OBSERVATIONS ON ENCEPHALOMYELITIS OF MICE (DA STRAIN)*

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Spontaneous encephalomyelitis of mice has been well and often described (1–5). There would be little point in adding another paper on the subject were it not that a strain isolated in this laboratory has certain characteristic effects which appear to have been overlooked in previous reports.

In brief these are the features which, we believe, merit emphasis and justify publication: (*a*) the occurrence of viremia prior to the onset of neurologic signs; (*b*) the occurrence in the lesions of peculiar spheroid bodies, to be described below; (*c*) the production of a chronic form of the disease, attended by marked myelin destruction in the spinal cord; (*d*) the production of skeletal myositis in sucklings, identical with that characterizing many strains of Coxsackie virus.

Material and Methods

The DA strain was recovered in 1948 from a mouse in the Harvard colony which had shown spontaneous paralysis over a period of 2 months. In other respects, it seemed in good health.

From brain suspensions of this animal, 8 passages were made by intracerebral inoculation of 3 week old mice. Following inoculation, there ensued paralysis of the extremities after incubation periods of 11 to 28 days. One or more mice in each passage remained free from paralysis or other signs of disease.

Further study was interrupted for a period of 19 months, during which time the virus was stored at −70°C. It was then passed rapidly by intracerebral inoculation through successive litters of suckling mice, the animals being killed for passage on the 7th day after injection, before the advent of neurologic signs. This procedure was suggested by the observations of Bodian and Cumberland (6) who had shown that virus is present in highest concentration in the cords in monkeys infected with poliomyelitis prior to the onset of paralysis. Brain, or brain and cord were harvested in the first passages. Beginning with the 5th infant passage, cords were invariably included in the material used for inoculation. The diluent used was 0.01 molar phosphate buffer. The weighed tissue was ground with alundum, and sufficient diluent added to make a 10 or 20 per cent suspension. After centrifugation at low speed for 10 minutes, the supernatant was glass-sealed and stored at −70°. Antibiotics

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were not used, but cultures were made from all passage material. Only bacteriologically sterile preparations were used.

For neutralization tests, one part of 10 per cent bovine albumen to one part brain suspension and one part serum dilution, was found to protect the virus for the 2 hour room temperature incubation period.

Blood for the study of viremia was collected by cutting the brachial artery of the lightly anesthetized mouse, taking up the blood in a capillary pipette, and promptly mixing with heparin.

When more than one litter of mice was to be used for inoculation, the infants were pooled and redistributed at random in order to make the groups more homogeneous.

Our study of the pathology is based upon the examination of some 140 mice of various ages infected by various routes and killed at various intervals after inoculation. Preparations from mice infected with other strains of Theiler's encephalomyelitis virus (GD-VII, TO, 4727) were available for comparison.

Bouin's fluid was used as routine fixative. In addition to hematoxylin-eosin, special stains (Giemsa, Loyez, phosphotungstic acid-hematoxylin, Bodian) were applied when indicated. Three coronal sections of brain and at least 4 transections of cord, taken at different levels and including the vertebral investment, were examined. In many of the animals, sections of the limbs and viscera were also taken.

Biological Properties of the Virus

Size.—The agent is smaller than 100 mμ. A 10^{-2} suspension in "hormone" broth was clarified by low speed centrifugation and then subjected to one cycle of 20,000 $\times$ *G* for 1 hour. This procedure is expected to sediment viruses of 100 mμ or more in diameter (7). The high speed supernatant was infective for infant mice in the original titer and was passed through a sintered glass filter, U. F. (ultrafine), without loss of titer.

Host Range.—The host range, so far as determined, is quite limited and compatible with that described for the Theiler group of viruses. Cotton rats, guinea pigs, and rabbits were not susceptible by the intracerebral route. The pathogenicity for monkeys and hamsters has not been determined.

The DA agent multiplies in the embryonated hen's egg. Two chick embryo lines have been established but the concentration of virus present and its distribution in various tissues has not been carefully studied. Amniotic fluid from 5th chick embryo passage killed 7 out of 9 3-day old mice with characteristic signs and lesions. By the most conservative estimate this material represents a 10^{-10} dilution of the mouse brain suspension used to initiate the chick embryo line.

Theiler strain GD-VII has been adapted to chick embryos by Dunham and Parker (8) and by Enright and Schultz (9). The FA strain was propagated in eggs by Gard (10) and by Riordan and Sá-Fleitas (11).

Infectivity.—The infectivity of 4th infant passage mouse brain and cord was determined by intracerebral inoculation of 3 week old mice and of infant mice. The virus now caused death of 100 per cent of mice inoculated with a 10 per cent suspension and in 3 week mice the LD_{50} (50 per cent lethal dose) was

$10^{-2.6}$ (12). The MPD_{50} (50 per cent mouse-paralyzing dose) was 10^{-3} or higher. In infant mice the LD_{50} was 10^{-3}.

In subsequent work, sucklings were used because the end-point was more sharply defined, there being no survivors with paralysis. 3 day old mice were found to tolerate the standard volume of 0.03 ml. used for intracerebral inoculation.

A titration of 5th passage material in 3 to 4 day old mice showed further increase in virulence without, however, any reduction in the time of incubation. The LD_{50} on three occasions ranged from $10^{-4.2}$ to $10^{-4.7}$. Its LD_{50} (also MPD_{50}) in 3 week mice was $10^{-3.2}$.

