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INTRODUCTION

For many years muscular dystrophy due to dietary insufficiency has been postulated. The investigational work has been for the most part concentrated on the factors, producing muscular dystrophy. Although some work has been done concerning the histopathology of nutritional muscular dystrophy, there has been no satisfactory work showing the complete picture. To fill this gap, guinea pigs were used as the experimental animal; attention was focused on the pathology of muscular dystrophy, cell differentiation by vital staining and the repair of damaged muscle fibers. The findings recorded should be useful in the explanation of pathology of the white muscle disease in lambs.

LITERATURE REVIEW

The production of generalized muscular lesions by dietary means has been achieved by many workers in different species of animals. Two factors have been discussed as being important in the production of experimental nutritional muscular dystrophy.

The first factor in question concerns the direct and toxic effect of cod liver oil in the production of muscular dystrophy. This effect of cod liver oil upon heart and skeletal muscles was demonstrated by Agduhr (1926, 1927) in white mice, rats, dogs, pig, cat, rabbits, and calves; by Hoejer (1927) and Hendriksen (1928) in young rats, by Malmberg (1929) in children and Wahlin (1931) in guinea pigs. These workers assumed that the cod liver oil fed those animals in large doses over a long period of time was noxious.

Supporting this view, Madsen, McCay, and Maynard (1935) demonstrated that cod liver oil added to a synthetic diet or natural diet resulted in dystrophy of skeletal and heart muscle in guinea pigs, rabbits, goats and sheep. They held the saponifiable fraction of the cod liver oil responsible for the development of the lesions.

They also thought that some factor in the synthetic diet increased the toxic effect of cod liver oil. Confirming these results concerning the harmfulness of the saponifiable fraction, McCay, Paul, and Maynard (1938) demonstrated that the hydrogenation of cod liver oil decreased the production of muscular dystrophy in guinea pigs. Later, Davis, Maynard, and McCay (1938) confirmed the results obtained by Madsen et al (1935); but denied the synergetic action of the synthetic diet in the production of dystrophy of skeletal muscles in guinea pigs.

Burack and Zimmerman (1937) reached the conclusion that cod liver oil in therapeutic doses could not exert the injurious effect in view of the small percentage of rats and mice affected and the large amount of oil used. A similar suggestion was made by Davis and Maynard (1938) who postulated that cod liver oil could be fed to dairy calves in the amount that might be required to supply vitamin D without harm.

The second factor involved in the production of dystrophy of skeletal muscle concerns the deficiency of vitamin E in the diet which resulted from the presence of rancid cod liver oil. The destructive effect of rancid cod liver oil on vitamin E also takes place in the digestive tract. This effect of cod liver oil may be considered as indirect in the production of dystrophy

of skeletal muscle. The destruction of vitamin E by oxidative reaction initiated by autoxidation of cod liver oil was first demonstrated by Cummings and Mattill (1931) and Mattill (1938 b) who postulated that the oxidative rancidity of animal fat may be the principal cause of muscular lesions and that the dystrophy can be prevented by the addition of two percent wheat germ oil to a synthetic diet containing no other source of vitamin E. Mattill again (1939) (cited by Shimotori et al 1940) explained the production of dystrophy in herbivora as follows: "Herbivorous animals have a large cecum where the food remains long enough for autoxidative changes to progress further and more rapidly than in omnivorous animals such as rats. From this point of view the long search for a toxic factor in Cod liver oil and for cures of the disorders produced thereby (cattle, rabbits, poultry) may have been following a wrong trail." With additional evidence, Mattill and Golumbic (1942) concluded that no distinction should be made between cod liver oil induced muscular dystrophy in rabbits and nutritional muscular dystrophy produced by lack of vitamin E. These workers disproved the theory that cod liver oil had a direct toxic action. However, Cox and Roos (1934) failed to produce lesions in rats even though they fed them on a diet containing high levels of cod liver oil.

It seems probable that nutritional muscular dystrophy is not due to a single dietary factor. Muscular dystrophy was produced in guinea pigs and rabbits with a diet containing no cod liver oil by Goettsch and Pappenheimer (1931), and Woodward and McCay (1932). These results would seem to exclude the fact that the toxicity of cod liver oil was the only factor in the production of nutritional muscular dystrophy.

In regard to vitamin E deficiency being responsible for muscular dystrophy, considerable work has been done. Muscular dystrophy was produced in rats reared on a vitamin E deficient diet by Olcott (1938), Evans, Emerson, and Telford (1938), Knowlton and Hines (1938), Pappenheimer (1939-1940) and by Mackenzie et al (1941 a) in the rabbit. Shimotori, et al (1940) confirmed these findings and reported that wheat germ, wheat germ oil and alpha-tocopherol protected the guinea pig against nutritional muscular dystrophy which can be produced in these animals when reared on the Madsen, McCay, and Maynard (1935) cod liver oil supplemented diet. Shimotori et al (1940) suggested that vitamin E was the specific factor which prevented nutritional muscular dystrophy when the vitamin B complex was adequately supplied. A similar suggestion was made by Mackenzie and McCollum (1940). Using the modified dystrophy producing diet 13 of Goettsch and Pappenheimer through the addition of ten percent defatted wheat

germ as a source of the water soluble factor, muscular dystrophy was produced in rabbits. The dystrophy was accompaniedby an increased creatin output which was considered an accurate index of the inception of dystrophy. The condition was cured by administration of alpha-tocopherol. Mackenzie and McCollum (1940) believed that the deficiency of fat soluble factor, vitamin E, was responsible for the dystrophy, and not water soluble factors. Mackenzie, Levine, and McCollum (1940) confirmed the previous finding that the water soluble factor in wheat germ was not necessary for the treatment of dystrophic conditions of skeletal muscles and furthermore that no quantitative or synergetic relationship existed between alpha-tocopherol and the water soluble factor. Recently Pappenheimer (1943) in an excellent literature review, stated that vitamin E was required in the normal metabolism of skeletal muscle.

Contrary to all these facts, Harris (1941) and Minot, and Frank (1944) reported no therapeutic effect following the use of various preparations of vitamin E in the treatment of progressive muscular dystrophy in man. These negative results obtained by Harris (1941), and Minot, et al (1944) were explained by Milhoret and Bartel (1945) who stated that the defect in the utilization of tocopherol in progressive muscular dystrophy was

due to a deficiency in the reaction of the condensation of tocopherol and inositol in the gastro-intestinal tract and that the degree of this deficiency appeared to determine the rapidity with which muscular dystrophy progressed. They also pointed out that in mild cases patients can synthesize sufficient amounts of the condensation product if large amounts of both inositol and tocopherol were given together, but those in which the disease progressed more rapidly would probably require the condensation product itself.

Goettsch and Pappenheimer (1931), Rogers et al (1931), Olcott (1938), and Pappenheimer (1939, 1940) found that the nutritional muscular dystrophy was confined to skeletal muscle. No alteration was found in the central nervous system, in the large peripheral nerve trunks, nor in terminal nerves and end plates.

It seems probable that other dietary constituents were involved in muscular dystrophy. Some evidence indicates that a vitamin C deficient diet can produce muscular dystrophy. Such results were observed by Dalldorf (1929) in guinea pigs reared on a vitamin C deficient diet and also by Hjarre and Lilleenngen (1936) in calves.

There may be other factors which produce muscular dystrophy in lambs and calves, so called "white muscle disease" or ("stiff lamb" disease). The heart and skeletal muscles were involved. This condition of young lambs three

to ten weeks old was first observed by Metzer and Hagan (1927) in this country. Marsh (1932) confirmed Metzer's observation and suggested that an improper calcium-phosphorous ratio in the diet played a role in this condition. Similar observations were made in lambs by Willman et al (1934), Vawter and Records (1939), Sholl (1939), Thorp (1942), Salyi (1942), Cheng (1945) and Cameron (1945). Hjarre and Lilleenngen (1936) and Vawter and Records (1947) reported a similar condition in calves of ten days to two months of age.

The white muscle disease of lambs was cured by Sholl (1939) and Willman et al (1946) by administration of wheat germ oil and vitamin E respectively. Recently, Vawter and Records (1947) reported that the condition in calves disappeared within a week to ten days after the diet of breeding cows was changed to green leafy alfalfa or green pasture. Both forms of forage considered good sources of vitamin E by Hathaway, et al (1932) and Hathaway, et al (1934).

Muscular dystrophy can be caused by factors other than dietary. Toxic, infectious, and traumatic agents can produce muscular changes. Stemmler (1914) listed the conditions under which muscular degeneration occurred: Typhus abdominalis, diphtheria, pneumonia, scarlet fever, tetanus, peritonitis, venom intoxication, and anaphy-

lactic conditions. Forbus (1926) observed muscular degeneration following pneumonia in man. Similar changes were observed by Steiner et al (1946) and by Clawson et al (1947) in rhuematoid and rhuematic arthritis of man.

Muscular degeneration was produced experimentally in various animals by Forbus (1926) following intra muscular injection of powerful irritants or by cutting off the blood supply; by Fishback and Fishback (1932 a, b) who used different types of trauma; Clark and Blomfield (1945), Clark (1946) by ligation of the artery to the muscle groups. Harman (1947) produced the condition by acute ischemia induced in the hind limbs of rabbits.

In this laboratory muscular dystrophy was produced in growing guinea pigs by feeding a synthetic diet according to the formula of Davis et al (1938)together with a supplement of 0.5 gram per animal daily of U. S. P. grade cod liver oil. The pathological changes might be attributed to the direct toxic effect of cod liver oil as many workers have pointed out or to the destructive effect on vitamin E in the diet by rancidity as many people have postulated, or finally to a synergism of both factors.

Our aim was to study the pathology of nutritional muscular dystrophy, not the etiology.

MATERIALS AND METHODS

The objectives of the experimentswere to induce the development of muscular dystrophy, attempt to differentiate the cells participating in the degenerative and regenerative processes and to study the process of regeneration of muscle cells at different stages in the developmental process.

The guinea pigs used in the experiments were divided into three groups of eight animals each. They weighed between 220 and 310 grams when placed on the experiments. Two guinea pigs from each group were kept as controls. The controls were kept in a pen and fed rabbit pellets,[x] lettuce, and grass. The other six guinea pigs were confined in individual cages with screen bottoms, no bedding being used. They were fed the basal synthetic diet used by Davis, Maynard and McCay (1938). It was made up as follows:

Casein	15 parts
Sucrose	15 "
Starch	33 "
Yeast	7 "
Lard	3 "
Salt mixture	4 "
[xx]Regenerated cellulose	20 "

x --- Rockland Rabbit Pellets
xx --- Provided through the courtesy of Dr. Ralph T. Cornwell, Sylvania Division American Viscose Corp. Fredericksburg, Va.

The salt mixture was a modification of Osborne and Mendel salt mixture prepared according to Hawk and Oser (1931). All guinea pigs were started on rabbit pellets. The diet was gradually replaced by the synthetic diet over a period of 3 to 4 days. The basal synthetic diet was kept in a refrigerator (approximately 40° F.) throughout the entire experiment in order to avoid the development of rancidity in the lard. The basal synthetic ration was fed ad libitum. The guinea pigs on the experiment received as a supplement 0.5 gram per animal daily of cod liver oil which had a potency of not less than 2,000 U. S. P. units (54,600 units per fluid ounce) of vitamin A per gram and 250 U. S. P. units (6,800 units per fluid ounce) of vitamin D per gram.

In addition, each animal, including control guinea pigs of Groups II and III received an ascorbic acid tablet, dissolved in one ml. of water, every other day. Each tablet contained 0.39 grains equivalent to 500 international units of vitamin C. The cod liver oil and aqueous solution of ascorbic acid were given the guinea pigs separately by pipette. The cod liver oil and ascorbic acid tablets were stored in the refrigerator. The guinea pigs were weighed every other day.

Group I was used to produce muscular dystrophy

with the dietary regimes just described. The guinea pigs were killed at different stages of the disease. The symptoms consisted of decreased activity, difficulty in walking and paralysis of fore and hind legs.

As an aid in the differentiation of the cells participating in degeneration and regeneration of muscle fibers, vital staining with trypan blue was carried out with guinea pigs of Group II. Following the technic of Menkin (1929) and Russell et al (1943) the trypan blue was given subcutaneously in the flank of the animal in a one percent concentration in physiologic solution of sodium chloride. The first injection of the dye was made on the 20th day of the experiment. At that time the animals began to show inactivity which may be considered as the first symptom of muscular dystrophy. The amount of dye given in each instance was determined by the weight of the animal and was administered on alternate days. The guinea pigs weighing from 400 to 450 grams were given 35 mg of the dye each time until a total of 175 mg had been administered. Only 30 mg of the dye was injected each time in guinea pigs weighing from 310 to 360 grams until a total 150 mg of the dye was introduced.

The plan was to kill the animals at definite intervals after the injections of trypan blue had been completed. However, one guinea pig died after 3 injec-

tions and two others succumbed after four injections. The remaining guinea pigs received five injections, and were killed one day and ten days later.

The Group III animals were used to study the regeneration of damaged muscle fibers at different stages of the disease. The guinea pigs were fed the same diet and supplements as used for the Group I and II until they began to show some difficulty in locomotion. The time of development of muscular dystrophy varied from one animal to another. Therefore, each guinea pig was closely observed. Whenever one began to show the first symptoms of decreased activity followed by slow locomotion, the diet was changed to that of the controls. Ascorbic acid was continued at this time but the cod liver oil was discontinued. The animals were killed at 1, 3, 6, 9, 12, 15 days after having been fed the normal diet.

Ether was used to kill the animals. Each animal was necropsied immediately after death or if living was killed. Materials were taken for histological study from the heart, abdominal muscles, tongue, masseter, and the various muscles of the front and rear legs. The specimens were fixed both in Zenker's fluid and formalin (10 percent). Formalin fixation was used only for vitally stained material and those to be frozen. Paraffin sections were cut 4 to 5 micra in thickness and stained

with Hematoxyline-eosin and Mallory's aniline blue stain. Eosin was used as a contrast in vitally stained sections. The frozen sections were cut 15 micra in thickness and stained with Sudan IV.

RESULTS OF EXPERIMENT I

The body weights of the guinea pigs are shown in Table I and Graph I. A study of the table and graph disclosed that there was a gradual increase in body weight. However, a drop in the body weight occurred during the first four days of the experiment. This resulted from the replacement of the rabbit pellet ration by the basal synthetic diet. The guinea pigs consumed very little of the ration during the first four days of the trial. Varying with individuals, the body weights declined again during the 22nd to 30th day of the experiment. The decrease in body weight occurred synchronously with the appearance of clinical symptoms, which were decreased activity, slow locomotion, difficulty in rising when placed on the back, lack of body tone, and finally paralysis of the fore and hind legs followed by death. However, some of the guinea pigs showed these symptoms and died without drop in body weight.

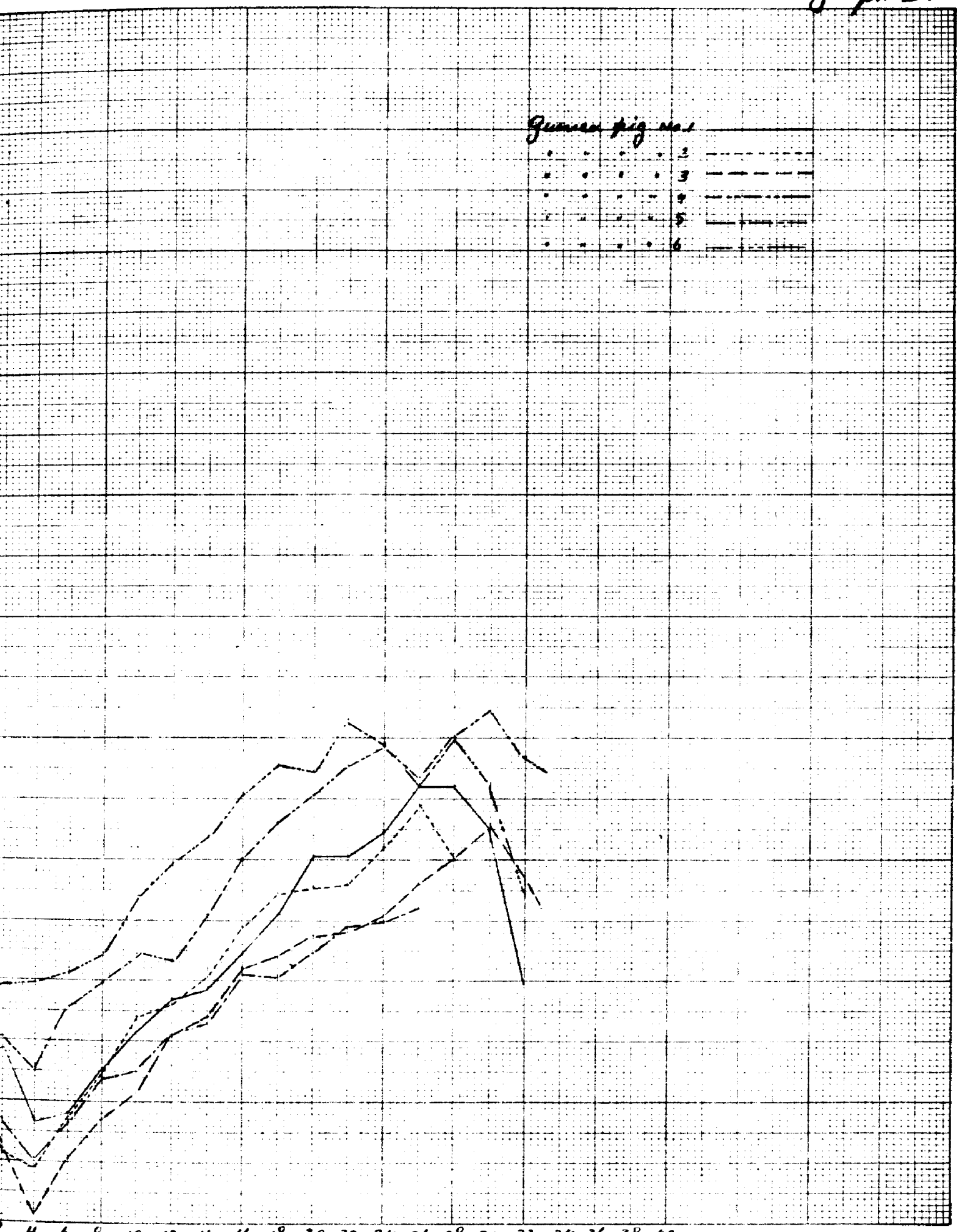
Unfortunately, a comparison could not be made between the body weights of the guinea pigs on the experiment and the controls. The controls did not show regular and gradual increases in weight because supplements of lettuce and grass were neglected for a few days. The body weights declined during this time. This was remedied by supplementing the diet with an ascorbic acid tablet, dissolved in one ml. of water, on alternate days.

Case reports of individual guinea pigs used in the experiment follow.

TABLE I: The Body Weight of the Guinea Pigs. (grams)

Days	Guinea pigs on the experiment					Controls		
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
Initial	293	289.6	294	301	291	310.6	288.4	295.5
2	297	263.4	264	301	274	318.4	301	306
4	273.8	258.6	242.6	290	253	319	296	271
6	276	275	261	311	274.5	322.4	295	270
8	291	290	274	319	288	329	271	253
10	303	308	283	329	290	348	268	237
12	314	312	302	327	302	359	262	223
14	317.6	321	208	342	306	368	247	211
16	329	338	324	360	323	382	268	221
18	342	349	328	372	322	391	288	244
20	361	351	335	381	329	289	308	260
22	361	352	336	391	338	406	323	283
24	369	364	342	397	340	398	348	313
26	384	378	352	387	344	384	353	341
28	384	360	360	400	died	400	376	359
30	370	killed	371	409		384	401	371
32	319		353	393		349	410	380
33	killed		344	390		killed	429	380
			killed	killed			killed	killed

Graph I.


Guinea pig no. 1
2
3
4
5
6
4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40

Case I

Group No.	Animal No.	Survival period (days)	Body Weight		
			Initial (grams)	Maximal (grams)	Final (grams)
I	5	26	291	344	344

Clinical observation:

Guinea pig 5 reared on the basal synthetic diet with supplements of 0.5 gram cod liver oil and Vitamin C gained weight until found dead in the cage. Toward the end of the experiment the guinea pig became inactive which was accompanied by general flabbiness, decreased body tone and difficulty in rising when placed on the back.

