MYOSITIS IN MICE FOLLOWING INTRAMUSCULAR INJECTION OF VIRUSES OF THE MOUSE ENCEPHALOMYELITIS GROUP AND OF CERTAIN OTHER NEUROTROPIC VIRUSES*

By ROBERT RUSTIGIAN, Ph.D.,‡ and ALWIN M. PAPPENHEIMER, M.D.§

(From the Department of Bacteriology and Immunology, Harvard Medical School, Boston)

Plates 5 to 7

(Received for publication, September 15, 1948)

In the course of certain experiments on attempted immunization, young mice were injected into the muscles of the leg with 0.2 cc. of centrifuged 10 per cent unfiltered suspension of brain containing active mouse encephalomyelitis virus, strain GD-VII. After a varying incubation period, usually 5 to 7 days, flaccid paralysis appeared first in the injected limb, later in the opposite limb, and often the forelegs. In most cases, death supervened. Because of the interesting lesions found in the muscles of the injected leg it was decided to make a detailed study of the effects of this and other neurotropic viruses upon the skeletal muscles.

In the literature concerned with the pathologic changes produced by mouse encephalomyelitis (Theiler's) virus, we have found no reference to the occurrence of local lesions following intramuscular injection.

However, Sabin and Olitsky (1), in 1938, working with the virus of vesicular stomatitis, found that young mice, after intramuscular injection, developed paralysis, appearing first in the injected limb; later they succumbed with signs of myelitis. As early as the 2nd day, there was noted an acute interstitial myositis, with necrosis of muscle fibers, and on the 3rd day, marked polymorphonuclear leucocytic reaction, wth phagocytosis of fragmenting muscle fibers. On the 5th day, the lesions were intense. There was hypertrophy and proliferation of sarcolemma nuclei. No inclusions were found, and the sciatic nerves showed no inflammatory reaction. The spinal cord in the lower lumbar region was the seat of severe lesions, confined to neurons of the anterior horns. The ganglion cells were in various stages of necrosis. There were early neuronophagia and a few perivascular accumulations of polymorphonuclears and lymphocytes. The meninges were not affected, and the spinal and sympathetic ganglia were also free from lesions This suggested that the virus entered the cord *via* the efferent axons of the anterior horn cells. The cervical cord was much less affected. Lesions were found in the ventral portion of the medulla, slightly cephalad to the decussation of the pyramidal tracts.

Intramuscular injecton of normal brain tissue produced an interstitial inflammatory reaction, but no necrosis of muscle fibers. Peck and Sabin (2) have recently

* Aided by a grant from The National Foundation for Infantile Paralysis.

‡ Fellow in Virus Diseases of the National Research Council.

§ Professor of Pathology, Emeritus, Columbia University.

found that, following intramuscular injection of St. Louis encephalitis virus in young mice, necrosis of muscle fibers was produced. In a personal communication to the authors from Dr. Sabin, he stated that he also observed muscle lesions following intramuscular inoculation with Eastern and Western equine encephalitis viruses. Dr. Dalldorf informed us that, in sections of the vertebral column of paralyzed hamsters inoculated with MM virus, he noted degenerative changes in the muscles of the back, and has kindly sent us sections illustrating these lesions.

In a recent note, Dalldorf and Sickles (3) reported the isolation from the feces of two children of an unidentified filterable agent. When injected intracerebrally into suckling mice, 3 to 7 days old, or into suckling hamsters, this agent produced paralysis and widespread lesions of the skeletal muscles. It did not affect the central nervous system and peripheral nerves.

Material and Methods

Stock virus suspensions were prepared as follows: pooled brains from sick or dead mice infected intracerebrally were ground with alundum and sufficient cold distilled water was added to give a 10 per cent suspension by weight except for the viruses of lymphocytic choriomeningitis, herpes simplex, and Eastern equine encephalitis which were suspended in isotonic phosphate buffer, pH 7.0–7.1. The suspensions were centrifuged at 2500 R.P.M. for 10 minutes, and the supernatant fluids were removed and considered as stock virus. They were stored in glass-sealed vials in a CO_2 cabinet, after rapid freezing in an alcohol dry ice mixture. When used, the frozen virus was thawed rapidly in a 37°C. water bath.

The strains of mouse encephalomyelitis and other neurotropic viruses employed in the present study, along with their properties following intracerebral inoculation of young mice, are summarized in Table I. Their characteristics in general conformed with previous descriptions. Additional comments, however, are necessary for certain of the viruses.

Mouse Encephalomyelitis Virus, Strain GD-VII.—Following intracerebral inoculation of the GD-VII virus, signs of encephalitis have predominated and in general conformed with the picture of the disease as described by Kearney *et al.* (4). However, we observed flaccid paralysis with either high or low concentrations of virus much less frequently than these workers who report its occurrence in practically 100 per cent of infected animals. For example, of 114 infected mice inoculated with infected mouse brain suspensions of different passages and observed carefully for obvious signs 29 per cent developed definite flaccid paralysis. With chick embryo-adapted GD-VII virus however, flaccid paralysis was more frequently observed; of 268 mice infected with egg passage virus 58 per cent became paralyzed.

Mouse Encephalomyelitis Virus, TO Type 4727 and FV.—Both these strains conformed to Theiler's original type (TO) of mouse encephalomyelitis virus (5). Some difficulty, however, was encountered in our laboratory in establishing the FV strain which was received approximately a year ago from Dr. Max Theiler. It was, at that time, described as having been recently isolated from the intestines of normal mice. Direct brain inoculations of a 20 per cent suspension of glycerinated brain (washed 4 times in saline) failed to produce signs of disease experiments. However, with one blind passage[1] of a 20 per cent suspension of brain and spinal cord from 5 mice sacrificed 6 days after intracerebral inoculation with the original material, 2 of 18 mice showed typical paralysis. One developed slight but definite paralysis on the 16th, and the other on the 22nd day. Further serial passage of brain and cord sus-

[1] It is realized that with blind passage, there is a risk of activating latent mouse encephalomyelitis virus.

pensions from infected mice resulted in an increase of pathogenicity. Even after 5 passages, however, 10 per cent suspensions of infected brain and spinal cord produced a morbidity of only 70 to 80 per cent. The severity of disease varied, some mice showing marked paralysis of all extremities followed shortly by death, while others showed only partial paralysis or weakness of one or more limbs, and subsequently recovered. No more passages were made

TABLE I

Viruses Studied and Their Observed Salient Characteristics Following Intracerebral Injection of Young Mice

Virus	No. of intracerebral passages in mice		Results following intracerebral inoculation		
	Previous studies	Present study	Cardinal symptoms	Incubation period*	Log virus concentration‡
				days	
Mouse encephalomyelitis					
Strain GD-VII	?	12	Encephalitis	2–4	5.8–6.3§
Strain FA	?	5	Encephalitis	2–4	5.2–5.8
Strain 4727	28	1	Paralysis	7–15	4.4
Strain FV	?	5	Paralysis	9–29	‖
SK virus, Jungeblut	380	2	Encephalitis	1	8.2
Lymphocytic choriomeningitis virus					
Mitchell strain	20	1	Encephalitis	6–8	5.0
Human poliomyelitis					
Lansing strain	?	3	Paralysis	2–12	3.0–3.5
Herpes simplex					
HF strain	?	1	Encephalitis	3–4	2.9
Eastern equine encephalitis	?	7	Encephalitis	1–2	8.0
JHM virus		42	Encephalitis	2–4	3.5–4.0

* Range of incubation period following intracerebral inoculation of 0.03 cc. of 10 per cent suspensions of infected brain or brain and spinal cord.

‡ M.I.D.$_{50}$ per 0.03 gm. infected brain or brain and spinal cord.

§ Range of virus concentration obtained for different suspensions.

‖ 10 per cent brain or spinal cord suspensions produced 70 to 80 per cent infection on intracerebral inoculation.

to determine whether its virulence could be further increased. Fifth passage virus, representing a TO strain of relatively low virulence, was employed for the study of the effects of intramuscular injection.

Lymphocytic Choriomeningitis Virus, Mitchell Strain.—This strain was obtained from Dr. Monroe D. Eaton who isolated it from the lungs of mice in the course of studies on human

respiratory viruses. It has been maintained in this laboratory as 10 per cent suspension of mouse brain in isotonic phosphate buffer, pH 7.0–7.1.

JHM Virus.—This agent, presumably a virus, was recovered from stock mice in the course of studies on diarrhea of suckling mice (6). Two mice, approximately 3 weeks old, were found to have spontaneous paralysis of the hind limbs. A 20 per cent suspension in infusion broth of the brains from these 2 mice was passed intracerebrally to 4 mice. One of the inoculated mice developed ruffled fur and paralysis on the 8th day. The other 3 mice failed to develop obvious signs of disease in 21 days. The paralyzed mouse was sacrificed on the 8th day and a 10 per cent suspension of its brain was then passed to 8 mice. All were hunched, ruffled, and quiet by the 5th and 6th days. Four were sacrificed at this time. Of the remaining 4 sick mice, 2 developed paralysis of the hind limbs on the 9th day and died on the following day. The 2 remaining mice showed no other signs for 21 days, and seemed to have recovered. Similar reactions were obtained with passage of 10 per cent brain suspension through the next 4 consecutive transfers. The activity of the virus appeared to become more stabilized from the 7th up to the present 42nd consecutive passage. During this time, the morbidity has been practically 100 per cent, and the incubation period has become reduced between 2 and 4 days. The disease has become more predominantly encephalitic in its obvious manifestations, with paralysis occurring rarely. This agent has not yet been identified. It is of unusual interest in that the underlying pathologic changes are characterized by extensive demyelinization of tracts in brain and cord, grey matter being relatively little affected. Its detailed study by Dr. F. Sargent Cheever and Dr. Orville Bailey is in progress, and their observations will be reported elsewhere.