The signs of disease in sucklings differed from those shown in weanlings. They became hunched, excitable, tremulous, with fur roughened and growth stunted, but death usually occurred before the onset of definite paralysis. Obviously the encephalitic signs dominated the picture.

Of 12 6 week old mice injected intracerebrally with a 10^{-2} suspension of infant brain and cord, 10 developed spasticity and partial paralysis of the hind legs, one of these dying spontaneously. 2 showed no signs of disease. A complete study of varying age susceptibility has not been made but it is obvious that the older animals respond to the infection less violently than do the newborn.

The virus may survive for at least 90 days in the CNS of intracerebrally inoculated mice. Thus mice inoculated at 6 weeks of age became paralyzed in the usual time. Some deaths occurred but the majority survived with varying degrees of paralysis. When these were sacrificed 90 days after inoculation, passage of CNS suspension into infant mice resulted in the usual signs of disease and the characteristic lesions.

Relation to Theiler Group of Viruses

As mentioned earlier the size of the DA agent, its infectivity, and host range are not incompatible with those of the less virulent members of the Theiler group of viruses (1, 2), TO and 4727.

Like the Theiler group the invasiveness of DA is much less by extraneural than by intracerebral inoculation. Weaned mice are completely resistant to intraperitoneal injection and even of newborn mice, one-third survive without signs. Only occasional animals develop the disease after intramuscular or intranasal inoculation.

Resistance to lethal infection increases with age. The results of one experiment are shown in Table I.

Efforts to show a serological relationship with known strains of Theiler's virus have been hampered, since the first requirement, homologous neutralization or protection, has not been satisfactorily met. Some cross-protection between DA and 4727 has been shown when survivors of groups of mice inocu-

lated intraperitoneally at 0 days of age are challenged intracerebrally 1 month later with heterologous virus.

Immunization of females by weekly intramuscular injections from the time of breeding throughout lactation did not sufficiently immunize the offspring to protect them from intracerebral challenge. Even when females were paralyzed as a result of intracerebral inoculation the result was the same.

TABLE I

Effect of Age on Outcome of DA Inoculation

Intracerebral inoculation of 0.03 ml. of 4th infant mouse brain and cord 10^{-2} (10 LD_{50} for 6 to 7 day old mice).

Age	No. inoculated	Killed for pathologic examination	Lesions	Deaths	Survival with paralysis	Survival without signs
3 days	16	2	+	14	0	0
7 "	6	2	+	3	1	0
14 "	7	2	+	5	0	0
6 wks.	12	1	+	1	8	2

TABLE II

Intracerebral Neutralization Tests

Serum, undiluted or diluted 1/5	Treatment of serum	LD_{50} DA	*in vitro* incubation	Prolongation of incubation period in mice
"Normal" 3 wk. old mice	Unheated	1000	2 hrs. room temperature	+
Paralyzed mice 3 mos. post DA, i.c.	"	1000	2 hrs. room temperature	+
"Normal" adult mice	56°C. ½ hr.	25	37°C. 1 ½ hrs.	−
Mice, intramuscular inoculation of DA, (repeated)	56°C. ½ "	25	37°C. 1 ½ "	−
Anti-GD-VII mouse serum*	56°C. ½ "	25	37°C. 1 ½ "	−
Anti-SK rabbit serum	56°C. ½ "	25	37°C. 1 ½ "	−

* The GD-VII and SK antisera were kindly supplied by Dr. Robert Rustigian of the University of Chicago.

Intracerebral challenge of 3 to 7 day old offspring with 20 to 100 LD_{50} of virus was invariably followed by paralysis and death. The minimum incubation period, however, was lengthened as much as 10 days.

Only partial neutralization could be shown in an intracerebral test with mouse serum. There was no demonstrable difference between serum from "normal" 3 week old mice and serum from mice which had been paralyzed for a period of 3 months after DA inoculation. The first serum, even in an infected colony, would be expected to have a low titer of antibody (15) and the second a high titer.

A summary of some intracerebral neutralization tests will be found in Table II.

Viremia

In our study of the pathology we have been impressed with the dominance of the vascular lesions in all stages of this disease. This suggested the possibility of early viremia and led to the following observation.

The first blood, taken after onset of paralysis, 15 days after intracerebral inoculation proved to be non-infective. However, when blood was obtained midway in the minimum incubation period of 11 days, virus was present. Early viremia was found not only in infections with DA strain but also in mice inoculated intracerebrally with three known Theiler strains, 4727, FA, and GD-VII. The results are summarized in Table III.

TABLE III

Viremia

Virus	Age of mice when inoc.	Route of inoculation	Bled: time post inoculation	Intracerebral subinoculation—3 day old mice		
				Signs	Killed for pathologic examination	Lesions
			days			
GD-VII 10^{-2}	3 days	i.c.	3	5/5*	1	+
FA 10^{-2}	3 "	"	6	7/7	2	+
4727 10^{-1}	3 "	"	6	8/8	1	+
DA 10^{-2}	3 "	"	6	11/25‡	6	+
					2	−§
" "	3 "	"	10	0/7	2	−
" "	7 "	"	15	0/4	None examined	
" 10^{-1}	0 "	i.p.	6	0/3	1	−
" 10^{-2}	3 wks.	i.c.	6	0/7	2	−

* Numerator = number with signs; denominator = number inoculated.

‡ Three experiments.

§ Killed before maximum incubation period.

