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HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON EXPERIMENTAL ALLERGIC MYOSITIS AND HUMAN POLYMYOSITIS

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ABSTRACT

Some histological and histochemical findings on the allergic experimental myositis of guinea pig and human cases with neuromuscular diseases were reported.

Innoculated animals showed hardly any clinical sings but histological changes of thigh muscle and diaphragma which were similar to those seen in human polymyositis. Diaphragma showed more remarkable changes than thigh muscle.

In human polymyositis, the increase of endo-, epi-, and peri-mysial connective tissue, round cell infiltration into interstitial tissue, phagocytosis with regenerating fibre and necrosis of muscle fibre were dominant findings.

Regarding histochemical findings, muscles from experimental animals showed reduction of phosphorylase activity, elevation of succinic dehydrogenase in early stage, slight reduction in late stage, and elevation of alkaline phosphatase in capillary elements.

In human polymyositis, specimen of muscle showed mildely reduced phosphorylase activity and little change of succinic dehydrogenase activity and in neuromuscular disease, succinic dehydrogenase activity was markedly reduced and phosphorylase activity was well retained.

Finally, SGOT values in experimental animals were elevated on early stage then gradually reduced and SGPT values varied from case to case though the values throughout the course were higher than normal ones.

CONTENTS

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PREFACE

Up to present, a little work of experimental study of allergic changes of muscular tissue has been done. Some reports indicating the subjects as one of serial organ allergies are available, but report regarding the allergic changes of muscular tissue itself are very few.

Chloa Tal (1656)[1] reported the pathomorphological changes of muscular tissue of rabbits and guinea pigs produced by an autoimmune process which was very similar to that of muscular dystrophy of human cases. Sobue *et al.* (1955)[2)-6)] indicated that specific muscular changes of guinea pig could be produced by repeated injections of homogenized muscular tissue or phosphatid extincted from muscle.

And recently, Nutahara (1960)[7] described the allergic changes of muscular tissue of rabbits from the view point of Arthus phenomena.

These reports hold barely morhological changes of muscular tissue and no report is available which has concerned with the histochemical features of experimental allergic muscular changes.

Recently, the pathogenesis of human polymyositis has been widely debated concerning to the similarity with autoimmune diseases.

Accordingly, a considerable amount of research has been reported on the pathomorphological studies of human polymyositis and its histochemical features have also been described by some authors[8)-11)].

In this respect, present study was designed to investigate the pathomorphological and histochemical changes of experimental myositis with some

serological studies, and consequently investigate the difference existing between experimental myositis and human polymyositis.

EXPERIMENTAL MATERIALS AND METHODS

Experimental animals: Hybrid guinea pigs both male and female were used.

Antigen: Muscles from the hind limb of normal dog were removed under sterile conditions and homogenized in a warning blender together with the physiological saline solution (30% wet weight), thereafter mixed with the same volume of complete Freund's adjuvant and in order to maintain sterile condition, few drops of Marsonin solutions were added; then homogenized perfectly. This Muscle-Adjuvant homogenate was used as antigen throughout the experiment.

Mode of antigen injections: The experimental animals were divided into eight groups according to their survival periods and all of them were given 0.1 ml. of antigen at weekly interval on their back subcutaneously. Eight groups of experimental animals, number of injection and the intervals between initial injection and death are shown on Table 1.

TABLE 1. Summaries of experimental animals

Animal uumbre		A-1	B-1	C-1	C-2	C-3	D-1	D-2	D-3	E-1	E-2	F-1	F-2	G-1	G-2	H-1
Injections		1	1	1	1	1	2	2	2	2	2	2	2	5	5	9
Time between 1st injection and death		12 (hr)	1 (day)	3	3	3	7	7	7	9	9	14	14	30	30	60
Pathological changes of tissue	Muscle	−	±	+	+	±	±	+	±	++	+	+	++	+	++	++
	Diaphragma	−	+	++	++	+	++	++	+	++	+++	+	++	++	+++	+++
Histological changes observed in skeletal muscle																
Large group of small fibre		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Change of fibre		−	−	±	−	±	±	−	−	+	+	±	+	+	+	+
Phagocytosis		−	−	−	−	−	−	−	−	−	−	−	+	+	±	+
Regenerating fibre		−	−	−	−	−	−	−	−	+	±	+	+	−	+	+
Nuclei	Hypolemmal	±	+	+	±	−	+	+	+	++	+	−	++	+	+	++
	Internal	=	+	+	−	+	−	±	−	+	+	−	+	−	+	+
Interstitial tissue	Fat	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
	Collagen	−	−	+	−	+	−	+	±	+	+	+	+	++	++	+
Cellular infiltration		±	+	+++	−	+	−	++	±	+	±	+	+	±	++	++
Ranges of fibre (μ)		40 ~ 55	42 ~ 60	35 ~ 70	35 ~ 70	35 ~ 60	32 ~ 60	30 ~ 70	35 ~ 65	32 ~ 65	35 ~ 75	28 ~ 75	10 ~ 80	7 ~ 90	15 ~ 80	15 ~ 85

Remarks: −: unaltered ±: questionably changed +: slightly changed
++: moderately changed +++: markedly changed

Two guinea pigs received Freund's adjuvant-saline solution mixture and served as controls. The mode of antigen injections were correponded to the experimental animals. The control animals were sacrificed on 50 days after initial injection, during which every experimental animal showed the remarkable changes in their muscles.

Subjects of experiments: The experimental animals were sacrificed on each stage shown on Table 1 by heart puncture and the blood was served for estimation of SGOT, SGPT and total protein values in serum.

The specimens of muscle from hind limb were histologically and histochemically investigated and those from diaphragma were histologically investigated.

Histological and histochemical prooedures: For histological investigatons, all of the specimens (ca. 1 cm^3) were fixed with 10% formol solution, embedded in paraffine, sliced into 6-8 micron thick sections and served for Haematoxyline-Eosin staing and PAS reaction.

Mucoplysaccharide reactions were also performed with those sections.

For the purpose of histochemical investigations, the specimens of muscle (ca 0.5 cm^3) were immediately frozen by means of dry ice saturated acetone from $-70°C$ to $-80°C$ and then sliced into 8 to 10 micron thick sections in Cryostat within 24 hours and served for staining of Succinic dehydrogenase, Acid phosphatase, Alkaline phosphatase, Acetylcholine esterase, Phosphorylase and Sudan Black stain.

Staining methods employed here were as follows:

1) Succinic dehydrogenase (Seligman and Nachtras's Method)
2) Acid phosphatase (Gomori's Method)
3) Alkaline phosphatase (Gomori's Method)
4) Acetylcholine esterase (Gomori's Method)
5) Phosphorylase (Takeuchi and Kuriaki's Method)
6) Sudan Black stain (Gomori's Method)
7) PAS reaction (MacManus' Method)
8) Reactions for Mucopolysaccharide:
   i) Methylen Blue extinction (Dempsey and Singer's Method)
   ii) Alcian Blue extinction (Steedman's Method)
   iii) Toluidine Blue Metachromaia(Pearse' Method)
   iv) Azur A stain (Spicer's Method)

Methods for estimation of SGOT, SGPT and Total protein values:

Estimation method for SGOT and SGOT values were followed to Reitman-Frankel's Method using Iatron's Kit for estimation of transaminase. Total protein value was determined by Optical protein meter.

HUMAN MATERIALS AND METHODS

Materials: Under local anesteia, specimen was taken from the muscle which showed strong atrophy in the patients with polymyositis and neuromuscular diseases.