Autopsy findings:

The carcass was in good physical condition. Practically all muscles of the fore and hind legs, especially the gracilis, adductors, biceps femoris, triceps brachii, suprascapularis as well as the back, abdominal and intercostal muscles were involved. However, the diaphragmatic and masseter seemed to be normal. The pathological change appeared to be degeneration of the skeletal muscles. The degeneration in some muscles was localized and involved only a part of the muscle, in others, the degeneration

was generalized and affected the entire muscle. The degenerated muscles were pale, yellowish gray streaked, patchy and had a cooked and moist appearance (Figs. 1, 2). They were easily torn and friable. The abdominal muscle were so thin and atrophied that the viscera could be observed through the abdominal wall. In the longitudinal view, the degeneration appeared as grayish streaks which were mottled or continuous throughout the length of the muscle. The grayish streaks and patches were distributed symetrically and bilaterally.

The right ventricle of the heart was dilated and the muscle was pale.

The liver appeared fatty and mottled. Pneumonia and congestion of the small intestine were present. The other viscera did not reveal any pathological changes.

Microscopic findings:

Primary changes consisted of coagulation necrosis in which the contractile substance acquired a hyaline, waxy appearance. (Fig. 3) The muscle fibers involved showed partial or total waxy degeneration. Regeneration of muscle fibers was taking place. The histological picture was not uniform throughout a single fiber.

The affected fibers became swollen, stained weak-

ly with eosin and were separated by edematous fluid. The nuclear activity of affected fibers was increased. The number of nuclei increased and were hyperchromatic. Muscle fibers, undergoing degenerative changes, lost first the cross and later the longitudinal striations, became homogenous, translucent, and stained deeply with cosin. Some affected fibers at this stage broke into small pieces, giving a granular appearance to the fiber. The granular mass filled the sarcolemma of the fiber when the sarcolemma was intact (Fig. 7, 8). Some other affected fibers did not break into granules but formed clumps which were homogenous and sometimes contained vacuoles (Fig. 4, 7, 10).

Nuclei of affectedfibers were pyknotic, staining dark blue with hematoxyline-eosin and many of them had undergone karyorrhexis and karyolysis. Usually, the sarcolemma was involved. In some instances, however, the destructive process was not severe --- the contractile substance was only hyalinated and the sarcolemma appeared not to be affected.

The changes stimulated infiltration of histiocytes, polymorphonuclear leucocytes, lymphocytes and plasma cells; proliferation of fibroblasts and giant cell formation, and finally multinucleated syncytial cell masses (Figs. 5, 9, 10, 12, 13).

The Histiocytes were numerous; playing an active role in removal of necrotic and hyalinated material. The necrotic part of the fiber became enveloped in giant cells which engulfed the tissue debris which was observed in these cells as blue-purple granules when sections were stained with Mallory's aniline blue stain. The multinucleated syncytial cell masses which were abundant and formed islands in the fibroblastic tissue appeared to be similar to the giant cells. As far as could be seen, the multinucleated syncytial cell masses did not engulf debris. They appeared to be nonphagocytic and possibly of muscular origin. Sarcolemma which escaped destruction were completely filled with muscle spindle cells, multinucleated syncytial masses, giant cells and histiodytes, giving rise to a "muskelzellenschlauche" of Waldyer (Fig. 10). The number of infiltrating polymorphonuclear leucocytes, lymphocytes and plasma cells was small. In some instance, the older degenerated tissues were partially calcified (Figs. 6, 11).

The regeneration of muscle fibers occurred synchronously with degenerative alteration of the contractile substances (Fig. 9). The plasmodial outgrowths were formed from the old damaged fibers. They retained their attachment to the old fiber. Where the sarcolemmal sheaths were preserved these outgrowths extended

through it in an even course, forming a cone-shaped mass with a pointed tip. The nuclei within the plasmodial outgrowths were arranged in rows parallel to the long axis of the old fiber and were centrally located. The nuclei were bladder shaped and contained one or two nucleoli. A granular cytoplasm surrounded the nuclei. These outgrowths were stained purplish with hematoxyline-eosin. Such outgrowths gave origin to new muscle fibers. Regeneration was a progressive process; therefore, the new fibers exhibited different stages of development. Some of them had only longitudinal striation and the nuclei were in rows centrally located. Others showed cross striation with peripherally located nuclei, in cross section, the new fibers were of different diameters.

When the pointed tip of the sarcoplasmic strands met an obstruction (necrotic tissue) in the growth process they became expanded and formed large multinucleated syncytial masses of sarcoplasm.

The complete regeneration of the muscle fibers necessitates the presence of at least a single muscle cell which developed new fibers by continuous growth of cytoplasm accompanied by division of nuclei. The nuclear division in regenerating muscle fibers took place by amitosis.

Although the diaphragmatic, the masseter, and the

tongue muscles were macroscopically normal, microscopically degenerative changes were revealed. However, these changes were not extensive, being confined to a small number of muscle fibers.

The heart muscle showed hydropic degeneration. Careful examination of the heart did not reveal hyaline degeneration.

Case 2

Group No.	Animal No.	Survival period (days)	Body Weight		
			Initial (grams)	Maximal (grams)	Final (grams)
1	2	28	289	378	360

Clinical observation:

Guinea pig 2 gained weight until the 26th day of the experiment. The body weight then declined from 378 grams to 360 grams in two days. At the same time there was a loss of activity, accompanied by weakness of the fore and rear leg muscles, slow locomotion, lack of body tone and finally difficulty in righting itself when placed on the back. The toes of the rear legs curled backward, indicating severe involvement of the extensor muscles. Respiration was abdominal and labored. The guinea pig vomited on 27th and 28th days of the experiment. The animal was killed on the 28th day.

Autopsy findings:

The carcass was in fair physical condition. The skeletal muscles on both sides of the body, especially the tibialis anterior, biceps brachii and pectoral muscles were light in color and appeared parboiled. Here and there some whitish patches were observed. The surface of the cross cut muscles was moist. The diaphragmatic, masseter and tongue muscles were apparently normal.

The right ventricle of the heart was dilated and flaccid.

The liver showed some fatty degeneration. The stomach and small intestine were highly congested, being involved with catarrhal inflammation. The Peyer's patches were swollen and projected into the lumen. The lung showed anthracosis.

Microscopic findings:

The skelctal muscles were primarily involved. The pathological changes were more or less similar to the changes described for Case 1. The dystrophy was well pronounced especially in the abdominal, quadriceps femoris, gracilis, and triceps brachii. The diaphragmatic, masseter and tongue muscles showed the least degenerative changes. The muscle fibers were involved partially or totally. Some fibers were swollen, stained weakly with

eosin, while others were shrunken. The cross and longitudinal striations were lost. However, in some fibers, the cross striations were accentuated and appeared close to each other, persisting even in the completely degenerated part of the muscle fiber, which had a homogenous appearance.

The nuclei of necrotic fibers were pyknotic or had disappeared. The presence of the sarcolemma was not constant. In some instances, the sarcolemma retained its integrity.

The necrotic fibers were invadedby histiocytes. A small number of polymormonuclear leucocytes, and lymphocytes were observed among the histiocytes. The process of removal of the necrotic areas was not as active as in Case 1. The giant cells, enveloping the necrotic tissues, and the fibroblastic activity of the interstitial connective tissue were also less. Multinucleated syncytial masses were rarely observed.

Myoregeneration was not pronounced. Here and there the formation of some new muscle fibers from the old fibers was observed.

Some of the cardiac muscle fibers were swollen and showed granular changes.

Case 3

Group No.	Animal No.	Survival period (days)	Body Weight		
			Initial (grams)	Maximal (grams)	Final (grams)
I	1	32	293	384	319

Clinical observation:

Guinea pig 1 was doing well and gained weight until the 27th day of the experiment. At this time the activity of the animal decreased. This condition became aggravated day by day. Three days later the guinea pig was scarcely able to walk and the toes of the rear legs curled backward and the animal could not reach the food and water containers. The body weight dropped sharply during this period of time (from 384 to 319 grams). The animal was killed on the 32nd day of the experiment.

Autopsy findings:

The carcass was in fair physical condition. The skeletal muscles on both sides of the body and the diaphragm were involved and lesions appeared as whitish patches or streaks scattered throughout the muscles. However, the masseter was apparently normal.

Both ventricles of the heart were dilated. The walls of the ventricles appeared thinner than normal,

and flaccid.

The liver showed fatty degeneration and was fragile. The small intestine was highly congested and showed catarrhal inflammation. The lung, spleen, kidney and other organs were apparently normal.

Microscopic findings:

Myodegeneration and well pronounced regeneration of muscle fibers were observed. The muscle fibers were swollen. The nuclei of the fibers were increased in number, were hyperchromatic, bladder-shaped, and located centrally and peripherally. The cross striation of some fibers was pronounced. There were other fibers which had lost the cross and longitudinal striations and were homogenous. Some degeneratedfibers were fragmented and contained deposits of calcium salts as evidenced by staining dark blue with hematoxyline. The nuclei were either pyknotic or karyolytic. The presence of sarcolemma was not constant. Where the sarcolemma existed it was filled with new muscle cells, histiocytes, giant cells and multinucleated syncytial masses.

The necrotic part of the muscle fiber was invaded by histiocytes and surrounded by large numbers of giant cells. Polymorphonuclear leucocytes, and lymphocytes were observed to a lesser extent. The interstitial con-

nective tissue was stimulated, giving rise to fibroblastic cells. Multinucleated syncytial masses were scattered throughout the fibroblastic tissue.

Myoregeneration was well pronounced. The plasmodial outgrowths from the remains of the fibers were at different stages of development. Some of these outgrowths were very young and did not show striation, stained basophilic and contained bladder-shaped nuclei which were centrally located and surrounded by a granular sarcoplasm. Others were more mature and showed longitudinal striations.

The heart showed degenerative changes with cellular infiltration (Fig. 14).

Case 4

Group No.	Animal No.	Survival period (days)	Body Weight		
			Initial (grams)	Maximal (grams)	Final (grams)
I	6	32	310	406	349

Clinical observation:

Guinea pig 6 gained weight gradually until the 21st day of the experiment at which time the body weight started to decline. At the same time, the activity and locomotion of the animal decreased. This condition was more aggravated

day by day. Finally the animal developed paralysis of the hind legs. The fore legs were still functioning. The guinea pig could not raise itself when placed on the back. On the 32nd day, paralysis was generalized and the animal refused to move. Respiration was labored and abdominal. The diarrhea that developed on the 29th day continued for 3 days. The animal was killed on the 32nd day and autopsied.

Autopsy findings:

The carcass was in fair physical condition. The whitish patches and streaks were scattered throughout the skeletal muscles with exception of the masseter. The changes were well pronounced in the intercostal, abdominal, pectoral, gracilis, adductors and the triceps brachii muscles. The diaphragmatic muscle showed a few whitish patches. The degenerative changes were bilateral and symmetrical.

Theright ventricle of the heart was dilated and flaccid. The wall of the right ventricle was thinner than normal.

The lung showed pneumonia. The liver appeared fatty and was friable. The stomach and small intestine were highly congested and showed catarrhal inflammation. The urinary bladder was distended with urine.

The other organs appeared normal.

Microscopic findings:

Practically all skeletal muscles were involved. The type of cellular changes were more or less similar to the cases described above. The extent of degenerative and regenerative processes varied from one muscle to another, even between fibers. In this animal the diaphragmatic and tongue muscles were more necrotic and showed more deposits of calcium salts than the other guinea pigs.

The pathological changes consisted of a coagulation necrosis characterized by a hyaline waxy appearance followed by myoregeneration. Muscle fibers underwent coagulation necrosis, breaking up into granular masses; in some instances the necrotic fiber became homogenous, translucent, and stained evenly with eosin. These necrotic fibers showed loss of nuclei and sarcolemma. Occasionally intact sarcolemma was observed. The necrotic tissues were invaded by histiocytes that developed into giant cells which engulfed the necrotic debris. When the sections were stained with Mallory's aniline blue, the engulfed debris took the dark blue stain. Such debris was easily demonstrable in the giant cells. The degenerative process stimulated fibroblast proliferation which was infiltrated by a small number of polymorphonuclear

leucocytes. This fibroblastic tissue contained a large number of multinucleated syncytial masses. As far as could be seen these cells had nothing to do with removal of the necrotic debris. They stained evenly with the Mallory's aniline blue stain. The cytoplasm was stained a dark red color. The nuclei were red in color. When the sarcolemma of the fiber was intact, it was completely packed with histiocytes, spindle shaped muscle cells, giant cells and multinucleated syncytial masses, giving rise to the "muskelzellenschlauche" of Waldyer.

In some instances, for example in the quadriceps femoris, the necrosis dominated the process of myoregeneration, while in other instances, in the biceps femoris and the biceps brachii, the myoregeneration predominated over the dystrophic changes. The myoregeneration took place synchronously with the degenerative alteration of the contractile substance. Here and there new plasmodial outgrowths sprouted from the old fibers. Some of them had longitudinal striations with bladder-shaped nuclei, centrally located.

The heart showed hyaline degeneration to some extent in the right ventricle.

Case 5

Group No.	Animal No.	Survival period (days)	Body Weight Initial (grams)	Maximal (grams)	Final (grams)
I	3	33	294	371	344

Clinical observation:

Guinea pig 3 gained weight until the 29th day of the experiment. Then, the body weight declined gradually during three days. The activity and locomotion of the animal decreased at the same time. At the 30th day the guinea pig was confined to the corner of the cage and did not walk. When the animal was placed on its back, it did not attempt to rise and kept this position for some minutes. Finally, general paralysis developed and the body lost tone. The respiration was abdominal and labored. The guinea pig was killed on the 33rd day of the experiment.

Autopsy findings:

Practically all skeletal muscles were involved and showed whitish streaks and patches throughout. However, the tongue muscles and the masseter appeared normal.

Both ventricles of the heart were dilated. The

walls of the ventricles were thin and flaccid.

The liver was friable and showed fatty degeneration. The lungs and small intestine were highly congested. The other viscera were apparently normal.

Microscopic findings:

The skeletal and diaphragmatic muscles were degenerated and showed coagulation necrosis. The masseter and the tongue muscles were also involved to a minor extent. Some necrotic fibers were broken into small pieces; others were homogenous; translucent and stained dark red with eosin. Calcium salts were deposited in some necrotic tissues as evidenced by the dark staining. On the other hand, some muscle fibers were swollen, the diameter being increased two or three times. The nuclei were active, hyperchromatic and increased in size. The removal of the necrotic part was in progress. The giant cells and histiocytes engulfed the necrotic debris which were noticeable in these cells as blue granules when stained with the Mallory's aniline blue. The formation of fibroblasts and multinucleated syncytial masses was very active where the removal of the necrotic tissues was almost complete.

The regeneration of new fibers occurred simultaneously. The other changes were similar to the cases de-

scribed above.

The heart showed fatty degeneration.

Case 6

Group No.	Animal No.	Survival period (days)	Body Weight		
			Initial (grams)	Maximal (grams)	Final (grams)
I	4	33	301	409	390

Clinical observation:

Guinea pig 4 increased in weight gradually until the 29th day. The animal showed decreased activity which became aggravated day by day. The body weight concurrently declined. On the 31st day the animal could hardly walk. Paralysis of the fore and hind legs developed. The guinea pig could not rise when placed on its back. Respirations were abdominal and labored. The animal was killed on the 33rd day.

Autopsy findings:

The animal was in good physical condition. All skeletal muscles and the diaphragmatic showed whitish patches and streaks. The masseter was apparently normal.

The liver was fatty and friable. The small intes-

tine was highly congested. Pneumonia was confined to the right apical lobe. The other organs were normal.

Microscopic findings:

The pathological changes consisted of hyaline degeneration and myoregeneration. The type of reaction was similar to that described for the previous cases. The only difference was the extensive necrotic changes in the skeletal muscles. The masseter was also involved. The regeneration was active, resulting in some new fibers at different stages of development.

The heart muscle showed hyaline degeneration.

RESULTS OF EXPERIMENT II

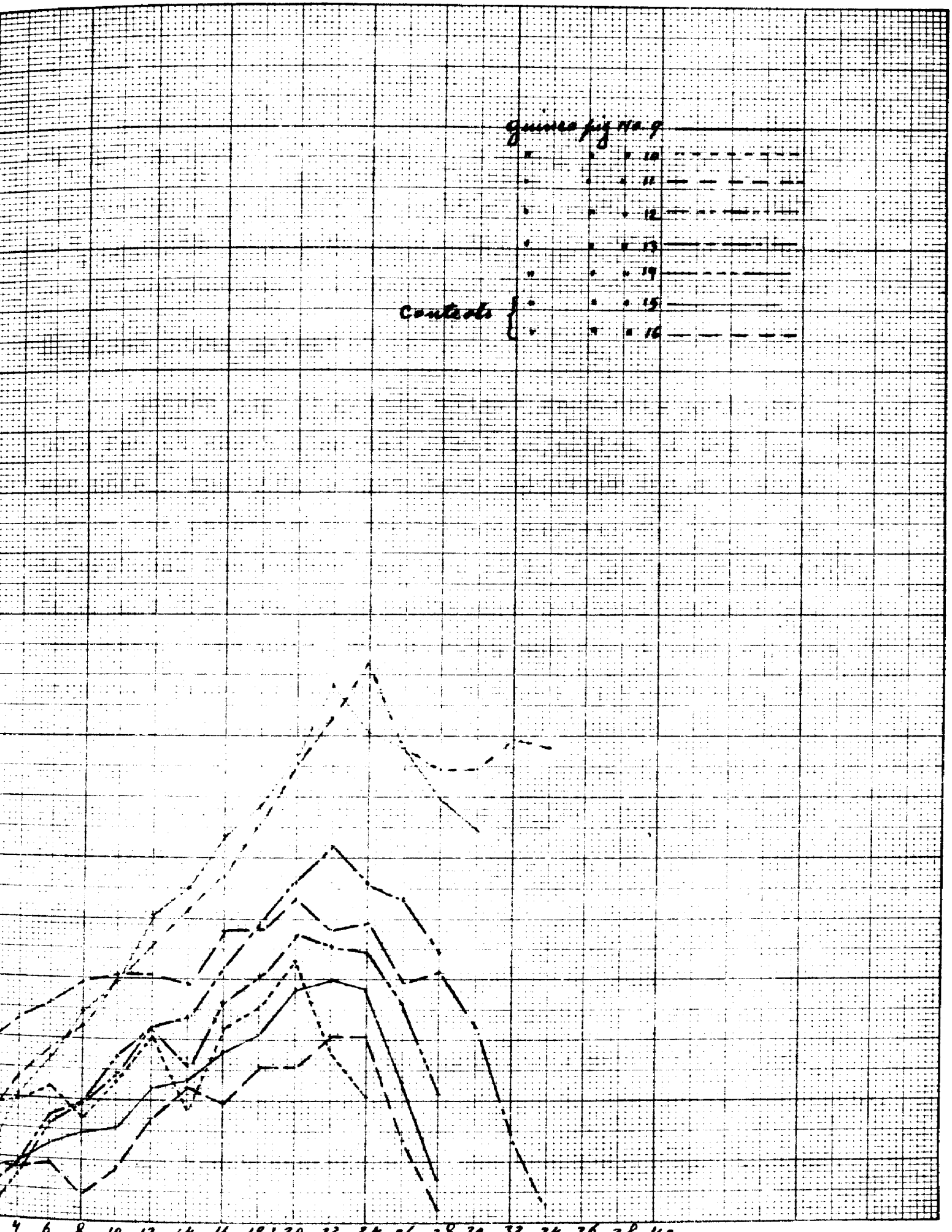
The body weights of all guinea pigs on this experiment are shown in Table II, and Graph II. It can readily be seen that the body weights increased gradually until the 20th day of the experiment. Increase in body weights of controls during the same period of time was more than that of the animals reared on the basal synthetic diet supplemented with cod liver oil and ascorbic acid.

Body weights declined sharply after the guinea pigs had received injections of a one percent solution of trypan blue. Each animal responded differently to the dye. Guinea pig 10 died after three injections, while guinea pigs 9 and 11 succumbed after four doses. Others remained alive after five injections had been given. The controls also responded to injections of the dye by losing weight. Due to the weight loss of the controls it might be concluded that trypan blue interfered with the gain in body weight, and was possibly toxic for the guinea pigs. Trypan blue hastened the death of the animals.