An attempt was made to assay the concentration of virus but blood, either whole, laked, or as plasma or washed cells, subsequently laked, was found to be non-lethal to infant mice when diluted more than 1:4. Although many mice injected with diluted blood appeared rough coated and stunted and showed some transient weakness, none of them had lesions of the central nervous system or other tissues.

Pathology

Lesions are found in the meninges, brain, spinal cord, anterior nerve roots, and sometimes in the peripheral nerves.

Meninges.—There is a patchy meningitis of varying severity, characterized by the infiltration of lymphocytes and large mononuclear cells. Some of the meningeal vessels are surrounded by dense mantles of lymphocytes. The choroid plexus is not involved.

Brain.—The lesions are sometimes rather sharply limited, but often more diffuse and scattered. The blood vessels are hyperemic. They contain no occlusive thrombi, but in some of the larger venules, one occasionally finds clusters of lymphocytes embedded in clumps of granular material, probably platelets. The endothelial cells are often swollen and distorted. About many of the capillaries, there is a sheath of proliferating adventitial cells amongst which mitotic figures are occasionally seen. Lymphocytes and plasma cells are present, but do not compose the major elements of the perivascular infiltrates, and most of the large cells are either histiocytic or microglial (Fig. 1). Often in the vicinity of the blood vessels, there is nodular proliferation of microglial cells with pale distorted nuclei (Fig. 2). Amongst them are many rod cells, with very long, beaded nuclei. These seem to be a characteristic feature of the lesions. There are no histiocytes, lymphoid or polymorphonuclear leucocytes. The ground substance is usually intact, except in sucklings inoculated at an early age in which one finds more extensive lesions accompanied by a certain amount of necrosis. It has not been possible to demonstrate an increase of neuroglia fibrils in the areas with the phosphotungstic acid–hemotoxylin stain.

The ganglion cells in general are not much affected, although individual cells in the vicinity of the microglial and vascular lesions may be found in various stages of necrosis. One has the impression that the disease is primarily a vascular one, and that the damage to nerve cells is secondary to the alterations in the blood vessels.

The lesions, as has been stated, are not always focal or limited to the vicinity of the blood vessels. There may be diffuse infiltration of microglial cells over a considerable area. In a few mice, which had survived the injection for a period of 3 weeks or more, there were seen large circumscribed areas of almost complete necrosis, with nuclear fragmentation and a purplish staining of the fibrillar ground substance, possibly beginning calcification. Such lesions were very exceptional, and in general, the changes were proliferative rather than necrotizing.

Distribution.—The lesions were scattered irregularly through various regions of the brain. They were almost invariably present in the medulla, beneath the floor of the 4th ventricle. The midbrain also was quite regularly affected, the cortex and hippocampus less often, and the cerebellum very rarely. Microglial nodules were found in the molecular layer of only one case.

Spinal Cord.—Usually, four blocks taken at different levels were examined. Lesions were found at all levels. They involved both the central grey matter, with a predilection for the anterior horns, and the penetrating vessels, extending out into the neighboring white matter. There was often quite marked lymphocytic infiltration of the meninges, especially about the vessels.

In a number of mice, though by no means in all, the anterior nerve roots have shown apparent loss of myelin, and increase in the sheath cells (Fig. 3). In favorably cut sections, this degeneration is seen to continue beyond the junction with the ventral root and ganglion. A Bodian preparation of one such cord showed quite striking swelling and beading of the neuraxons. The peripheral nerves were not examined as a routine, but in one mouse recently studied, which had been inoculated at the age of 6 days with a 10^{-2} suspension, and was sacrificed 8 days later, a large nerve trunk in the leg showed, in its central portion, very definite destruction of myelin, swelling and fragmentation of the neuraxons, and increase and swelling of the Schwann cells (Fig. 4). Degeneration of small intramuscular nerves was a frequent finding.

Whether such changes in the ventral nerve roots and peripheral nerves are to be attributed to a direct effect of the virus, or whether they are secondary to the degeneration of the motor cells of the cord, is uncertain. However, degenerating nerve roots may be found at levels where the ganglion cells are normal and the grey matter free from inflammatory lesions. The dorsal roots and ganglia are never affected.

Lesions of a somewhat different and interesting character have been found in the spinal cord of older mice, and merit special description. Mouse 5459 received an intracerebral injection of 5th passage virus (10^{-2}), followed by four weekly intramuscular injections of 0.2 ml. each. It was sacrificed 71 days after the first injection, at which time it appeared somewhat stiff and spastic and uncertain in its movements, but was not paralyzed.

Minimal lesions were found in the brain,—occasional perivascular lymphoid infiltration of meningeal and deeper arterioles, slight focal or more diffuse microglial accumulation in adjacent brain tissue. Sections stained with the Loyez myelin method did, however, disclose areas of microcystic degeneration, with loss of myelin, astrocytosis and gliosis in medulla and pons.

The spinal cord was the seat of striking and unusual lesions, largely restricted to the white fiber tracts. The location varied at different levels. Lateral, ventral, and even posterior columns were affected (Figs. 5 and 6). There was extensive destruction of myelin, with formation of microcysts, together with proliferation of microglia, hypertrophy of astrocytes, and apparently some increase or condensation of neuroglia fibers. The grey matter was relatively unaffected, the lesions restricted to occasional perivascular cuffing, and to slight focal accumulations of microglia. There was no extensive destruction of ganglion cells.

Some of the ventral nerve roots also showed loss of myelin and microcyts. The dorsal root ganglia were not affected.

Comparable lesions were found in 3 other mice treated in the same way.