They were thirteen patients with polymyositis altogether including one dermatomyositis (Table 2, case 1), three acute cases (case 2, 3, 4) nine chronic cases (case 5, 6, 7, 8, 9, 10, 11, 12, 13). Among chronic cases, three showed muscle induration (case 9, 10, 11), one showed muscle contracture (case 12), and one was suspected a neurogenic interference by means of Electromyography (case 13). The muscle from two spinal progressive muscular atrophy and one amyotrophic lateral sclerosis were also served for histochemical investigations.

TABLE 2. Summaries of human polymyositis

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Age (year)	10	24	30	51	31	15	30	26	18	17	13	18	53
Age of onset (year)	9	24	30	51	30	13	24	23	17	13	13	5	53
Clinical matters	Der. My.	Acute cases		Chronic cases					with induration			Con-tructure	Neur. int.
Muscle	Del.	Gas.	Del.	Del.	Del.	Gas.	Tib. an.	v. lat.	Gas.	Bra.	Gas.	Gas.	Gas.
Histological findings													
Large group of small fibre	±	+	+	−	−	++	++	−	−	+	+	−	+++
Changes of muscle fibre	+	++	++	+	+	+	+	+	+	+	±	−	+
Phagocytosis	+	++	++	+	+++	+	+	+	+	+	±	−	+
Regenerating fibre	−	++	++	−	+	++	++	+	+	+	+	−	+
Change of nuclei: Hypolemmal	++	±	0	+	0	+	0	++	±	+	+	0	0
Change of nuclei: Internal	+	±	+	+	+	+	+	+	±	+	+	+	+
Interstitial tissue: Fat	+	0	0	+	+	0	+++	+	0	0	+++	+++	++
Interstitial tissue: Collagen	++	+	++	+	+	+++	+++	++	+	++	++	+	++
Cellular infiltration	+++	+	+	++	±	+++	+++	+	+	+++	+++	−	+
Largest fibre (μ)	30	98	60	70	75	165	105	90	112.5	90	?	97.5	105
GOT	45		53		95	81	18	33	40	419		40	48
GPT	45		36		43	20	21	31		125		10	45

Remarks: Der. My.: dermatomyositis Neur. int.: neural interference
Del.: deltoideus Gas.: gastocnemius Tib. ant.: tibialis anterior
Bra.: brachialis
0: normal ±: questionably changed +: slightly changed
++: moderately changed +++: markedly changed

Four specimens from Deltoideus, six from Gastrocnemius, one from Tibialis anterior, one from Vastus lateralis, and one from Brachialis were histologically investigated. Out of these muscles three specimens from Deltoideus in the patients with polymyositis and two specimens from Frachialis in the patients

with spinal progressive muscular atrophy and one from Tibialis anterior in the patients with amyotrophic lateral sclerosis were histochemically investigated.

Methods: Cubes of muscle (ca 1 cm^3) were removed with minimum trauma, fixed with 10% formol solution and served for histological investigation. For the purpose of hisochemical investigations, the removed cubes of muscle were immediately frozen by means of dry-ice saturated acetone and served for stainings of Succinic dehydrogenase, Acid phosphatase, Alkaline phosphatase, Acetylcholine esterase, Phosphorylase and PAS staining.

The staining methods applied here followed to the same method which were described in "Experimental methods".

RESULTS

I. *Clinical observations on experimental animals*: The remarkable loss of weight of experimental animals was not observed except for slight weight loss in two months after initial injection. Clinically neither muscular wasting nor ataxia was observed until two months after initial injection when slight decrease of muscular strength of hind limb developped in some cases. Control animals failed to show any of those clinical signs.

II. *Macroscopic observations*: Almost of muscles appeared to be unaltered whereas some of them either swallen or atrophic. Even the musle tissue which showed these changes preserved their normal colouration.

All the muscles from diaphragma showed unaltered appearance with normal colouration.

III. *Microscopic observations*:

A) *Histological changes*:

i) *Muscle from hind limb*: Pathological changes revealed on each guinea pig varied from one by one. Generally, histological findings by means of Haematoxyline-Eosin stain were outlined as follows:

The muscle tissue of A group (12 hrs after initial injection) revealed almost normal findings except slight cellular infiltration around capillaries.

On the muscle tissue from B group (1 day after initial injection), proliferation of hypolemmal nuclei with elongation, small round cell infiltration around the capilaries were clearly observed. But mnsle fibre in this stage failed to show any changes.

Interstitial tissue except capillaries remained intact.

The remarkable pericapillaritis and the small round cell infiltration around small arteries besides marked proliferation of elongated hypolemmal nuclei were seen in C group (3 days after initial injection). Some muscle fibres lost their striation and stained homogenously by Eosin and interstitial tissue were

swelled slightly.

The proliferation of interstitial tissue and the increase of hypolemmal nuclei became remarkable in the muscle tissue of D group (7 days after initial injection) (Plate 1).

Some elongated and enlarged nuclei showed such arrangement as like chain along the fibre. The enlargement of capillaries with cellular infiltration were the familiar features in this stage.

Regarding the fibre, one guinea pig of this group revealed slightly mixing feature in their size, namely some of them were swelled and others were normal size, while the atrophic fibre could not be seen in this stage.

In the muscle of E group (9 days after initial injection) some of atrophic fibre were observed and homogenous stainability of the fibre increased in number.

The muscle fibre from F group (14 days after initial injection) revealed phagocytosis otherwise the same as E group (Plate 2).

The pathological changes of muscle tissue such as uneveness of muscle fibre, cellular infiltration into interstitial tissue, increase of elongated hypolemmal nuclei with dominant nucleoli, loss of striation, flocular degeneration and the proliferation of interstitial tissue seemed to be marked on the stage of G group (30 days after initial injection). Moreover, muscle tissue from G group showed slight fat infiltration into interstitial tissue. Even on this stage so far as it could be observed, intrafusal fibre remained intact.

In the muscle of H group (60 days after initial injection) almost the same pictures as that of G group were observed (Plate 3, 4).

ii) *Muscle from Diaphragma*: Though normal muscle tissue from diaphragma showed much individual differences, summarized different points from skeletal muscle were insisted in smaller diameter of fibre and more abunndant number of nuclei than the skeletal muscle.

Now, regarding the pathological changes of diaphragma muscle tissue, the same process and the character of changes were observed except the remarkable atrophy of muscular fibre, but in diaphragma, the inflamatory changes of interstitial tissue were observed in earlier stage and severer degree than the skeletal muscle.

Proliferation of nuclei in the muscle of diaphragma appeared more rapid and stronger than in the skeletal muscle.

iii) *Muscle from human polymyositis*: One of the most characteristic histological features of polymyositis was the increase in the endo-, epi-, perimysial connective tissues (Table 2 case 1-13). Although eight cases showed the large group of small fibre, the muscle fibres were separated from each other by connective tissue (case 1, 2, 3, 6, 7, 10, 11, 13) and the amount of

increased connective tissue was seemed to be more abundant in chronic case (case 6, 7, 8, 10, 11, 13). In some cases of more advanced and/or chronic case (case 7, 11, 12), epimysial connective tissue was replaced by fat tissue.

Round cell infiltration in the interstitial tissue was seen in all cases except case 12.

The changes of muscle fibre such as vacuolar degeneration or necrosis were also present in the majority of muscle examined but it could not be correlated with the course of disease.

Phagocytosis and regenerating fibre were observed in the muscle from both of acute and chronic cases (cases 2, 3, 5, 6).