TABLE II: The Body Weights Of The Guinea Pigs.
(grams)

	Guinea pigs on the experiment						Controls	
Days	No. 9	No. 10	No. 11	No. 12	No. 13	No. 14	No. 15	No.
Initial	282	281	272	280	316	272	279	292
2	276	298	277	270	318	262	281	293
4	279	299	277	279	327	274	301	307
6	286	304	279	294	332	292	314	317
8	289	294	269	299	339	299	329	324
10	291	306	278	314	341	309	339	341
12	304	321	294	324	341	324	861	352
14	307	297	304	327	339	312	370	363
16	316	324	299	344	356	332	387	374
18	322	331	311	359	357	341	397	391
20	337*	346*	311*	372*	367*	354*	414*	411*
22	340	317	321	384	357	351	437	426
24	337	301	321	372	359	349	421	444
26	306	died	287	367	339	332	416	416
28	274		264	349	342	302	399	409
30	died		died	killed	324	killed	389	409
32					289		killed	419
34					266			416
					killed			kill

*The guinea pigs received the first injection of trypan blue.


Guinea pig no. 9
10
11
12
13
14
Contents
15
16
4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40

Case 7

Group No.	Animal No.	Survival period (days)	Body Weight Initial (grams)	Maximal (grams)	Final (grams)
2	10	26	281	346	301

Clinical observation:

Guinea pig 10 gained weight gradually until the 20th day at which time the first injection of the dye was made. The body weight declined after which the activity and appetite of the animal decreased. After two injections, the guinea pig stayed in a corner of the cage, developed diarrhea which continued until the guinea pig succumbed. The skin, conjunctiva and feces were stained intensely blue. This was accompanied by flabbiness of the muscles decreased activity and difficulty on rising when placed on its back. The animal received three injections of trypan-blue and died on the 26th day of the experiment.

Autopsy findings:

The carcass was in fair physical condition. The skin, conjunctiva, all skeletal muscles and viscera were stained blue in color. The lungs were purplish. Blue coloration in the heart was confined to the sulci coronaria.

The right ventricle of the heart appeared dilated.

The liver was friable and appeared fatty. The stomach and small intestine were inflammed.

Microscopic findings:

All skeletal muscles were more or less involved. The changes consisted of coagulation necrosis in which the contractile substance acquired a hyaline waxy appearance. The degree of degeneration varied from one muscle to another. The pathological picture was similar to findings recorded for experiment I. Muscle fibers were swollen and weakly stained. Cross and longitudinal striations had disappeared. In some of the fibers, cross striations were accentuated, becoming closer to each other. The cross striations persisted in some of the necrotic fibers. The contractile substance became homogenous, translucent, breaking up into small pieces. Nuclei of the affected fibers were either pyknotic or karyolysed. The sarcolemma of the fibers was involved, however, it sometimes persisted. These changes stimulated the infiltration of histiocytes, polymorphonuclear leucocytes and lymphocytes. The two last mentioned cells were few in number. Hyaline clumps were invaded by histiocytes and often enveloped in giant cells. The fibroblastic activity increased. Spindle shaped muscle cells and multi-nucleated syncytial masses were formed. When the sarcolemma of an affected fiber persisted it was completely filledby

histiocytes, giant cells, spindle shaped muscle cells, and multinucleated syncytial masses. These cells phagocytosed the injected dye (Figs. 15, 16, 17). However, not all spindle shaped muscle cells and multinucleated syncytial masses engulfed the trypan blue (Fig. 18). The dye was present in these cells in the form of fine granules. In addition to these cells endothelial cells of the capillary vessels and fibroblasts also engulfed the trypan blue (Fig. 19).

Myoregeneration was taking place at the same time, but it had not progressed very far. Some plasmodial outgrowths were observed, showing nuclei arranged in rows and granular sarcoplasm.

The heart muscle had undergone hydropic degeneration.

Case 8

Group No.	Animal No.	Survival period (days)	Body Weight		
			Initial (grams)	Maximal (grams)	Final (grams)
2	9	28	282	340	274

Clinical observation:

Guinea pig 9 lost activity on the 22nd day and developed general flabbiness, slow locomotion and difficulty

in righting itself when placed on the back. At the same time, the body weight declined. This condition became progressively worse and the animal died on the 28th day of the experiment. The guinea pig received four injections of the dye, which was followed by intense coloration of skin and conjunctiva. The feces voided were also blue in color.

Autopsy findings:

The carcass was in poor physical condition. Practically all skeletal muscles and viscera were stained blue. The masseter and the latissimus dorsi were dark purple in color. The right axillar lymph node was found to be swollen. A small abscess was present in the right masseter region.

Both ventricles of the heart appeared dilated.

The viscera were apparently normal.

Microscopic findings:

All skeletal muscles showed more or less dystrophic changes. In comparison with case 7, it was found that necrosis of the muscle fibers was more advanced. Otherwise the changes were similar to case 7. Histiocytes, giant cells, some spindle shaped muscle cells and a few of multinucleated syncytial masses phagocytosed the trypan

blue (Figs. 15, 16, 17, 18, 19). The coarse granules of the dye were observed in these cells. The sections of the popliteal lymph node showed that the reticulocytes, small lymphocytes and macrophages had also absorbed the dye. (Fig. 22).

The heart showed no pathological changes.

Case 9

Group No.	Animal No.	Survival period (days)	Body Weight		
			Initial (grams)	Maximal (grams)	Final (grams)
2	11	28	272	321	269

Clinical observation:

Guinea pig 11 received four injections of the dye. After two injections the increase in body weight ceased and declined on the 24th day. The decline in weight was accompanied by loss of activity, paresis of the hind legs and backward curling of the toes of rear extremities. The animal stopped eating after the 3rd injection of the dye, developed diarrhea which was greenish colored and of an offensive odor. The guinea pig died on the 28th day.

Autopsy findings:

All skeletal muscles and viscera were stained blue. The gracilis and abdominal muscles appeared atrophied and friable.

The heart was flaccid and the walls were thinner than normal. It was stained blue along the sulci coronariae. The small intestine showed catarrhal inflammation.

Microscopic findings:

Practically all muscles, especially the quadriceps femoris, gracilis, gastrocnemius, triceps brachii, abdominal muscles and the tongue muscles were involved. The pathological changes consisted of extensive coagulation necrosis of the muscle fibers. Cellular reaction and myoregeneration were minor in extent. Histiocytes and giant cells which phagocytosed the dye were rarely observed. The dye granules which appeared in these cells were fine. The spindle shaped muscle cells and multinucleated syncytial masses were also scarce. No trypan blue could be seen in the cytoplasm of these cells.

Myoregeneration was almost nil.

The heart showed hyaline degeneration to a limited extent. None of the cells engulfed the dye.

Case 10

Group No.	Animal No.	Survival period (days)	Body Weight		
			Initial (grams)	Maximal (grams)	Final (grams)
2	12	28	280	384	349

Clinical observation:

The body weight of guinea pig 12 increased gradually until the 22nd day of the experiment. The animal lost weight after two injections were applied. At this time the activity of the guinea pig decreased, and locomotion became slow. The animal received 5 injections of the dye and was killed one day after the last injection was made.

Autopsy findings:

The carcass was in good physical condition. The skin, conjunctiva, and skeletal muscles were tinged blue. The muscles especially the abdominal, gracilis, and adductors muscles appeared atrophied and were easily torn.

The heart was dilated and stained blue along the sulci coronariae.

The viscera were blue in color. The feces were stained blue.

The liver was enlarged and friable.

Microscopic findings:

The skeletal muscles showed hyaline degeneration. The degenerative processes were not extensive, being confined to a small number of fibers which were surrounded by fairly normal muscle fibers. The character of the degenerative change was similar to the cases heretofore mentioned.

Histiocytes and giant cells were filled with the dye. (Fig. 15, 16). The engulfed dye appeared in coarse granules. Some spindle shaped muscle cells and a few multinucleated syncytial masses phagocytosed trypan blue (Figs. 16, 18). When the sarcolemma of fibers persisted it was packed by these cells giving rise to the "muskelzellenschlauche" of Waldyer (Fig. 16).

In addition to these cells, histiocytes, some fibroblastic cells of interstitial tissue and endothelial cells of capillaries engulfed the dye. A large number of histiocytes were observed around blood vessels. (Fig. 19, 20).

Myoregeneration was in progress.

The heart showed hyaline degeneration and was infiltrated by histiocytes which had engulfed the trypan

blue (Fig. 21).

Case 11

Group No.	Animal No.	Survival period (days)	Body Weight Initial (grams)	Maximal (grams)	Final (grams)
2	14	28	272	354	302

Clinical observation:

The increase in body weight of guinea pig 14 continued gradually until the 20th day of the experiment. The animal started to lose weight after the first injection of trypan blue. This condition continued and became aggravated when the animal had received four injections of the dye. The skin, conjunctiva and feces were stained blue. Decreased activity and slow locomotion became apparent. The respirations were abdominal and labored. The animal was killed one day after the fifth injection had been administered.

Autopsy findings:

The carcass was in good physical condition. The skeletal muscles, skin and conjunctiva were stained blue. The abdominal and pectoral muscles took on a deep blue color. They were thin and atrophied. The masseter was

dark purple in color.

Both ventricles of the heart were dilated and blue stain was present along the sulci caronariae.

The viscera were tinged blue. The liver was enlarged and friable. The other organs appeared normal.

Microscopic findings:

The skeletal muscles, especially the abdominal, quadriceps, femoris, gastrocnemius, intercostal and triceps brachii were primarily involved. The changes consisted of coagulation necrosis of the muscle fibers and was similar to the cases described heretofore. The necrotic tissues were infiltrated by histiocytes and surrounded by giant cells. These cells had phagocytosed the trypan blue which was coarsely granular. Some spindle shaped muscle cells and a few multinucleated syncytial masses appearedto engulf the trypan blue. The dye granules were also observed in the cytoplasm of fibroblasts, histiocytes of connective tissue and endothelial cells of capillaries.

The heart showed hyaline degeneration and was infiltrated with histiocytes, and showed absorbed dye (Fig. 21).

Case 12

Group No.	Animal No.	Survival period (days)	Body Weight		
			Initial (grams)	Maximal (grams)	Final (grams)
2	13	34	316	367	302

Clinical observation:

Guinea pig 13 gained weight gradually until the 20th day of the experiment. The body weight declined and the activity of the animal decreased on the days following the first injections of the trypan blue. The skin, conjunctiva, and feces were stained blue. The animal had difficulty in rising when placed on the back and locomotion was slow. Decrease of the body weight continued 6 days after the five injections had been completed. The animal was killed on the 34th day of the experiment.

Autopsy findings:

The carcass was in poor physical condition. The skin, skeletal muscles and conjunctiva were stained blue. The abdominal and rear leg muscles showed atrophy. The madibula was easily fractured.

The right ventricle of the heart was dilated. The heart was stained deeply blue along the sulci coronariae.

The viscera were blue. The lungs showed pneumonia. The liver was enlarged and friable.

Microscopic findings:

The skeletal muscles, especially the quadriceps femoris, semitendinosus, gastrocnemius, triceps brachii, abdominal, and diaphragmatic muscles showed coagulation necrosis which acquired a hyaline and waxy appearance. These changes were similar to the changes described in detail for the cases heretofore reported. The only difference being the extensive necrosis of muscle fibers and large depositions of calcium salts. Myoregeneration was not active. Here and there some histiocytes and giant cells with engulfed fine granules of trypan blue were observed. A small number of spindle shaped muscle cells and multinucleated syncytial masses were scattered in the fibroblastic tissue. Some of these cells had phagocytosed the dye. The dye also had accumulated in the necrotic tissues.

The heart showed necrosis and deposition of calcium salts. Histiocytes infiltrated the degenerated areas of the cardiac muscle.

RESULTS OF EXPERIMENT III

The body weights of all guinea pigs on this experiment are shown in Table III and Graph III. It can readily be seen that the body weight of animals increased gradually. The difference between the final body weights of the guinea pigs on the experiment and that of the controls was not significant. It was noticed that the course of the disease in this experiment was much longer than in previous ones. The early symptoms which consisted of decreased activity, slow locomotion and difficulty in rising when placed on the back, appeared between the 37th and the 44th day of the experiment.

The long duration of the course of the disease in this experiment might be attributed to difference in susceptibility of individuals and outside temperature because the same basal synthetic diet, cod liver oil and ascorbic acid were used throughout the three experiments. For this reason, the dietary factors were constant. The experiments I, II were conducted during August and October. Experiment III was set up during November and continued until the first week of February. The hot season possibly caused decreased peristalsis of the intestine and food stayed longer in gastro-intestinal tract in contact with the separately supplemented cod liver oil which probably resulted in autoxidative destruction of vitamin E in the

diet. This vitamin E deficient diet and cod liver oil together shortened the course of the disease and resulted in early appearance of symptoms in the experiments I and II.

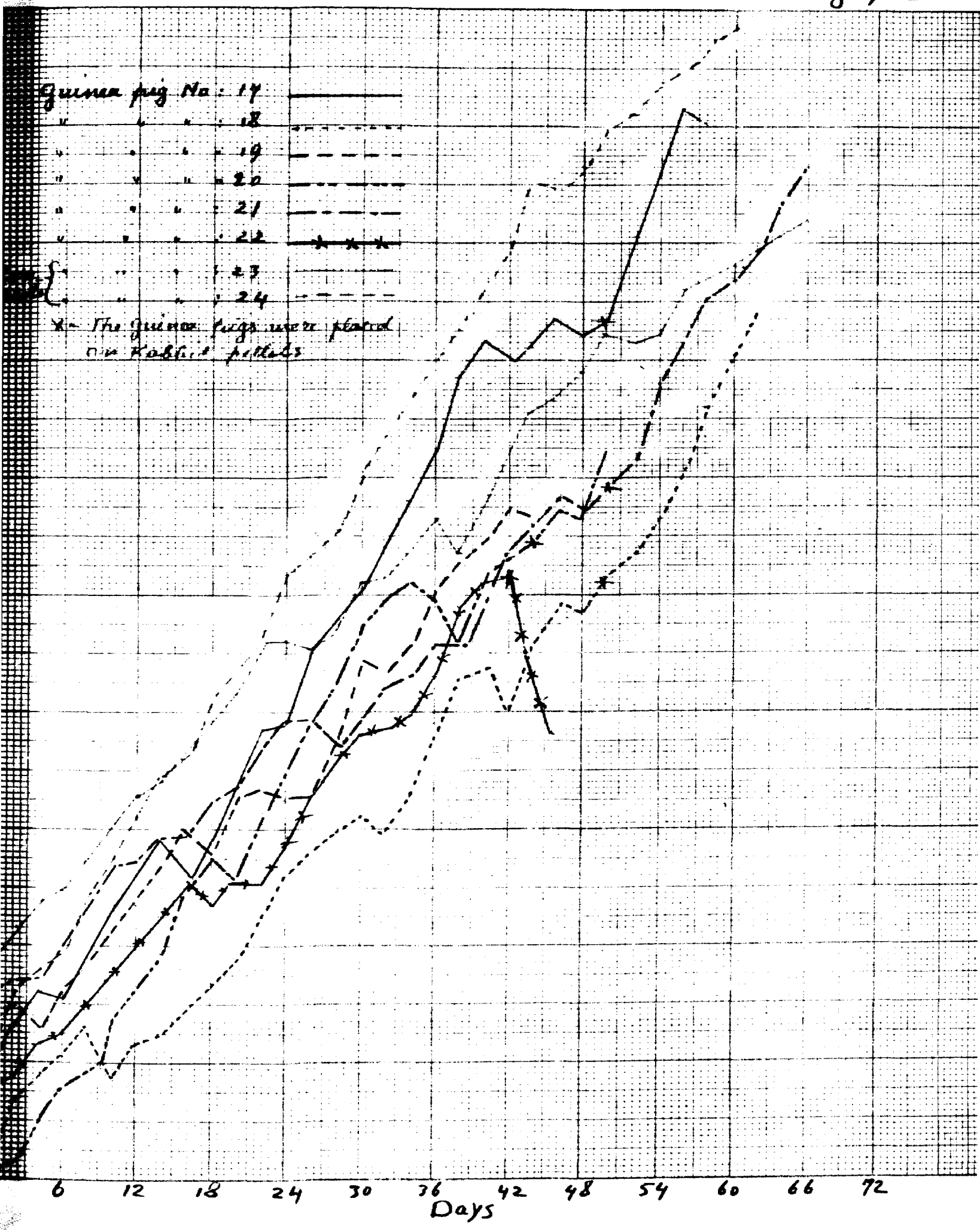
Case reports of guinea pigs used in this experiment follow.

TABLE III. The Body Weights of the Guinea Pigs.
(grams)

Days	Guinea pigs on the experiment						Controls	
	No. 17	No. 18	No. 19	No. 20	No. 21	No. 22	No.23	No.24
Initial	259	225	274	222	284	251	294	262
2	272	246	279	226	288	255	306	284
4	284	256	272	242	289	266	315	291
6	282	262	284	251	300	270	319	297
8	296	272	293	257	312	279	329	315
10	312	255	306	275	327	288	343	327
12	324	266	312	285	329	301	351	344
14	337	269	326	295	336	311	356	356
16	324	279	340	317	339	322	369	367
18	336	287	332	331	349	314	377	384
20	356	296	351	324	354	322	386	393
22	374	311	353	346	369	321	404	402
24	376	324	351	359	377	334	404	427
26	401	331	351	375	377	352	401	432
28	410	329	372	387	369	369	407	442
30	422	344	398	409	378	372	424	462
32	439	339	394	416	387	374	426	477
34	452	349	403	424	394	380	434	489
36	469	371	421	418	403	392	446	497
38	494	390	431	404	403	416	434	510
40	507	394	439	426	422	424	454	527
42	501	369	449	432	434	426*	471	537
44	504	402	447	438*	444	399	481	561
46	514	417	died	449	454	372	486	559
48	509	414		446	449	killed	496	566
50	514*	426*		469	459*		509	579
52	542	434		killed	466		506	584
54	569	446			492		509	595
56	586	463			506		524	600
58	581	484			521		527	610
60	killed	502			527		532	615
62		516			537		539	killed
64		killed			557		542	
66					566		547	
					killed		killed	

*The guinea pigs were placed on rabbit pellets, supplemented by ascorbic acid, the cod liver oil was discontinued.

Graph III


Guinea pig No: 17
" " " 18
" " " 19
" " " 20
" " " 21
" " " 22
" " " 23
" " " 24
X- The guinea pigs were placed on Koch's phlox
6
12
18
24
30
36
42
48
54
60
66
72
Days

Case 13

Group No.	Animal No.	Survival period (days)	Body Weight Initial (grams)	Body Weight Final (grams)
3	19	44	274	447

Clinical observation:

Guinea pig 19 gained weight gradually until the 20th day of the experiment. The increase of body weight was almost insignificant during the 20th to the 26th days. Then, the animal started to gain weight again until it was found dead on the 46th day. However, the animal walked slowly and its activity decreased.

Autopsy findings:

The skeletal muscles, especially the pectoralis, intercostalis, tibialis anterior andthe triceps brachii muscles showed whitish streaks. Some degenerative whitish patches were also observed in the abdominal, gracilis and the adductors muscles.

The right ventricle of the heart appeared dilated and flaccid.

The liver was fatty. The other organs were apparently normal.

Microscopic findings:

The skeletal muscle fibers had undergone coagulation necrosis and showed a hyaline, waxy appearance. These changes were more or less similar to previous cases heretofore described. The necrosis of muscle fibers was extensive. The fibers lost striations, nuclei and sarcolemma. However, in some instances, the cross striations were accentuated and the sarcolemma persisted. Some necrotic fibers appeared as small granules, others showed a homogenous appearance. Cellular reaction was not active. Here and there some histiocytes, polymorphonuclear leucocytes and lymphocytes were observed in necrotic tissues. Myoregeneration was insignificant.

The cardiac muscle showed hyaline degeneration and was infiltrated by histiocytes, polymorphonuclear leucocytes and lymphocytes.

Case 14

Group No.	Animal No.	Survival period (days)	Body Weight Initial (grams)	Body Weight Final (grams)
3	22	46	251	372

Clinical observation:

Guinea pig 22 increased in body weight gradually until the 42nd day of the experiment. The body weight declined from 426 grams to 372 grams during the last four days. The animal showed slow locomotion and difficulty in rising when placed on the back the 37th day and developed diarrhea on the 38th day. This condition disappeared on the 42nd day of the experiment. Then, the guinea pig was placed on rabbit pellets, supplemented by ascorbic acid, and the cod liver oil was discontinued. The animal was killed on the 46th day of the experiment.

Autopsy findings:

The skeletal muscles, especially the adductors, triceps brachii, quadriceps femoris, tibialis anterior, subscapularis, biceps brachii, pectoralis muscles, abdominal, and intercostal muscles showed extensive whitish streaks. The gluteus, semimembranosus, semitendinosus and biceps femoris muscles were light in color. The masseter and diaphragmatic muscles were apparently normal. The degenerative changes were symmetrical and bilateral.

