NEUROPATHOGENICITY OF GROUP A COXSACKIE VIRUSES

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PLATES 5 AND 6

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The two characteristics of the Group A Coxackie viruses that are most important in isolation and identification are the susceptibility of immature mice and hamsters and the generalized destruction of the striated muscles following infection (1). These have been invariable features of all the strains we have studied but in recent years it has become evident that they are not the whole picture. The present report provides evidence that Group A strains may cause poliomyelitis in adult mice and monkeys as well as myositis in newborn mice.

It was early noted (2) that while 10 to 12 gm. mice inoculated with brain suspension of Coxsackie virus, Group A, Type 1 exhibit no recognizable signs of infection those inoculated with muscle suspension are sometimes paralyzed. Attempts at that time to adapt the virus to 10 to 12 gm. mice by alternate passage through suckling mice or serially failed.

For a period of years no paralysis was seen in routine tests in young adult mice inoculated intracerebrally with many strains of Coxsackie virus Group A including all types. A number of these were brain suspensions used with the dual purpose of identifying the Coxsackie virus and excluding other mouse pathogens, and many others were muscle-bone suspensions.

Recently, within a comparatively short period, paralysis was noted in 10 to 12 gm. mice inoculated with muscle-bone suspensions of strains of Group A of three different types: A Type 1 stock strain received from Europe with a request to check its type; a strain from another laboratory which had the histopathological characters of Coxsackie Group A but was not neutralized by sera of A 1-19. The third strain was the representative strain of Group A, Type 14.

The paralysis resembled that induced by the Lansing strain of poliovirus or mouse encephalomyelitis. It was not increased by intracerebral passage from paralyzed mice. The infection induced in suckling mice by brain, or brain and cord, of paralyzed adult mice was neutralized by the homologous immune serum. For this reason and because similar lesions, but no overt paralysis, had at times been associated with Group A infections in young guinea pigs, it was suspected to be due to the Coxsackie virus infection. An effort was therefore made to investigate this property. High titered suspensions of infected, suckling mouse leg tissue, representing each of the three types, were prepared and inoculated into large groups of mice by various routes.

The A-14 strain induced paralysis in a number of animals in the first trials just described and the paralysis-producing property has since been successfully transferred through subsequent generations. The other two types of virus have not caused paralysis and no lesions were detected in mice sacrificed at various times for histologic examination.

Materials and Methods

The mice were from the Albany colony. The monkeys (*Macaca cynomolgus*) were young adults.

The methods used in the preparation of the A-14 antiserum, the mouse neutralization test, and the preparations of virus seed have recently been described in detail (3). The intraspinal inoculations followed the technic of Habel and Li (4).

The poliomyelitis antisera were supplied by The National Foundation for Infantile Paralysis, Inc. Type 1 (Brunhilde) was Lot 3-1, Type 2 (Lansing) was Lot 4-5, and Type 3 (Leon) was Lot 1A-2.

The GD VII hamster and the MM monkey antisera were from animals hyperimmunized by means of repeated injections of infected mouse tissues. Both are of high potency and the GD VII antiserum effectively neutralizes all of the encephalomyelitis viruses we have studied (5).

The A-14 monkey serum was prepared from blood drawn 16 days after inoculation with infected suckling mouse leg suspension. This serum has been tested for complement-fixing antibodies using EEE, WEE, St. Louis encephalitis, herpes simplex, mumps, and LCM antigens. In each case the titer was less than 4. The serum had a titer of 32 when tested with measles antigen.

EXPERIMENTAL

First Adult Mouse Generation.—

Twelve 8-day-old mice were inoculated subcutaneously in the region of the spine, using 0.05 ml. of a 10 per cent suspension of infected suckling mouse leg as seed. All these animals were paralyzed 3 days later and showed extensive hyalin degeneration of their skeletal muscles.