Elongation and increase of hypolemmal nuclei as well as internal nuclei were observed in about a half of examined muscles.

In these changes, increase of internal nuclei were more dominant features than that of hypolemmal nuclei.

The muscles of all cases showed a marked variation in fidre size. Fibres of varying stage tend to be scattered at randam througnout the section though some of them showed grouping of fibres (case 1, 2, 3, 6, 1, 10, 11, 13) most of which were separated by connective tissue.

Extremely large fibres (over 100 microns) were observed in the muscles from chronic cases (case 6, 7, 9, 13).

The clinical and histological features are summarised in Table 2.

B) *Histochemical changes*:

i) *Muscle from hind limb*:

a) *Succinic dchydrogenase* (S.D.H.)

Normal muscle tissue: In the normal muscle tissue three kinds of fibres could be classified, that is, those having strong, intermediate and less enzyme activities. Generally, the small fibre showed strong activity and large fibre showed less activity and those of mediate diameter showed intermediate activity. The deposition of diformazen was scattered evenly in sarcoplasma but some of them showed "check board" appearance i.e. the part of sarcoplasma which was closely attached to sarcolemmal sheeth showed stronger enzyme activity (Plate V).

Pathological tissue: The muscle tissue from B, C, and D group (1 day, 2, 7 days after initial injection respectively) showed stronger enzyme activity than normal. The distribution of diformazan deposit exhibitted "check board" type in majority of fibre (Plate VI).

In the muscle from D and E group (7, 9 days after initial injection) the diformazen deposit around some of hypolemmal nuclei was observed.

The muscles from G and H group (30, 60 days after initial injection) showed weaker enzyme activity with perinuclear diformazan deposit than those from

the other stages (Plate 7).

b) *Acid phosphatase* (*Ac. Ph.*)

Normal mucle tissue: The Ac.Ph. activity in normal muscle tissue was localized in peripheral nerve fibres and neither muscle fibre nor connective tissue showed any activity (Plate 8).

Pathological muscle tissue: In the muscle tissue from A and B group, no changes were observed. But the muscle from C group (3 days after initial injection) showed Ac.Ph. activity around some of hypolemmal nuclei which showed suddanophilia by Sudan Black stain.

In the muscle from F and G group (14, 30 days after initial injection), a few muscle fibre showed the enzyme activity most of which were atrophied ones (Plate 9). The activity of this enzyme in muscle fibres was not extend along the fibre but took a scattered feature.

c) *Alkaline phosphatase* (*Alk.Ph.*)

Normal muscle tissue: The Al.Ph. activity in the normal muscle were localized in some capillary walls, endothelium of venule, and intime of arteries. The muscle fibre lacked in activity (Plate 10).

Pathological muscle tissue: The muscle from A group (12 hrs. after initial injection) were within normal limit.

But in the muscle from D to H group (7 days to 60 days after initial injection), increased number of capilaries showed stronger activities than normal. Enzyme activities of muscle fibres remained normal in all group (A to H) (Plate 11).

d) *Acetylcholine esterase* (*Ach.E.*)

Normal muscle tissue: With the method employed here, the activity was localized in endoplates of the nerves. However, few muscle fibres showed slight activity in sarcoplasma closed to endoplates (Plate 12).

Pathologlcal muscular tissue: Ach.E in pathological muscle showed neither changes of activity nor localization,

Generally, the activities of Ach.E. in pathological muscle sustained little change (Plate 13, 14).

e) *Phosphorylase* (*PhR*)

Normal muscular tissue: Normal muscular fibres showed reverse features to that of S.D.H. activity, that is, large fibre showed stronger activity than small ones. And also intermediate fibres were also observed (Plate 15).

Pathological muscular tissue: PhR activity in the pathological muscle were observed at D and E group (7 days and 9 daays after initial injectiion). PhR

activity of the muscle in D group were moderately reduced, even in unatrophied fibres. The muscular fibre in E group also showed greately reduced activity (Plate 16).

f) *PAS reaction*

Normal muscular tissue: Glycogen was evenly stained within the fibres, although the glycogen content in large fibre was richer than that of in small fibre (Plate 17).

Pathological muscular tissue: The PAS reaction in the muscle from E group revealed marked reduction in fibre but increased in the interatitial tissue (Plate 18).

The atrophied fibres avoided mostly to PAS reaction.

g) *Sudan Black Staining* (S.B.S.)

Normal muscular tissue: Normal muscle revealed mixed feature of sudanophilia. The large fibres were less sudanophilic than small fibres (Plate 19). The materials which was stained by Sudan Black were mitochondria and lipid droplet in the fibre.

Pathological muscular tissue: In the muscles from A to C group failed to show any differences from normal tissue.

The more course advanced, the finer sudanophilic droplet became. And in the muscle from D to H group (7 to 60 days after initial injection), some of hypolemmal nuclei showed sudanophilia. The atrophied fibres revealed condenced feature of sudanophilic droplets (Plate 20).

h) *Acid mucopolysacoharide*:

For the purpose of investigating the changes of ground substances of interstitial tissue preceding to the morphological changes, some histochemical reactions such as Methylen Blue extinction, Toluidine Blue metachromasia, Alcian Blue, Azur A stain were done. The results of these observations were summarized in Table 3.

1) *Methylen Blue extinction*: This staining was employed at various ionic concentrations, *i.e.* at pH 2.6, 3.8, 4.9, 6.9 and 7.9. As Table 3 shows, in the normal muscle, nuclei and hyaloplasma were more strongly stained at higher ionic concentrations and hyaloplasma was more intensely stained. Connective tissue showed also same tendency with muscle fibres.

Regarding the pathological muscles, connective tissue showed metachromasia according to the course.

At earlier stage (A, B and C group), it showed beta-metachromasia and at later stage (F, G and H group) gamma-metachromasia.

TABLE 3. Summaries of changes of ground substances

			M.B. 2.6	M.B. 3.8	M.B. 4.9	M.B. 6.9	M.B. 7.9	A.B. 2.2	A.B. 4.8	T.B. W	T.B. 2.5	T.B. 4,5	T.B. 7.0	Az.A 1.5	Az.A 3.0	Az.A 4.5
Normal Tissue	Mucles fibre	Nucleus	±	+	+~++	+	+	−	++	+++	+	++	++	±	++	+++
		Hyaloplasma	−	−	+	++	+++	−	++	+	−	−	+++	−	−	−
	Ccnnective tissue		−	−	±β	+	+	−	++	++γ	−	++	++β	−	−	−
A-Group	Muscle fibre	Nucleus	±	+	++	++	++	−	+	Not examined				±	++	+++
		Hyaloplasma	−	−	−	+++	+++	−	+~−					−	−	−
	Connective tissue		−	−	±	+β	±	−	++					−	−	±β
B-Group	Muscle fibre	Nucleus	±	+	++	++	++	−	++	+++	+	++	++	±	++	+++
		Hyaloplasma	−	−	+	+++	+++	−	+~±	+~++	−	−	+++	−	−	−
	Connective tissue		−	−	++γ	++	+	−	++	++γ	−	±β	++β	−	−	−
C-Group	Muscle fibre	Nucleus	±	+	++	+	+	−	+	+++	+	++	++	±	++	+++
		Hyaloplasma	−	−	+	+++	+++	−	+	++	⊕Atr	−	+++	−	−	−
	Connective tissue		−	±β	±β	+β	±β	−	++	+β	−	γ ±~++	β ++	−	−	−
D-Group	Muscle fibre	Nucleus	±	+	++	++	+	−	+	+++	+	++	++	Not examined		
		Hyaloplasma	−	−	+	+++	+++	−	+	+	−	−	+++			
	Connective tissue		±	±	±γ	+γ	++	−	++	++γ	−	±γ	+β			
E-Group	Muscle fibre	Nucleus	Not examined							+++	+	++	++	±	++	+++
		Hyaloplasma								+	−	−	+++	−	−	−
	Connective tissue									++γ	−	++	++β	±	−	−
F-Group	Muscle fibre	Nucleus	±	+	++	+		Necr −~+	++	+++	+	++	+	+	++	+++
		Hyaloplasma	−	−	+	+++		−	+	++	−	−	+++	−	−	−
	Connective tissue		±	+	±γ	+		−	±	++γ	−	±γ	+γ	−	−	±γ
G-Group	Muscle fibre	Nucleus	±	+	++	+	+	−	+	+++	+	++	+		++	+++
		Hyaloplasma	−	±(++)	+	+++	++	−	+	++	−	−	+++		−	−
	Connective tissue		+	+	+γ	+γ	+	−	±	++γ	−	+γ	+β		−	+γ
H-Group	Muscle fibre	Nucleus	Not examined													
		Hyaloplasma														
	Connective tissue															