The right ventricle of the heart appeared dilated. The liver was fatty. The small intestine was highly congested. The lungs showed pneumonia.

Microscopic findings:

The pathological changes of the muscle fibers consisted of coagulation necrosis and myoregeneration which were more or less similar to the findings heretofore described. Necrosis of muscles fibers was extensive. Some necrotic fibers were broken into small pieces, giving rise to a granular appearance in the regenerated areas. Others were homogenous and translucent, staining evenly with eosin. The nuclei of necrotic fibers were either pyknotic or karyolytic. Persistence of sarcolemma of affected fibers was not constant. The cellular reaction was very active. Fibroblast proliferation, infiltration of histiocytes and giant cell formation were active. The necrotic fibers were invaded by histiocytes and enveloped in giant cells. The removal of tissue debris was taking place by these last two types of cells. This process was followed by active myoregeneration. However, degenerative and regenerative processes went hand in hand. Regeneration was a progressive process; therefore, the new fibers exhibited different stages of development. The most successful regeneration took place in those instances where only the contractile substance was destroyed while sarcolemma and the peripheral layer of sarcoplasm with nuclei were intact.

The first sign of formative activity of muscle

fibers after injury appeared in the muscle nuclei of the old damaged fiber. This process was followed by formation of plasmodial outgrowths from the stumps of old fibers. These plasmodial outgrowths containing nuclei arranged in rows retained their attachment to the old fiber and usually showed pointed tips which penetrated into the fibroblastic tissue (Fig. 23). The pointed tips became pear-shaped in those instances where the tips met obstructions (possibly a necrotic tissue). The thickened tips gave rise to multinucleated syncytial masses which lost their attachment to the old fibers and were observed in fibroblastic tissue as islands morphologically resembling giant cells.

The nuclei within plasmatic strands became hypertrophied, bladder shaped and multiplied by continuous division. The divisional process was limited to nuclei not sarcoplasm. The nuclear division took place mostly by amitosis. Here and there some mitotic figures were observed. Nuclei were centrally located in the plasmodial outgrowths and were formed in rows parallel to the axis of the old fiber. In some instances, the nuclei were separated from each other by vacuoles.

Cytoplasm of the plasmodial outgrowth appeared granular in the early stages and was basophilic. As the new muscle fiber approached maturity the basophilic

staining characteristic of cytoplasm decreased and they stained more intensely with eosin. Myofibrils appeared in the cytoplasm at early stages of growth, and gave the long striated appearance to the cytoplasm. The myofibrils occupied the periphery of plasmatic strands in the beginning. During later stages the nuclei moved toward the periphery, so that the central parts became occupied by myofibrils. The appearance of myofibrils in the cytoplasm did not take long after the plasmatic strands had been pushed out from the old fibers. The longitudinal striations were followed by the appearance of cross-striations in the fibers.

The newly formed muscle fibers were parallel to each other and they followed the usual muscle pattern.

The regenerative processes were pronounced in the abdominal, pectoral and gastrocnemius muscles. However, the fibers formed were at different stages of development. The diameter of the new fibers was still small.

The cardiac muscle showed hydropic degeneration.

Case 15

Group No.	Animal No.	Survival period (days)	Body Weight Initial (grams)	Body Weight Final (grams)
3	20	50	222	469

Clinical observation:

Guinea pig 20 gained weight gradually until the 50th day of the experiment and developed diarrhea on the 25th day which continued for three days. The difficulty in rising when placed on the back, and slow locomotion appeared on the 38th day. This condition became aggravated day by day until the 44th day at which time the animal was placed on rabbit pellets, supplemented by ascorbic acid and the cod liver oil was discontinued. The guinea pig was fed this diet for six days and was killed on the 51st day of the experiment.

Autopsy findings:

The carcass was in good physical condition. The skeletal muscles appeared light in color. Some whitish patches were observed in the pectoral and rhomboideus muscles.

The right ventricle of the heart was dilated.

The other organs were apparently normal.

Microscopic findings:

The skeletal muscles were involved. Coagulation necrosis of the muscle fibers and myoregeneration were taking place. Necrotic fibers showed similar changes as

heretofore described, but to a minor extent. The removal of necrotic debris was apparently taking place by histiocytes and giant cells. The number of these cells was very small in those cases where the removal of debris was accomplished. The fibroblastic tissue filled the gap left behind. Here and there some polymorphonuclear leucocytic and lymphocytic infiltration was observed.

Regenerative processes dominated the necrotic changes. The regenerating fibers were in different stages of development. The old damaged fibers sprouted, giving rise to plasmodial outgrowths which penetrated the fibroblastic tissue. The nuclei of the plasmodial outgrowths were arranged in rows parallel to the long axis of the original muscle and were located in the central part. The sarcoplasm was granular (Fig. 24). The central part of the sarcoplasm showed some vacuoles which extended between adjacent nuclei. Some fibers showed longitudinal striation at this stage. Others disclosed a faint cross striation. Nuclei had migrated toward the periphery (Fig. 24); myofibrils occupied the central portion of the fibers. The number of nuclei in these fibers was still abundant. The end part of the regenerating fiber was usually pointed. Nuclear division was active in this part. Every once in a while, a pointed tip became thickened and pear-shaped,

and contained many nuclei which were arranged in the form of clusters (Fig. 25). The multinucleated syncytial masses were disconnected from the old fibers. It was not uncommon to find that the end part of the regenerating fiber showed bifurcation. In such fibers two rows of nuclei were present and located at the periphery as though they were going to give rise to two separate fibers.

The regenerating fibers were parallel to the old ones and retained their attachment to the stump of pre-existing fibers. In cross sections the newly formed fibers were of different diameter.

No noticeable changes were found in the myocardium.

Case 16

Group No.	Animal No.	Survival period (days)	Body Weight Initial (grams)	Body Weight Final (grams)
3	17	58	259	581

Clinical observation:

The body weight of guinea pig 17 gradually increased. The animal showed difficulty in righting itself when placed on the back and walked slowly on the 44th day of the experiment. This condition became aggravated during the

following days. On the 50th day of the experiment the guinea pig was placed on rabbit pellets, supplemented by ascorbic acid. The cod liver oil was discontinued. The disorders mentioned above disappeared in three days. The animal was killed 9 days after the diet had been changed.

Autopsy findings:

The skeletal muscles, especially the pectoralis, triceps brachii, quadriceps femoris and biceps femoris appeared uniformly light in color. The abdominal muscle showed some whitish patches.

The heart and viscera were apparently normal.

Microscopic findings:

The skeletal muscles, especially the abdominal and quadriceps femoris showed degenerative and regenerative changes. Degenerative change was minor in extent. Here and there some limited necrotic areas were observed. The histiocytes and giant cells were very scant. Myoregenerative processes were very active. The new muscle fibers extended into the fibroblastic tissue and were parallel to each other. The cross-striation was pronounced. The nuclei in some fibers were still numerous and formed rows and they migrated more or less to the

peripheral part of the fibers. However, some others looked more mature. The nuclei had decreased in size and number, being found under the sarcolemma (Fig. 26).

The heart muscle did not show any change.

Case 17

Group No.	Animal No.	Survival period (days)	Body Weight Initial (grams)	Body Weight Final (grams)
3	18	62	225	516

Clinical observation:

The body weight of guinea pig 18 increased gradually until the end of the experiment. However, the animal showed difficulty in rising when placed on the back and walked slowly on the 46th day. These disorders were aggravated until the 50th day and disappeared in three days after the animal was placed on rabbit pellets, supplemented by ascorbic acid and the cod liver oil discontinued. The guinea pig was fed this diet for twelve days and it was killed on the 62nd day of the experiment.

Autopsy findings:

The skeletal muscles appeared light in color.

No whitish patches or streaks were found in the muscles.

The heart showed some dilatation. The other organs were apparently normal.

Microscopic findings:

Some necrotic changes of the muscle fibers were observed after a long search of the sections. Regenerative processes were active. The muscle fibers were mature and extended into the fibroblastic tissue. The cross striations of new fibers were pronounced. The nuclei in these fibers were large and abundant. They were located peripherally. Some of them were in the central part of the fiber. (Fig. 27). The diameter of new fibers were different.

The heart muscle showed no changes.

Case 18

Group No.	Animal No.	Survival period (days)	Body Weight Initial (grams)	Body Weight Final (grams)
3	21	66	284	566

Clinical observation:

Guinea pig 21 gained weight gradually and showed

difficulty in righting itself when placed on the back and walked slowly on the 47th day of the experiment. This disorder almost disappeared in three days after the animal had been placed on rabbit pellets supplemented by ascorbic acid and the cod liver oil discontinued on the 50th day. The guinea pig was killed 15 days after substituting the new ration.

Autopsy findings:

The skeletal muscles were apparently normal. The pectoral and biceps brachii muscles were lighter in color than the others.

The heart and viscera appeared to be normal.

Microscopic findings:

The muscle fibers exhibited necrotic and regenerative changes. Here and there small necrotic areas were observed. The regenerative processes were active. The newly formed muscle fibers were of different diameter (Fig. 28). The fibers were more or less mature, but some fibers contained a large number of nuclei. The muscle fibers were extending into fibroblastic tissue and were parallel to each other. There were certain areas in which fibroblastic activity dominated over the formation of new fibers. The gap left after the necrotic fibers had been removed was filled by fibroblastic

connective tissue in which fat lymphocytes, and some polymorphonuclear leucocytes had infiltrated. These cells were often observed around small vessels.

The heart did not reveal any microscopic changes.

DISCUSSION

Skeletal muscular dystrophy was produced in growing guinea pigs by feeding a synthetic diet according to the formula of Davis et al (1938) together with a supplement of 0.5 gram per animal daily of U. S. P. grade cod liver oil.

The experimental design was not concerned particularly with the etiology of muscular dystrophy. However, the pathological changes produced might be attributed to the direct toxic effect of cod liver oil as pointed out by Agduhr (1926, 1927), Hoejer (1927), Hendriksen (1928), Malmberg (1929), Wahlin (1931), Madsen et al (1935), McCay et al (1938), and Davis et al (1938), or to the destructive effect of cod liver oil on vitamin E in the diet by rancidity as pointed out by Cummings et al (1931), Mattill (1938 b, 1939), and Mattill et al (1942), or finally to a synergism of both factors. An attempt was made to minimize the development of rancidity in the fat by keeping the synthetic diet and cod liver oil in the refrigerator. Although the cod liver oil was given separately, no steps were taken to prevent mixing of the cod liver oil and diet in the gastro-intestinal tract and subsequently to minimize the probable destruction of vitamin E. Under these circumstances, we were not able to hold any particular substance responsible for the condition produced. Irre-

spective of whether cod liver oil acted directly or indirectly there was evidence that the addition of cod liver oil to the synthetic diet resulted in muscular dystrophy in guinea pigs which confirmed the results obtained by Madsen et al (1935) and Davis et al (1938). These changes in skeletal muscles were not specific for a cod liver oil containing synthetic diet. The muscular changes were also produced in guinea pigs and rabbits with a diet containing no cod liver oil by Goettsch and Pappenheimer (1931), Woodward and McCay (1932), in rats reared on a vitamin E deficient diet by Olcott (1938), Evans et al (1938), Knowlton and Hines (1938), Pappenheimer (1939, 1940) and in the rabbit by Mackenzie et al (1941 a).

Some evidence indicates that a vitamin C deficient diet can also produce muscular dystrophy. Such results were observed by Dalldorf (1929) in guinea pigs and by Hjarre and Hilleennggen (1936) in calves.

There may be other factors which cause muscular dystrophy in lambs and calves, the so called "white muscle disease". This condition was observed by Metzer and Hagan (1927), Marsh (1932), Willman et al (1934), Vawter and Records (1939), Sholl (1939), Thorp (1942), Cheng (1945) and Cameron (1945) in lambs; by Hjarre and Lilleennggen (1936) and Vawter and Records (1947) in calves.

In addition muscular dystrophy can be caused by factors other than dietary, that is, by toxic, infectious and traumatic agents. Such instances were reported by Stemmler (1914) in different --- diseases, Forbus (1926) in pneumonia, Steiner et al (1946) and Clawson et al (1947) in rhuematoid and rhuematic arthritis of man. The condition was also produced in various animals by Forbus (1926) following intramuscular injection of powerful irritants or by cutting off the blood supply, by Fishback and Fishback (1932 a, b) with different types of trauma, by Clark and Blomfield (1945), Clark (1946) and Harman (1947) following ligation of the artery to the muscle groups.

Under the conditions described above, the guinea pigs fed the synthetic diet, supplemented by 0.5 gram cod liver oil daily per animal and ascorbic acid on the alternate days gained weight gradually. After a period of growth which ranged from 22 to 36 days there was usually a decline in weight which occurred synchronously with the appearance of clinical symptoms. The clinical response consisted of decreased activity, slow locomotion, difficulty in rising when placed on the back, which sometimes preceded the decline in body weight, lack of body tone, and finally paralysis of the fore and hind legs followed by death. As long as the administration of cod liver oil was continued no spontaneous recovery took place. However, if dystrophic

changes had not progressed too far, and the animals were placed on rabbit pellets, supplemented by ascorbic acid on alternate days, and the cod liver oil discontinued, a remarkable quick alleviation of clinical symptoms and recovery, followed by increase in body weight, occurred in a short period of time without using wheat germ, wheat germ oil and alphatocopherol as suggested by Shimotori et al (1940) and Mackenzie and McCollum (1940) for the treatment of the condition. The course, duration, and intensity of the condition varied considerably in each animal. The duration of the course of the disease might be influenced by outside temperature as observed in experiments I and II, if other dietary factors were kept constant. The hot season seemed to shorten the course possibly by causing decreased peristalsis of the intestine which makes food stay longer in the gastro-intestinal tract in contact with separately supplemented cod liver oil which probably results in autooxidative destruction of vitamin E in the diet if Mattill's hypothesis (1939) was correct. The vitamin E deficient diet and cod liver oil together resulted in the early appearance of clinical symptoms.

Pappenheimer (1939) attributes the dystrophic changes produced in the young female rat by restricted vitamin E diet to excessive contraction of the fiber with a segmental rupture and subsequent necrosis, a direct and selective toxic action and finally to angiospastic occlusion which resulted in anoxemia and infarction. During the

degenerative process of skeletal muscles the myofibrills undergo dystrpphic changes first. They lose their integrity and become homogenous. Of course, these changes interfere with the normal function of the muscle fiber, in other words, inability in contraction of contractile substance developed. Furthermore it is hard to believe that fibers contract excessively when they lose their ability for contraction. Not all muscle fibers undergo degenerative changes, some of them are partially involved. It seems improbable that angiospastic occlusion results in a necrosis of one part of a fiber and leaves another part of the same fiber sound. Pathogenesis so described is not clear. It seems reasonable to assume that there was a disturbance in metabolism of muscle cells due to the toxic effect of cod liver oil and lack of vitamin E in diet as a result of rancidity. Hawk et al (1947) stated the importance of vitamin E in the oxidative processes in muscle cells.

The pathological changes appeared to be confined primarily to skeletal muscles. In advance cases, practically all skeletal muscles were more or less involved. However, the abdominal, intercostal, pectoralis, gracilis, adductors and triceps brachii muscles were affected before the others. The lesions in the muscles were similar to those described by Forbus (1926) in pneumonia, Goettsch and Pappenheimer (1931) following the feeding of diet 13, Madsen et al (1935), Davis et al (1938) by feeding a synthetic diet, supplemented

by cod liver oil, Evans et al (1938), Pappenheimer (1939, 1940) by a vitamin E deficient diet. There was some similarity between the lesions produced in this laboratory and those described by Metzer and Hagan (1927), Nieberle and Cohrs (1931), Willman et al (1934) Hjarre, Lilleenngen (1936), Salyi (1942), Cheng (1945), Vawter and Records (1947) in so called white muscle disease of lambs and calves. The pathological changes consisted of skeletal muscle degeneration. The degeneration in some muscles was localized and involved only a part of the muscle; in others, the degenaration was generalized and affected the entire muscle. The muscles were pale, yellowish gray streaked, patchy and showed a cooked, moist appearance (Figs. 1, 2). They were easily torn, friable and atrophied. In the longitudinal view, the degeneration appeared as grayish streaks which were mottled or continuous throughout the length of the muscle. The grayish streaks and patches were distributed symmetrically and bilaterally as stated by Madsen et al (1935). However, Pappenheimer (1939) stated that all muscles were involved in the degeneration produced in young rats on a restricted vitamin E diet, but were not necessarily symmetrical.

The histological picture found on examination of the skeletal muscles depended on the intensity and progress of the degenerative process. Primary changes consisted of coagulation necrosis in which the contractile substance ac-

quired a hyaline, waxy appearance followed by myoregeneration. Muscle fibers involved showed partial or total waxy degeneration (Fig. 3). The histiological picture was not uniform throughout a single fiber.

The affected fibers became swollen, stained weekly with eosin and were separated by edematous fluid, while others were shrunken. The nuclear activity of affected fibers was increased. The number of nuclei were increased and appeared hyperchromatic. Muscle fibers first lose the cross and later the longitudinal striations. Although Millar (1933) stated that during degenerative processes both the Q and the j bands seemed similarly affected and disappeared at an early stage in the dystrophic change while the Z line or Krause's membrane remained intact even in advance stages of hyalinization. At this time the muscle fibers broke into granules. The author concluded that the Z line was the most resistant component of the cell as far as starvation was concerned. As far as could be seen in our sections the Z line seemed to disappear with the Q and the j bands. However, in some fibers, the cross striations were accentuated and appeared close to each other, persisting even in the completely degenerated part of the muscle fiber. This was observed by Clark (1946) and Harman (1947) in muscle necrosis induced by ligation of the artery to the muscle groups. The muscle fibers became homogenous, translucent and stained deeply with eosin. Some affected fibers at

this stage broke into small pieces, giving a granular appearance to the fiber. The granular mass filled the sarcolemma of the fiber when this sheath was intact (Figs. 7, 8). Other affected fibers did not break into granules but formed clumps which were homogeneous and sometimes contained vacuoles (Fig. 4, 7, 10).

The nuclei of affected fibers were pyknotic, staining dark blue with hematoxylin-eosin and many of them had undergone karyorrhexis and karyolysis. Usually the sarcolemma was involved. In some instances, however, the destructive process was not severe, the contractile substance was only hyalinated and the sarcolemma appeared not to be affected.

The changes stimulated the infiltration of polymorphonuclear leucocytes, lymphocytes, plasma cells and histiocytes, also the proliferation of fibroblasts, giant cell formation and development of multinucleated syncytial cell masses (Figs. 5, 9, 10, 12, 13). The number of the first three cells was few and they were observed in the interstitial tissue. The histiocytes were numerous and invaded the necrotic fibers, playing an active role in the removal of necrotic material. The necrotic part of the fiber became enveloped in giant cells which engulfed tissue debris. The multinucleated syncytial cell masses which appeared to be similar to giant cells morphologically were of muscular origin. Where the pointed tip of a regenerating fiber met an obstruction, the fiber

became pear shaped, thickened and contained many nuclei in cluster form. The multinucleated syncytial masses were disconnected from the old fibers. As far as could be seen the multinucleated syncytial masses did not engulf debris. In some instances, the older degenerated areas were partially calcified (Figs. 6, 11).

The sarcolemma which escaped destruction were completely filled with muscle spindle cells, multinucleated syncytial masses, giant cells and histiocytes, giving rise to "Muskelzellenschlauche" of Waldyer (Fig. 10).

There was no uniformity of opinion concerning the origin of the phagocytic cells in the "Muskelzellenschlauche" of Waldyer which invaded the necrotic material.

Forbus (1926) conducted an experimental study on myodegeneration and myoregeneration using vital staining. As a result, he confirmed his previous work and stated "the phagocytic cells found within the persistent sarcolemma, which together with the muscle cells derived from the muscle nuclei form the "Muskelzellenschlauche" of Waldyer, are wandering cells of extramuscular origin and have no connection with the muscle cells, although he mentioned that "muscle cells as well as phagocytic cells of the "Muskelzellenschlauche" are capable of being stained by intravenous injection of vital dyes (Carmin or trypan blue) during their development, and hence such vital staining alone was insufficient

for differentiating between cells of muscle origin and cells of extramuscular origin". We found that the "Muskelzellenschlauche" of Waldyer contained histiocytes, giant cells which phagocytosed the dye in large amounts. The spindle-shaped muscle cells and multinucleated syncytial masses were not actively phagocytic; however some of them engulfed the dye to a limited extent (Figs. 15, 16, 17, 18). Fibroblasts and endothelial cells of capillaries took up the trypan blue (Fig. 19). On this basis it was difficult to draw an accurate conclusion concerning the origin of these cells.