That repeated intramuscular injections are not requisite for the production of such changes in the white tracts is indicated by their occurrence in 3 mice (5490, 5491, 5492) which had been treated as follows. When 6 to 7 days old, they had been injected with brain suspension of 4th passage material intracerebrally in concentrations ranging from 10^{-3} to 10^{-6}. The LD_{50} of this suspension was 10^{-3}. The 28 survivors of the 32 inoculated mice showed no paralysis during the ensuing 33 days. They were then challenged with 5th passage mouse brain suspension, 10^{-2} intracerebrally. Paralysis of the hind limbs was first noted 12 days later, and persisted. 3 of the mice were observed for a period of 3 months, and were still paraplegic when killed at that time.

Again, the lesions were almost entirely limited to the white matter of the cord, in which were found large patches of myelin destruction, with microglial cells, hypertrophied astrocytes, and some large vacuolated cells, evidently containing lipids. The meningeal vessels were cuffed with lymphocytes, and many of the ventral nerve roots were depleted of myelinated fibers.

Bodian preparation of longitudinal cord sections through the areas of myelin destruction showed that the axons, though pushed apart and distorted, were for the most part well preserved (Fig. 7).

Brain suspensions from 2 mice of this group were injected intracerebrally into 3-day-old sucklings. They developed encephalitis after the usual incubation period of 11 days or more and were found to have the characteristic lesions. The virus was therefore still present in these chronic cases—a fact which speaks against a non-specific "allergic" causation of the demyelinating lesions.

The distribution of these lesions through various tracts, and the minimal changes found in the central grey matter make it unlikely that they are secondary to destruction of ganglion cells. Whether they are due to a direct and continuing action of the virus, or to an allergic effect such as has been produced in mice by repeated intramuscular injections of normal brain tissue with the aid of adjuvants (14, 15) can be determined only by further experiments.

So far as we are aware, destructive, demyelinating lesions of the white matter have not previously been described in association with viruses of the Theiler group. The JHM virus characteristically produces extensive demyelinating lesions in the cord (16, 17), but induces

other histopathologic changes—giant cell formation, liver necroses, etc., which have not been seen in these animals.

Myositis.—After intramuscular injection, there is produced a myositis of moderate intensity, limited to the injected limb. Groups of fibers or single fibers undergo necrosis. There is a profuse histiocytic reaction about the degenerated fibers, and regenerating fibers are seen in the vicinity (Fig. 8). There are no intranuclear inclusions in these such as are regularly seen in myositis caused by injection of the GD-VII strain of Theiler virus. The lesions are comparable in intensity with those produced by the TO and 4727 strains of Theiler virus, but are much less widespread and severe than those elicited by GD-VII or Columbia SK strains (18).

Myositis involving limb and back muscles has also been noted in a number of suckling mice inoculated intracerebrally with 0.03 ml. of amniotic fluid or with heparinized blood, obtained on the 6th day after injection of 3 day old mice, and before the onset of neurologic signs. The muscle lesions were identical in their histopathology with those produced by many strains of Coxsackie virus (Fig. 9) although less widespread.

Similar muscle lesions were also found in 3 day old mice inoculated intracerebrally with the Theiler strain 4727 (Fig. 10), and sacrificed 6 days later, before the advent of signs, or lesions of central nervous system. Areas of myositis in back and limb muscles were likewise seen in 3 day old mice inoculated intracerebrally with 6 day blood of FA strain infected mice (Fig. 10). It will be noted from Table IV that the muscle lesions were found as early as the 6th day after inoculation with 4727 and FA strains, whereas they were absent in 3 mice infected with DA virus and sacrificed on the same day. After 13 to 16 days, when encephalomyelitis was pronounced, myositis was present in 7 mice. Muscle lesions were never found in mice inoculated at the age of 3 weeks or more.

After intracerebral or intraperitoneal injection of GD-VII virus no lesions of skeletal muscles have yet been noted. This is interesting in view of the intense myositis produced locally after intramuscular injection of this strain (14).

Fuchsinophile granules, such as have been described in the muscle lesions caused by the Conn.-5 and other strains of Coxsackie virus (19), could not be demonstrated. On the other hand, the larger spheroidal bodies noted in the lesions of the brain and cord occurred also in and about the degenerating muscle fibers, though in sparse numbers.

There have been no significant lesions of the abdominal or thoracic viscera. The fetal adipose tissue of infant mice was not affected as it is with many strains of Coxsackie virus.

Spherical Bodies.—A feature of the lesions which has attracted our attention is the occurrence of spherical bodies in and about the lesions. These vary greatly in size, ranging roughly from 1 to 5μ, and rarely even larger (Fig. 11). They are frequently located within the cytoplasm of a microglial, adventitial, or endothelial cell, but occur most commonly free in the fibrillar ground substance. Occasionally they have been seen within the lumen of a venule or capillary (Fig. 12). Usually they are surrounded by a narrow unstained halo, but there is no limiting membrane, and the halo is not always present.

While the shape of these structures is most often spherical, some of them may be slightly irregular and even crescentic. Occasionally they are paired, but nothing to suggest budding was seen.

After prolonged staining with buffered Giemsa, these bodies are colored purplish blue, in contrast to the deep blue of the nucleoli of the ganglion cells which they resemble in size and shape. They are well stained with thionin or dilute methyl violet. They do not retain the Gram stain. In hematoxylon-eosin preparations, they can usually be distinguished from the chromatin of the nuclei, but the intensity of the staining varies, and they are less easy to identify than in Giemsa or eosin-thionin preparations. They do not stain black like myelin with sudan black. The Feulgen reaction is positive.