Intramuscular Injections.—The viruses were injected into the calf muscles of mice approximately 3 weeks old. The usual dose was 0.2 cc. of 10 per cent brain or brain and spinal cord suspensions. Normal mouse brain suspensions prepared in the same manner were inoculated intramuscularly as controls. All mice used in the experiments were bred in the laboratory and derived from the Schwentker strain of Swiss albinos.

EXPERIMENTAL OBSERVATIONS

Intramuscular Inoculation of Normal Brain Suspensions

Symptoms.—Seventeen mice inoculated intramuscularly with 0.2 cc. of a 10 per cent normal brain suspension prepared from 20 pooled brains of young mice did not show any signs of disease. Twelve mice inoculated intracerebrally with 0.03 cc. also failed on inspection to exhibit any abnormal signs.

Pathology.—In attempting to interpret the significance of the muscle lesions caused by injection of brain suspensions infected with various viruses, it was essential to define the histologic reaction which followed the introduction of normal brain tissue suspension. For this purpose muscle tissue was taken 3, 6, 10, and 24 hours, 2, 4, and 6 days after injection. This material caused no recognizable disease following inoculation.

Examination of Stained Sections.—It was found that injection of 0.2 cc. of the preparation elicited an early and often quite intense local inflammatory reaction. During the early period, there occurred an increasingly intense edema and cellular infiltration of the intramuscular connective tissue and fat. Polymorphonuclear leucocytes predominated during the first 24 hours; later, large mononuclear cells prevailed. The muscle fibers contiguous to the inflamed areas often were necrotic, but there were no diffuse changes in the main mass of muscle tissue. After 2 or 3 days, the necrotic fibers were replaced by young regenerating fibers, which soon increased in size and regained their myohemoglobin, but were still distinguishable from the original unaffected fibers by their smaller size and by the central location of their nuclei. The presence of particulate brain matter was not essential for the production of the lesions since

even after centrifugation at 10,000 R.P.M. for 30 minutes, in the cold, the clear supernatant evoked a similar reaction.

Not all normal brain suspensions produced even this superficial necrosis of muscle fibers. A second lot of normal brain suspension prepared from 50 brains and tested in the same manner failed to cause necrosis of muscle fibers adjacent to the intramuscular septa; the initial inflammatory reaction in the fat and connective tissue had virtually disappeared by the 4th day. Thus the early reaction evoked by normal brain tissue subsides within a few days, leaving no permanent damage to the muscles. This effect is quite different from that produced by the injection of brain suspensions containing certain viruses as will be shown by the results of the experiments to be described.

Intramuscular Inoculation of Various Viruses Studied

The symptoms and pathologic results of intramuscular inoculation of the viruses studied are summarized in Table II.

Mouse Encephalomyelitis Viruses, Strains GD-VII and FA.—

Symptoms.—Intramuscular inoculation of these mouse encephalomyelitis strains in amounts representing about 600,000 M.I.D.$_{50}$ intracerebral doses produced practically 100 per cent morbidity and mortality. The incubation period was usually 5 to 7 days with a mean of about 6 days; occasionally signs of disease were noted as early as 4 days or as late as 9 days. While infection consistently followed intramuscular inoculation with high concentration of virus, there was marked difference in sensitivity between the intramuscular and intracerebral routes. For example, the titer of one suspension of GD-VII virus given intramuscularly was 0.2 gm. $\times 10^{-1.8}$ as calculated by the method of Reed and Muench (7); when inoculated intracerebrally it was 0.03 gm. $\times 10^{-6.3}$. Similar results were obtained with the FA virus.

In contrast to the encephalitic disease produced by intracerebral injection, infection by the intramuscular route resulted in paralysis. Up to the present, over 300 intramuscular injections have been made with the GD-VII virus in the course of various experiments. The typical picture has been that of an ascending type of paralysis with subsequent death. The onset of infection was characterized by the gradual development of flaccid paralysis of the injected leg. The opposite hind leg than became paralyzed and later one or both front legs. Occasionally at this stage, paralysis of the trunk was also observed. Some of the animals looked sick, had ruffled fur, and occasionally conjunctivitis. Death invariably occurred 1 or 2 days after paralysis became generalized. The duration of the illness ranged from 1 to 6 days, but usually ran its course in 2 to 3 days.

Slight variations from this sequence of events were occasionally noted. In a few mice, after the development of paralysis of the injected limb, paralysis of the front leg occurred before that of the opposite hind leg; in a few mice, death followed paralysis of the hind legs before the forelegs became affected. Finally in a few mice, paralysis appeared in another limb before the injected left limb became affected. Rarely, the mice had generalized convulsions preceding the paralysis. With the FA virus, a few mice survived the paralytic phase and went on to apparent recovery, retaining only slight residual paralysis or deformity.

The above description applies to mice 3 weeks of age. Of 21 fourteen week old mice, observed for 30 days, only 4 developed paralysis and one of these survived. The incubation period for the 4 paralyzed mice averaged 9.7 days as compared to 6.2 days for the 3 week old mice in the control series. This increased resistance with age has been previously demonstrated in mice inoculated by other routes (8).

Pathology.—The histologic effect of injecting brain suspensions containing GD-VII and certain other viruses into the muscles of the leg was different from what happened with in-

jection of normal brain suspension. This difference was well shown in the following experiment in which 0.2 cc. of GD-VII virus brain suspension was injected into the muscles of

TABLE II

Effect of Intramuscular Inoculation of Young Mice with 0.2 Cc. of 10 Per Cent Central Nervous System Tissue Suspensions of Mouse Encephalomyelitis and Other Neurotropic Viruses

Virus	Log virus concentration* of suspensions injected intramuscularly	No. with obvious reactions‡	Infected	Cardinal symptoms	Average incubation period	Myositis
			per cent		*days*	
Mouse encephalomyelitis						
Strain GD-VII	5.8–6.3§	124/127	97	Paralysis	5.8	++++
Strain FA	5.2–5.8	62/63	98	Paralysis	6.6	++++
Strain 4727	4.4	4/20	20	Paralysis	8.5	++
Strain FV	‖	1/41	2	Paralysis	9.0	+
SK virus, Jungeblut	8.2	20/20	100	Encephalitis and paralysis	1.3	+++++
Lymphocytic choriomeningitis						
Mitchell strain	5.0	15/16	94	Edema, injected site	7.5	++++¶
Human poliomyelitis						
Lansing strain	3.0–3.5	0/19	0			+
Herpes simplex						
HF strain	2.9	3/20	15	Encephalitis	7.3	+
Eastern equine encephalitis	8.0	26/26	100	Encephalitis	2.0	+
JHM virus	3.5–4.0	5/25	25	Paralysis	8.0	+
Normal brain		0/17	0			+¶

* M.I.D.$_{50}$ per 0.03 gm. infected brain or brain and spinal cord.

‡ Numerator = number positive; denominator, number inoculated.

§ Range of virus concentration of suspensions employed for intramuscular studies.

‖ 10 per cent brain or spinal cord suspension produced 70 to 80 per cent infection on intracerebral inoculation.

¶ See text.

the right leg and an equivalent amount of normal brain suspension into those of the left leg (Table III).

Muscle Lesions Caused by the GD-VII and FA Viruses.—The following description is based

on the study of 169 mice injected into the muscles of the calf with brain suspensions containing one or the other of these viruses. The number examined and the interval following the injection are recorded in Table IV.

Gross Lesions.—During the first few days, the muscles of the infected limb appeared swollen and edematous, in comparison with those of the opposite side. Later they became opaque and dry, with whitish yellow streaks and patches. All contractility was lost. These changes

TABLE III

Histologic Effect of Injecting GD-VII Virus Brain Suspension in Right Leg and Normal Brain Suspension in Left Leg

Mouse No.	Time after injection	Myositis	
		R	L
	days		
642	7	++++	−
643	7	++++	−
644	7	++++	−
656	8	+	−
657	8	+++	−
669	9	++++	−
670	9	+++	−
673	10	+++	−
681	11	+++	−

−, muscle normal; + to ++++, different degrees in intensity of myositis.

TABLE IV

Time of Examination and Number of Animals Studied for Myositis Following Intramuscular Injection of Mouse Encephalomyelitis GD-VII and FA Viruses

	Hours				Days															
Time	1/4	3	6	12	1	2	3	4	5	6	7	8	9	10	11	13	15	30	69	73
No. of mice	5	3	3	3	6	21	6	25	13	19	14	10	17	11	3	1*	1	7	1	1

* The mice which survived beyond 10 to 11 days had received smaller doses, or were older than 3 weeks and therefore less susceptible.

involved the muscles of the calf and thigh, sometimes the glutei, but did not extend to the muscles of the back or abdomen, nor to the muscles of the opposite leg.

Microscopic Lesions.—These were characteristic of an intense acute myositis. Many of the muscle fibers were necrotic, segmented into eosin-staining masses without structure, and often fragmented further into irregular clumps. Between the necrotic fibers, there was a profuse inflammatory exudate; the participating cells included polymorphonuclear leucocytes, lymphocytes, and large mononuclear histiocytes (Fig. 1). As the process progressed, the polymorphonuclears showed nuclear pycnosis and fragmentation, and their proportion diminished in comparison with the mononuclear elements. Often the necrotic fibers became invaded by polymorphonuclears and histiocytes, which were seen buried in the necrotic masses,

and in various stages of disintegration. Phagocytosis of small clumps of necrotic muscle substance by the invading histiocytes was often apparent.