Goettsch and Pappenheimer (1931) reported their results on the study of nutritional muscular dystrophy in guinea pigs and rabbits. They agreed with Forbus as to the origin of the so called invading "histiocytes". They also found large multinucleated plasmatic masses, lying against the necrotic remains of the muscle substance. In fact, according to our interpretation the large multinucleated plasmatic masses are nothing more than giant cells.

In the same year, Nieberle and Cohrs (1931), in the study of hyaline degeneration of muscle, observed the proliferation of histiocytes in the "muskelzellenschlauche," but they did not state anything about the origin of the cells and also pointed out that the granulation tissue surrounding the necrotic tissues was infiltrated by polymorpho-

nuclear leucocytes and foreign body giant cells. These cells were also observed at the end of the muscle fiber, forming hood-shaped masses which according to them lost their attachment to the fibers. It seemed to us that the masses formed at the end of the fiber were similar to our multinucleated syncytial masses described above which were formed when the growing end of a regenerating fiber met an obstruction (possibly necrotic material) and are not actively concerned with the removal of necrotic material. However some of these cells phagocytize the dye to a minor extent.

Madsen et al (1935) produced muscular dystrophy in various animals with a cod liver oil containing diet and found myodegeneration followed by an active myophagocytosis. They did not mention, however, the type of cells responsible for myophagocytosis.

In the study of the pathological changes of skeletal muscles induced in rats by vitamin E restricted diet, Pappenheimer (1939) found that the segments of necrotic muscle fibers became enveloped in plasmatic multinucleated masses and pointed out that these plasmatic multinucleated masses possibly were derived from the persisting myocytes. One year later the same author (1940) conducted another experiment and produced muscular dystrophy in guinea pigs and rabbits by a vitamin E deficient diet and stated that the necrotic fibers became invaded by polymorphonuclear leuco-

cytes and histiocytes which often fused to form plasmatic multinucleated masses about the degenerated remains. It is probable that both of these cells are giant cells which originated from histiocytes and were active in the removal of necrotic material. In our sections the multinucleated syncytial masses, originating from muscle cells were not actively phagocytic, even though some of these cells engulfed the trypan blue to a slight degree.

Cheng (1945), in the study of white muscle disease of lambs, stated that "by means of aniline blue and eosin stain, the morphology of nuclei and the staining affinity of both giant cells and regenerating muscle fibers were exactly the same, indicating clearly that they were all derived from the same parent tissue, i. e., muscle and were not of extra-muscular origin", and also added that "the function of the muscle giant cells was to remove the dead muscle tissue and that of the regenerating muscle fibers to fill the gaps left by the dead tissue". Using the same staining method we found that the giant cells concerned with removal of foreign material showed engulfed debris. However, as far as could be seen the spindle shape muscle cells and multinucleated syncytial masses (called by Cheng muscle giant cells) did not reveal engulfed debris. It seemed to us that the staining method suggested by Cheng was not conclusive regarding the origin of the cells.

Clark (1946), working on regeneration of mammalian striped muscle in rabbits, did not accept the view concerning the phagocytic function of regenerating muscle cells and their active role in the removal of necrotic material. However he mentioned that in certain circumstances the remains of a damaged muscle fiber might give rise by budding to independent cellular elements and he added that there was no evidence that these independent cells formed myoblastic tissue or that they played any part in the removal of necrotic muscle fibers.

Maximow and Bloom (1947) assumed that the sarcoblasts possessed phagocytic functions and digested the degenerated fibers.

Confirming the finding of Forbus (1926) Altschul, and Friesen (1947) reported that dissociating muscle cells of damaged, but not completely necrotic, muscle fibers phagocytosed the dye following the insertion of catgut impregnated with either trypan blue, carmine or hematoxylin. He also found that in the rabbit which received trypan blue intraperitoneally, the proliferated muscle nuclei did not show granules of trypan blue in the juxtanuclear plasma and he assumed that either the sarcolemma prevented the penetration of the dye or that the myocytes were not sufficiently individualized. It seemed to us that the spindle shaped muscle cells and multinucleated syncytial masses have the same origin, and some of them phagocytose the vitally injected

dye to a minor extent. Histiocytes and fused forms of histiocytes, i. e., giant cells, engulf the dye in large amounts and are extra muscular in origin. However, Chevremont (1940) reported the direct transformation in vitro of myoblasts into typical histiocytes.

Regeneration of muscle fibers occurred synchronously with degenerative alteration of the contractile substance as observed by Forbus (1926), Goettsch and Pappenheimer (1931), Madsen et al (1935), Davis et al (1938), Pappenheimer (1939) and Cheng (1945). The most successful regeneration took place in those instances where only the contractile substance was destroyed, the sarcolemma and the peripheral layer of sarcoplasm with nuclei remained intact. This observation was also stated by Nieberle and Cohrs (1931). The first sign of formative activity of muscle fibers after injury appeared in the muscle nuclei of the old damaged fibers. This process was followed by formation of plasmodial outgrowths from the stumps of old fibers. These plasmodial outgrowths retained their attachment to the old fiber and usually showed pointed tips which penetrated the fibroblastic tissue (Fig. 23). Bifurcation of the tip was not uncommon. However, the pointed tip became thickened and pear-shaped when it met an obstruction, giving rise to a multinucleated syncytial mass (Fig. 25). Similar observations were made by Lewis (1943) in transplants of rhabdomyosarcomma and by Clark (1946) in the study of the regeneration of muscle in

rabbits. Nuclei within plasmatic strands multiplied mostly by amitosis. However here and there some mitotic figures were also observed. This finding confirmed Kitt (1929) and Clark's (1946) observations.

Maximow and Bloom (1947) stated also that "The nuclei, during the gradual growth of the muscle fibers, increased in number by mitosis and in later stages, perhaps by amitosis". Contrary to our finding, Forbus (1926), Goettsch and Pappenheimer (1931), Speidel (1938), Pappenheimer (1939), Cheng (1945) claimed that the nuclear division in regenerating muscle fibers was mitotic. Nuclei were centrally located in young plasmodial outgrowths and surrounded by a granular cytoplasm which stained basophilic (Fig. 24). As the new fibers approached maturity the basophilic staining characteristic of the cytoplasm decreased and they stained more intensely with eosin. Myofibrills occupied the periphery of plasmatic strands in the beginning. During later stages the nuclei moved toward the periphery, so that the central parts became occupied by myofibrills (Figs. 26, 27). The appearance of myofibrills in the cytoplasm did not take long after the plasmatic strands had pushed out from the old fibers. The longitudinal striations appeared first and were followed by cross-striation of the fibers. Regenerating fibers arose from the stumps of preexisting fibers by continuous outgrowth as observed by Clark (1946). There was no evidence to indicate that muscle cells originated from fibroblastic tissue. Levander (1945) claimed

that myoblastic elements arose by differentiations of mesenchymatous tissue by a process of induction. Also, Clark and Blomfield reported (1945) that fibroblastic elements might contribute to myogenesis, but they did not mention whether fibroblasts originated from new muscle fibers or not. It seems to us that the fibroblastic tissue formed strengthens weakened muscle due to necrosis and it helps in the formative process.

The origin of sarcolemma has been discussed by many workers. Forbus (1926) stated that "of the origin of a new sarcolemma, we are able to say nothing", whereas Kitt (1929) believed that the sarcolemmal sheath probably was formed by connective tissue which enveloped the new fiber. In study of living muscle of the frog (tadpoles), Speidel (1938) found that the sarcolemma was derived from myoblasts. It seemed that sarcolemma was formed by muscle cells.

The new fibers formed were parallel to each other. The fibers formed were at different stages of growth. Therefore we were not able to measure the rate of growth. In a study of regeneration of mammalian striped muscle, Clark (1946) found that the regenerating muscle fibers grew at the rate of at least 1 - 1.5 mm. per day. Confirming previous findings, Clark and Wajda (1947) reported that the average rate of growth was of the order of 1.2 mm. a day from the

4th to 14th day and in some cases this rate reached as much as 1.7 mm. per day, and also added that immobilization of the part severely impaired the process of repair and regeneration. Myoregeneration appeared complete.

SUMMARY AND CONCLUSIONS

1 - Dystrophy of skeletal muscles was produced in growing guinea pigs by feeding a synthetic diet according to the formula of Davis et al (1938) together with a daily supplement of 0.5 gram per animal of U. S. P. grade cod liver oil.

2 - The set up of the experiments did not permit a conclusion concerning etiology. However, the pathological changes might be attributed to a direct toxic effect of cod liver oil or to the destructive effect of cod liver oil on vitamin E in the diet by rancidity, or to a synergistic action of both factors.

3 - Pathological changes consisted of coagulation necrosis of skeletal muscles which acquired a waxy, hyaline appearance, followed by myoregeneration.

4 - Not all spindle shape muscle cells and multinucleated syncytial masses, of muscular origin phagocytosed vitally injected trypan blue dye during their development.

5 - Regeneration of muscle was complete. Regenerating fibers arose from the stumps of pre-existing muscle fibers by continuous outgrowth. In some instances, the pointed tip of regenerating fibers became thickened and pear-shaped giving rise to multinucleated syncytial masses.

6 - Nuclear division in regenerating muscle fibers took place

mostly by amitosis.

7 - The sarcolemma appeared to be a product of regenerating muscle cells.

8 - Hyaline degeneration of cardiac muscle did not occur in all cases studied.

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Fig. 1. Case 4. (Autopsy No. 9461)

Whitish patches and streaks are scattered throughout the muscle of the hind legs and the abdomen.

Fig. 2. Case 4. (Autopsy No. 9461)

Whitish patches and streaks are seen in the abdominal and intercostal muscles.

P L A T E 1

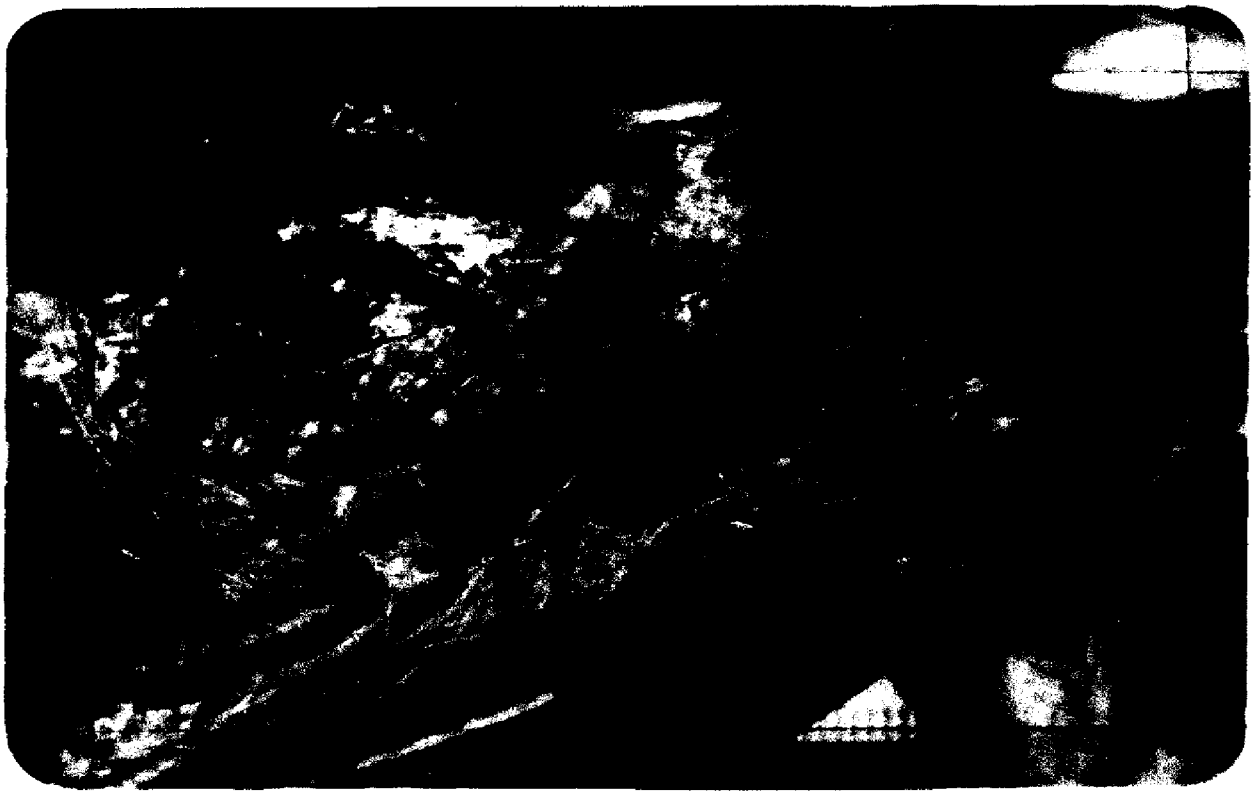


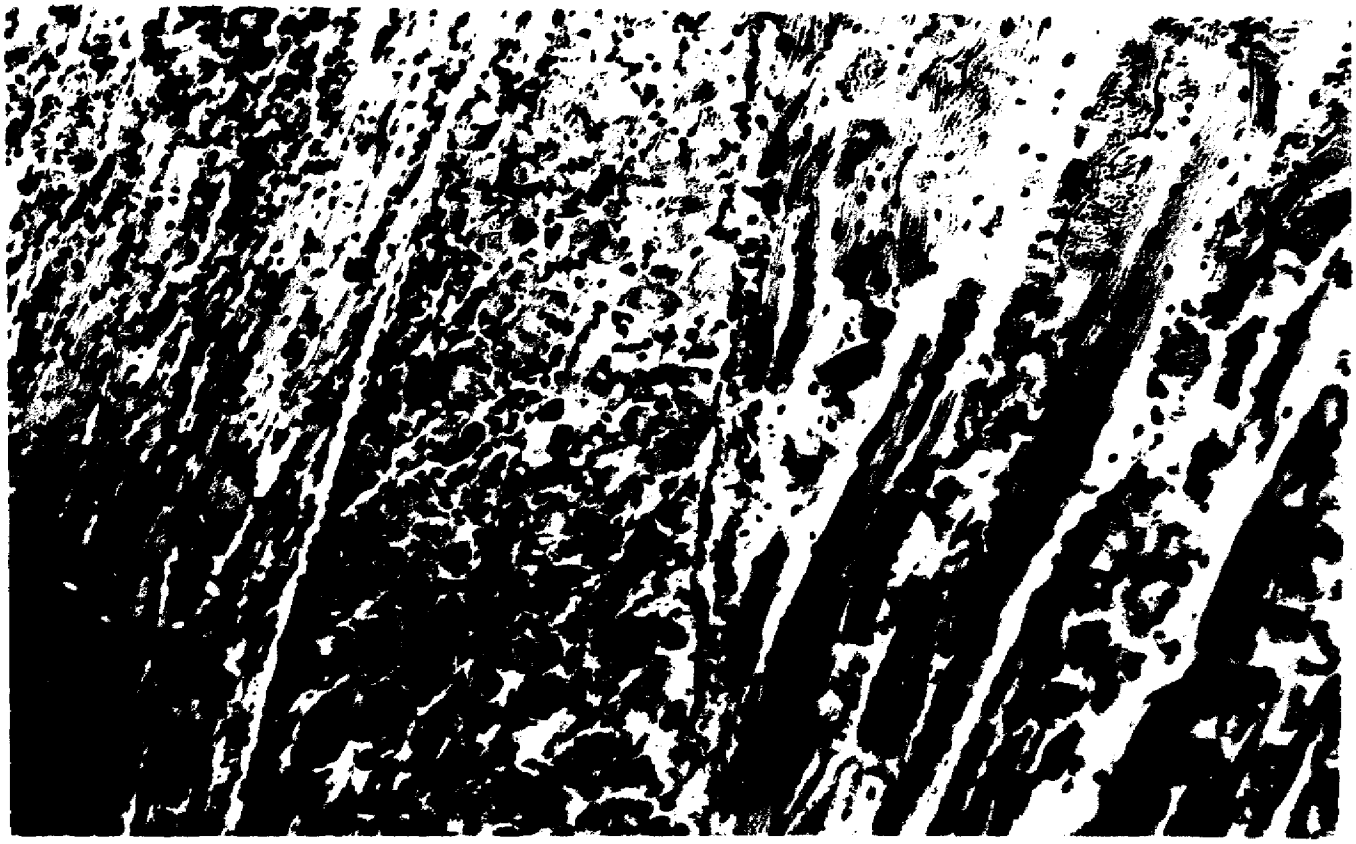
Fig. 3. Case 6. (Autopsy No. 9455)

Coagulation necrosis of the biceps femoris muscle. Infiltration with phagocytic cells is seen (H&E), x130.

Fig. 4. Case 6. (Autopsy No. 9455)

Extensive coagulation necrosis of the quadriceps femoris muscle. The hyaline clumps and granular breakdown of necrotic fibers are seen in the center of photomicrograph. (H&E), x103.