TABLE IV

Myositis Produced by Various Strains of Theiler Virus after Intracerebral Inoculation

Mouse No.	Strain of virus	Material inoculated	Age when inoculated	Time after inoculation	Myositis	Remarks
			days	*days*		
5494	DA	Plasma	6	15	+	Encephalitis +++
5420	"	Blood	3	16	+	" +++
5406	"	"	3	14	++	No encephalitis Myelitis ++
5405	"	"	3	14	+++	No encephalitis Myelitis +++
5404	"	"	3	14	+	Encephalitis +++ No myelitis
5239	"	Amniotic fluid	3	13	+++	Encephalomyelitis ++++
5238	"	Amniotic fluid	3	13	+	" +++
5462	" 10^{-2}	Mouse CNS	3	9	−	Encephalitis ++ Cord +
5463	" "	" "	3	6	−	Encephalitis + Myelitis −
5464	" "	" "	3	6	−	Myelitis − Encephalitis +
5465	" "	" "	3	6	−	No encephalomyelitis
5457	4727 10^{-1}	" "	3	6	++	No lesions of cord
5456	" "	" "	3	6	++	Congenital hydrocephalus
5455	" "	" "	3	6	+	No encephalomyelitis No lesions of cord
5454	" "	" "	3	6	+++	Beginning encephalomyelitis
5505	Fa 10^{-2}	" "	3	11	+	Encephalitis +++
5488	" "	" "	3	6	+	No lesions of CNS
5487	" "	" "	3	6	+	" " " "
5486	" "	" "	3	6	+	" " " "
5573	GD-VII 10^{-2}	" "	3	4	−	Encephalomyelitis +++
5574	" " "	" "	3	4	−	" ++++
5575	" " "	" "	3	4	−	" ++++
5579	" " "	" "	3	6	−	Encephalitis ++++

Identical structures are seen in the lesions of mice infected with 4727 strain of Theiler virus.

We have been able to arrive at no satisfactory conclusion as to the nature of these bodies. They may be merely pycnotic chromatin fragments from the nuclei of necrotic cells but often

they are found at a distance from any degenerating cellular elements, and they have been seen within the lumina of blood vessels, as well as within the cytoplasm of lining endothelial cells and adventitial cells. Although they frequently occur within cells, it is more probable that they have been phagocyted than that they are in the nature of primary cytoplasmic inclusions, since they are so frequently found free at a distance from any cellular element. Still, it is conceivable that they may have originally been intracellular, and either that they have been liberated from the cell or that the cell itself which contained them may have undergone necrosis and disappeared.

Quite recently, Harford and Hamlin (20) have described and pictured spherical, *basophilic* cytoplasmic bodies in the bronchial epithelium of mice which had been injected intratracheally with influenza virus. These resemble closely in size, appearance, and staining the structures which we have described. They also give a positive Feulgen reaction. The authors describe a pinching off of the cytoplasm containing these inclusions, with their discharge into the lumen of the bronchus.

DISCUSSION

The first question which arises concerns the identity or relationship of the DA strain with other members of the Theiler group of murine encephalomyelitis. It resembles the Theiler group in its host range, small size, development of resistance with age, and in its manifestations of disease when inoculated into mice by different routes. Failure to obtain neutralization in intracerebral tests provides another similarity.

von Magnus (13) has reported that intracerebral neutralization tests with TO fail to reveal antibody, although this is demonstrable in high titer in Theiler-free mice by the intraperitoneal route. The latter procedure is unsuitable for tests with the DA agent at present because of its low invasiveness by extraneural routes in our mice.

It is highly probable that our mouse colony is Theiler-infected, as spontaneous paralysis occurs occasionally. The presence of Theiler antibody does not alter the response to intracerebral challenge but has a marked influence on challenge by extraneural routes, as shown by von Magnus (13) using TO strain. It seems likely that a satisfactory neutralization test would result, should we extend our work to Theiler-free mice as has been done by von Magnus, and by Dean (21).

As was shown in Table III, we failed to demonstrate virus in the blood of 3 week old mice. This is in accord with early observations of Theiler (1), who found that blood obtained at intervals of 1 to 8 days after intracerebral injection was non-infective.

The occurrence of viremia in infant mice after intracerebral inoculation is of particular interest in the light of recent reports that early viremia is a frequent finding during the incubation period of experimental poliomyelitis in chimpanzees and *cynomolgos* monkeys. Horstmann (22) found virus in the blood of these animals after feeding Lansing- and Brunhilde-type human poliomyelitis strains.

As regards the pathologic changes induced by this agent, we have found them to differ in some respects from those elicited by the 4727 and other strains of Theiler virus. The original description of Theiler (1) is based on the five strains first isolated by him, all of which produced paralysis in adult mice after incubation periods ranging from 7 to 30 days. Only 10 per cent of sucklings, in which the virus caused 100 per cent mortality, became paralyzed. His brief account of the pathology stresses the necrosis of ganglion cells, especially in the anterior horn cells of the spinal cord, and the perivascular lymphoid infiltrations. Olitsky and Schlesinger (5) have given a somewhat more detailed account of the pathology. The lesions, according to them, are of two basic types: (*a*) mesodermal-glial, consisting of perivascular and round cell infiltration, swelling, and proliferation of vascular endothelium in smaller vessels; (*b*) neuronal, varying from early stages of tigrolysis to complete disappearance of ganglion cells with resulting vacuolization of stroma.