Ten 4-week-old mice were inoculated intramuscularly in the left thigh, using 0.03 ml. of the same suspension. Nine of the 10 became paralyzed between the 3rd and 9th day. The 10th died on the 4th day. Five were examined histologically and each was found to have an extensive poliomyelitis of the spinal cord and brain stem. Focal myositis was found at the site of the inoculation in 4 of the 5. Three mice were killed and their spinal cords and medullae harvested and prepared as an inoculum for newborn mice. The suspension was found to contain approximately 3000 ID_{50} per ml.

Thirty-eight 4-week-old mice were inoculated intraspinally. Five were immediately paralyzed by the inoculation. Of the remaining 33, 15 became paralyzed between the 2nd and 8th day. A suspension of the brains of 3 paralyzed on the 3rd day was tested in newborn animals and found to contain more than 30,000 ID_{50} per ml. Three of the intraspinally inoculated mice were examined histologically and each was found to have extensive lesions of the cord and, in two, minute muscle lesions as well.

Second Adult Mouse Generation.—

Two 10 per cent suspensions prepared from the intraspinally inoculated mice were used for passage. Thirty 4-week-old mice were inoculated intraspinally with 0.02 ml. of the first suspension. Of these, 4 were traumatized and discarded. Two became paralyzed, one on the 7th and the other on the 11th day.

Ten 4-week-old mice were inoculated intracerebrally (0.03 ml.) and of these 6 were paralyzed between the 4th and 16th day. In each of these extensive myelitis was identified microscopically.

Ten mice injected intramuscularly remained well.

Sixteen 4-week-old mice inoculated intraspinally with the second suspension consisting of pooled cerebella, medullae, and spinal cords of the 3 mice of the first generation that were paralyzed on the 7th day responded rather similarly. Four of these developed flaccid paralyses. Their brains and spinal cords were selected for passage.

Third Adult Mouse Generation.—

Groups of 4-week-old mice were inoculated by various routes, intraspinal, intracerebral, intramuscular, and both intraspinal and intramuscular with 10 per cent suspension. The latter group showed paralyses in 4 of the 8 animals on the 8th day and the cords and brains of these animals were taken for passage. Half of the mice inoculated intracerebrally also became paralyzed but only after longer intervals. These mice all showed extensive lesions of their cords and no anatomic evidence of disease in their muscles. Paralysis was late and infrequent in the animals inoculated intramuscularly or intraspinally.

Six 10-day-old mice that received the same suspension by the intracerebral route were prostrate on the 4th day. Each was found to have extensive lesions of the striated muscles and minute foci of inflammation in the grey matter of the spinal cords.

A single litter of 4-day-old mice was also inoculated. In this case the injection was into the peritoneal cavity. All succumbed to muscular paralysis on the 5th day.

Fourth Adult Mouse Generation.—

Six of 14 4-week-old mice inoculated intraspinally and intramuscularly with a 10 per cent suspension of brains and cords of the third generation were paralyzed on the 7th day and their brains and spinal cords were harvested. A group inoculated only by the spinal route remained well.

All of 9 4-week-old mice inoculated intracerebrally were paralyzed between the 6th and 15th day. These animals were held for observation. Two died. The others showed a considerable recovery of function. Seven weeks after infection, paralysis could be recognized in only one although others were still somewhat awkward in their movements. They had also largely regained their normal size. On the 16th day of the experiment these mice weighed, on the average, 25 per cent less than their unparalyzed controls.

The suspension used in this generation was titrated in 5-day-old mice and found to contain approximately 40,000 ID_{50} per ml.

Fifth Adult Mouse Generation.—

Suspensions harvested from the mice injected both intraspinally and intramuscularly were tested in several dilutions in mice of different ages. In this comparative testing the intracerebral route was used since intraspinal inoculation proved impractical in the infants. The responses of these animals are shown in Table I.

The suspension was also tested in newborn mice with standard typing sera to determine the identity of the virus. The results of this test appear in Table II.