PLATE 2



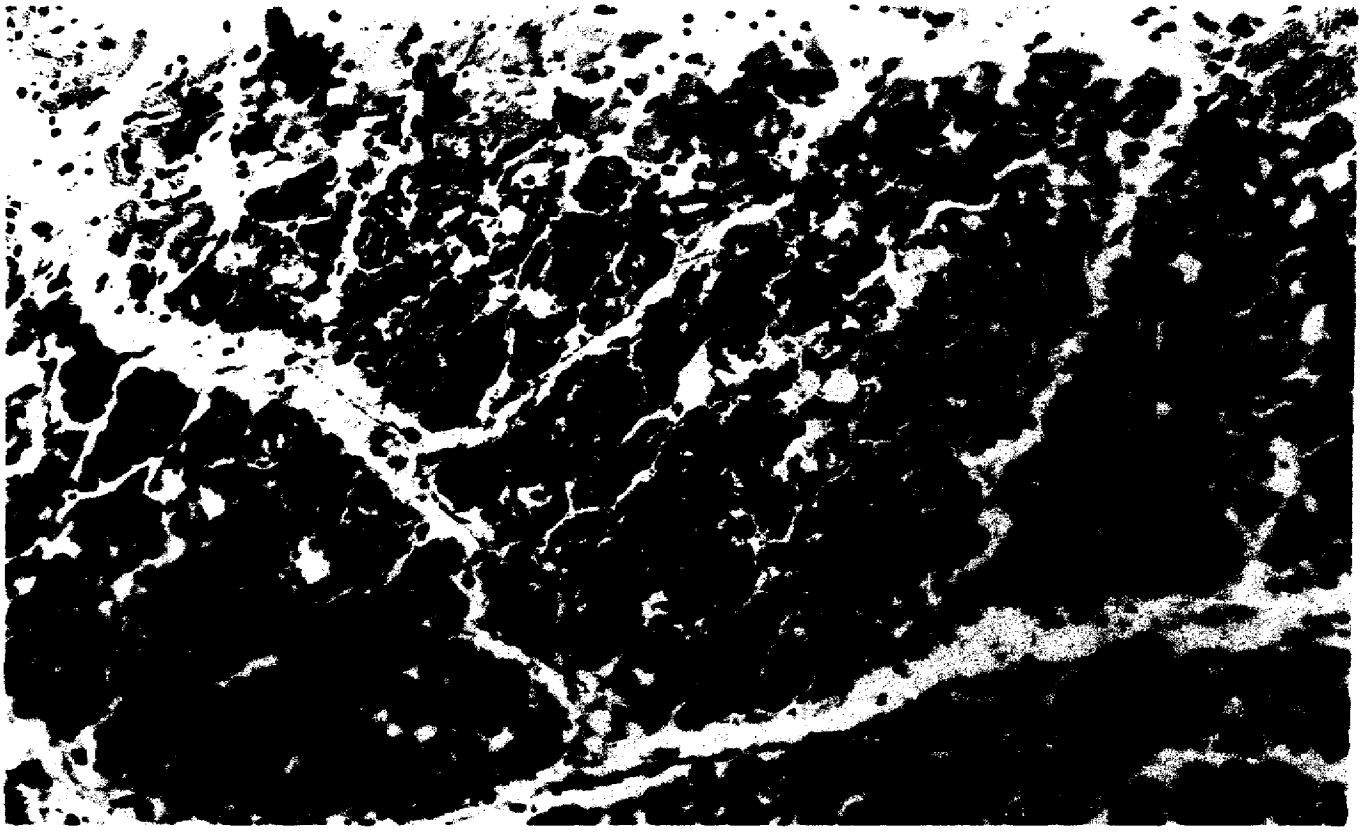


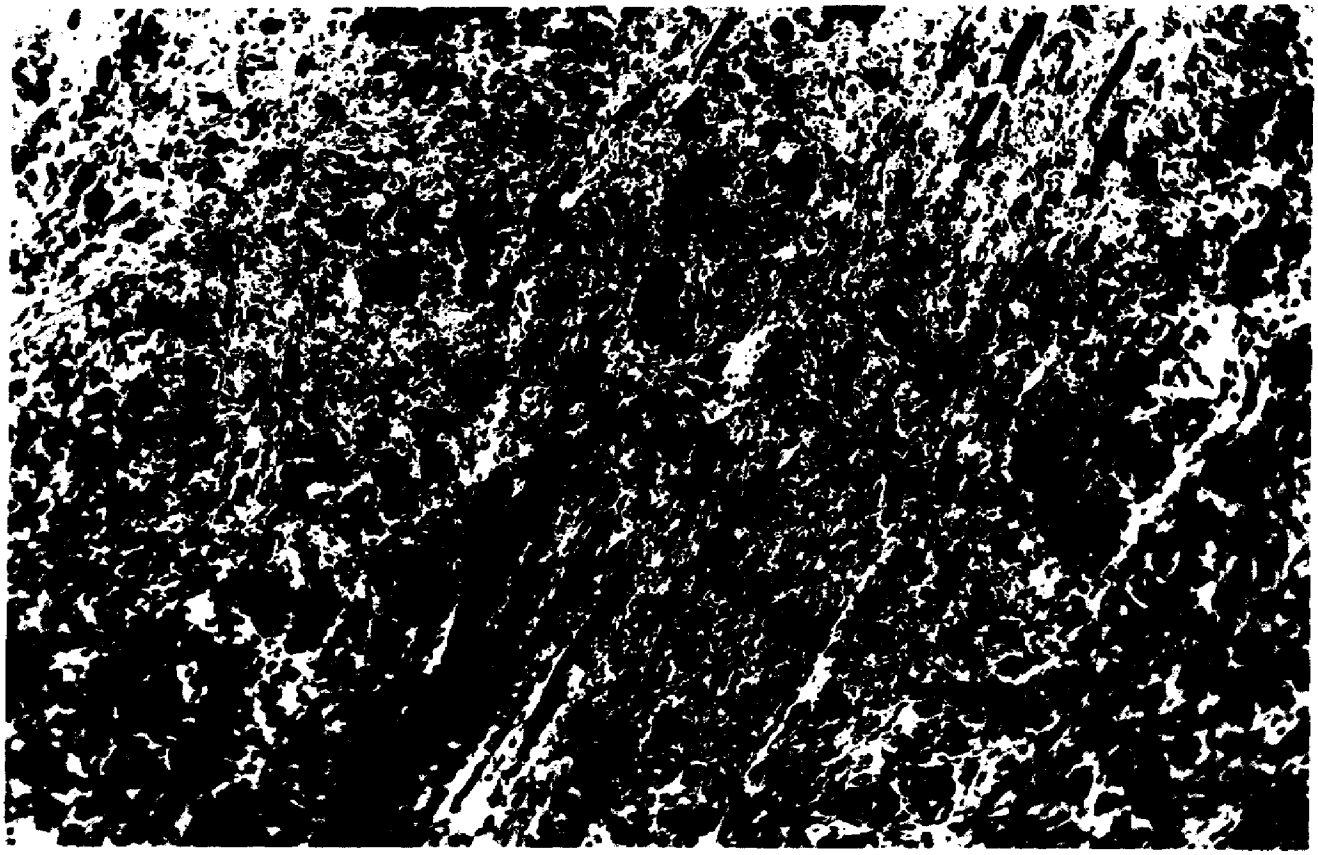
Fig. 5. Case 4. (Autopsy No. 9461)

The muscle fibers of the quadriceps femoris were replaced by fibroblastic tissue into which had infiltrated histiocytes and giant cells. Necrotic islands are also observed. (Mallory's aniline blue), x103.

Fig. 6. Case 1. (Autopsy No. 9454)

Deposits of calcium in necrotic tissue of the pectoral muscle. (H & E), x103.

PLATE 3



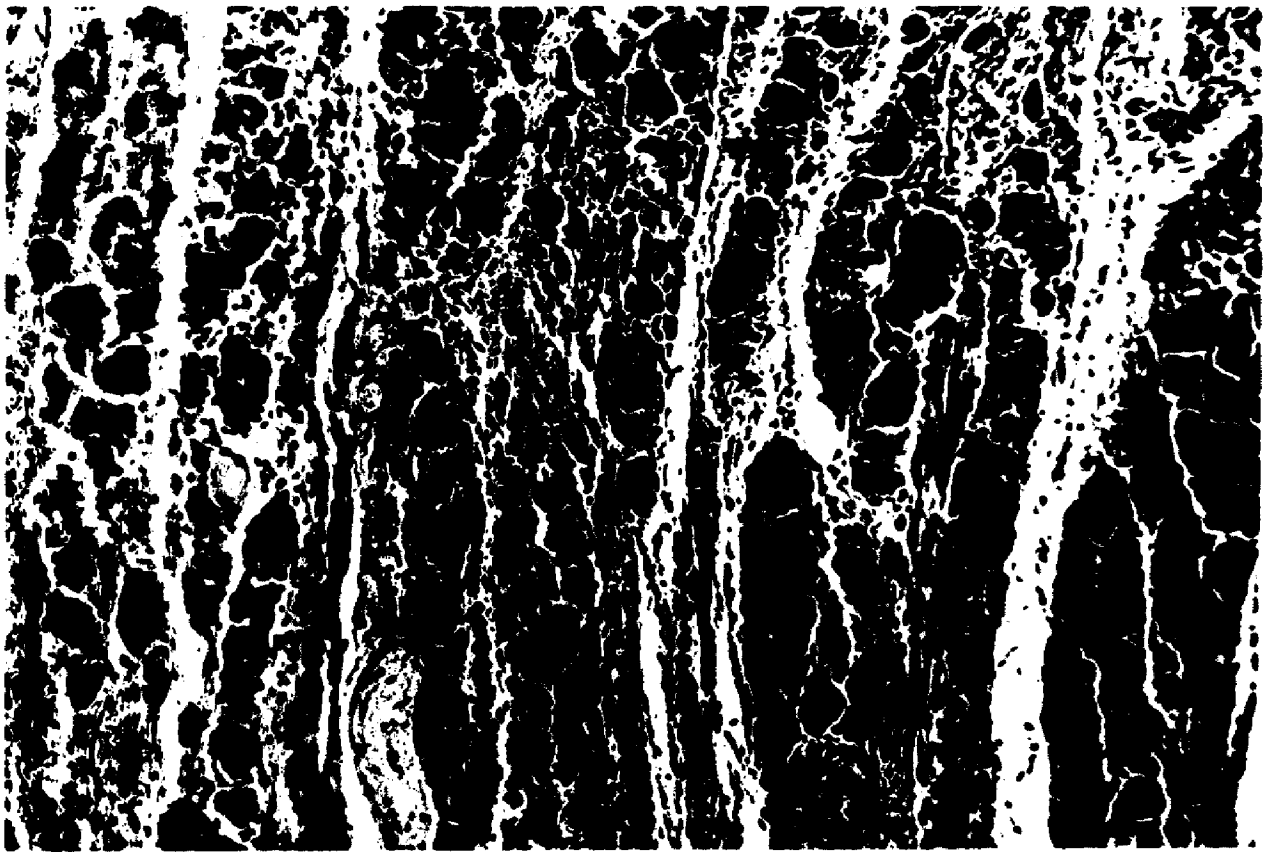


Fig. 7. Case 1. (Autopsy No. 9454)

Vacuolization of degenerated fibers of the abdominal muscle, and hyaline clumps surrounded by phagocytic cells (H&E), x103.

Fig. 8. Case 6. (Autopsy No. 9455)

The fibers of the quadriceps femoris show coagulation necrosis which assumed a granular appearance. Histiocytes with engulfed debris are seen. (H&E), x625.

PLATE 4

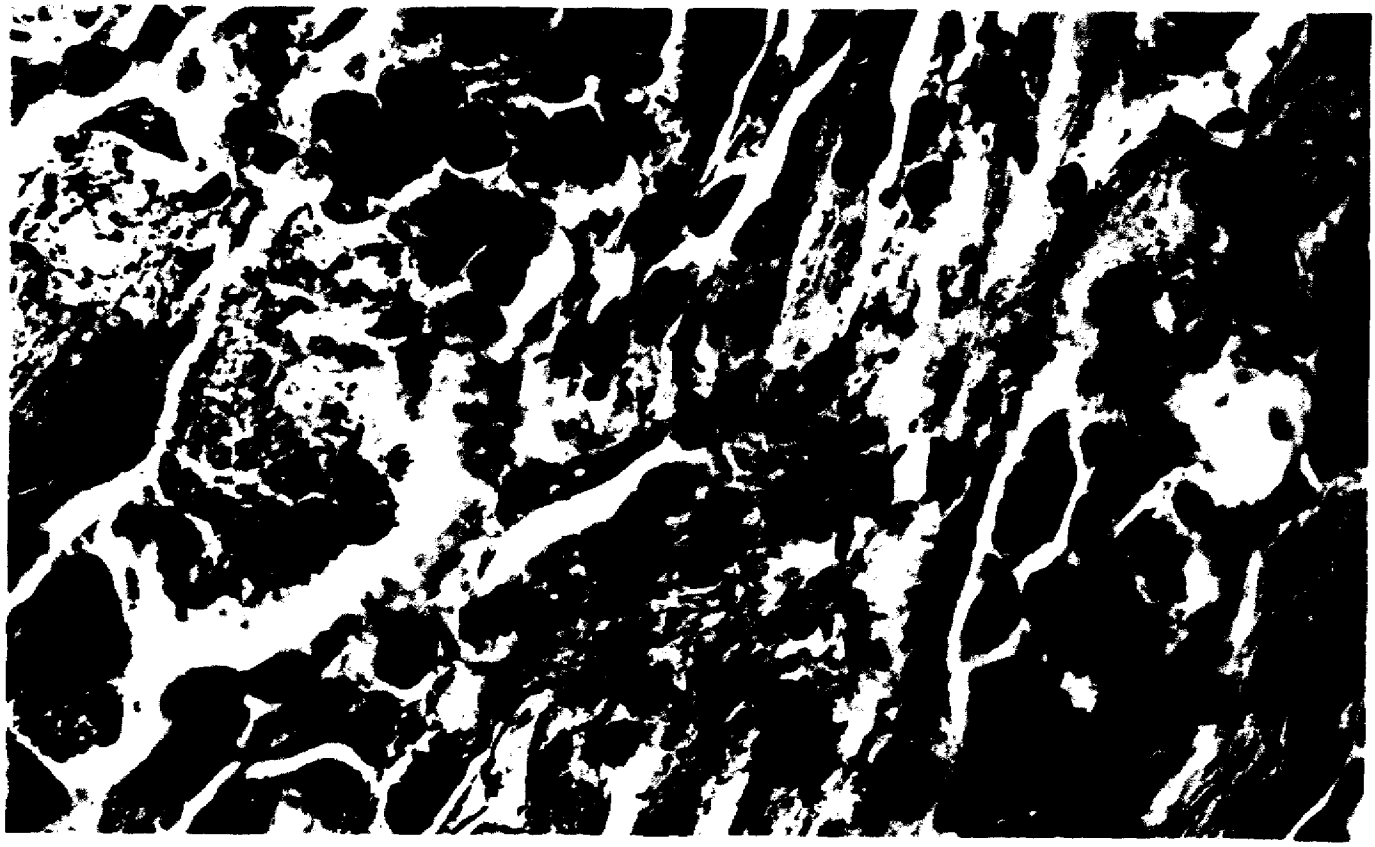
Fig. 9. Case 6. (Autopsy No. 9455)

Coagulation necrosis of the fibers of the biceps femoris muscle together with histiocytes, engulfed debris, and formation of some muscle cells (H&E), x625.

Fig. 10. Case 1. (Autopsy No. 9454)

Vacuolization of degenerated fibers of the abdominal muscle and cells with staining characteristics of the "Muskelzellenschlauche" of Waldyer are seen. Muscle cells and multinucleated syncytial masses possess pale nuclei with indefinitely outlined cytoplasm. Histiocytes and giant cells have deeply stained nuclei with definite outline. (H&E), x625.

PLATE 5



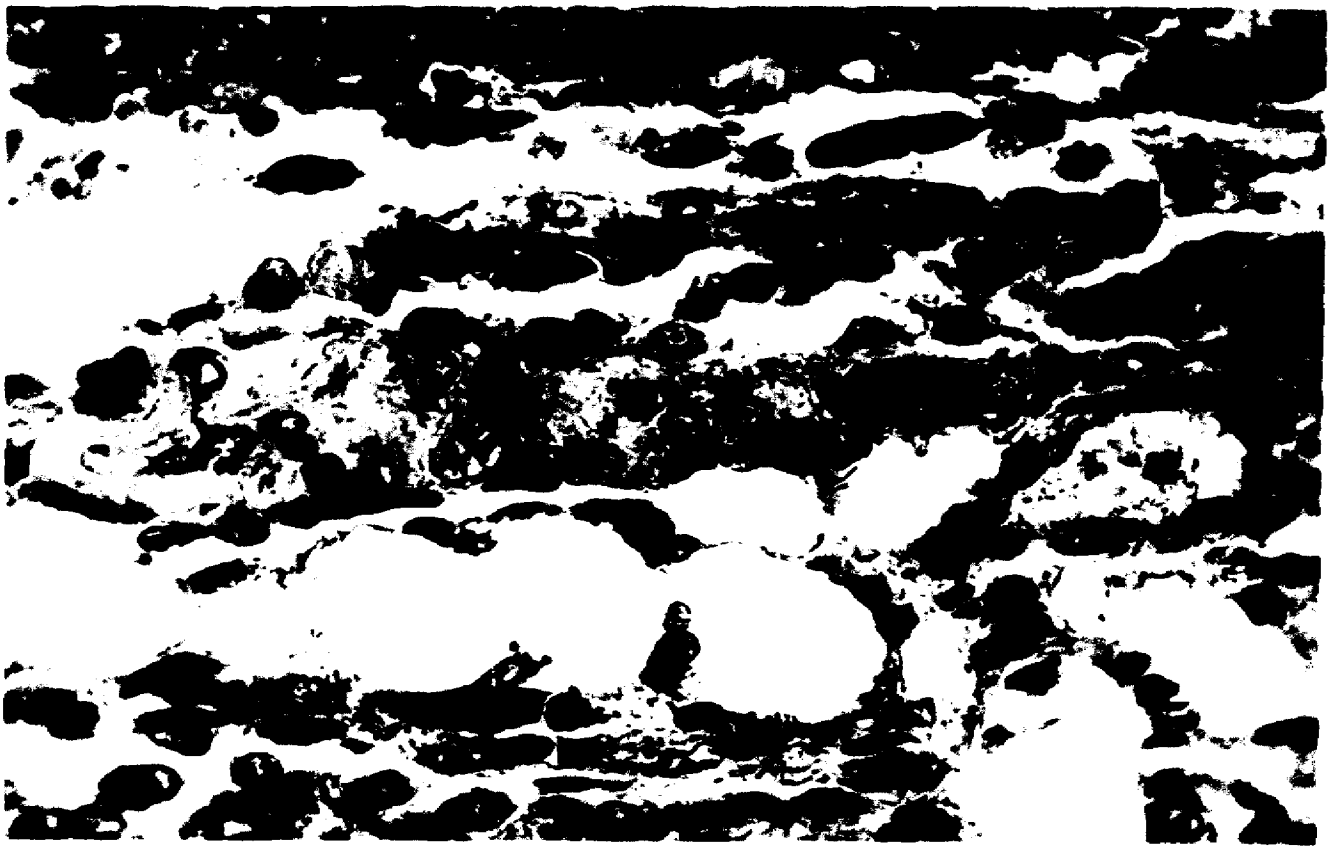


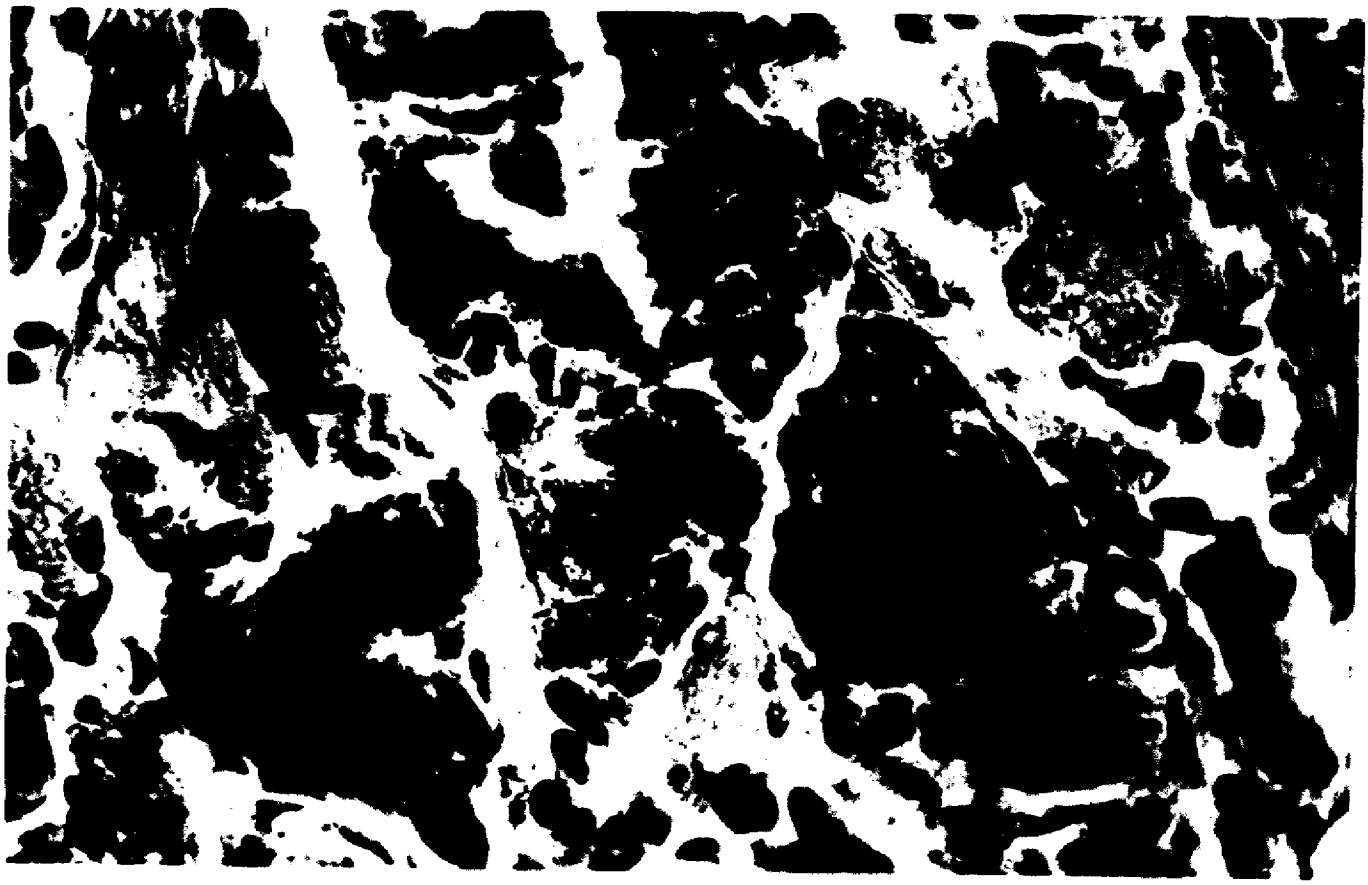
Fig. 11. Case 1. (Autopsy No. 9454)

Deposits of calcium in the necrotic tissue of the pectoral muscle and persistence of cross-striation in the degenerated fiber are seen. (H&E), x625.

Fig. 12. Case 1. (Autopsy No. 9454)

Deposits of calcium, giant cells, and histiocytes are present in the center of photomicrograph. (H&E), x625.