Our findings with the DA strain supplement, and, to a minor degree, differ from those of the authors cited. Necrosis of ganglion cells is a conspicuous feature of the lesions only in the newborn, in which after repeated passage, there is produced a severe and often diffuse type of encephalitis in which one may find areas of complete necrosis. Death frequently occurs with little or no involvement of the spinal cord. In older animals, however, the perivascular and microglial reactions dominate the picture, and although occasional neuronal degeneration may be seen in the more severely affected areas, one has the impression that the lesions are primarily vascular, and that destruction of ganglion cells is decidedly less widespread than with other strains of Theiler virus.

We have not found in previous reports, reference to degeneration of anterior nerve roots. Although this was not found in all preparations, it occurred with sufficient frequency to deserve mention. Thus in 51 mice with myelitis, degeneration of ventral nerve roots was recorded as present in 25, absent in 26. The posterior roots, in contrast, were never affected, nor were lesions seen in the dorsal ganglia.

In order to ascertain whether this is a peculiar feature of the lesions due to DA strain, we have carefully reviewed the spinal cords of 19 cases of myelitis due to the 4727 strain of Theiler virus, 5 due to FA strain, and 11 due to GD-VII. Despite the fact that these agents bring about great destruction of anterior horn cells, degeneration of ventral nerve roots, comparable to that noted with the DA virus, was not found. One is forced to the conclusion that this lesion of motor nerve roots is distinctive of infection with this particular strain.

Another lesion to which we have found no reference in published descriptions of the pathology of Theiler encephalomyelitis, is the extensive destruction of myelin in the white tracts of the spinal cord. This occurs in irregular patches and in its distribution, bears no obvious relation to blood vessels. Although the

mice with these late lesions had received multiple injections, there is little reason for interpreting them as "allergic." A point against this is the persistence of the virus in the brain and cord of these animals for at least 90 days after the last inoculation. Theiler, in 1937, had reported the recovery of virus in mice which had been paralyzed for several months. In a number of mice sacrificed in earlier stages, we have encountered inflammatory foci in the white tracts which might well have been precursors of the demyelinated patches.

Although "allergic encephalomyelitis" has been produced in mice by Olitsky and Yager (14, 15) by repeated intramuscular injections of normal brain, supplemented by killed tubercle bacilli and liquid petrolatum, and the lesions were accompanied by demyelination in some animals, injections of normal brain alone failed to give rise to lesions. We have examined the spinal cords of 5 mice which had been given 4 weekly intramuscular injections of 0.1 ml of infected mouse brain. When sacrificed 41 days later, none of these had signs of disease or lesions of the cord.

We must conclude therefore, that in some animals it is possible by intracerebral inoculation with the DA strain to produce a chronic form of encephalomyelitis characterized by destructive lesions in the white matter. Whether other strains of Theiler virus are capable of eliciting similar changes, remains to be investigated.[1]

The spheroidal bodies described above present another puzzling problem. We have found them to be invariably present, not only in the lesions of the central nervous system, but also in the muscle lesions of sucklings. Their occurrence is not restricted to lesions caused by the DA strain, since identical structures are present in the lesions produced by 4727 and other strains of Theiler virus.

One's first impression is that these bodies are merely pycnotic chromatin particles from necrotic cells, but this is not borne out by further study. Their occurrence remote from cells undergoing necrosis, and often within the cytoplasm of endothelial and adventitial cells, or free within the lumen of blood vessels, speak against this simple explanation. Since we have been able to arrive at no satisfactory interpretation as to their origin or significance, we must be content merely to record their presence in the lesions as a characteristic, if not pathognomonic feature.

The production of skeletal myositis in suckling mice has come to be regarded as a distinctive feature of the disease caused by certain strains of Coxsackie virus (23, 24). It was interesting, if not entirely unexpected, to find that the DA strain of spontaneous murine encephalomyelitis, as well as other Theiler strains (4727, FA) were capable of eliciting similar lesions of skeletal muscle.

[1] We have recently found demyelinating lesions in a mouse inoculated intracerebrally with strain 4727 at the age of 5 weeks. It was sacrificed after having been paralyzed in hind legs for over a month.

It has been previously pointed out (25) that the EMC and Columbia SK viruses share this property; and it has recently been shown by Skinner (26) that several strains of foot and mouth disease virus produce severe myositis in suckling mice. The obvious conclusion from these observations is that lesions of the skeletal muscles in suckling mice may be caused by a variety of agents, and cannot be looked upon as pathognomonic of a well defined group of viruses isolated from human sources.

SUMMARY

A new agent, DA, has been isolated from a spontaneously paralyzed mouse. Its biological properties and the pathology of lesions in experimental infection indicate close relationship with the Theiler group of encephalomyelitis viruses.

Serological studies were inconclusive. The intracerebral neutralization test failed to reveal measurable antibody and other routes of inoculation were unsuitable because of low invasiveness of the agent. Repeated vaccination of females did not render their offspring resistant to homologous intracerebral challenge.

The occurrence of early viremia is reported following intracerebral inoculation of the DA strain and also the known Theiler strains, 4727, FA, and GD-VII.

The pathology of mice experimentally infected with DA and with known members of the Theiler group is described. Attention is called to the demyelinating lesion of the cord in mice surviving for several months and to the persistence of virus in the CNS of such animals.