Finally, the suspension was tested in 4-week-old mice using antisera prepared with the three types of poliovirus, the GD VII strain of mouse encephalomyelitis virus, the MM strain of encephalomyocarditis virus, the serum of a monkey bled 16 days after infection with the A-14 strain and the hyperimmune hamster antiserum. The outcome of this experiment is shown in Table III. Only the A-14 hamster antiserum afforded striking protection although the monkey convalescent serum also exhibited some neutralization.

It seems quite clear that the virus responsible both for the myositis in the suckling mice and the poliomyelitis in adults was serologically similar and was, in both cases, neutralized by A-14 antisera and not by antisera for those viruses known to cause similar lesions in mice.

It was also evident that the virus, in its 5th passage in adult mice, had retained a high virulence for newborn animals (in which the response was limited

TABLE I

Pathogenicity of the Fifth Adult Mouse Generation

Ages of mice	Dilution of CNS	No.	Response of mice	
			Frequency of paralysis	Incubation
			per cent	*days*
1 day	10^{-1}	15	100	3.6
	10^{-3}	14	100	4.6
	10^{-5}	14	36	5.2
10 to 14 days	10^{-1}	16	56	7.4
	10^{-3}	16	56	9.1
	10^{-5}	16	12	10.0
Weanling	10^{-1}	14	50	7.4
	10^{-3}	16	25	9.0
	10^{-5}	15	6	11.0
4 wks.	10^{-1}	16	69	9.0
	10^{-3}	16	36	9.6
	10^{-5}	15	0	—
3 mos.	10^{-1}	16	75	10.0
	10^{-3}	16	6	11.0
	10^{-5}	16	0	—

The results are the composite of duplicate tests, using in one case spinal cords and in the other mouse brains. Groups of 8 mice were inoculated intracerebrally (0.03 ml.). The animals that died within 24 hours have been excluded.

to the skeletal muscles), and a considerable, though lesser virulence for older mice (in which the response was a flaccid paralysis associated with poliomyelitis). The infectivity titer of the suspension diminished with the age of the test animals, although the frequency of paralysis in mice receiving the 10 per cent suspension was somewhat greater in the 4- and 12-week-old mice than in those 10 to 21 days of age. The interval between inoculation and paralysis increased with the age of the animals.

The results summarized in Table I represent equal groups inoculated sepa-

rately with brain and spinal cord suspensions. They have been combined in the table because there was no significant difference in the outcome in the two series. The titers were similar. The incubation period in those that received the spinal cord suspension was somewhat briefer but the difference was less than 1 day.

Histologic examination was undertaken of representative mice including control groups in which paralysis was not noted. The lesions were identical with those seen earlier. This was true of the 3-month-old animals as well as the young adults. Animals protected by A-14 antisera were free of lesions. Repre-

TABLE II

Neutralization Test in Suckling Mice

Adult mouse passaged virus

Serum		Coxsackie virus A-14 No. 52113					
Hamster	Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Normal hamster	Undiluted		8/8	8/8	0/8	2/8	1/8
Group A Pool 1	*	8/8					
" " " 2		8/8					
" " " 3		8/8					
" " " 4		0/8					
A-13 No. 5359	Undiluted	8/8					
A-16 No. 52109	Undiluted	8/8					
A-12 No. 51204	1:5	8/8					
A-18 No. 52112	1:5	8/8					
A-19 No. 53157	1:5	8/8					
A-14 No. 52113	1:40	0/8					
A-14 No. 52113	1:80	0/8					
A-14 No. 52113	1:160	0/8					

Virus: brain and cord suspension, generation 6, adult mice. Test animal: suckling mice, 2 days old. Dose: 0.05 ml., intraperitoneally.

* Group A serum pools 1 to 4 include the sera for the various A types in final dilution of 1:4 or 1:5. Group A pool 4 contains serum of A-12, -14, -18, -19.

sentative lesions are shown in Figs. 1 *a* and 1 *b*. The poliomyelitis was usually diffuse although focal changes were frequently found that could be correlated with the site of the paralysis. The changes consisted of perivascular and interstitial accumulations of lymphocytes and glial cells and degeneration and necrosis of neurones in the anterior and lateral horns. The process commonly extended into the medulla and pons where it was distributed irregularly. The cerebral hemispheres were but slightly affected and the cerebral cortex was not altered. The cerebral lesions amounted to small, scattered foci of perivascular infiltration with inconspicuous interstitial reactions. The cerebella were not notably altered.