PLATE 6



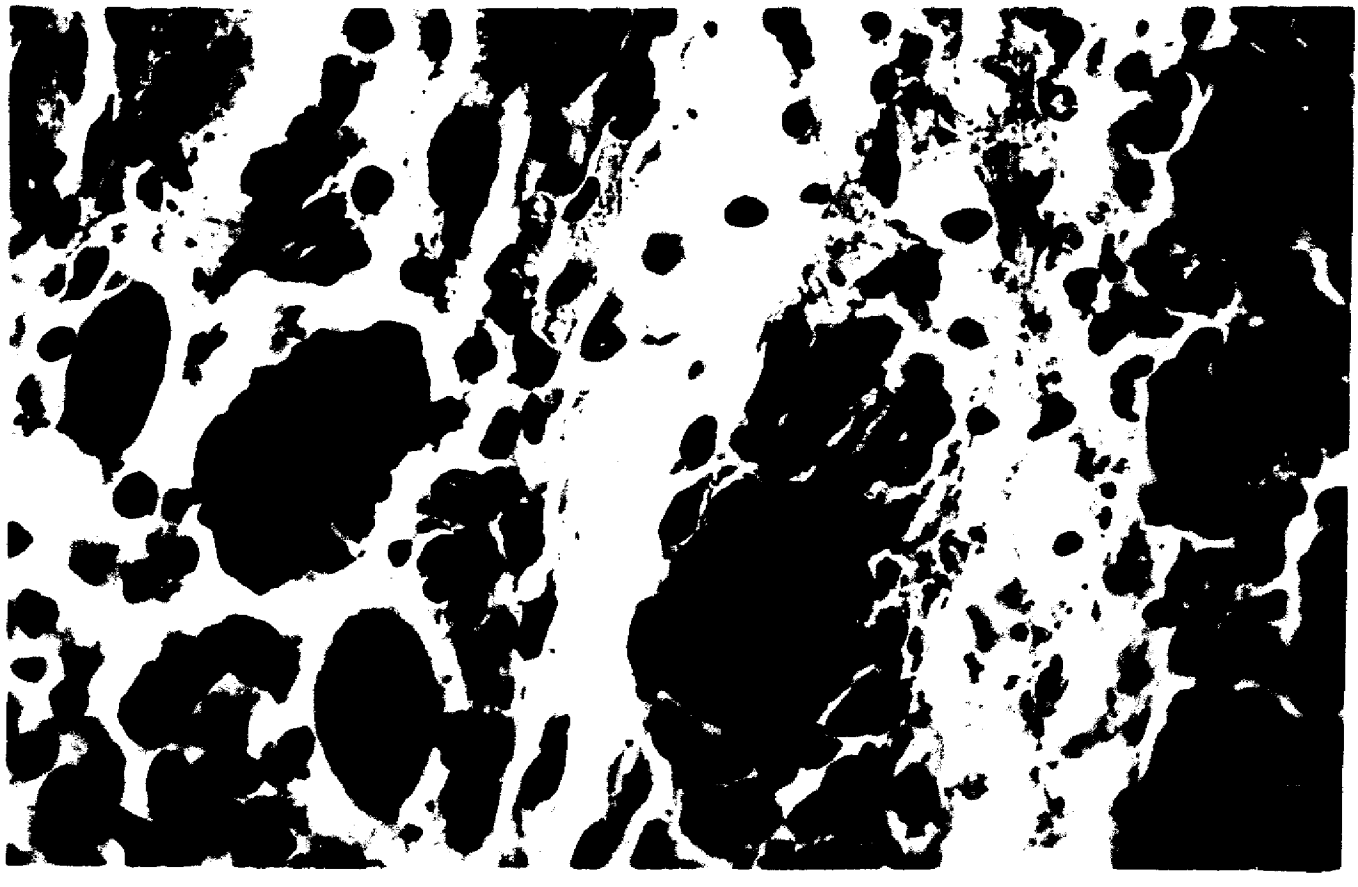


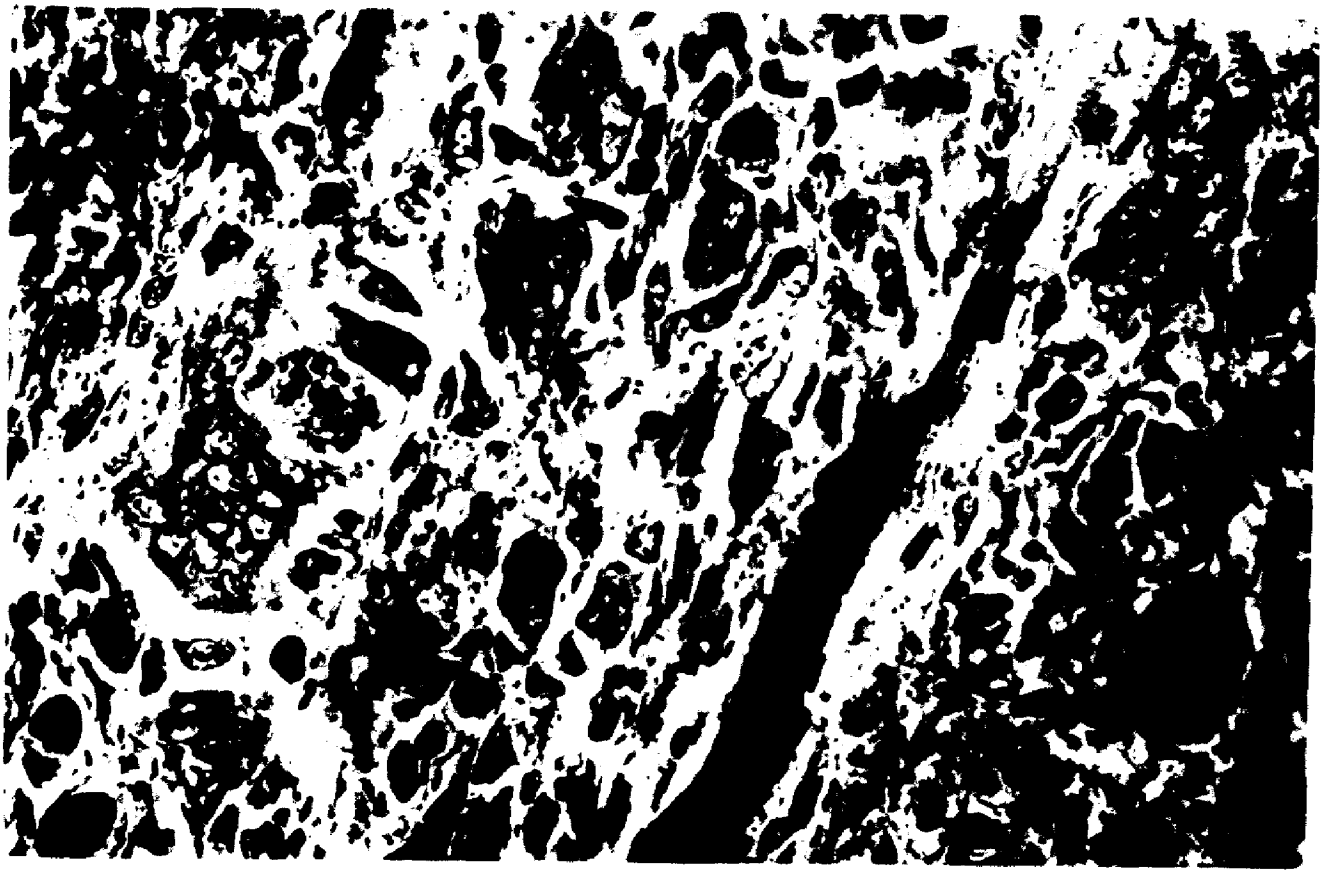
Fig. 13. Case 4. (Autopsy No. 9461)

Giant cells with engulfed debris are present. (Mallory's aniline blue), x625.

Fig. 14. Case 3. (Autopsy No. 9462)

The heart showed degenerative changes and cellular infiltration. (H&E), x625.

PLATE 7



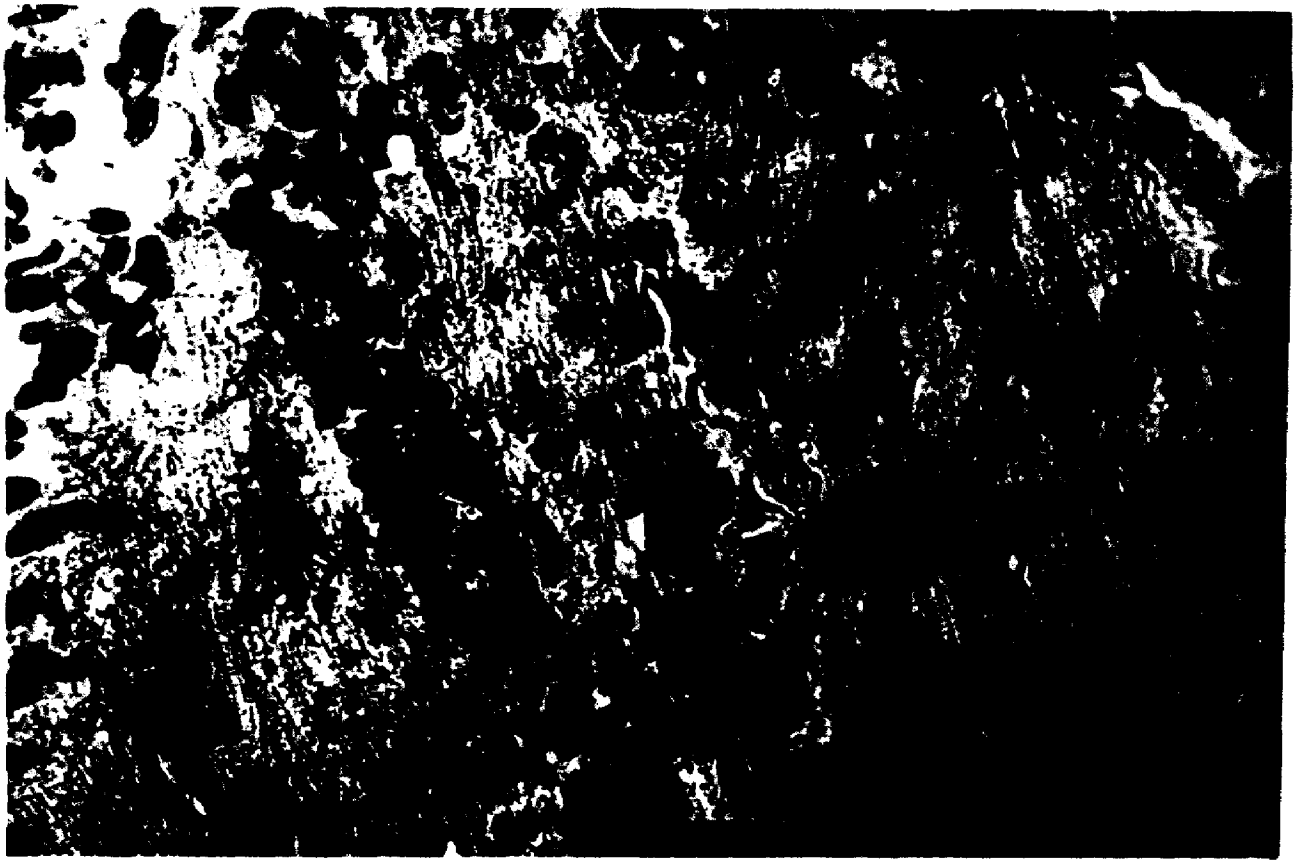


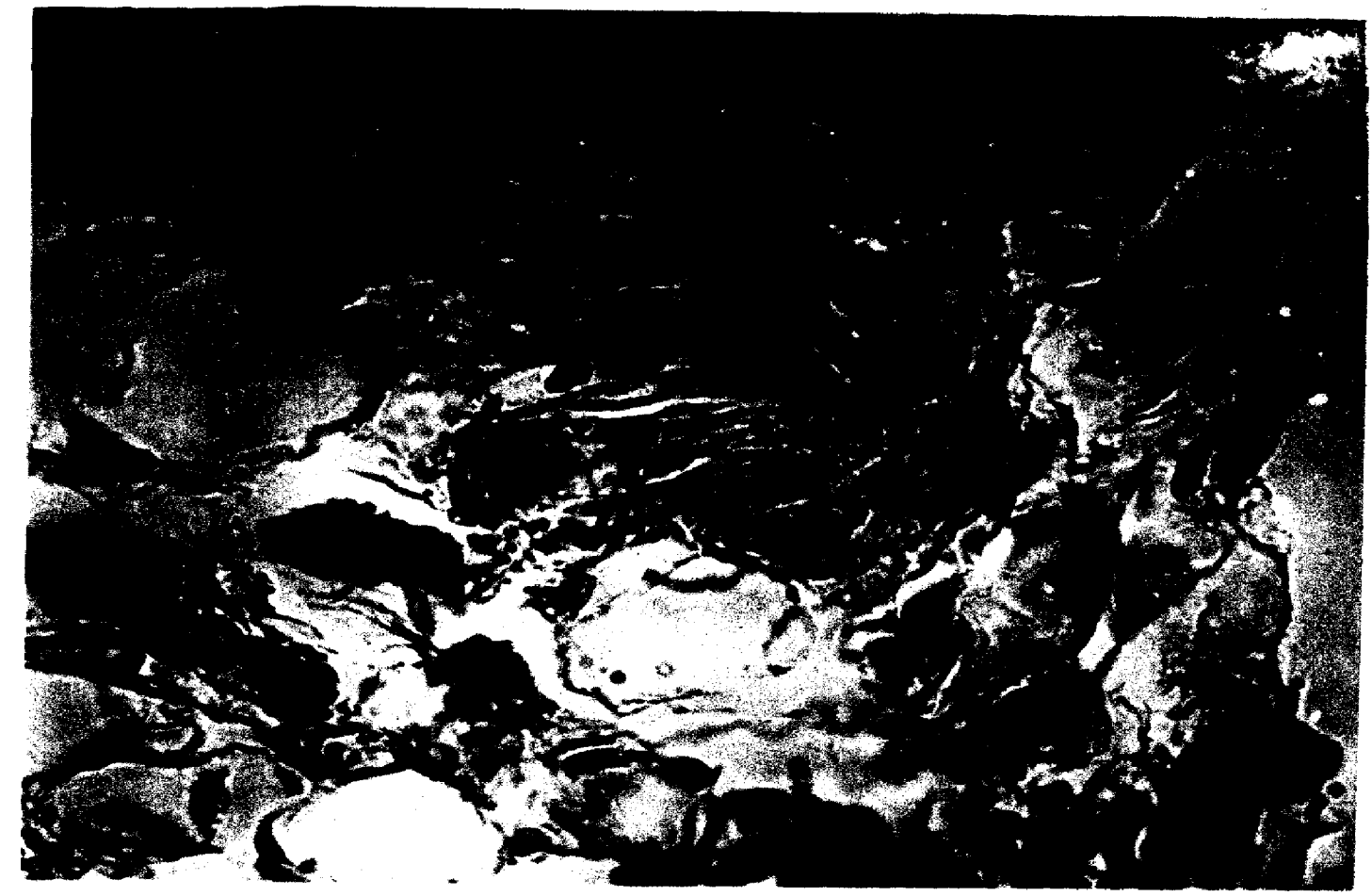
Fig. 15. Case 10. (Autopsy No. 9559)

Histiocytes and giant cells show trypan blue granules which are scarce in spindle shaped muscle cells and multi-nucleated syncytial masses. The later two cell types are seen in the center of photo-micrograph (vital staining with trypan blue & eosin contrast), x800.

Fig. 16. Case 10. (Autopsy No. 9559)

Histiocytes and giant cells engulfed the trypan blue. (Vital staining with trypan blue & eosin contrast), x1020.

PLATE 8



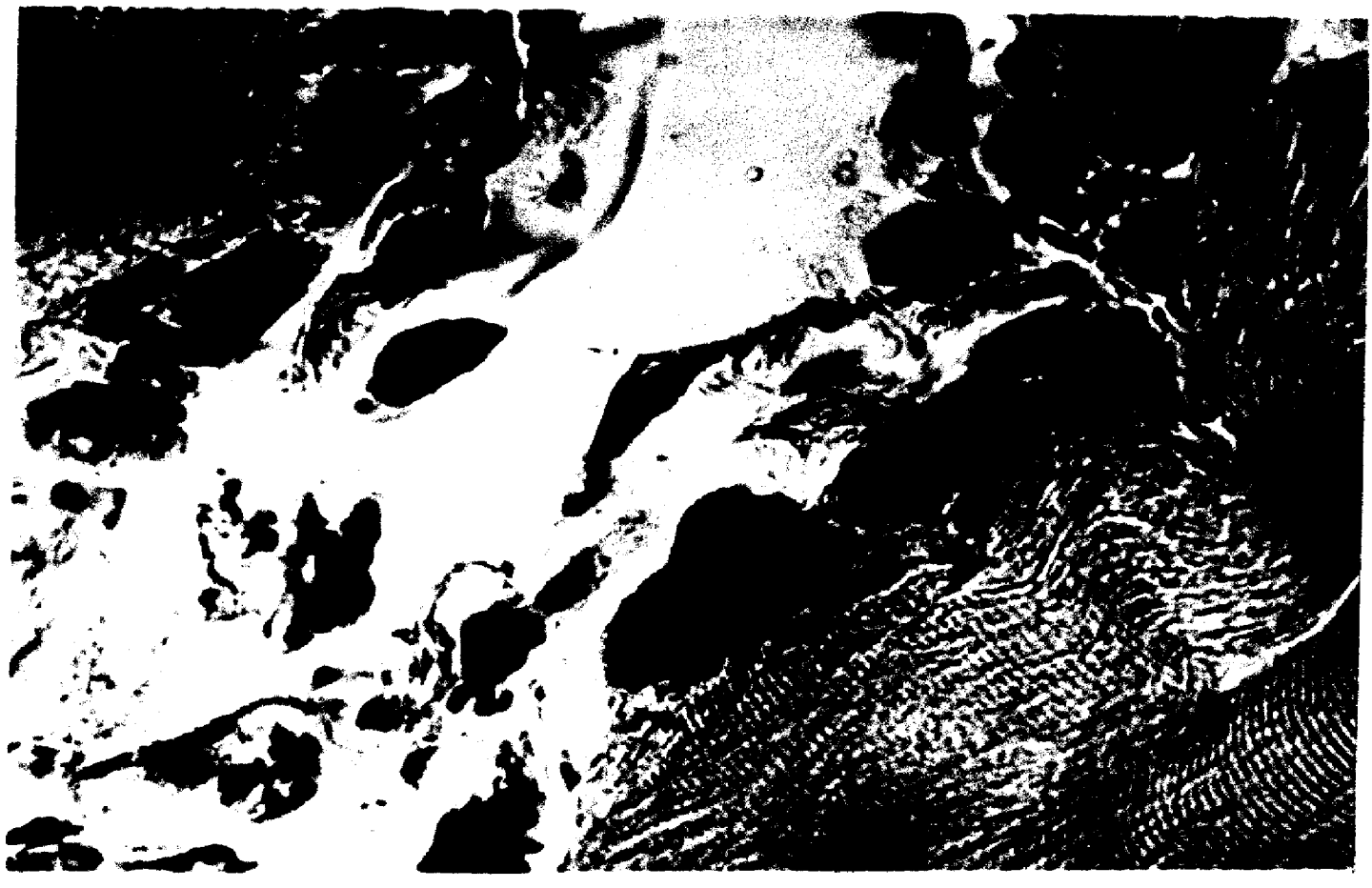


Fig. 17. Case 7. (Autopsy No. 9556)

Giant cells and histiocytes engulfed trypan blue. They are seen in the center of photomicrograph where the muscle fibers had undergone necrosis (vital staining with trypan blue & eosin contrast), x625.

Fig. 18. Case 10. (Autopsy No. 9559)

The degenerated part of the muscle fibers were infiltrated by histiocytes and giant cells. The muscle nuclei have increased in numbers, forming single and multinucleated syncytial masses. The former phagocytosed trypan blue in large amounts. Some of the muscle cells and multinucleated syncytial masses also engulfed the dye to a lesser extent. (Vital staining with trypan blue & eosin contrast), x1020.