The inflammatory process was not limited to the muscles, but affected also the intermuscular fat and fibrous tissue. No thromboses or other changes were seen in arteries or veins, and there was little or no hemorrhage. The sciatic nerve and the large intermuscular branches showed no lesions recognizable with the hematoxylin-eosin stain, although they were often surrounded by inflammatory exudate (Fig. 2). In Cajal preparations the axones of the nerve bundles and the epilemmal portions of the motor neurites were excellently preserved. Branching filaments could be traced into the end-plates of segmented and necrotic muscle fibers. From a purely morphological point of view it would seem that the destruction of the muscle substance takes place without any antecedent damage to the terminal neurites.

A striking feature of the lesions was the early regeneration of new muscle fibers, beginning on the 4th day. The myolemmal nuclei with their adjacent sarcoplasm, often escaped destruction. They took on the character of myoblasts, the nucleus becoming large and vesicular and the sarcoplasm basophilic (Fig. 3). They aligned themselves end to end, myofibrils developed on their surface, and later the staining changed from purplish blue to red with the regeneration of myohemoglobin, and cross-striations reappeared. But for a long time the regenerated fibers could be identified by their smaller caliber and by the central location of their nuclei which were often aligned in long chains. The mooted question as to whether these chains of nuclei result from amitotic division, need not be discussed here. Mitotic figures were not infrequently found, and in some cases at least one could be certain that the dividing cells were myoblasts and not histiocytes.

Calcification of the necrotic fibers occurred occasionally but was not a regular feature of the process (Fig. 4). With the subsidence of the acute inflammatory reaction, fibroblasts appeared among the leucocytes and gave rise to a certain amount of residual fibrosis.

Intranuclear Inclusions.—The nuclei of the regenerating muscle fibers in almost all cases contained eosinophilic inclusions. These were in the form of irregular clumps replacing the basichromatin, which was crowded against the nuclear membrane. Often the nucleus was occupied by a single spherical body surrounded by a clear halo, resembling inclusions of the herpes type (Fig. 5). Often only a single nucleus of a row contained an inclusion, the others being normal or somewhat hydropic in appearance. With the Laidlaw acid fuchsin–orange G stain, the inclusions did not retain the fuchsin stain as do the usual virus inclusion bodies.

We have come to no conclusion as to the significance of these structures. The lesions produced by Theiler's virus in the central nervous system are not accompanied by inclusion bodies, and their occurrence only in the regenerating muscle fibers and not in any of the degenerating fibers or wandering cells is puzzling. They resemble in their general appearance and staining, small clumps of coagulated, necrotic muscle substance, and it is possible that such material may become incorporated within the nucleus during the reconstruction of the fibers from the myoblasts. We have actually seen nuclei partially surrounding such necrotic fragments, but have not been able to accept this explanation of their origin. Whatever their significance, these intranuclear inclusions appear to be characteristic features of the lesions.

The resemblance of the muscular lesions to those produced in young rats (9), mice (10), and other animals (11), by deficiency of vitamin E is very striking. Here, too, one finds similar components of necrotic and regenerating fibers and an intense polymorphonuclear and histiocytic inflammatory reaction. We have reexamined preparations of rat muscle showing nutritional muscular dystrophy, and have been unable to find similar intranuclear inclusions in the regenerating fibers. This perhaps would speak against the idea that they represent incorporated necrotic fragments of muscle, since the histological reactions in the two conditions are identical.

Residual Lesions.—Seven four week old mice which had survived the initial injection were killed on the 30th day. While none at this period showed active myositis, there was distinct

evidence of previous damage, such as has been noted in other mice injected at this age. Many of the muscle fibers had centrally placed nuclei, often forming long strings, and were obviously derived from regenerated fibers. Small groups of calcified fibers were also present. There was no interstitial change, but perivascular lymphocytic accumulations were seen.

One mouse surviving intramuscular injection of FA virus, and still showing slight residual paralysis, was killed on the 69th day. There were no lesions except a single Waldeyer "*Muskelzellschlauche;*" *i.e.*, a necrotic fiber replaced by a column of histiocytes and polymorphonuclear leucocytes. This may be taken as an indication of minimal, but still persistent activity. A mouse, 14 weeks old, which had developed paralysis, but survived following inoculation of GD-VII virus, was killed after 73 days. There were a few atrophic fibers and slight perivascular infiltration of lymphocytes. The cord showed degeneration of some motor nerve roots, and slight microcystic degeneration of the white tracts.

The muscle lesions, except in two instances, were strictly limited to the injected limb. That there is a correspondence between the lesions and the presence of active virus is shown in the following experiment. Eight mice, which had received intramuscular injections of 0.05 cc. of 10 per cent of GD-VII brain suspension into the left leg were sacrificed on the 7th and 8th days, when both hind limbs had become paralyzed. Dissected muscles from the right and left legs of 5 mice were separately ground, suspensions prepared, and titrated for virus by intracerebral injection. It was found that the muscle suspension from the injected limb titrated to $10^{-6.3}$ M.I.D.$_{50}$ while that from the opposite limb was less than 10^{-1} M.I.D.$_{50}$. In accordance with these findings, the injected muscle was the seat of intense myositis, whereas the muscles from the paralyzed, but uninjected limb, were normal.

The muscles of the back which were included as routine in the spinal cord section were not affected. The myocardium was also sectioned in a number of paralyzed mice, and no lesions were found. Furthermore, several animals injected directly into the heart did not develop lesions of the heart muscle, although they became paralyzed, and typical lesions were found in the brain and spinal cord.

Resistance of Older Mice.—As previously stated, mice, 14 weeks old, show a striking resistance to intramuscular injection. It was interesting to find that local lesions were either mild or absent. We have examined 9 mice, sacrificed in groups of 3 after 6, 8, and 14 days. One killed on the 6th day had diffuse myositis of moderate intensity; the remaining mice showed at most a mononuclear and lymphocytic cell infiltration of the intermuscular connective tissue. After 2 weeks there were no recognizable lesions. Similar differences in local as well as general susceptibility between young and old mice to the virus of vesicular stomatitis were found by Sabin and Olitsky (1).

Lesions of the Central Nervous System Following Intramuscular Inoculation.—In nearly all cases, sections of the brain and spinal cord at various levels were studied. In contrast to the results of intracerebral inoculation, lesions of the brain were absent, or when present, limited to medulla, pons, and the white matter or cerebellum. Necrosis of the fasciculus dentatus, which is found so regularly after intracerebral injection, did not occur in these mice. On the other hand, myelitis was found in 86 per cent of mice after the 4th day. The lesions were most marked in the lumbar cord, and were sometimes unilateral.

4727 Virus.—

Symptoms.—Following intramuscular injection of 3 weeks old mice with this TO type of mouse encephalomyelitis virus, ascending paralysis was produced in 4 of 20 animals. With 2 weeks old mice, 6 of 10 became affected. As with the GD-VII and FA strains, paralysis first appeared in the injected limb. The incubation period was from 7 to 11 days. There were no survivors among the paralyzed mice not used for pathologic study.

Pathology.—This virus gave rise to a rather mild form of myositis in both 2 and 3 weeks

old mice. The lesions, during the first 4 days, were not unlike those produced by suspensions of pooled normal brains. Later (6 to 11 days), however, they differed in that there was continuing necrosis of individual fibers, with a cellular inflammatory reaction largely limited to these necrotic fibers, (Fig. 6) and to the intercellular connective tissue, where it was superseded by abundant growth of fibroblasts. Often these formed compact granulomatous masses in the walls of the lymphatics. In the early stages, they included many polymorphonuclear leucocytes. There was considerable individual variation in the intensity and distribution of the lesions. In none of the cases did the lesions attain the severity of those resulting from the injection of GD-VII or FA virus.

At 6 days, when signs of nervous disorder were not apparent, no lesions were found in the brain or cord, although myositis of the type described was present in the injected limb. After 8, 11, and 13 days, however, when the particular mice taken for examination had become paralyzed, lesions were present in cord and brain stem. Surviving mice, which had shown no paralysis, were killed after 33 days. No lesions were found in muscle, brain, or cord.

FV Virus.—

Symptoms.—Only 1 of 40 mice injected by the intramuscular route developed paralysis with this weakly paralytic type strain. Virus recovered from the spinal cord of this mouse, produced typical paralytic infection when inoculated intracerebrally into other mice.

Pathology.—No significant myositic reactions were found after intramuscular injection in 9 mice sacrificed and examined at varying intervals between 2 and 12 days. The one mouse which developed paralysis (9th day) was killed on the 12th day when paralysis had become general. The leg muscles of all the mice examined showed mononuclear infiltrations of the intermuscular septa, especially in the walls of the lymphatics. This was rather striking in the 2 and 4 day animals, but subsided thereafter. These lesions were essentially similar to those resulting from injection of normal brain suspensions, and since they were not progressive, we are inclined to regard them as non-specific.

In the mice without obvious signs the central nervous system was not affected, but in the paralyzed mouse, of which only the brain was studied, encephalitic foci were found in the basal ganglia and brain stem—degenerating ganglion cells, collections of microglia, and perivascular cuffing.

Other Viruses.—

The studies with other viruses were made to determine whether the local lesions following intramuscular injection of mice were a general property of other neurotropic viruses or whether such reactions might be specific for only certain species or types of viruses.

Jungeblut's SK Virus.—

Symptoms.—The virus was highly virulent by the intramuscular route. In one experiment, 0.1 cc. of a 10^{-7} suspension infected 6 out of 6 mice, while the same amount of a 10^{-8} dilution produced infection in 4 out of 6 mice, with brain suspension which titered $10^{-8.2}$ by intracerebral injection of 0.03 cc. High virulence of this virus by other routes has been reported (12). Following intramuscular injection of the virus in high concentration, a fulminating disease ended in death within 36 to 48 hours. At this time there was weakness but no frank paralysis. With lower concentrations of virus, the incubation period was extended to 3 or 4 days, and paralysis was evident.