Remarks: −: no reaction ±: questionably stained +: slightly stained ++: moderately stained +++: strongly stained
Atr: Atrophied fibre Necr: necrotized fibre γ: gamma-metachromasia β: beta-metachromasia
M.B.: methylen blue A.B.: alcian blue T.B.: Toluidin blue Az.A.: azur A

TABLE 4. Histology and values of GOT, GPT and total protein in experimental myositis

Animals No.	Histology		Values of		
	Muscular fibre	Connective tissue	T.P	SGOP	SGPT
A-1	0	±		Not examined	
B-1	0	±		Not examined	
C-1	0	+	5.1	180	23
C-2	0	±	5.5	172	32
C-3	±	+		Not examined	
D-1	±	+	4.2	88	20.5
D-2	+	+	4.3	88	11.5
D-3	±	+	4.5	120	15.5
E-1	++	++	4.6	100	18.1
E-2	+	++	5.0	100	15.0
F-1	+	+	4.8	120	12.0
F-2	++	++	4.6	132	18.0
G-1	+	++	4.2	110	10.0
G-2	++	++	5.0	80	8.0
H-1	++	++	4.2	130	18.0
Normal-1	0	0	5.2	72	15
Normal-2	0	0	5.5	83	18
Normal-3	0	0	5.1	62	11

Remarks: 0: normal ±: considerably changed +: moderately changed ±: questionably changed

2) *Toludine Blue*: This stain was done at different ionic concentrations such as pH 2.5, 4.5, 7.0 and aqueous solution.

In the normal tissue, nuclei were stained at pH 2.5 lightly and at pH 7.0 moderately, and hyaloplasma was strongly stained at pH 7.0. Connective tissue showed Beta-metachromasia at pH 7.0.

In the pathological muscle tissue, gamma-metachromasia of connective tissue became more dominant in group D to G at pH 4.5 but the hyaloplasma of some muscle fibres showed stronger ortho-chromatic charactor from A to G group.

3) *Alcian Blue*: This staining was observed in pH 2.2, and 4.8 solutions. In the normal muscle no part of the tissue was stained at pH 2.2 and connective tissue showed stronger orthochromatic reactions than muscular fibres at pH 4.8.

In pathological muscle, any part of tissue was not stained at pH 2.2 except some necrotized fibres which showed slight orthochromasia. By pH 4.8 solution, hardly any change of metachromasia was observed.

4) *Azur A*: This staining was observed at pH 1.5, 3.0 and 4.5 respectively. In the normal muscle, nuclei were more strongly orthochromatically stained at high pH solutions, and connective tissue was not stained at all.

In the pathological muscle, some part of connetive tissue showed gamma-metachromasia on later stage (F and G group).

On the early stage (A group) exceptionally beta-metachromasia was observed at pH 4.5.

ii) *Nuscles from human polymyositis and neuromuscular diseases*:

The results of histochemical observations on human polymyositis and neuromuscular diseases were summarized in Table 5.

TABLE 5. Summaries of histochemical findings of human cases

Case No.	Site of tissue	S.D.H.	Pn.R.	Al.Ph.	Ac.Pn.	PAS
			Polymyositis			
1	Muscle fibre	N	↓	0	0	N ~ ↓
	Interstitial tissue	0	0	N	0	N
6	Muscle fibre	↓	N ~ ↓	0	0	↓
	Interstitial tissue	0	0	↑	0	N ~ ↑
8	Muscle fibre	N	↓	+(small fibre)	0	↓
	Interstitial tissue	0	0	N	0	N
			Neurogenic muscular diseases			
S.P.M.A 1	Muscle fibre	↓↓	0	0	0	N
	Interstitial tissue	0	N	N	0	N
S.P.M.A 2	Muscle fibre	↓	N ~ ↓	0	0	N
	Interstitial tissue	0	0	N	0	N
A.L.S. 1	Muscle fibre	↓	N	+	0	N
	Interstitial tissue	0	0	N	0	N

Remarks: N: Normal activity +: reduced ↓↓: considerably reduced
0 : Activity was not detected. +: Unusually activity was detected.
↑ : elevated

The muscle from two out of three patients with polymyositis showed reduced activity of PhR while S.D.H. activity was well retained (Plate 21, 22). In the muscles from neurogenic muscular diseases, PhR activity was well retained and S.D.H. activity was greatly reduced in every muscular fibres (Plate 26, 27).

Regarding the Al.Ph. activity, every muscles examined showed normal activity except one muscle from polymyositis (case 8) which showed positive Al.Ph. reaction in muscular fibre (Plate 24).

No changes of Ac.Ph. activity was observed in human cases.

PAS reactions in muscle from polymyositis were rather weak than normal. Comparing this with that of neuromuscular diseases, the muscle from neuromuscular diseases held well PAS reactions in muscle fibres.

IV) *Changes of SGPT, SGOT and total protein values*:

These values of normal and pathological animals were shown in Table 4. The normal SGOT and SGPT values of guinea pig were 72.3 and 14.7 respectively. These were the average values of 3 guinea pigs of hybrid both sexes.

Regarding the experimental animals, SGOT value was markedly elevated on the stage of C group and then gradually reduced.

However, the value on the stage of H group (60 days after initial injection) was about two times higher than normal.

Concerning the SGPT value, it varied from case to case with no definite correlation te experimental stage.

Total protein value did not show so characteristic changes, generally it inclined to decrease.

In human cases with polymyositis, SGOT and SGPT values varied considerably from patient to patient and they seemed to have no relations to histological changes. These values are summarized in Table 2. It was notable that one chronic case showed extremely high SGOT and SGPT values such as 419 and 125 respectively.

DISCUSSIONS

A) *On the histological findings*: A, Chloa Tal and E. Liban reported that the changes obtained by an autoimmune process were similar to those of muscular dystrophy. This study presented that the histological changes of allergic myositis of guinea pig were similar to those seen in human polymyositis.

The histological changes such as complete necrosis fibres, round cell infiltration in interestitial tissue, and regenerating fibre, which were seen in the muscles from G and H group (30 and 60 days after initial injection), will allow to conclude that the changes obtained here are myositic changes but not dystrophic one.