PLATE 9

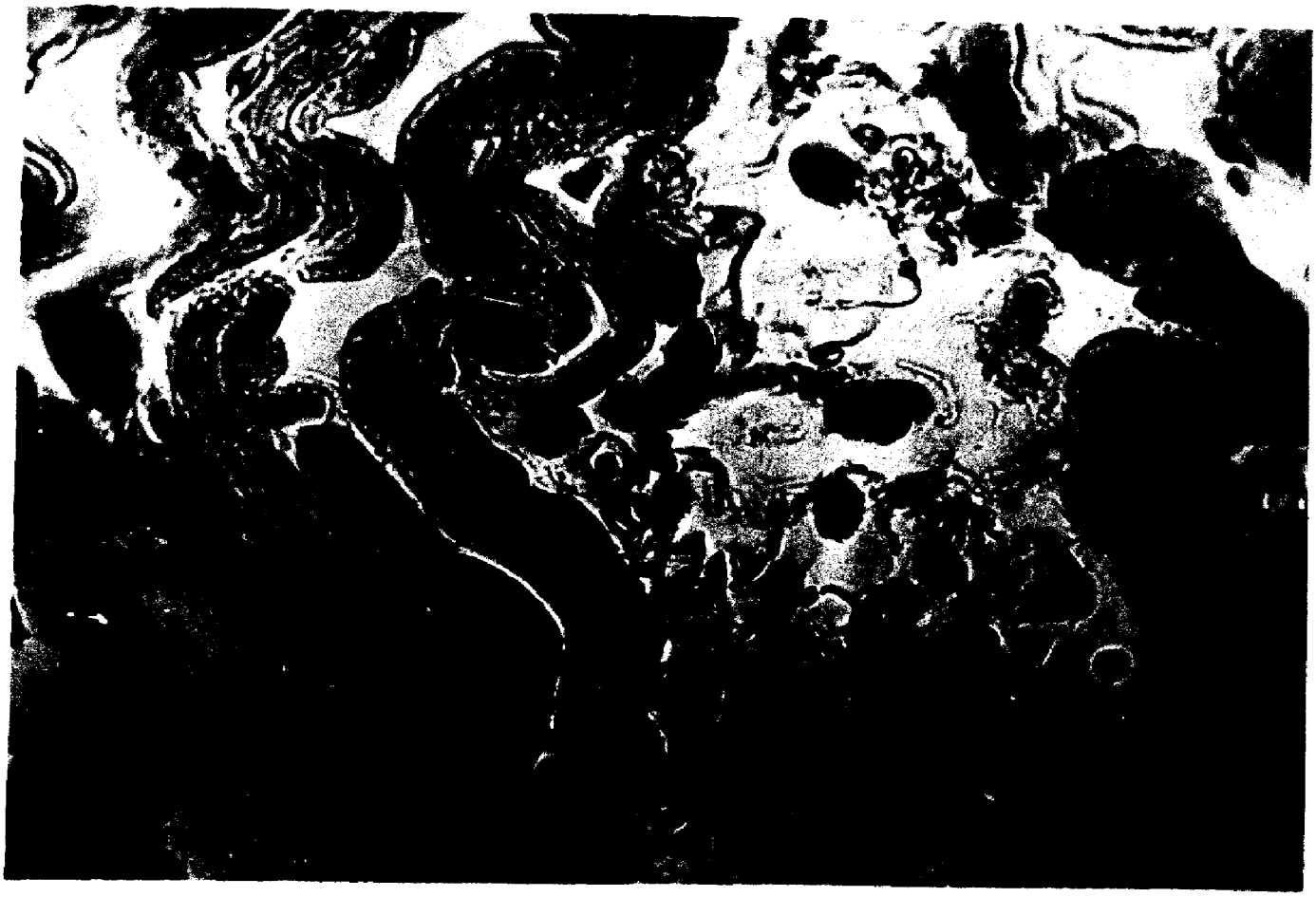


Fig. 19. Case 10. (Autopsy No. 9559)

Histiocytes, fibroblasts, and endothelial cells show engulfed trypan blue. (Vital staining with trypan blue & eosin contrast), x1050.

Fig. 20. Case 10. (Autopsy No. 9559)

Histiocytes (which engulfed the dye) are observed around the blood vessel. (Vital staining with trypan blue & eosin contrast), x565.

PLATE 10

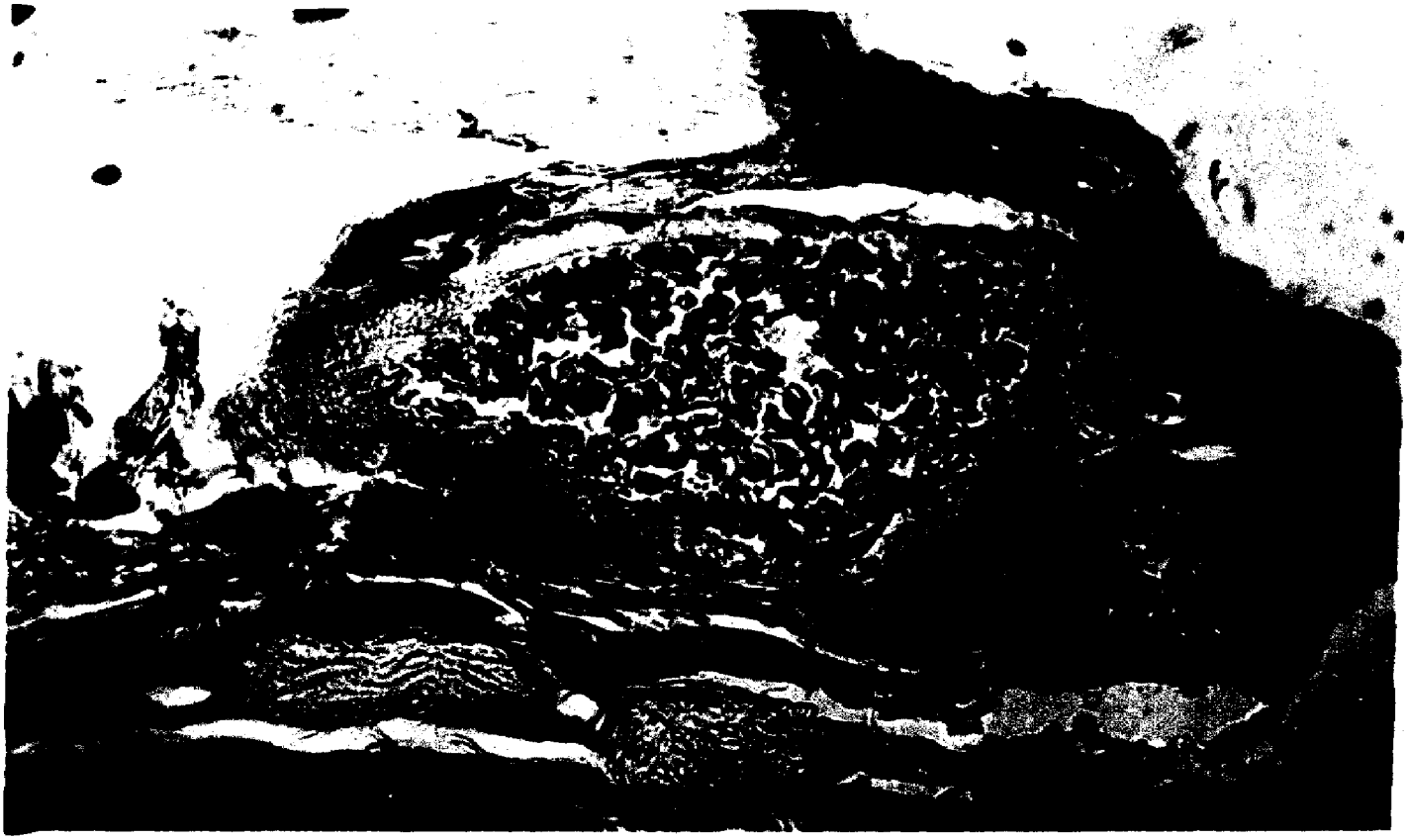


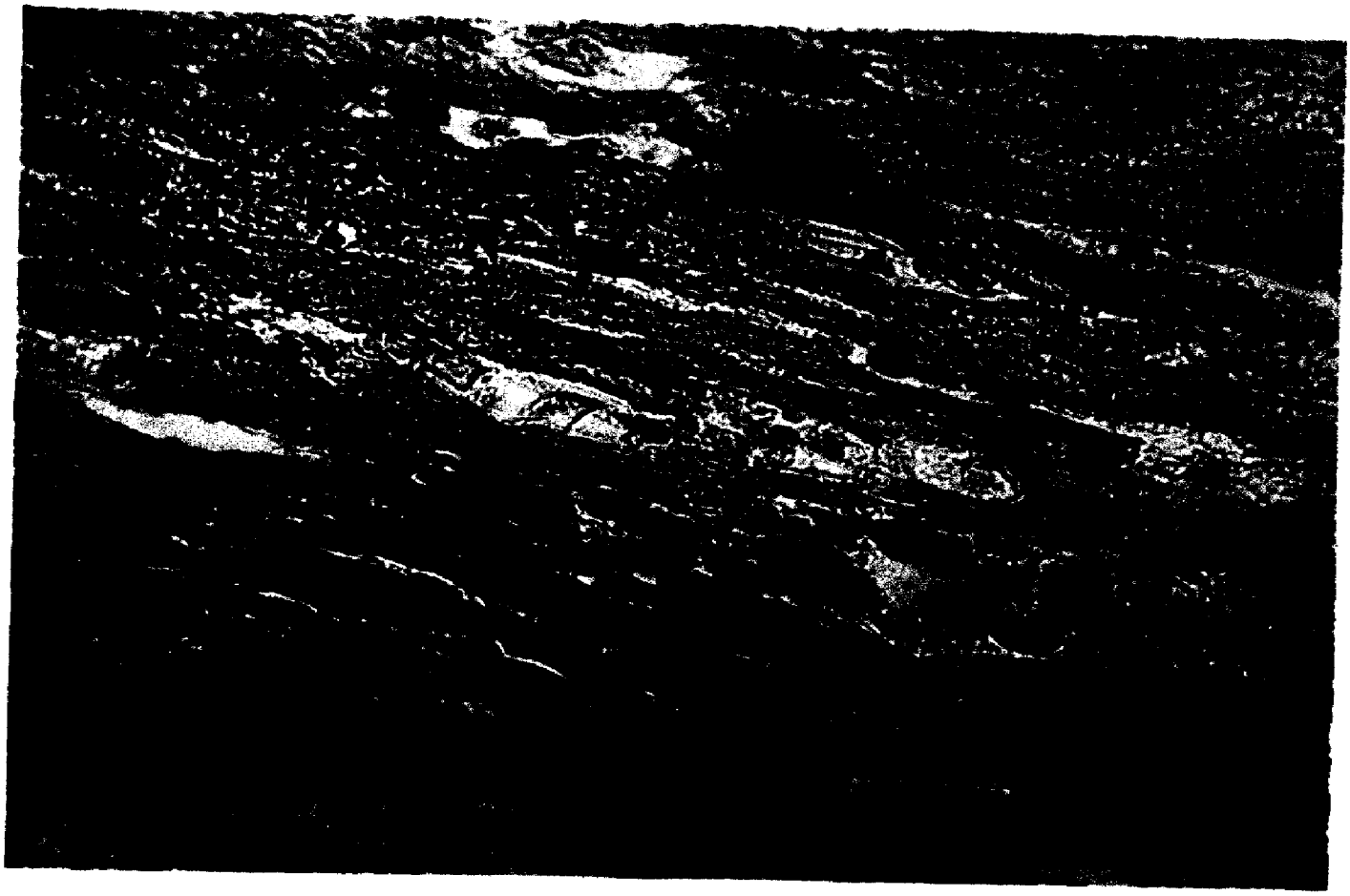
Fig. 21. Case 10. (Autopsy No. 9559)

The heart shows hyaline degeneration and is infiltrated with histiocytes which had engulfed trypan blue. (Vital staining with trypan blue & eosin contrast), x640.

Fig. 22. Case 8. (Autopsy No. 9556)

The popliteal lymph node. The reticulocytes, small lymphocytes and macrophages took up the dye. (Vital staining with trypan blue & eosin contrast), x1035.

PLATE 11



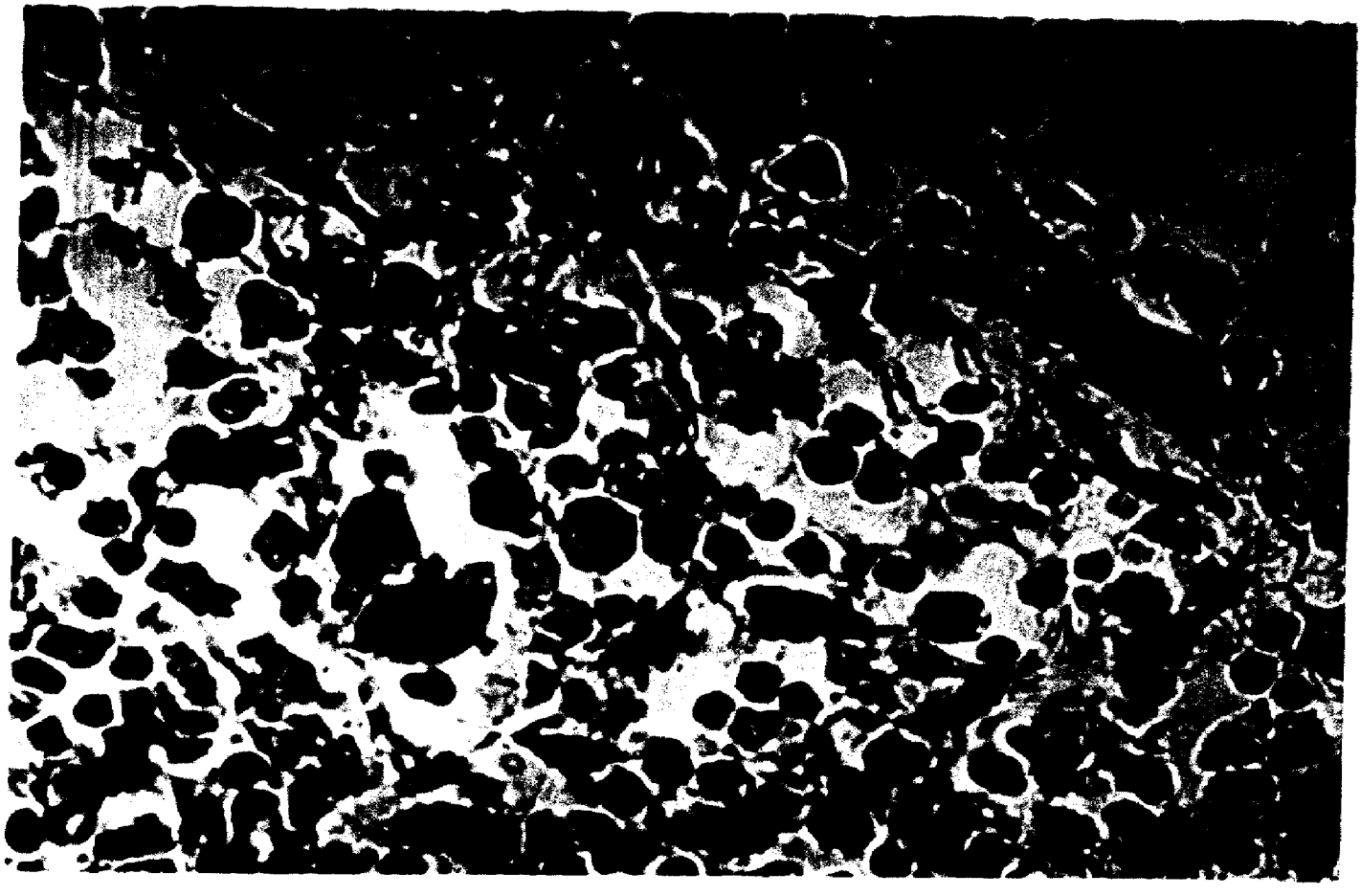


Fig. 23. Case 14. (Autopsy No. 9719)

Plasmodial outgrowths originate from the stumps of old preexisting muscle fibers. The picture was taken three days after cod liver oil had been discontinued. (H&E), x625.

Fig. 24. Case 15. (Autopsy No. 9724)

Young muscle fibers are showing longitudinal striations. Nuclei are centrally located and surrounded by granular cytoplasm. The picture shows regeneration of muscle fibers 6 days after cod liver oil had been discontinued. (H&E), x625.

PLATE 12

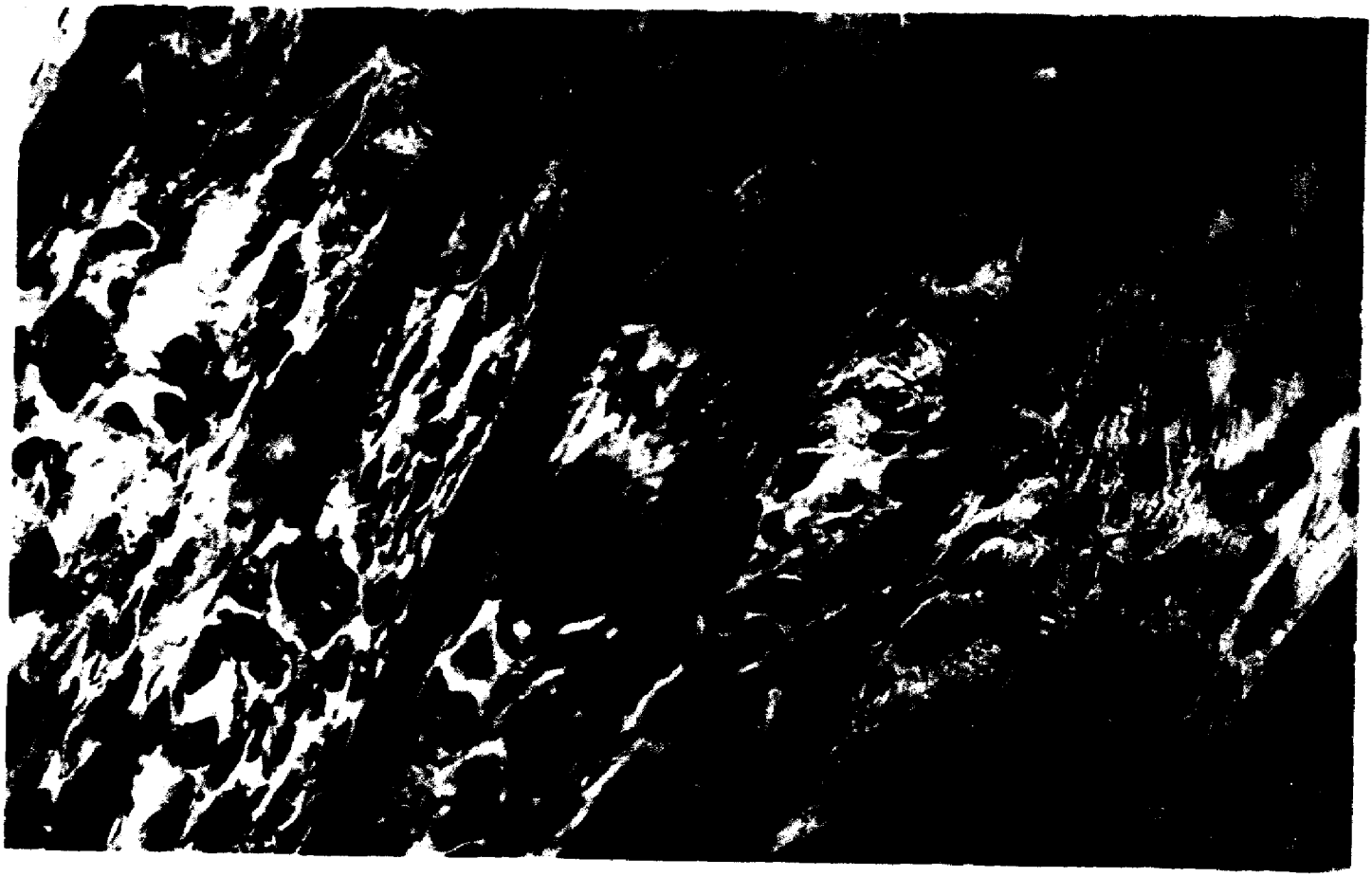


Fig. 25. Case 15. (Autopsy No. 9724)

Young muscle fibers at different stages of development. Multinucleated syncytial masses at the end of the fiber are seen. The picture was taken 6 days after cod liver oil had been discontinued. (H&E), x625.

Fig. 26. Case 16. (Autopsy No. 9752)

Young muscle fibers at different stages of growth. They are still multinucleated, located centrally and peripherally. The cross-striations are pronounced. The guinea pig was killed 9 days after the cod liver oil had been discontinued. (H&E), x625.

PLATE 13

Fig. 27. Case 17. (Autopsy No. 9759)

The muscle fibers are mature. The cross-striations of new fibers are pronounced. The number of nuclei are decreased. The guinea pig was killed twelve days after the cod liver oil had been discontinued. (H&E), x625.

Fig. 28. Case 18. (Autopsy No. 9786)

The cross section shows different diameters of new muscle fibers. The guinea pig was killed fifteen days after the cod-liver oil had been discontinued. (H&E), x625.

PLATE 14