Pathogenicity for cynomolgus Monkeys.—

The starting material of the series just described (10 per cent suspension of infected suckling mouse leg tissue) was inoculated into two young *cynomolgus* monkeys. Inoculation was intracerebral (0.2 ml.) and intramuscular (0.1 ml.). The animals were observed for 16 days during which no certain signs of illness were noted. They were then bled and their organs sampled for histologic examination. Scattered areas of focal poliomyelitis were found in the spinal cords of both animals. The lesions were essentially the same as those in the mice. They were limited to the grey matter of the cord. Ten per cent suspensions of minced and pooled samples of the spinal cords of these monkeys were tested in newborn mice and found to be non-infectious.

Groups of three adult *cynomolgus* monkeys were inoculated with the starting material representing the other two Group A strains that had caused paralysis in an occasional mouse

TABLE III

Neutralization Test in Adult Mice

Adult mouse passaged virus

Serum	Response of mice
Normal monkey	5/7
Encephalomyocarditis monkey	7/8
Type 1 poliomyelitis monkey	7/7
Type 2 " "	6/6
Type 3 " "	5/8
GD VII hamster	6/8
A-14 monkey conv.	4/8*
A-14 hamster 1:5	0/8
A-14 hamster 1:50	0/8

Numerator indicates number of mice paralyzed. First day deaths have been excluded. The virus suspension (10 per cent mouse brain) was prepared from mice of the 4th adult mouse passage. The poliomyelitis monkey sera were diluted 1:10, the dilutions of the A-14 hamster serum are shown in the table. The other sera were not diluted.

* Three after prolonged incubation.

but which failed to do so in the present experiments. The monkeys showed no signs of disease. When they were sacrificed, 2 weeks following inoculation, no histologic evidence of infection was found. Their central nervous systems were intact.

The 5th adult mouse passage of A-14 was injected intracerebrally (0.2 ml.) and intramuscularly (0.1 ml.) into 3 additional monkeys. The 10 per cent suspension of adult mouse brain served as the inoculum. One of the animals was febrile on the 3rd and 4th day, one on the 4th day, and the third on the 5th. Paralysis was not noted. All were sacrificed on the 6th day. Suspensions of pooled, minced samples of their spinal cords were tested in newborn mice. Ten per cent suspensions were in each case infectious and in one of the three, typical deaths followed injections of a 10^{-3} dilution as well. In each of these monkeys frank poliomyelitis was found on histologic examination. Representative lesions are shown in Figs. 2 *a* and 2 *b*.

DISCUSSION

The most interesting aspect of the experiments to us has been the disclosure that a Group A virus has the property of causing poliomyelitis in mice and

monkeys. It has, of course, been known for a long time that these viruses apparently proliferate in the central nervous system. Infected mouse brain can be used as virus seed although the muscles of the newborn are more infectious (2). In the absence of direct observations of the morbid process in man the disease in animals provides our only present clues to the pathogenesis of Group A infections. It is probable that several at least of these viruses do invade the central nervous system of man as well as mice for several types have been isolated from the cerebrospinal fluid of patients with aseptic meningitis (6). The lesions in the monkeys infected with the A-14 virus may be a clue to what occurs in man under such circumstances.

Other viruses than the established types of poliovirus are known to cause poliomyelitis in mice and monkeys. Mouse encephalomyelitis is a classical example and it is sometimes called mouse poliomyelitis. Encephalomyocarditis virus causes poliomyelitis in both mice and monkeys (7) but while the histologic changes are similar the natural history of the disease is evidently rather different and the virus is apparently not enteric nor as widely distributed as are the Coxsackie and polioviruses. Thus the lesions of poliomyelitis, as presently defined, are not specific of an infection by a particular agent and it is only by careful analysis of the distribution of the lesions that more or less characteristic patterns can be determined. Since these may owe a good deal to the host, to the virulence of the strains, and to the extent of the adaptation as well as the route of inoculation, histologic differentiations require comprehensive comparisons.