Pathology.—The virus, when introduced intramuscularly even in high dilutions, proved to

be of extraordinary pathogenicity, and the muscle lesions were of an extent and intensity greater than those produced by any of the other viruses studied. Material from 38 mice was examined, the animals being sacrificed at intervals ranging from 15 minutes after injection to 4 days.

With the standard dose of 0.2 cc. of a 10 per cent brain suspension definite lesions were recognized even after 15 minutes. There was edema, not only of the intramuscular septa, but separating the muscle fibers themselves, and an occasional polymorphonuclear leucocyte was present between the fibers. Actual necrosis of the muscle fibers as evidenced by hyalinization and segmentation, was not yet present, but after 3 hours, the fibers adjacent to the intermuscular septa were quite evidently necrotic. After 6 hours, there was further progression of the lesions, and at 12 hours there was maximal necrosis of the fibers, with fragmentation and very marked edema and leucocytic reaction (Fig. 7). Many of the dead fibers were being invaded by polymorphonuclear leucocytes (Fig. 8). At 24 and 48 hours, the picture was about the same. It was interesting to find that although the contractile portions of the fibers had completely lost their striations, and had undergone fragmentation, the myolemmal nuclei were still preserved, and even appeared enlarged and were occasionally found undergoing mitosis. At this stage, no actual regeneration of new fibers had taken place, and since, with the dosage used, the mice did not survive beyond 48 hours, there was no further opportunity for regeneration to occur.

In Cajal preparations, terminal neurites were well preserved, and delicate fibrils and even end-plates could be found among the segmented, necrotic fibers (Fig. 9). So far as one can draw conclusions from the histologic evidence, it would seem that necrosis of the fibers is not preceded by alterations in the terminal motor nerves or end-organs.

The remarkable pathogenicity of this virus is illustrated by the lesions found in 4 mice which had been injected with 0.1 c. and 0.2 cc. of a dilution of 10^{-7}, and killed on the 4th day when they were paralyzed and moribund. The muscles of the injected leg, and even those of the back as far as the interscapular region were the seat of maximal necrosis, with edema and a sparse inflammatory reaction. The spinal cord in the cervical region showed massive unilateral necrosis of the grey matter.

With the usual intramuscular dose of 0.2 cc. of 10 per cent brain suspension, lesions were found in the spinal cord and brain on the 2nd day. They were severe, and characterized by extensive necrosis of all elements in the affected areas. A detailed description of the neuropathology has been given by Wolf (13).

Lymphocytic Choriomeningitis Virus—Mitchell Strain.—

Symptoms.—Ten animals observed for 30 days after intramuscular inoculation did not show any signs indicating disease of the central nervous system. However, marked swelling of the injected leg appeared 7 to 8 days after injection; this gradually subsided without apparent ill effects. Dr. Eaton has informed us that this particular strain has produced fatal infections in mice inoculated intraperitoneally, in contrast to the indefinite, non-fatal illness usually obtained by this route with other strains of lymphocytic choriomeningitis virus (14).

Pathology.—Twelve mice, killed 2, 4, 7, and 8 days after intramuscular inoculation, provided material for a study of the lesions. These were intense, but fundamentally different from those following injection of Theiler's GD-VII, FA and 4727 viruses, and the SK virus.

It was noted in the gross that there had occurred edematous swelling of the leg, easily recognized in the intact animal. After dissecting off the skin, the muscles appeared pale, watery, and translucent.

Microscopically, there was intense edema, affecting especially the areolar tissue of the intermuscular septa. There was a profuse cellular reaction. The predominant cell type was the small lymphocyte, occasional plasma cells, large mononuclear histiocytes, and only an oc-

casional polymorphonuclear leucocyte. The lymphoid cells were most abundant in the intermuscular connective tissue, but loose collections were found also between the muscle fibers, and scattered through the adipose tissue (Fig. 10). The popliteal lymph node was large and hyperplastic and contained an increased proportion of immature lymphocytes. There was profuse migration of the lymphocytes into the surrounding fat.

In contrast to the necrosis induced by GD-VII, FA, and SK viruses, the muscle fibers were not attacked by the LCM virus. They showed no recognizable alteration, despite the intense edema and cellular reaction in the interstitial tissue. The small nerves appeared edematous, and the nuclei of the Schwann cells and of the perineurium were swollen. The condition of the terminal neurites and end-plates was not investigated. Histologic examination disclosed no significant lesions in brain or spinal cord.

Additional Viruses Studied.—

Symptoms.—The mouse-adapted Lansing virus of human poliomyelitis failed to produce any paralysis by the intramuscular route; one mouse out of 19 inoculated became ill and died on the 4th day after inoculation; there was mild myelitis. Similar results were obtained with the HF strain of herpes simplex virus; 3 of 20 mice became sick without paralysis and died between the 5th and 12th days. The virus was recovered from their brains. The strain of Eastern equine encephalitis virus gave rise to a rapidly fatal disease with high concentration of virus (0.2 cc. of 10 per cent infected brain suspension). It was characterized by convulsions and prostration, and terminated in death within 48 hours. Infection was also produced with 10^{-3} and 10^{-5} dilutions of brain suspension, but the incubation period was delayed ranging from 3 to 6 days. Paralysis was not noted. The JHM virus produced an ascending paralysis in 5 of 25 mice inoculated intramuscularly with late passage virus (37th to 42nd) but not in any of 42 mice injected with earlier passage virus (2nd to 21st). The incubation period for the paralyzed mice was from 7 to 9 days.

Pathology.—Relatively little local reaction was observed following intramuscular injection of these viruses. The Lansing, herpes, and JHM viruses produced a transient interstitial inflammatory reaction with limited necrosis of fibers adjacent to intermuscular septa, followed by early replacement with regenerated fibers. The Eastern equine virus also elicited a similar inflammatory reaction, but the fibers contiguous to the inflamed connective tissue were unaffected. With higher dilutions of this virus which produced signs of infection, no local lesions were found. Since the reactions for these viruses were indistinguishable from those which resulted from the injection of normal brain, they may well have been non-specific lesions resulting from brain tissue. Our negative results with the Eastern equine encephalitis virus are thus not in accord with Sabin's observations (personal communication). The discrepancy may be due to the use of a different strain of virus or of mice.

The central nervous system of the mice inoculated with the Eastern equine encephalitis virus and the JHM virus was also examined histologically. With the Eastern equine virus the lesions in the central nervous system were confined to the basal ganglia, pons, and medulla and consisted of small areas of focal necrosis with spongy rarefaction of the tissue, necrosis of ganglion cells and glia, and slight polymorphonuclear leucocytic reaction. No lesions were found in the spinal cord. The JHM virus produced a characteristic type of meningoencephalitis featured in its later stages by extreme demyelinization of the spinal cord tracts (Fig. 11).

Evidence for Local Multiplication of Virus

The progressive character of the lesions suggested a regional multiplication of the virus. In order to obtain quantitative evidence on this point, a series of young mice was inoculated intramuscularly with the GD-VII virus, and another with the SK virus.

The doses used were sufficient to cause infection, but not early muscle lesions. The attack rate among the controls was 78 per cent in one experiment and 67 per cent in another with the GD-VII virus, and 100 per cent with the SK. At various intervals, 6 mice were killed with ether; the pooled legs minus the feet and cut off at the hip joint, and the spinal cords and brain

TABLE V

Demonstration of Local Multiplication of GD-VII and Jungeblut SK Virus Following Intramuscular Inoculation, and the Occurrence of Myositis as a Function of Virus Concentration

Virus	Interval after inoculation	Obvious reactions	Pathology and virus concentration						
			Leg			Spinal cord		Brain	
			Amount virus*		Myositis	Amount virus	Myelitis	Amount virus	Encephalitis
			Exp. 1	Exp. 2	Exps. 1 and 2				
GD-VII	15 min.	0	3.6	3.4	0	0‡		0	
	1 day	0	2.6	3.5	±	0	0	0	0
	2 days	0	3.8	3.7	±	0	0	0	0
	3 days	0	4.5	4.6	±		0	0	0
	4 days	0	4.8	4.9	++	4.8	+§	2.9	0
	5 days	0	5.4	5.1	+++	0	0	0	0
	6 days	+P	6.3	5.6	++++	5.2	+	3.7	0
	8 days								
	Exp. 1	+CPS	5.5			6.5		4.8	
	Exp. 2	+PH		4.0	+++R	6.2	+	4.5	0
	12 days								
	Exp. 2	+CPS		3.8	Advanced regeneration	4.2	+	4.1	+
SK	15 min.	0	<1.0		0	0		0	
	6 hrs.	0	1.5		±	0	0	0	0
	12 hrs.	0	1.8		±	0	0	0	0
	1 day	0	3.5		±	0	0	0	0
	2½ days	0	6.2		+++	6.1	+§	5.6	0
	3 days	+SP	6.5		++++	7.7	+++	7.6	+++

+P = paralysis, injected limb; +CPS = complete paralysis and sick; +PH = paralysis, hind limbs; +SP = sick, paralysis. Blank = not tested. R = regeneration.

* Log virus concentrations as M.I.D.$_{50}$ intracerebral doses per 0.03 gm. infected tissue.

‡ Results with spinal cord and brain in 15 minutes through 6 days for Experiment 1.

§ Myelitis in one of three mice.

from 3 of them were titrated by intracerebral injection for virus activity. The other 3 mice were examined histologically. The experiment was repeated with the GD-VII virus and essentially similar results were obtained. The results are summarized in Table V.