Regarding the process of histological changes of experimental myositis, cellular infiltrations in interstitial tissue (including inflamatory change of capillary) were observed first, and the increase in endomysial connective tissue were seen at early stage (C and D group) preceding to the changes of muscle fibre such as uneveness of fibre size or degeneration.

Also in human cases, the changes of connective tissue and inflamatory changes of interstitial tissue were dominant findings.

Extremely large fibre, which was seen in human chronic cases, could not be observed in allergic myositis; however the observation period being elongated, it will have a possibility of appearance. Because the extremely large fibre might be considered to be compensatory hypertrohy of the fibre to maintain muscle strength.

The vacuolar and hyaline degeneration, and complete necrosis of fibre were

seen in the muscles of majority of the cases.

The muscles from acute cases showed stronger change than chronic cases, and the former showed more abundant phgocytosis with regenerating fibre than the latter. This suggest that the process to recovery may be well taken place in acute cases.

In this report, it has also noted that the muscle from diaphragma of experimental myositis showed severer changes than that from skeletal muscle, though the differences between these two tissues were not qualitative but quantative.

Little work has been done to study histological changes of diaphragma in human polymyositis. As it is naturaly considered from the results of this study that the diaphragma in human polymyositis may show some interesting changes, the further investigation of diaphragma should be recommended.

Up to present, there are some reports on the histological relation between human polymyositis and experimental myositis[2)-6)], but little work has been done to study histochemical relations between those two. Hence, we shall discuss on this problem in next paragraph.

B) *On histochemical findings*:

1) *Changes of enzyme activity*:

It is widely known that the normal striated muscle fibres are divided into three groups. John M. Stein and Helen A. Padykula[12)] reported these three categories of fibre according to the amount of succinic dehydrogenase, adenosine triphosphatase, and glycogen contained in each muscular fibre. Those are Type A, B and C fibres. Type A fibre contains usually rich glycogen and scanty Succinic dehydrogenase, which is corresponding to "white" muscular fibre after Ranvier, and also have large diameter, rich phosphorylase activity and scanty lipid.

Recently it was clarified that "white" muscle was a rapid contracting, tonic and extrapyramidal fibre. Type B and C fibre correspond to "Red" muscular fibre after Padykula.

"Red" muscular fibre riches in succinic dehydrogenase, lipid, and glycogen. These two B and C fibres in "Red" muscular fibre are classified by various phase in a cycle of glycogen deposition and release.

In present study we intended to clarify the correlation between disordered muscular fibres and their categories but as far as we could obtaine the results, any special correlations were not detected.

The reduction of phosphorylase activity was universal in the muscular tissue at each stage of the course, and it was very rapid. While succinic dehydrogenase activity in muscular fibre was slightly elevated at early stage (B, C and D group) and then gradually reduced as the course advanced.

In the atrophied fibres in later stage, none of them kept phosphorylase

activity but some of them held a little amount of succinic dehydrogenase activity.

Although the species speciality must be taken into cinsideration to evaluate this results, it may be suggested that phosphorylase activities are more easily affected than succinic dehydrogenase activites on the course of allergic myositis.

Regarding perinuclear diformazan deposit, it remained obscure whether it means pathological changes or not.

In human cases, as we see on Table 5, changes of phosphorylase and succinic dehydrogenase activities in the muscles from patients with polymyositis were similar to those of experimental myositis except one case (case 6) which revealed well retained phosphorylase activity (Plate 23), while the muscles from the patients with neurogenic muscular diseases showed reversed changes to the muscle of polymyositis, *i.e.* succinic dehydrogenase activity was markedly reduced and phosphorylase activity was well retained.

Uono (1965)[9][13] stated that in neurogenic muscular diseases such as amyotrophic lateral sclerosis, spinal progressive muscular atrophy and neural progressive muscular atrophy, the reduction of succinic deehydrogenase activity was remarkable: while, in progressive muscular dystrophy, the phosphorylase activity was markedly reduced, with no correlation between atrophied or hypertrophied fibre and phosphorylase activity.

Tokuomi (1964)[14] observed that in the muscle from polymyositis strong phosphorylase activity was seen in small fibre and strong oxidative enzyme reaction in large fibre.

From the results obtained in this study, we could agree with considerable reduction of succinic dehydrogenase in neurogenic muscular diseases but not necessarily agree with strong phosphorylase activity in small fibre in polymyositis.

PAS reaction in muscular fiberes revealed decreasing tendency unrelated to catogories of fibre size.

This fact suggests that it might be due to decrease of phosphorylase activity.

Biological functions of Alkaline phosphatase has not been clarified yet. Some authors suggested that this enzyme takes parts in fibrous protein formation and metabolic passage through cell menbrane[15]. The meaning of elevation of this enzyme around capillaries in this study may be supposed that it has a relation to elevated permiability of capilaries, which causes edema of interstitial tissue in early stage (D group) and fibrous protein formation in later stage. The meaning of strong activity of this enzyme in muscular fibre from patients with polymyositis remaind obscure though there is a concept that this enzyme might break down the high energy phosphate bond in muscular fibre[16].

The elevation of acid phosphatase activity in nuclei seemed to coincide

with the deposition of lipid around the nuclei.

This suggests the degenerating process of the nuclei[15], however it may be noted that long incubating time employed here often causes a diffusion artifact.

Hence, the histochemical results in this study might not give any clue to clarify the histochemical character of human polymyositis as the cases examined are insufficient number and the process of histochemical changes in experimental myositis could not be directly applied on that of human cases. However, we should like to mention that the foci of histochemical problems on experimental myositis and human polymyositis should be placed on the rapid and marked reduction of phosphorylase activity and a little change of succinic dehydrogenase activity.

2) *Changes of ground substance in interstitial tissue*:

In our study, T.B.M. (Toluidine Blue Metachromasia) was seen in the tissue later than C group (3 days after initial injection) which was the stage showing the swelling of interstitial tissue.

The part showing T.B.M. positive revealed Methylen Blue metachromasia positive (at pH 4.9, 6.7), Azur A metachromasia positive (at pH 4.8) and positive PAS reaction fast to diastase.

Asuming from this results, main pathological component in the interstitial tissue which was revealed by serial metachromasia may be hyaluronic acid. It is very difficult to give a meaning to the incrrease of hyaluronic acid in connective tissue.

On this respect Mihashi (1959)[17] reported that marked reaction of mncopolysaccharide was found in the early granulation tissue which is on the way of fibre formation. Further Amano (1958)[18] stated that mucopolysaccharide does not participate in fibre formation as chemical component of collagen fibre but as promoting factor of this process. These concepts might be applied to evaluate the results of our study.

C) *On the changes of SGOT and SGPT values*:

Thompson (1959)[19] reported that seven out thirteen polymyositis cases showed slightly higher values of SGPT and SGOT, and acute cases showed more elevated values than chronic cases.

Pearson (1957)[20] reported that in some acute cases, one showed extremely high SGOT value that was 10 times higher than normal.

It is wldely known that the acute case shows higher SGOT and SGPT values than chronic case. In this study exceptionally one chronic case showed extremely high SGOT and SGPT value but generally any correlation between SGOT and SGPT value and duration of diseases could not be obtained.

The otherhand, in experimental myositis high SGOT and SGPT values were estimated at the stage of C group (3 days after initial injection) then

gradually reduced.

The mechanism of elevation of transferase activity in myopathy has not been clarified. But judjing from the results of our study, it was considered that the elevation of emzyme values might not be owed only to the change of muscular metabolism.

SUMMARY

Some histological and histochemical findings on the allergic experimental myositis and human cases with neuromuscular diseases were reported.