The anatomic material we have examined leads us to suspect that the lesions caused by the A-14 virus in mice may be separated from those of the other forms of poliomyelitis with which we are familiar but a much more extensive experience would be needed to justify a complete characterization.

In view of the occasional paralysis associated with similar lesions that we have noted in mice infected with the two other Group A viruses and the occurrence of similar lesions in monkeys (but not thus far in adult mice!) infected with three strains of A-7 Coxsackie virus (8), it can be concluded that the ability to cause poliomyelitis is shared by several Coxsackie viruses.

It will be noted that the histologic observations supplement the serologic tests in indicating that the pathogenic properties, the ability to destroy striated muscle and nervous tissue, are antigenically identical and that the age of the animals determines the outcome. Thus, while muscle lesions alone were found in the newborn mice and only poliomyelitis in the mice 1 month old or older, the 10- to 14-day mice exhibited both lesions to some degree and an occasional focus of myositis was seen in weanling animals. The susceptibility of the neuraxis may well be present in the immature as well as the adult mice since the former do not survive long enough to allow such lesions to become manifest. It is noteworthy that the striated muscle of young (15 day) mice under-

goes hyalin necrosis following direct exposure to vesicular stomatitis virus while the muscle of older mice remains intact (10).

The experiments resemble somewhat earlier ones in which a Group B virus was adapted to adult mice (9), in that the anatomic expression of the infection in the mature mice was in both cases limited to a single lesion and the disease in the adults was less severe than in the young. In both instances the influence of the age of the animals was modified but not abolished by the adaptation. To some extent the Coxsackie characteristics survived the adaptation.

SUMMARY

Coxsackie virus, Group A, Type 14, has been adapted to adult mice and monkeys and induces in them poliomyelitis-like lesions and, in the case of the mice, flaccid paralysis.

Dr. Frances E. Barnhart and Miss Marie Mutterer were of great help to me in these experiments.