With the GD-VII, a significant increase in the virus content of the leg was obtained on the 3rd and 4th days, while by the 5th and 6th days, the increase

was about 100- to 1000-fold. On the 8th day and 12th days when the animals were completely paralyzed, or had paralysis of both hind limbs, the virus concentration of the leg was significantly lower than on the 6th day. Three animals which did not become paralyzed were killed after 28 days. No virus was recovered from brain, cord, or leg. Although these results are based on titration of suspensions of the entire limb, similar virus increases were obtained when suspensions were prepared from muscles dissected free of bone and sciatic nerve. The muscle lesions were progressive and showed a maximum intensity by the 6th day. On the 8th and 12th days when the virus concentration had decreased, the acute reaction was subsiding and regeneration advanced. Virus appeared in the brain and cord on the 4th day.

Because of the greater virulence of the SK virus, a smaller initial dose could be used, and the multiplication of the virus demonstrated in even more striking fashion. Thus there was found an increase of the order of 100,000 or more in the leg at 3 days, when the animals were sick and moribund.

It was of interest to see whether a virus which did not give rise to significant lesions of the muscles, also multiplied locally. A suspension of the JHM virus titering $10^{-3.5}$ M.I.D.$_{50}$ was inoculated intramuscularly, and leg suspensions titrated in a similar manner. The 15 minute control series showed a virus concentration of less than 10^{-1} M.I.D.$_{50}$, while after 2 days it had risen to $10^{-2.9}$ M.I.D.$_{50}$ followed by a decrease to $10^{-2.4}$ M.I.D.$_{50}$ on the 4th day, and to less than 10^{-1} M.I.D.$_{50}$ on the 6th day. In spite of this transient rise in concentration, the muscles showed no significant lesions. It is of interest that with a similar concentration of the virus GD-VII, only minimal changes were seen in the muscles. Further studies are necessary to determine whether the absence of myositis with the JHM virus is merely due to its relatively low concentration or whether it is an indication of a fundamental difference in its pathogenicity for muscle tissue.

Muscle to Muscle Passage of GD-VII Virus

Further evidence for the local multiplication of GD-VII virus was secured by muscle to muscle passage. Muscle dissected free of the sciatic nerve and bone was ground, and centrifuged 10 per cent suspensions injected in the amount indicated in Table VI.

Since it is obvious that the muscle suspensions are not entirely free from nervous elements, even when the main trunk of the sciatic nerve is excluded, one is not justified in concluding that the multiplication of the virus takes place within the muscle fibers themselves. Further experiments will be needed to decide this point.

In contrast to these results with leg or muscle tissue, are the comparable observations of Theiler and Gard (8). With the closely allied FA strain, these workers found no multiplication in the nasal mucosa after intranasal inoculation, nor in the abdominal viscera after intraperitoneal inoculation.

Effects of Sciatic Nerve Section

As has been pointed out, there is close resemblance of the virus lesions to those produced in suckling rats (9) and mice (10) by deficiency of vitamin E. Because of the fact that the muscular dystrophy can be prevented by section of the sciatic nerves (15), it was interesting to ascertain whether nerve section would also prevent the development of the virus myositis. The operation was performed on a number of mice and the GD-VII virus, in 0.05 cc. dosage, injected after 5 to 6 days into the gastrocnemius of each leg, the unoperated limb serving as control. No consistent difference was found between the lesions on the operated and unoperated sides. Nor did nerve section, in a series of animals injected only on the operated side, prevent the development of myelitis.

TABLE VI

Demonstration of Local Multiplication of GD-VII Virus by Muscle to Muscle Passage

Material injected	Passage No.	Dose	Interval after inoculation	Obvious reactions*	Myositis	Amount of virus‡
Brain		0.2 cc., 10 per cent suspension	6 days	15/15	+++	
Muscle	1	0.2 cc., 10 per cent suspension	6 days	16/16	++++	
Muscle	2	0.2 cc., 10 per cent suspension	6 days	10/10		
Muscle	3	0.2 cc., 10 per cent suspension	6 days	16/16	++++	
Muscle	4	0.05 cc., 10 per cent suspension	15 min.	0/20	−	4.1
			1 day	0/20	±	4.5
			2 days	0/20	±	4.4
			4 days	1/20	+++	6.1
			6 days	19/20	++++	6.0

Blank = no test.

* Numerator = number positive; denominator = number inoculated.

‡ Log virus concentration as M.I.D.$_{50}$ intracerebral doses per 0.03 gm. infected tissue.

However, it could be shown by India ink injection of equivalent amounts, that the injected material passes rapidly up the intermuscular fascial planes to the inguinal region, so that it may well come in contact with nerve branches above the point of section of the sciatic. One cannot therefore conclude that the virus does not reach the central nervous system by way of nerves. The minimal infective dose of the GD-VII virus by the intramuscular route is so large that it is impossible to limit the injected material to the gastrocnemius muscle.

Similar experiments have recently been completed with the SK virus in which 0.01 cc. of a dilution of 10^{-3} of infected mouse brain was injected into the left gastrocnemius muscle a week after section of the left sciatic nerve. When the animals were examined 48 hours later, it was found that the lesions were less widespread and intense in the operated mice than in the unoperated controls. After 72 hours, both operated and control mice were dead or mori-

bund and there was little difference in the intensity of the lesions in the two groups. It is clear from these experiments that section of the sciatic nerve affords no protection against the spread of the virus to the central nervous system; nor does it do more than delay the development of the muscle lesions.

Protection against Myositis with Specific Antiserums

Since there was evidence which demonstrated a direct relationship between myositis and local virus multiplication, it was of considerable interest to determine whether the myositic reactions could be neutralized specifically with antiserums.

Antiserums were prepared against the GD-VII and SK viruses by intravenous injections of rabbits.[2] Neutralization of the virus by the test and control serums was performed by mixing equal amounts of undiluted serum with 20 per cent brain suspnsions of GD-VII virus for tests by the intramuscular route and varying concentrations of virus by the intracerebral route. The anti-SK rabbit serum was tested only by the intramuscular route using varying concentrations of virus against a constant amount of antiserum. The serum-virus mixtures were then held at 3–5°C. for approximately 18 hours prior to inoculation.

Preliminary experiments on the neutralization of GD-VII virus when tested by the intracerebral route with two prepared anti-GD-VII rabbit serums revealed no significant amounts of antibody. A definite degree of neutralization of the virus was obtained, however, when tested by the intramuscular route. Thus 16 of 18 mice inoculated intramuscularly with 0.2 cc. of anti-GD-VII rabbit serum–virus mixture were protected against the obvious manifestations of the disease. On the other hand, normal rabbit serum under the same test conditions showed no protection in 51 of 53 mice. Similar results have been obtained recently with anti-GD-VII serum prepared in a *rhesus* monkey by a series of intramuscular injections over a period of $2\frac{1}{2}$ months. The neutralization index of this serum by the intracerebral route gave a value of only 10 and would be classified as highly doubtful for specific antibodies (16). But 10 of 10 mice did not develop infection when injected intramuscularly with 0.2 cc. of the same serum-virus mixture. In contrast, none of 10 mice injected with normal monkey serum-virus mixture and none of 6 mice inoculated with anti-lymphocytic-choriomeningitis monkey serum-virus mixture were protected. These results indicate that with the GD-VII virus the intramuscular route is more sensitive than the intracerebral route for the detection of antibodies and are in accord with Lennette and Koprowski's findings (17) with certain other neurotropic viruses that extraneural routes are more suitable than the intra-

[2] One-tenth cubic cm. of 10 per cent brain suspension of GD-VII virus was given on the 1st day and gradually increased to 1.0 cc. in 10 days. An additional 1.0 cc. was injected on the 18th day and a week later the animals were bled. An anti-SK rabbit serum was prepared in a similar manner except that the injections were made at shorter intervals in the course of 9 days.

cerebral route for the demonstration of antibodies. The anti-SK rabbit serum protected against signs of the disease with all of the final dilutions of SK virus employed (10^{-4} through 10^{-9}) and represented a neutralization of at least 10,000 intramuscular doses.

TABLE VII

Protection of Mice against GD-VII Myositis with Anti-GD-VII Rabbit Serum

Virus without serum				Virus + normal rabbit serum				Virus + anti-LCM* serum				Virus + anti-GD-VII rabbit serum			
Mouse No.	Time	Myositis	Obvious reactions	Mouse No.	Time	Myositis	Obvious reactions	Mouse No.	Time	Myositis	Obvious reactions	Mouse No.	Time	Myositis	Obvious reactions
	days				*days*				*days*				*days*		
1061	2	++++	−	1188	2	±	−					1191	2	±	−
1062	2	++	−	1189	2	−	−					1192	2	±	−
1063	2	++	−	1190	2	±	−					1193	2	±	−
1091	2	++	−	1605	2	±	−								
1092	2	++	−	1606	2	±	−								
1093	2	+++	−	1607	2	±	−								
1611	2	±	−												
1612	2	±	−												
1613	2	+++	−												
1048	4	++++	+	1206	4	±	−					1209	4	−	−
1099	4	+++	+	1207	4	±	−					1210	4	−	−
1100	4	++++	+	1208	4	+	−					1211	4	−	−
1631	4	+++	+	1625	4	+++	−								
1632	4	+++	−	1626	4	+++	−								
				1627	4	+++	−								
1110	6	++++	+	1230	6	+++	+	1224	5	++++	+	1231	6	−	−
1111	6	++++	+	1653	6	++++	+	1225	5	+++	+	1232	6	−	−
1112	6	++++	+	1654	6	++++	+	1226	5	++++	+	1233	6	−	−
651	8	++++R	+	1244	8	+++	+	1242	8	++++	+	1243	8	−	+
652	8	++R	+	1248	11	+++	+					1249	11	−	+
				1375	28	+++RC	+					1378	28	−	−
				1376	28	+RC	+					1379	28	−	−
				1377	28	±	−					1380	28	−	−

RC = regeneration and calcification; R = regeneration.

