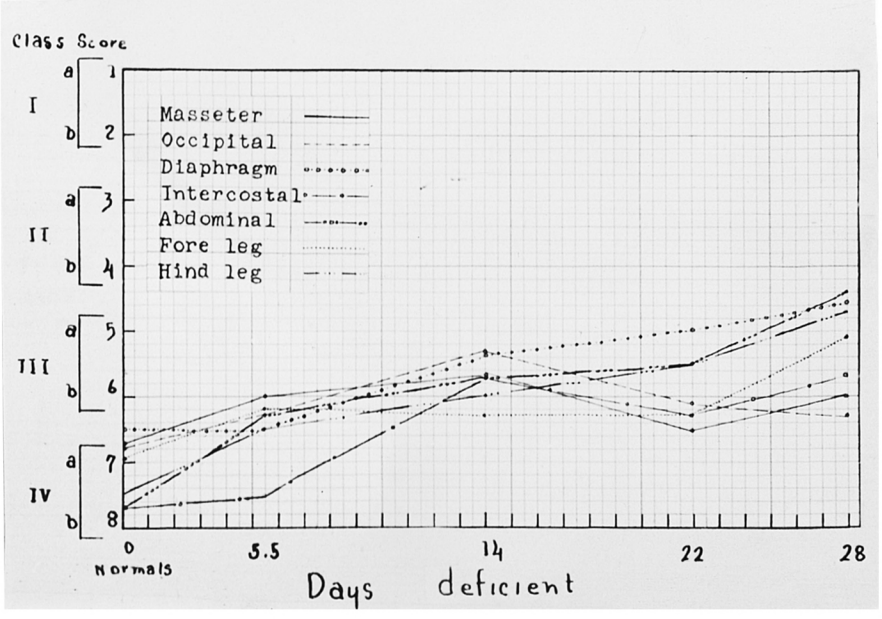

Fig. 1

EXPLANATION OF PLATE IX

Fig. 1. This is a graphic representation of the trend in deficiency of individual muscles from the animals of lot four. The two extreme peaks at 10.5 and 17.5 are accounted for by the fact that at these points animals were necessarily killed before their allotted time because of their dying condition.

PLATE IX

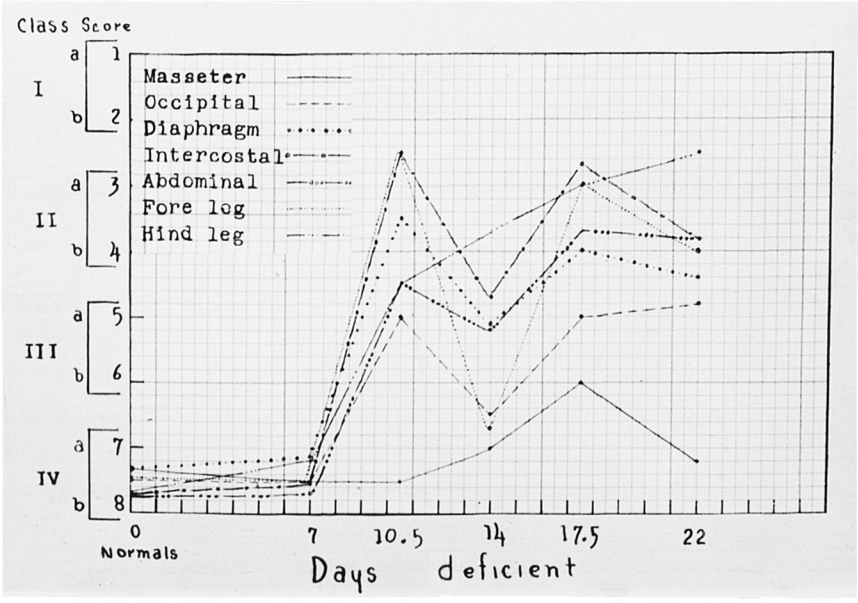

Fig. 1

EXPLANATION OF PLATE X

Fig. 1. This graph shows regression lines calculated for the animals of lots three and four. Homogeneity tests of different groups of muscles showed that in lot four the intercostal, foreleg, abdominal, diaphragm, and occipital muscles reacted similarly to the deficiency. The hindleg, because of its significantly extreme degeneration, and the masseter, because of its small degree of degeneration, could not be included in this homogeneous group.