Another characteristic feature of the pathology was the degeneration of ventral nerve roots and in some mice of the peripheral nerves. Similar changes were not seen with strains of Theiler virus other than DA.

Spheroidal bodies of undetermined significance were found in the lesions.

Finally the occurrence of myositis after intracerebral inoculation, not only mice infected with the DA virus, but also with the 4727 and FA strains, is described and discussed.

Addendum.—Since submitting this paper, we have examined several mice receiving a single injection of virus and allowed to survive for 2 to 4 months. Lesions of the white matter of cord and brain stem identical with those described were found. Virus was recovered from brain suspensions of these animals. This is taken as evidence that the demyelinating process is a direct reaction to the virus rather than a sensitization phenomenon.

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EXPLANATION OF PLATES

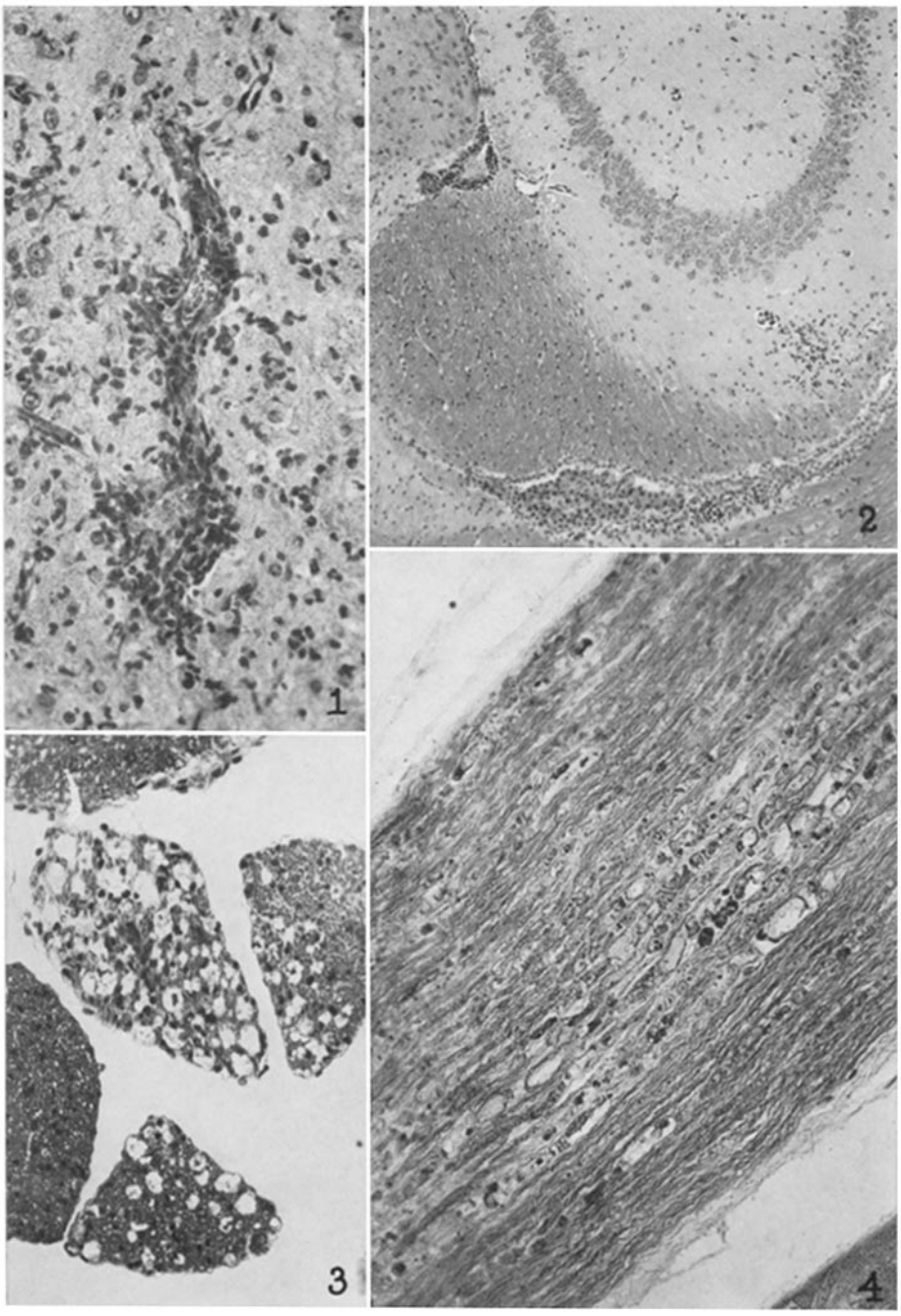
PLATE 22

FIG. 1. Mouse 5182. 6 days old when inoculated i.c. with 10^{-2} 4th passage material. Killed 13 days later. Capillary showing perivascular infiltration and multiplication of sheath cells. Hematoxylin and eosin. × 246.

FIG. 2. Mouse 3392. Inoculated i.c. with 1st passage material. Glial nodule in hippocampus, in vicinity of blood vessel. Lymphocytic infiltration about meningeal vessels. Hematoxylin and eosin. × 84.

FIG. 3. Mouse 5043. Inoculated at age of 3 weeks with 4th passage material i.c. On 20th day, paralysis of both hind limbs, followed by partial recovery. Killed on 28th day. Degeneration of anterior nerve roots of cord; dorsal roots are unaffected. Hematoxylin and eosin. × 211.