* Lymphocytic choriomeningitis.

To determine the effect of anti-GD-VII rabbit serum in protecting against myositic lesions, a series of mice was inoculated intramuscularly with the same amount of the test and control serum-virus mixtures and then sacrificed at

various intervals. A similar experiment was made with the anti-SK rabbit serum and normal serum from the same rabbit except that the tests were performed by inoculating various dilutions of virus with a constant amount of undiluted serum. The results are summarized in Tables VII and VIII.

TABLE VIII

Protection of Mice against SK Myositis with Anti-SK Rabbit Serum

Virus + normal rabbit serum					Virus + anti-SK rabbit serum				
Amount virus per inoculum*	Mouse No.	Time	Myositis	Obvious reactions	Amount virus per inoculum*	Mouse No.	Time	Myositis	Obvious reactions
		days					*days*		
10^{-4}	1731	3	+++	+	10^{-4}	1741	3	−	−
	1734	3	++	+		1742	3	−	−
	1736	3	+++	+		1743	3	−	−
	1737	3	+	+					
10^{-5}	1738	4	−	+	10^{-5}	1749	4	−	−
	1744	4	++++	+		1750	4	−	−
	1745	4	++++	+		1751	4	−	−
10^{-6}	1732	3	±	+	10^{-6}	1752	4	−	−
	1733	3	−	+		1753	4	−	−
	1735	3	++	+		1754	4	−	−
	1739	3	+++	+					
	1740	3	+	+					
10^{-7}	1746	3	−	+	10^{-7}	1769	6	−	−
	1764	5	−	+		1770	6	−	−
	1765	5	−	+		1771	6	−	−
	1766	6	−	−					
	1767	6	−	−					
	1768	6	−	−					
10^{-8}	1747	3	−	+	10^{-8}	1884	7	−	−
	1881	7	−	−		1885	7	−	−
	1882	7	−	−		1886	7	−	−
	1883	7	−	−					

* Dilution of infected brain suspension per 0.1 cc. inoculum.

Fourteen mice inoculated with the anti-GD-VII rabbit serum–GD-VII virus mixture and sacrificed for histological examination at intervals of 2 to 28 days showed no myositis or regenerative changes in the injected muscle. Furthermore, 2 mice in the control series which had developed obvious signs and which had characteristic lesions in the spinal cord were free from muscle lesions in the injected limb. The slight reactions observed in 2 days can readily be con-

sidered as non-specific. In contrast, mice injected with normal rabbit serum–GD-VII virus and anti-lymphocytic choriomeningitis serum–GD-VII virus mixtures demonstrated marked myositis and regenerative changes in 4 days and thereafter (Fig. 12, *a* and *b*). It is interesting to note (Table VII) that the moderate lesions generally observed to occur on the 2nd day after injection of virus suspensions in distilled water did not occur with virus treated with normal rabbit serum, either unheated or heated (60°C. for 20 minutes). In addition this normal rabbit serum in two different control series prolonged significantly the average incubation period when compared to the saline or normal and anti-LCM monkey serum controls.

The anti-SK rabbit serum also afforded protection against myositis as well as against the signs of nervous disease, while the normal rabbit serum failed to do so (Table VIII). Several mice, however, which had signs indicating involvement of the central nervous system, showed no local muscle lesions. As has been stated, this occurred also in 2 mice inoculated with anti-GD-VII serum–GD-VII virus mixture. In recent experiments, no cross-protection was obtained with anti-GDVII and anti-SK serums, either as regards morbidity or local myositic lesions.

DISCUSSION

While it will prove interesting to extend these observations to other viruses, it is already clear that certain "neurotropic" viruses have marked pathogenicity for skeletal muscle. Of the viruses tested, the most intense lesions were produced by Jungeblut's SK virus. Even though the taxonomic position of this virus is not clear, the early and widespread necrosis of skeletal muscle fibers appears to be a striking effect of this agent. Since it takes place as early as 6 hours after injection it must, with all probability, be ascribed to a direct effect of the virus upon the muscle fiber, rather than an indirect one through the mediation of the nervous elements. There are other reasons for attributing the muscle lesions to a direct action of the virus on the muscle fibers. Even the most severe damage to neurites or end-plates should not produce massive necrosis and fragmentation of muscle fibers, such as occurs within a short period after injection of SK virus. Normal appearing neurites and end-organs may be found in Cajal preparations to persist amongst the necrotic fibers. There is thus no histologic evidence of early damage to the nervous elements. When the sciatic nerve is severed and the virus injected 1 week later into the gastrocnemius, lesions can still be produced in the muscles with both the SK and GD-VII viruses. The virus has now been transmitted through seven muscle to muscle passages. If one were to assume that the virus were restricted to the nerve elements alone the dilution would be so great as to bring the dosage far below the known infectivity level.

The muscle lesions produced by the GD-VII and closely related FA strains

of mouse encephalomyelitis are slower to develop. Although these viruses likewise produce widespread necrosis of muscle fibers, the animals survive long enough for this to be accompanied by a profuse cellular inflammatory reaction. Moreover since the injury to the muscle fibers does not necessarily entail death of the muscle nuclei and their surrounding cytoplasm, regeneration of new fibers takes place *pari passu* with the destruction of the original fibers, and is a very conspicuous feature of the lesions after the 4th day. We have called attention to the regular occurrence of intranuclear inclusion bodies in the regenerating fibers. They have been found only in the lesions produced by the GD-VII and FA viruses, and not in the regenerating fibers which appear in the superficial portions of the muscles after injection of normal brain suspensions nor in the dystrophic muscle of suckling rats on vitamin E–deficient diet. We are therefore inclined to regard them as a specific feature of the lesions associated with the presence of these particular viruses.

Whereas the muscle lesions obtained with mouse encephalomyelitis virus strain 4727 were not nearly so intense as those resulting from the "encephalitic" GD-VII and FA strains, nevertheless the production of mild myositis by this "paralytic" type indicates that such tissue reactions are not solely produced by "encephalitic types" of mouse encephalomyelitis viruses. The ability to attack muscle tissue may be a function of the virulence for members of this group. If virulence can be estimated to some extent from the final concentration attained in central nervous system tissue, then there is some correlation for the strains studied between their respective degrees of infectivity and their ability to produce myositis. Thus the GD-VII and FA strains which produce a severe muscle reaction are active in the order of 10^{-6} dilution per 0.03 gm. of infected central nervous system tissue by the intracerebral route. Virus 4727 which is a relatively active strain for TO types, nevertheless was about a 100-fold less pathogenic by the intracerebral route and correspondingly produced less intense lesions in the muscle. Finally, the TO type FV strain which, in high concentration, did not even produce 100 per cent infection by the intracerebral route, failed to elicit significant lesions in muscle. Moreover from the data on local multiplication following intramuscular inoculation, it would appear that a certain threshold concentration of virus may be necessary before myositis is evident. With the GD-VII virus significant lesions were present at the time when the local virus concentration had attained levels of about 10^5 M.I.D.$_{50}$ intracerebral doses.

In spite of these relationships it is not intended to imply that because certain strains of mouse encephalomyelitis virus have the same titer by the intracerebral route they necessarily possess equal pathogenicity for other tissues. Although data are lacking for this group of viruses, there is evidence from the study of other viruses that variation in the ability of strains to attack diverse tissues cannot be ascribed solely to differences in concentrations. Moreover,

even extensive multiplication in tissues may not necessarily result in marked pathologic changes (18, 19). It should also be recalled that with both the GD-VII and FA viruses about 50,000 times more virus was necessary to produce a 50 per cent morbidity rate by the intramuscular than by the intracerebral route. Jungeblut's SK virus, on the other hand, proved almost equally virulent by the intramuscular as by the intracerebral route. It is therefore possible that all mouse encephalomyelitis viruses may not have the inherent quality to multiply locally following intramuscular injection as was the case with the GD-VII strain. But if other members of the group, whether they be of the "paralytic" or "encephalitic" type, are capable of such local multiplication the possibility exists that the occurrence of myositis may be directly related to a given concentration of virus necessary to interfere with the normal metabolism of muscle. Hence, until further studies are made including tests with additional strains, it may be unwise to consider the failure of certain TO types to produce myositis or the production of a delayed mild reaction by others, as a fundamental feature differentiating them from the "encephalitic" types.

Insofar as the other known characteristics which differentiate the "encephalitic" from the "paralytic" types of mouse encephalomyelitis virus are concerned they might also have to be considered with some reservation as representing fundamental differences. Differences in the manifestations of the disease are marked following intracerebral inoculation, and the "encephalitic" types appear to have a greater predilection for brain tissue. Furthermore these latter types are more virulent as evidenced by greater invasiveness and higher infectivity and mortality rates. But such differences should not be overemphasized. The disease produced by intramuscular inoculation of "encephalitic" strains is cardinally a flaccid paralysis with typical lesions in the spinal cord. Also with intraperitoneal inoculation of the FA virus flaccid paralysis is usually produced (8) and we have obtained similar results by subcutaneous and intraperitoneal inoculations with the GD-VII virus. The GD-VII virus, when first isolated and studied, produced symptoms referable to lesions in the cord more predominantly than to lesions in the brain (8). Bearing on this are Melnick and Riordan's recent studies (20) on the occurrence of latent encephalomyelitis in which they show that the central nervous system tissue from five primary cases of flaccid paralysis produced encephalitis on intracerebral passage. The shorter incubation period following intracerebral inoculation for the "encephalitic" types may be a more fundamental difference. But even this criterion is difficult to define since the incubation period is lengthened with lower concentrations of virus for both the "encephalitic" and "paralytic" types.