In lot three the abdominal, diaphragm, hindleg, foreleg, and intercostal muscles formed a homogeneous group with the exclusion of the masseter and occipital muscles which reacted only slightly to the deficiency. Also here the more sudden and extreme degeneration of the muscles from the preconditioned animals of lot four is illustrated.

PLATE X

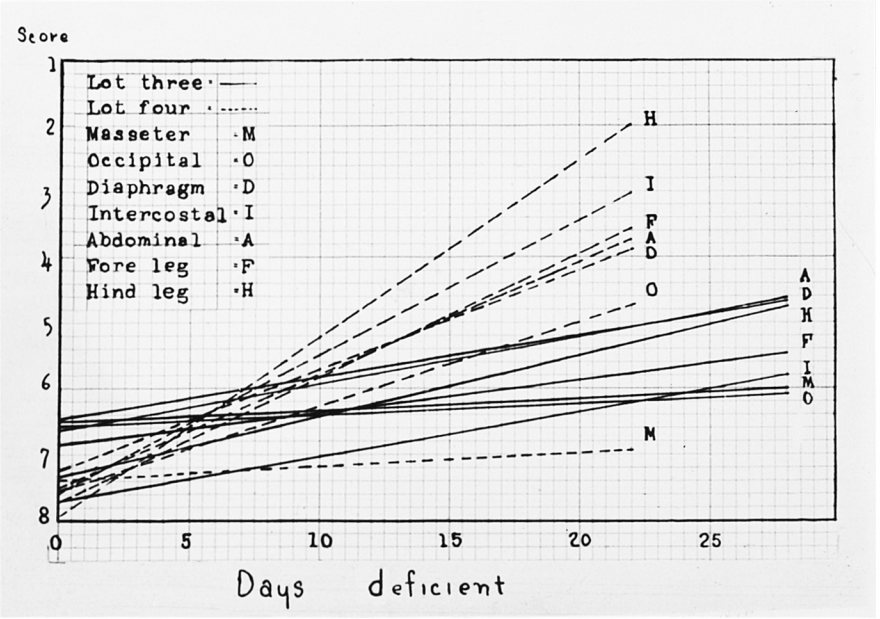
Score
1
2
3
4
5
6
7
8
Lot three
Lot four
Masseter ·M
Occipital ·O
Diaphragm ·D
Intercostal ·I
Abdominal ·A
Fore leg ·F
Hind leg ·H
H
I
F
A
D
O
A
D
H
F
I
M
O
M
0
5
10
15
20
25
Days deficient

Fig. 1

SUMMARY

1. Muscles from vitamin C-deficient guinea pigs showed a gradual degeneration of muscle fibers, increasing with the number of days the animals were on a deficient diet.

2. Degeneration was manifested first in the thinning or wrinkling of the muscle fibers followed by the complete breaking down of the ribrillar structure of the muscle.

3. Nuclear proliferation which paralleled the degenerating process, for the most part, appeared to be due to the close proximity of the nuclei in the thinning fibers.

4. Striations were evident at all times in the fibers except when complete degeneration had occurred.

5. Degeneration was found in all muscles studied regardless of use. The masseter and occipital showed the least response to the deficiency, while the hindleg muscles showed the most extreme response. The intercostal, diaphragmatic, abdominal, and foreleg muscles reacted similarly.

6. Histological effects of the vitamin deficiency on connective tissue were minor in comparison to the extreme effects on the muscle substance.

ACKNOWLEDGMENTS

Acknowledgment is made to Dr. Mary T. Harman, professor of zoology who suggested the problem and supervised the work, and to Mary F. Schroller, who assisted in much of the technique work and who cared for the experimental animals. Appreciation is also expressed to Dr. H. C. Fryer, associate professor of mathematics, for his criticism of the statistical analysis of the data.

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