The allergic muscular changes of guinea pig could be produced by this way.

Innoculated animals showed hardly any clinical signs but histopathological changes of thigh muscle and diaphragma which were similar to those seen in human polymyositis. Diaphragma showed more remarkable changes than thigh muscle. In human polymyositis, the increase of endo-, epi-, and peri-mysial connective tissue, round cell infiltration into interstitial tissue, phagocytosis and necrosis of muscular fibre were dominant findings.

Hisochemical findings revealed in allergic myositis of guinea pig are summarized as follows:

1) S.D.H. activity showed elevation in early stage, thereafter reduced.

2) Phosphorylase activity showed reduction in muscular fibre throughout experimental stage.

3) Alkaline phosphatase activity was elevated in capillary elements.

4) Acid phosphatase activity was positive in nuclei in some muscular fibre on rather early stage, with sudanophilic character and in a few muscular fibre in later stage.

5) PAS reaction was reduced in muscular fibre, meaning the decrease of glycogen and elevated in connective tissue, suggesting the increase of mucopolysaccharide.

In human polymyositis, specimen of mnscle showed mildely reduced PhR activity and little change of S.D.H. activity and in neurogenic muscular disease, S.D.H. activity was markedly reduced and PhR activity was well retained.

SGOT values in experimental animals were elevated on early stage then gradually reduced and SGPT values varied from case to case though the values throughout the course were higher than normal ones.

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EXPLANATION OF PLATES

PLATE 1. Haematoxyline-Eosin stain.
Muscle from D group (7 days after initial injection)
Note: Increase of hypolemmal nuclei and round cell infiltration into interstitial tissue and around capillary.

PLATE 2. Haematoxyline-Eosin stain.
Muscle from F group (14 days after initial injection)
Note: Increase in connctive tissue and uneveness of fibre.

PLATE 3. Haematoxyline-Eosin stain.
Muscle from H group (60 days after initial injection)
Note: Necrosis of fibre, increase of endo-, peri-mysial connective tissue and round cell infiltration into interstitial tissue. Intrafusal fibre remain intact.

PLATE 4. Haemtoxyline-Eosin stain·
Muscle from H group (60 days after initial injection)
Note: Necrosis of fibre.

PLATE 5. S.D.H. activity.
Normal muscle.
Note: Small fibre shows stronger activity than large fibre. Fibre showing intermediate activity are also seen.

PLATE 6. S.D.H. activity.
Muscle from D group (7 days after initial injection)
Note: Activity is elevated comparing with normal muscle.

PLATE 7. S.D.H. activity.
Muscle from G group (30 days after initial injection)
Note: Activity is slightly reduced. Perinuclear diformazan deposit are observed.

PLATE 8. Ac.Ph. activity (Nuclei are stained by haematoxyline)
Normal muscles.
Note: Activity is showed on peripheral nerve fibre. Muscle fibre does not show any activity.

PLATE 9. Ac.Ph. activity (Nuclei are stained by haematoxylin)
Muscle from F group (14 days after initial injection)
Note: Some of nuclei show activity and muscle fibre shows partly positive reaction.

PLATE 10. Al.Ph. activity.
Normal muscle.
Note: Some capillaries show activity while muscle fibre fails to show activity.

PLATE 11. Al.Ph. activity.
Muscle from H group (60 days after initial injection)
Note: Increased activity on capillary elements.

PLATE 12. ACHE activity.
Nrmal muscle.
Note: Endoplates shows activity and sarcoplasma close to endoplates also show slight activity.

PLATE 13. AchE activity.
Muscle from G group (30 days after initial injection)
Note: Activity is localized in endoplate but the strength is the same with normal.

PLATE 14. AchE activity.
Muscle from H group (60 days after initial injection)
Note: Even in necrotized fibre the activity is well retained.

PLATE 15. RhR activity.
Normal muscle.
Right side shows transverse section and left side shows longitudinal section.
Note: Large fibres show strong activity.

PLATE 16. PhR activity.
Muscle from F group (14 days after initial injection)
Note: Marked reduction of activity unrelated to fibre size.

PLATE 17. PAS reaction.
Normal muscle.
Note: Large fibre contains rich glycogen. Cross striation is demonstaable.

PLATE 18. PAS reaction.
Muscle from F group (14 days after initial injection)
Note: Marked reduction of the reaction in muscle fibre and elevation in connective tissue.

PLATE 19. S.B.B.
Normal muscle.
Note: Three categories of fibre are demonstrable accoding to their sudanophilia.

PLATE 20. S.B.B.
Muscle from D group (7 days after initial injection)
Note: Sudanophilic droplets become fine and sudanophilia of some nuclei are observed.

PLATE 21. PhR activity.
Muscle from human polymyositis (case 8)
Note: PhR activity is reduced universely in the section.

PLATE 22. S.D.H. activity.
Muscle from human polymyositis (case 8)
Note: Activity is well retained and atrophied fibres keep some activity.

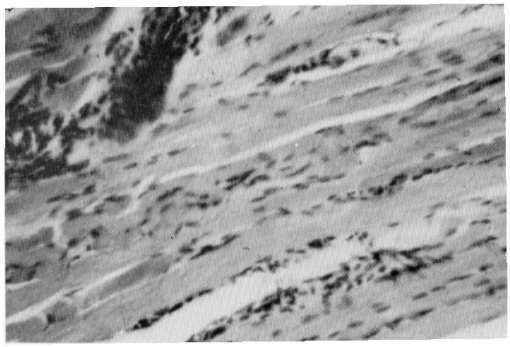
PLATE 23. PhR activity.
Muscle from human polymyositis (case 6)
Note: Activity is well retained even in atrophied fibre.

PLATE 24. Al.Ph. activity.
Muscle from human polymyositis (case 8)
Note: Some of muscle fibres show the activity.

PLATE 25. PAS reaction.
Muscle from human polymyositis (case 8)
Note: PAS reaction in muscle fibre is weaker than normal.

PLATE 26. PhR activity.
Muscle from human spinal progressive muscular atrophy (neuromuscular disease case 1)
Note: Activity is well retained, atrophied fibres show slight activity.

PLATE 27. S.D.H. activity.
Muscle from the same patient with Plate 26.
Note: Activity is markedly reduced.



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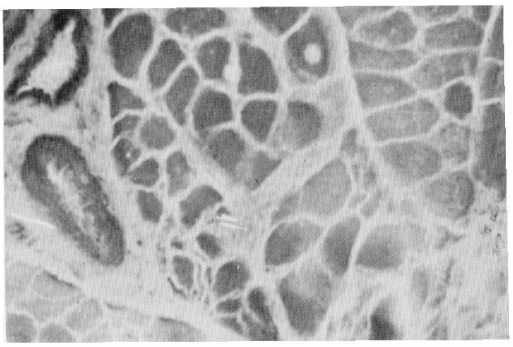

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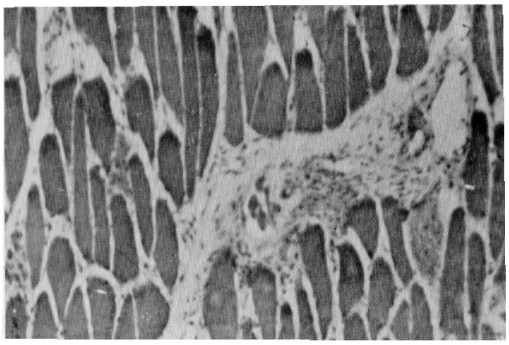