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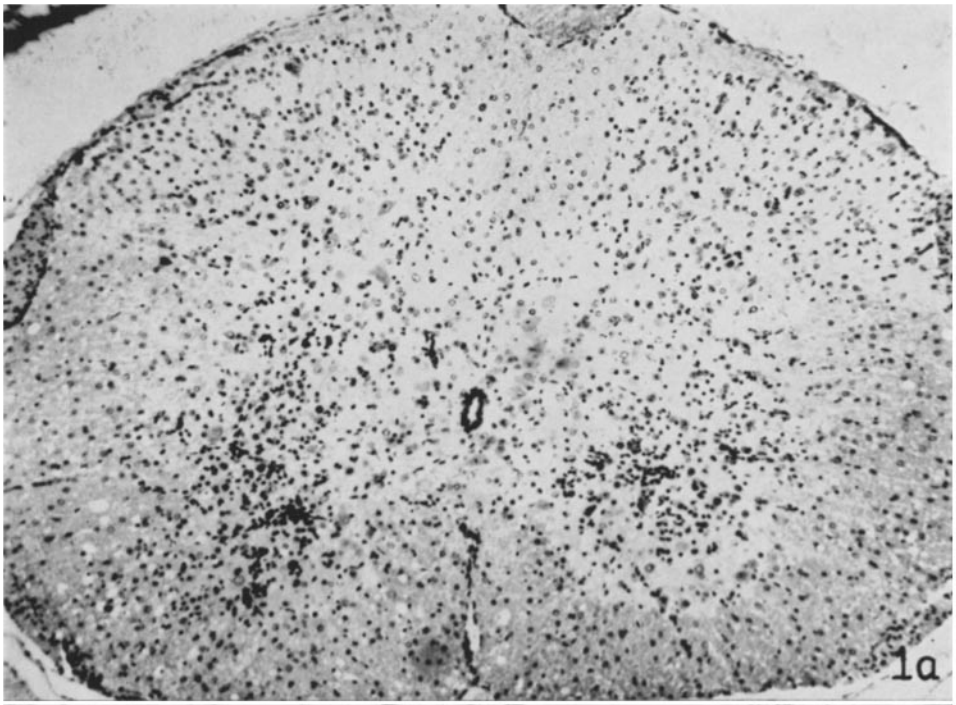
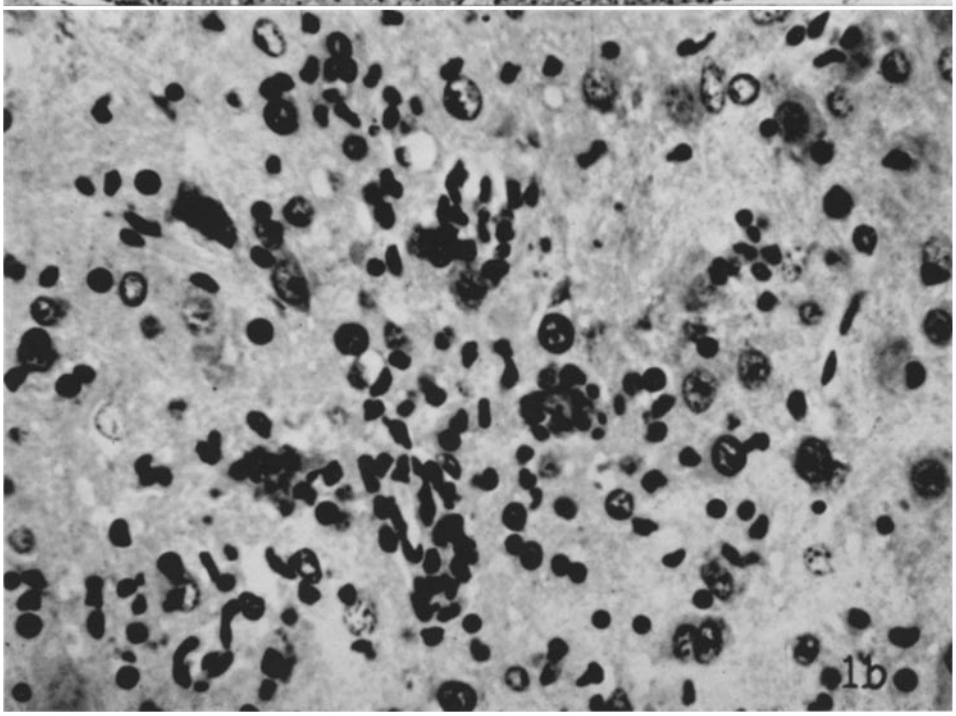
EXPLANATION OF PLATES

PLATE 5

FIGS. 1 *a* and 1 *b*. Spinal cord (lower thoracic) of a 10 to 14 gm. mouse inoculated intracerebrally with the fifth adult mouse passage of A-14. The mouse was paralyzed 5 days later. The animal was sacrificed 7 days after paralysis was first seen. Hematoxylin-eosin.

Fig. 1 *a*. Conspicuous lesions of both anterior horns. × 90. Fig. 1 *b*. Nature of the cellular infiltration, here largely pericapillary. One pycnotic neurone can be seen in the upper left. × 420.