On the other hand the "encephalitic" and "paralytic" types of mouse encephalomyelitis appear to have the same size, stability, and other physical and chemical properties (8, 21). These characteristics may be more indicative of

their fundamental relationships. Thus we are inclined to accept the "encephalitic" types as more highly pathogenic strains (22) or cerebral forms (21) of mouse encephalomyelitis virus.

Following intramuscular injection in the leg the mouse encephalomyelitis viruses GD-VII, FA, Jungeblut's SK virus, and occasionally mouse encephalomyelitis viruses 4727 and FV, and the unidentified JHM virus sooner or later invade the central nervous system. Sabin and Olitsky (1) have suggested that infection with the vesicular stomatitis virus takes place by way of the motor nerves, since the lesions after intramuscular injection are usually most intense in the gray matter of the lumbar region of the cord. The virus of Eastern equine encephalitis produces no myelitis and the lesions are restricted to medulla, white matter of cerebellum, and basal ganglia. There is therefore a possibility that this virus reaches the central nervous system through the circulation rather than *via* the peripheral nerves. With the SK virus, we have found that small inocula into the gastrocnemius muscle may be followed by lesions of brain and cord, even when the sciatic nerve has been previously resected.

SUMMARY

A study has been made of the local effects following intramuscular injection of various neurotropic viruses.

Early massive necrosis of muscle fibers accompanied by edema and acute inflammatory reaction is produced by Jungeblut's SK virus even in low concentrations.

Similar but more slowly developing lesions follow the introduction of mouse encephalomyelitis GD-VII and FA strains. Strain 4727 (TO type) produces inflammatory changes with fibrosis in the intermuscular septa and necrosis of scattered individual fibers. The relatively avirulent FV strain (TO type) was not pathogenic for skeletal muscle.

The Mitchell strain of lymphocytic choriomeningitis virus gives rise to a profuse lymphocytic and monocytic infiltration of the fat and connective tissue but does not cause necrosis of muscle fibers.

No significant lesions resulted from intramuscular injection of the murine-adapted human poliomyelitis Lansing virus, the HF strain of herpes, a strain of Eastern equine encephalitis virus, or a still unidentified demyelinating mouse virus.

Evidence is presented that the mouse encephalomyelitis virus GD-VII and Jungeblut's SK virus multiply locally in the injected limb. The GD-VII virus has been passed through four muscle to muscle passages and muscle lesions have been elicited at the same time.

Specific and complete protection against myositis was obtained by anti-GD-VII and anti-SK rabbit sera.

The authors are grateful to Dr. John F. Enders for the GD-VII strain of mouse encephalomyelitis virus and the Lansing strain of human poliomyelitis virus, to Dr. Max Theiler for the FA and FV strains of mouse encephalomyelitis virus, to Dr. Gilbert Dalldorf for the 4727 strain, to Dr. C. W. Jungeblut for the SK virus, and to Dr. W. McD. Hammon for the Eastern equine encephalitis virus. We also wish to express our appreciation for the technical assistance of Miss Mary Brown, Miss I. K. Michelson, and Miss Sheila Richardson.

BIBLIOGRAPHY

1. Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1938, **67,** 201.
2. Peck, J. L., Jr., and Sabin, A. B., *J. Exp. Med.*, 1947, **85,** 647.
3. Dalldorf, G., and Sickles, G. M., *Science*, 1948, **108,** 61.
4. Kearney, E. B., Pond, W. L., Plass, B. A., Maddy, K. H., Elvehjem, C. A., and Clark, P. F., *J. Bact.*, 1948, **55,** 89.
5. Theiler, M., *J. Exp. Med.*, 1937, **65,** 705.
6. Cheever, F. S., and Mueller, J. H., *J. Exp. Med.*, 1947, **85,** 405.
7. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27,** 493.
8. Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72,** 79.
9. Pappenheimer, A. M., *Am. J. Path.*, 1939, **15,** 179.
10. Pappenheimer, A. M., *Am. J. Path.*, 1942, **18,** 169.
11. Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54,** 145.
12. Jungeblut, C. W., Sanders, M., and Feiner, R. R., *J. Exp. Med.*, 1942, **75,** 611.
13. Wolf, A., *J. Exp. Med.*, 1942, **76,** 53.
14. van Rooyen, C. E., and Rhodes, A. J., Virus Diseases of Man, London, Oxford University Press, 1940.
15. Pappenheimer, A. M., On Certain Aspects of Vitamin E Deficiency, Springfield, Illinois, Charles C. Thomas Company, 1948.
16. Paul, J. R., The filtrable viruses, in Laboratory Methods of the United States Army (J. S. Simmons and C. J. Gentzkow, editors), Philadelphia, Lea and Febiger, 5th edition, 1944.
17. Lennette, E. H., and Koprowski, H., *J. Immunol.*, 1944, **49,** 375.
18. Feller, A. E., Enders, J. F., and Weller, T. H., *J. Exp. Med.*, 1940, **72,** 367.
19. Enders, J. F., Mumps. Diagnostic Procedures of Virus and Rickettsial Diseases, New York, American Public Health Association, 1948.
20. Melnick, J. L., and Riordan, J. T., *J. Immunol.*, 1947, **57,** 331.
21. Gard, S., *Acta med. Scand.*, 1943, suppl. 143.
22. Theiler, M., *Medicine*, 1941, **40,** 443.

EXPLANATION OF PLATES

All of the sections were stained with hematoxylin and eosin except that of Fig. 9.

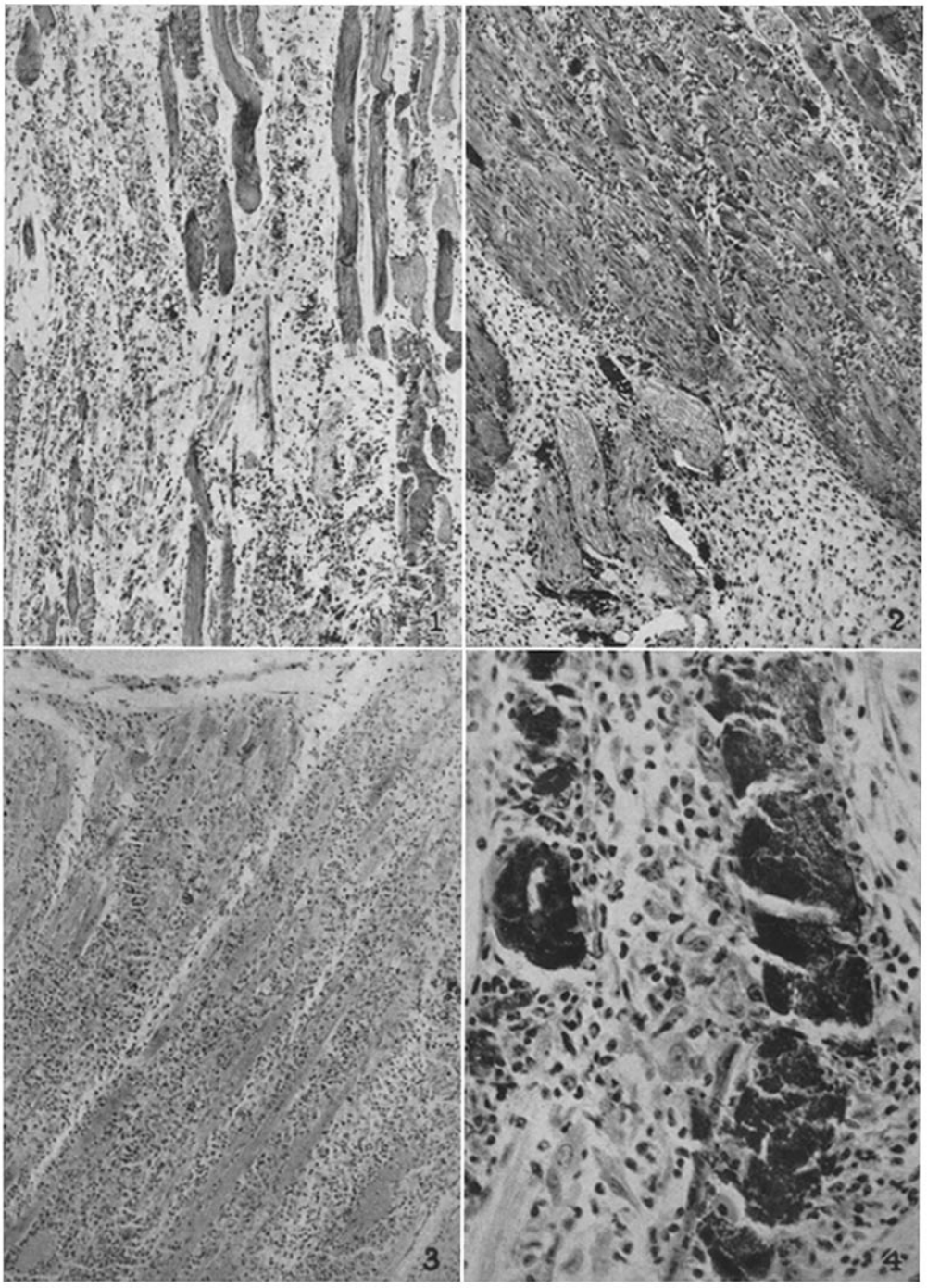
PLATE 5

FIG. 1. Virus GD-VII. Mouse 1100. Acute myositis following intramuscular injection of 0.2 cc. infected brain suspension into leg. Necrosis of muscle fibers. Intense interstitial inflammatory reaction. 4 days after intramuscular injection. × 125.

FIG. 2. Virus GD-VII. Mouse 655. Later lesion. Mononuclear cell infiltration of intramuscular connective tissue. Several intact nerve branches are shown. There is regeneration of new muscle fibers. 6 days after intramuscular injection. × 125.