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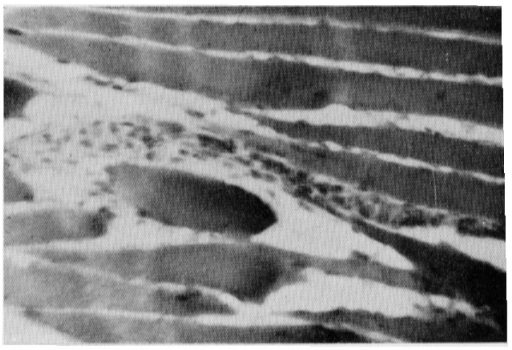

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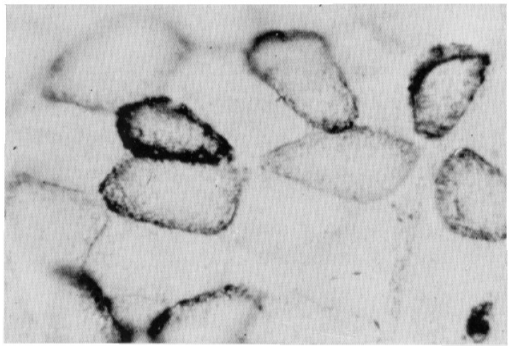

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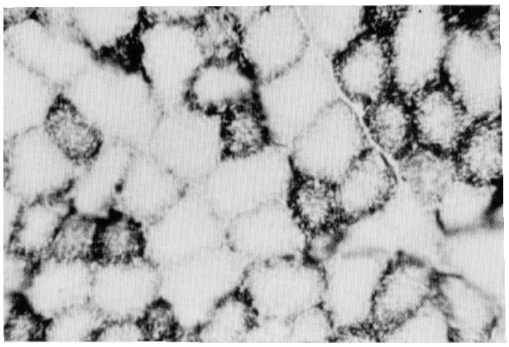

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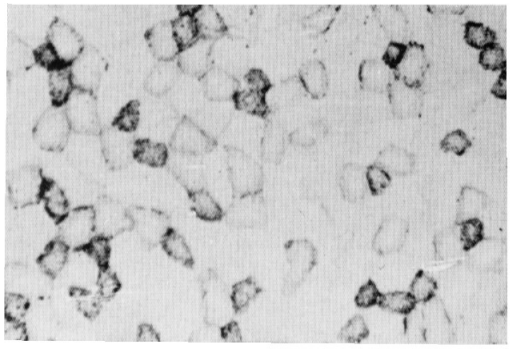

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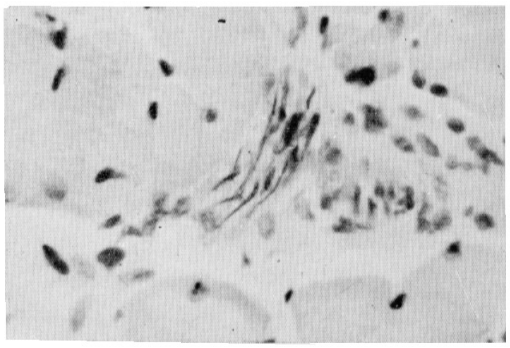

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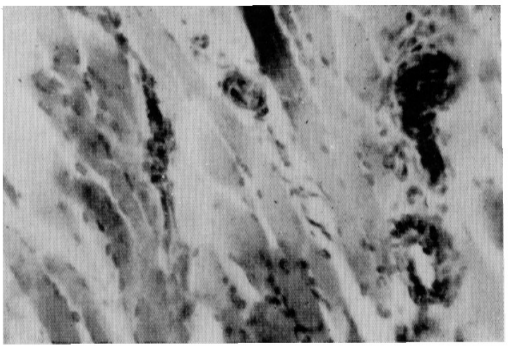

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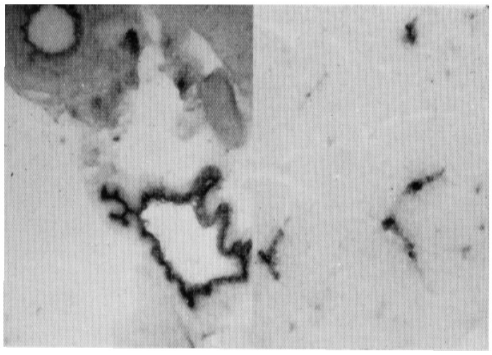

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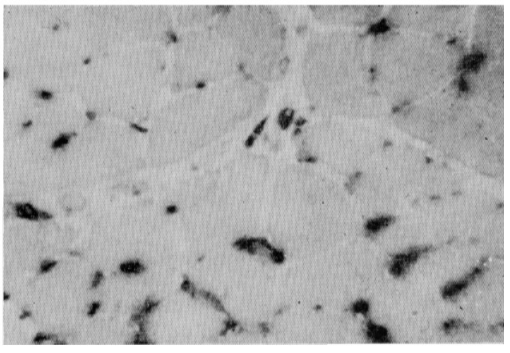

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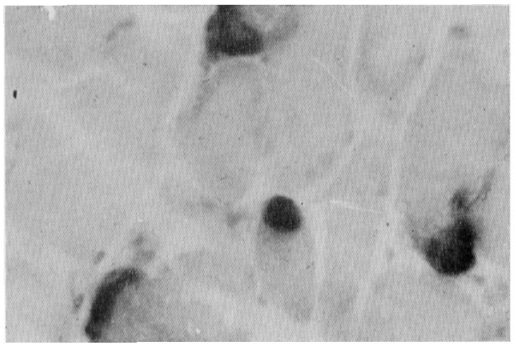

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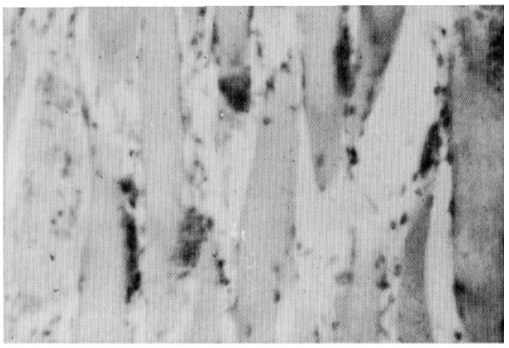

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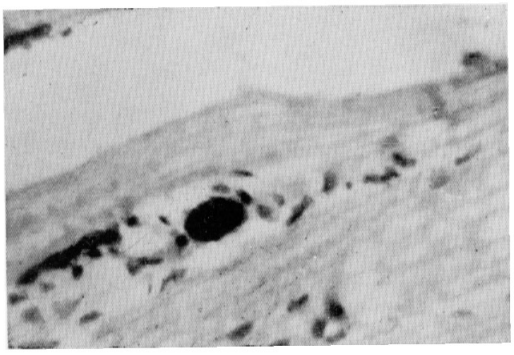

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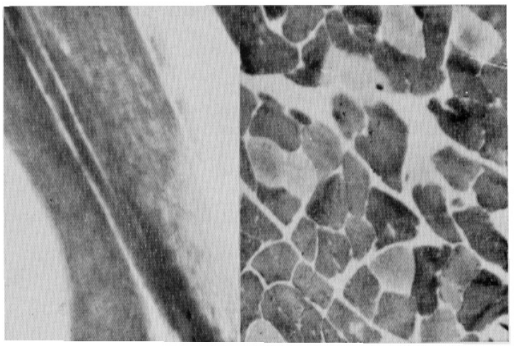