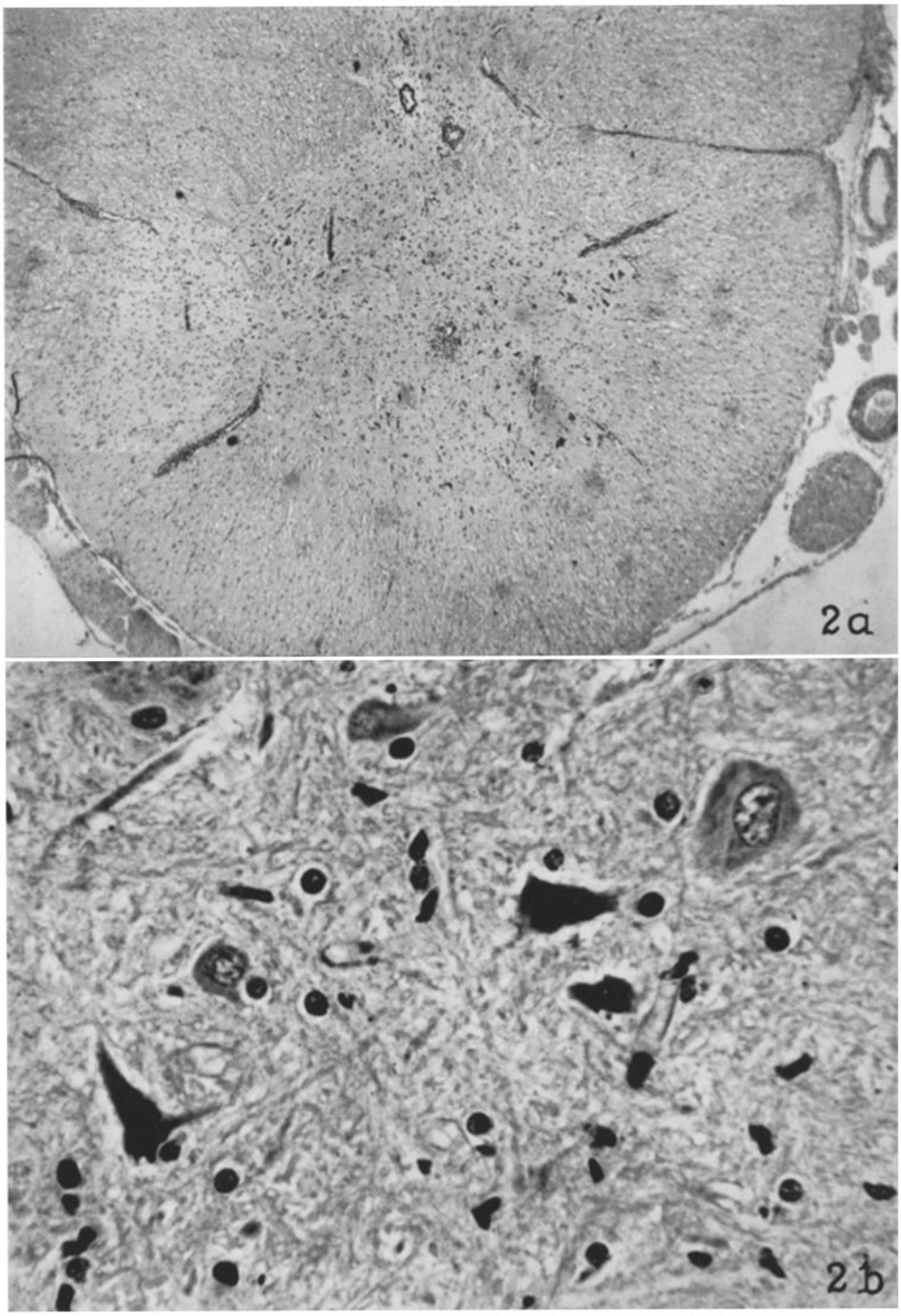
(Dalldorf: Neuropathogenicity of Group A Coxsackie viruses)

PLATE 6

FIGS. 2 *a* and 2 *b*. Lumbar spinal cord of a monkey (No. 3-61) inoculated intracerebrally and intramuscularly with the A-14 Coxsackie virus and sacrificed 16 days later. Giemsa stain.

Fig. 2 *a*. Distribution of the myelitis. × 25.

Fig. 2 *b*. Several motor neurones in various stages of decay. × 420.



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