FIG. 3. Virus GD-VII. Mouse 642. Myositis accompanied by extensive regeneration of muscle fibers. 7 days after intramuscular injection. × 75.

FIG. 4. Virus GD-VII. Mouse 1112. Calcification of necrotic muscle fibers. 7 days after intramuscular injection. × 450.



(Rustigian and Pappenheimer: Myositis in mice)

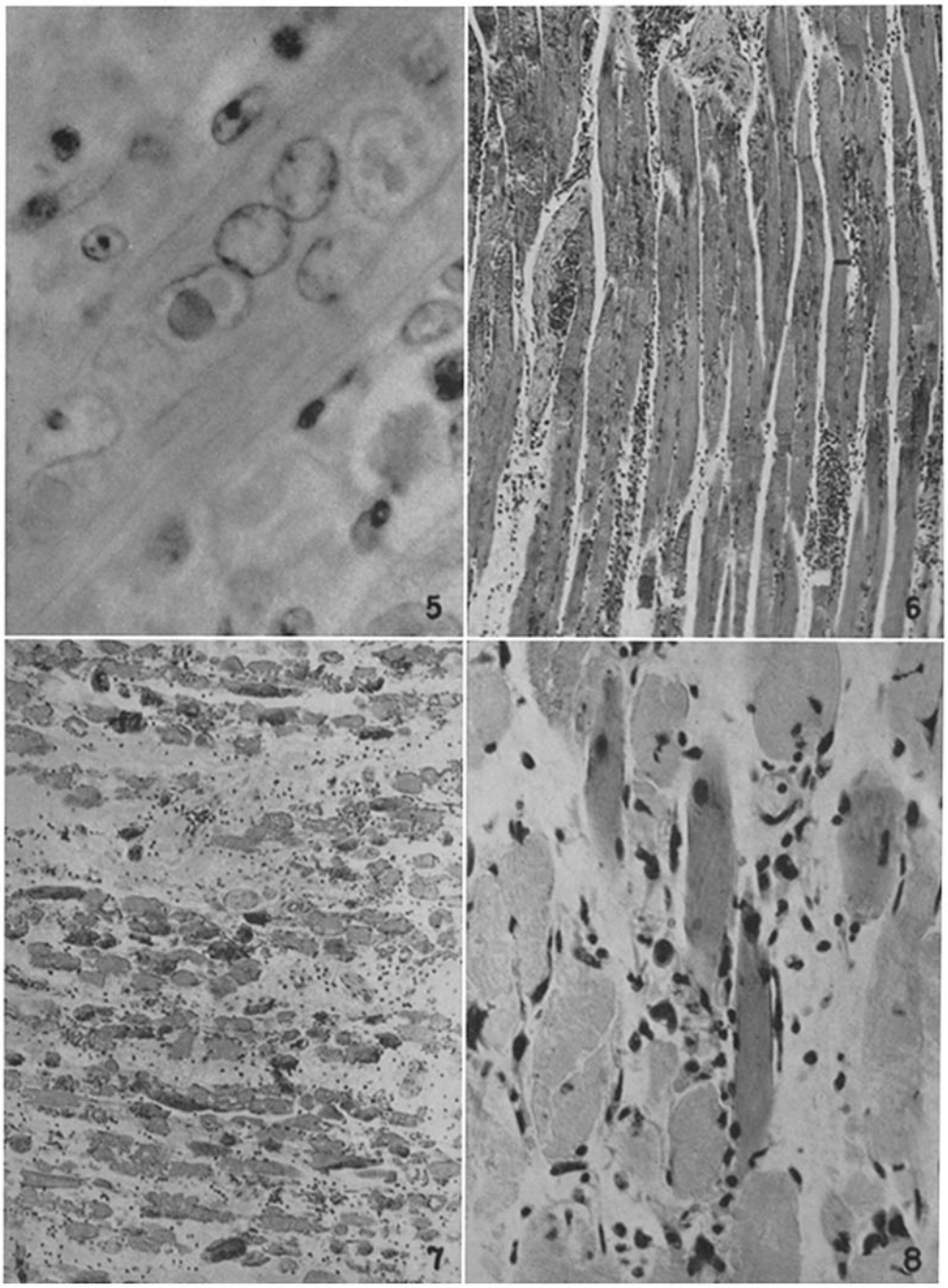
PLATE 6

FIG. 5. Virus GD-VII. Mouse 655. Intranuclear eosinophilic inclusion in regenerating muscle fibers. × 1200.

FIG. 6. Virus 4727. Mouse 1466. Necrosis with leucocytic invasion of discrete muscle fibers. 6 days after intramuscular injection. × 125.

FIG. 7. Virus SK. Mouse 1078. Massive segmental necrosis of muscle fibers with interstitial edema and early inflammatory reaction. 12 hours after injection. × 125.

FIG. 8. Virus SK. Mouse 1088. Hyaline necrosis of fibers, with early inflammatory reaction. 48 hours after intramuscular injection. × 450.



(Rustigian and Pappenheimer: Myositis in mice)

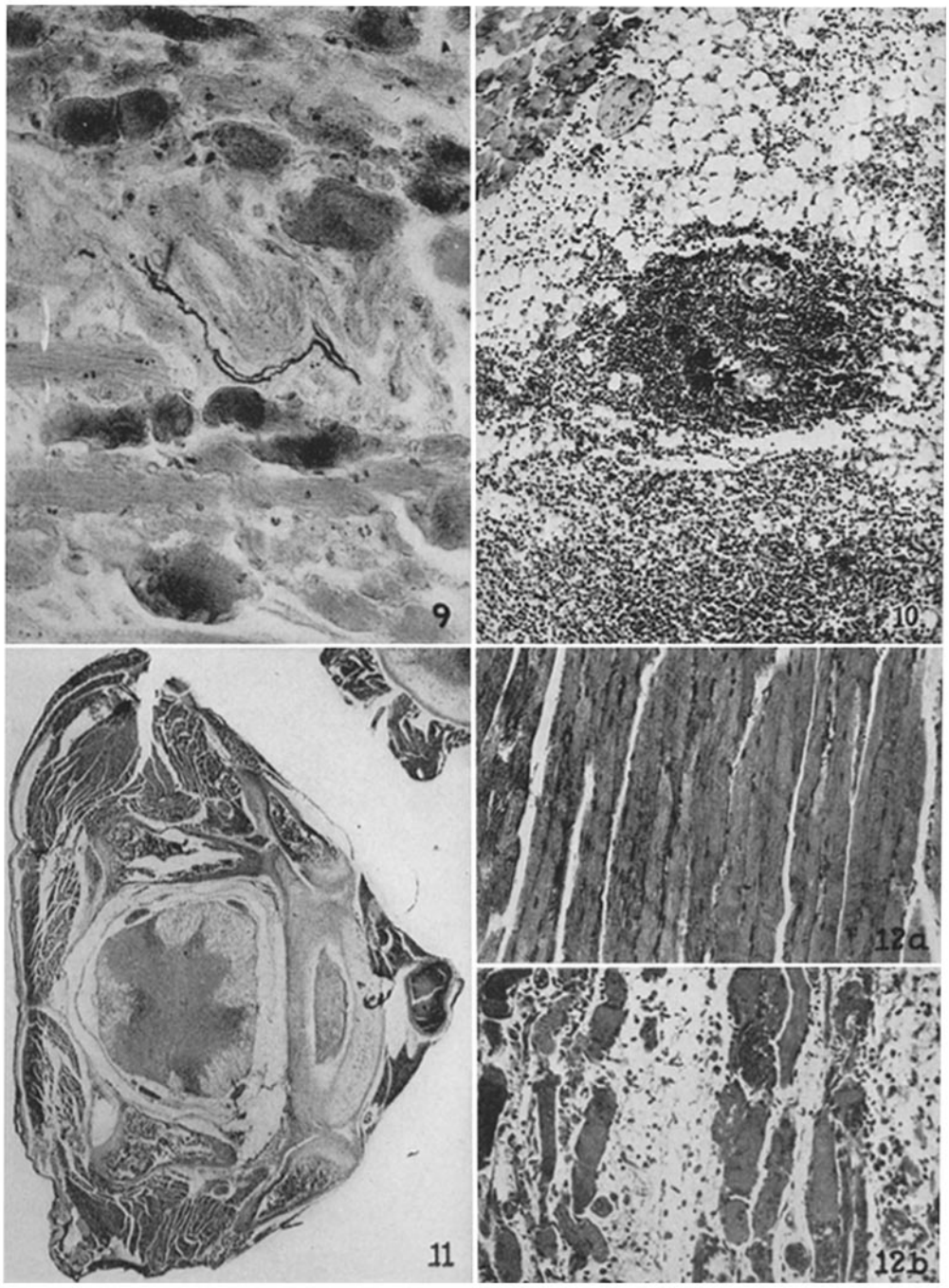
PLATE 7

FIG. 9. Virus SK. Mouse 1089. Preservation of terminal neurites between degenerated muscle fibers. Cajal stain. × 450.

FIG. 10. Lymphocytic choriomeningitis virus. Mouse 1124. Intense mononuclear and lymphocytic infiltration of intermuscular fat and connective tissue. Muscle fibers (shown in upper right hand corner) are not necrotic. 8 days after intramuscular injection. × 125.

FIG. 11. Virus JHM. Mouse 960. Characteristic demylinating lesions in lumbar cord. 7 days following intramuscular injection. × 16.

FIG. 12. (*a*) Anti-GD-VII rabbit serum + GD-VII virus. Mouse 1233. Muscle 6 days after injection. (*b*) Normal rabbit serum + GD-VII virus. Mouse 1650. Muscle 6 days after injection. × 125.



(Rustigian and Pappenheimer: Myositis in mice)