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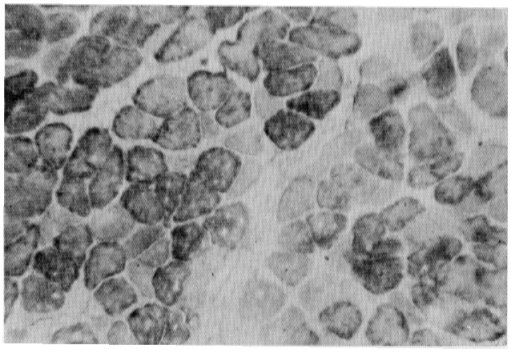

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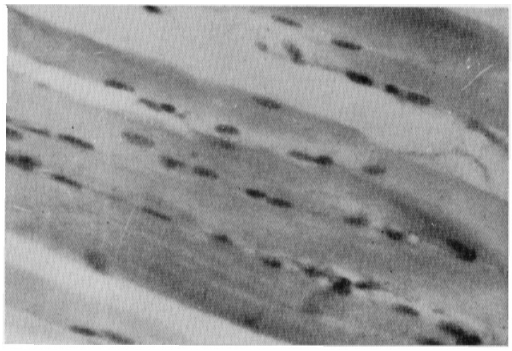

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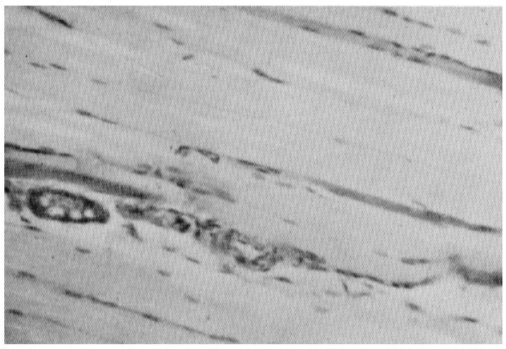

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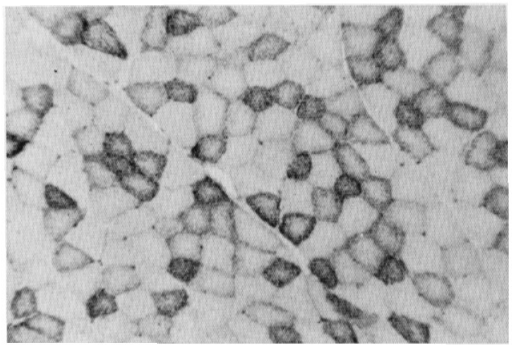

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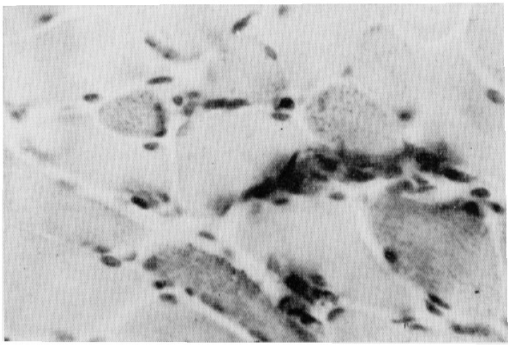

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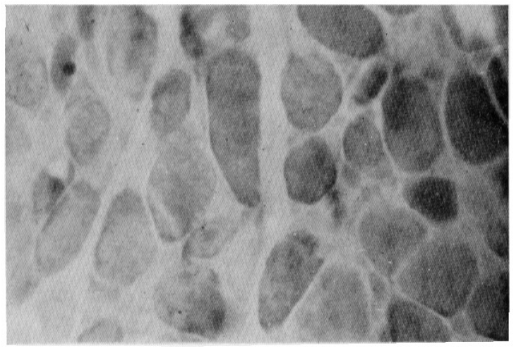

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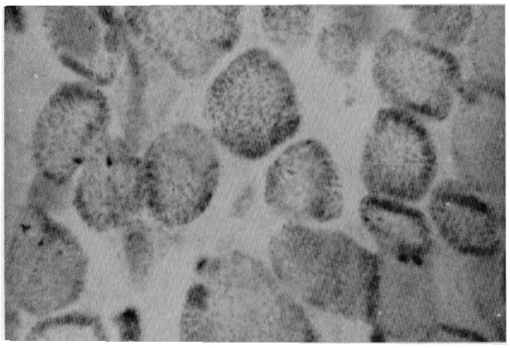

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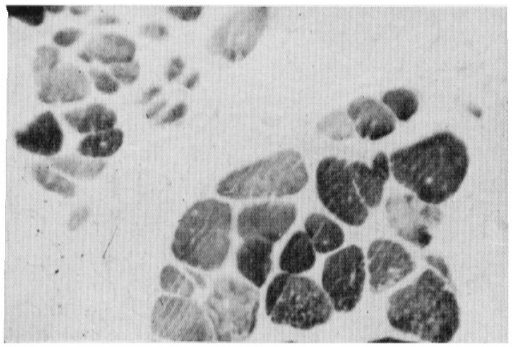

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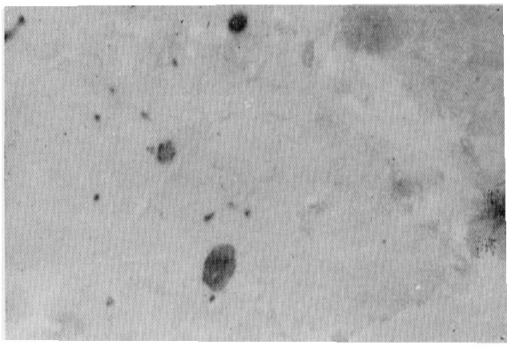

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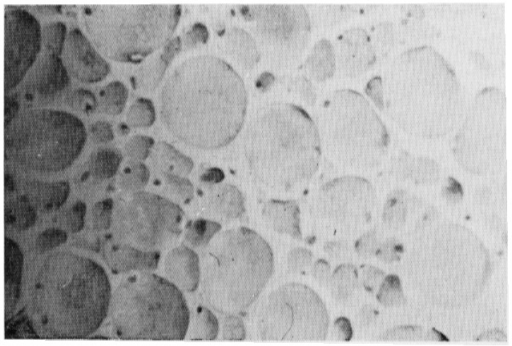

PLATE 25

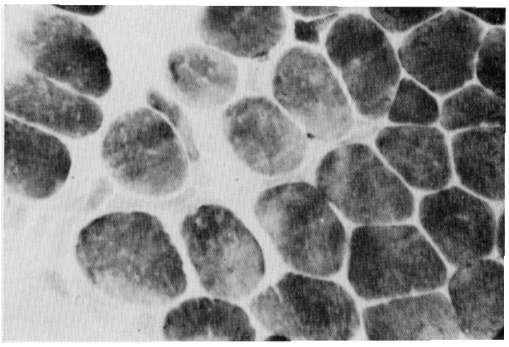

PLATE 26

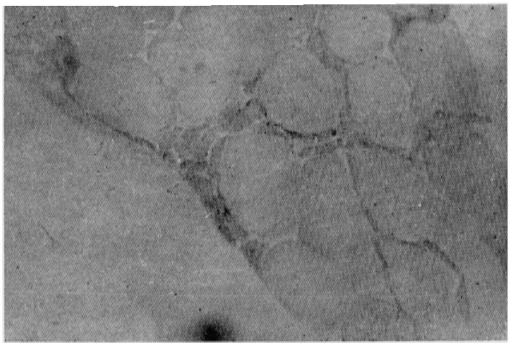

PLATE 27