FIG. 4. Mouse 5328. Inoculated at 6 days i.c. with 10^{-2} suspension of infected mouse brain. Sacrificed 8 days later. Branch of sciatic nerve showing degeneration with microcysts and loss of myelin in central portion. Sudan black–myelin stain. × 197.



(Daniels *et al.*: Encephalomyelitis of mice)

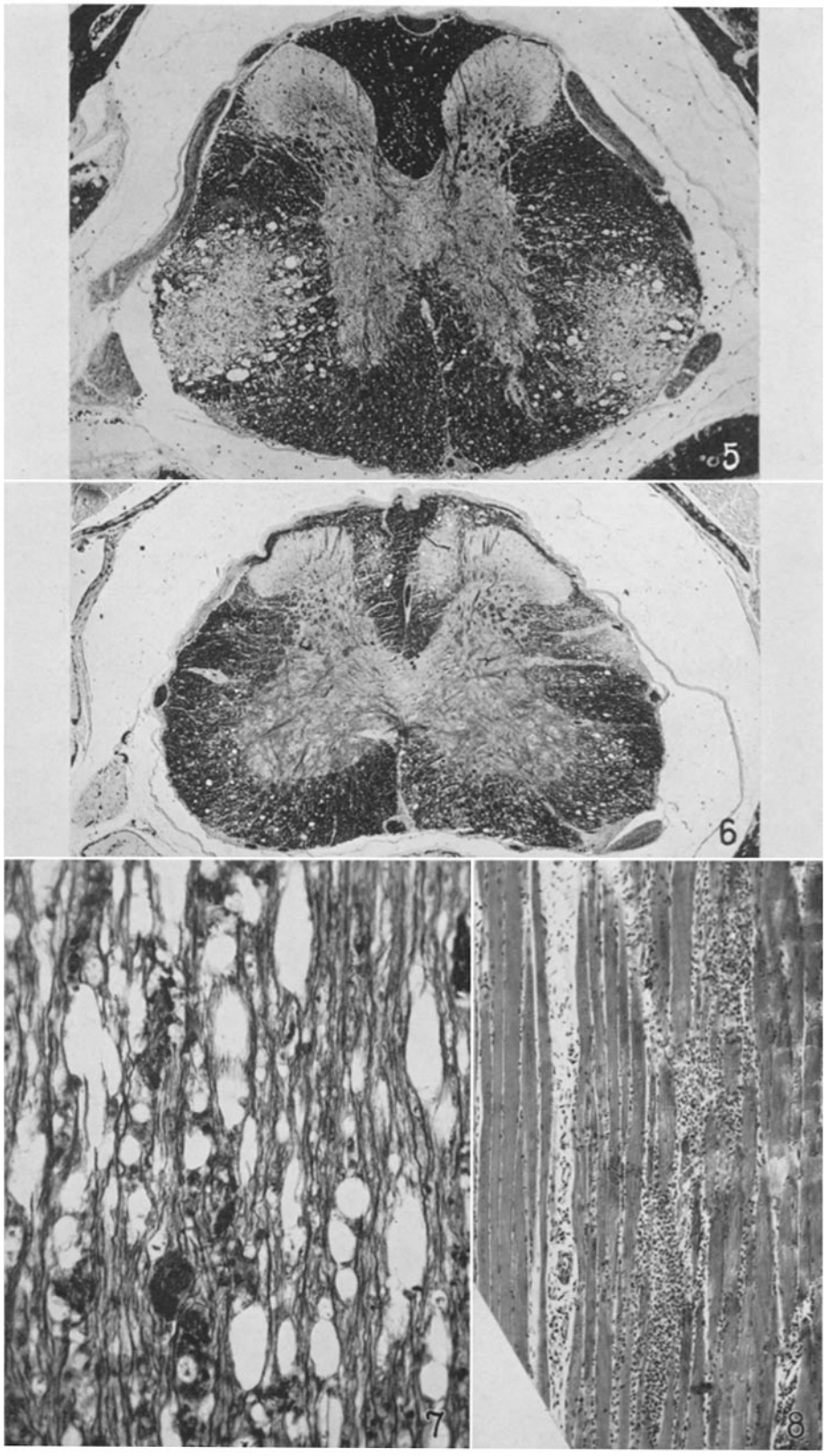
PLATE 23

FIG. 5. Mouse 5459. Received 1 i.c. and 4 intramuscular injections of 5th passage material. When sacrificed on 71st day after inoculation, there was almost complete spastic paraplegia. Dorsal cord—large areas of myelin destruction in lateral pyramidal tracts. Loyez stain. × 57.

FIG. 6. Mouse 5460. Same treatment and signs as mouse 5459. Cervical cord, showing patches of myelin destruction in dorsal columns, as well as slighter lesions in lateral and anterior tracts. Loyez stain. × 35.

FIG. 7. Mouse 5492. Received 1 i.c. injection of 10^{-5} virus suspension at age of 6 days. This caused no signs of illness during ensuing 33 days. It was then given an i.c. injection of 10^{-2} suspension. It became partially paralyzed 12 days later, and was killed after 3 months. There were extensive areas of myelin destruction in the spinal cord. Bodian stain, showing preservation of neurites in degenerated areas. × 190.

FIG. 8. Mouse 5025. Injected at age of 3 weeks intramuscularly with 20 per cent infected amniotic membrane. Killed 13 days later. Focal myositis at site of injection. Hematoxylin and eosin. × 95.



(Daniels *et al.*: Encephalomyelitis of mice)

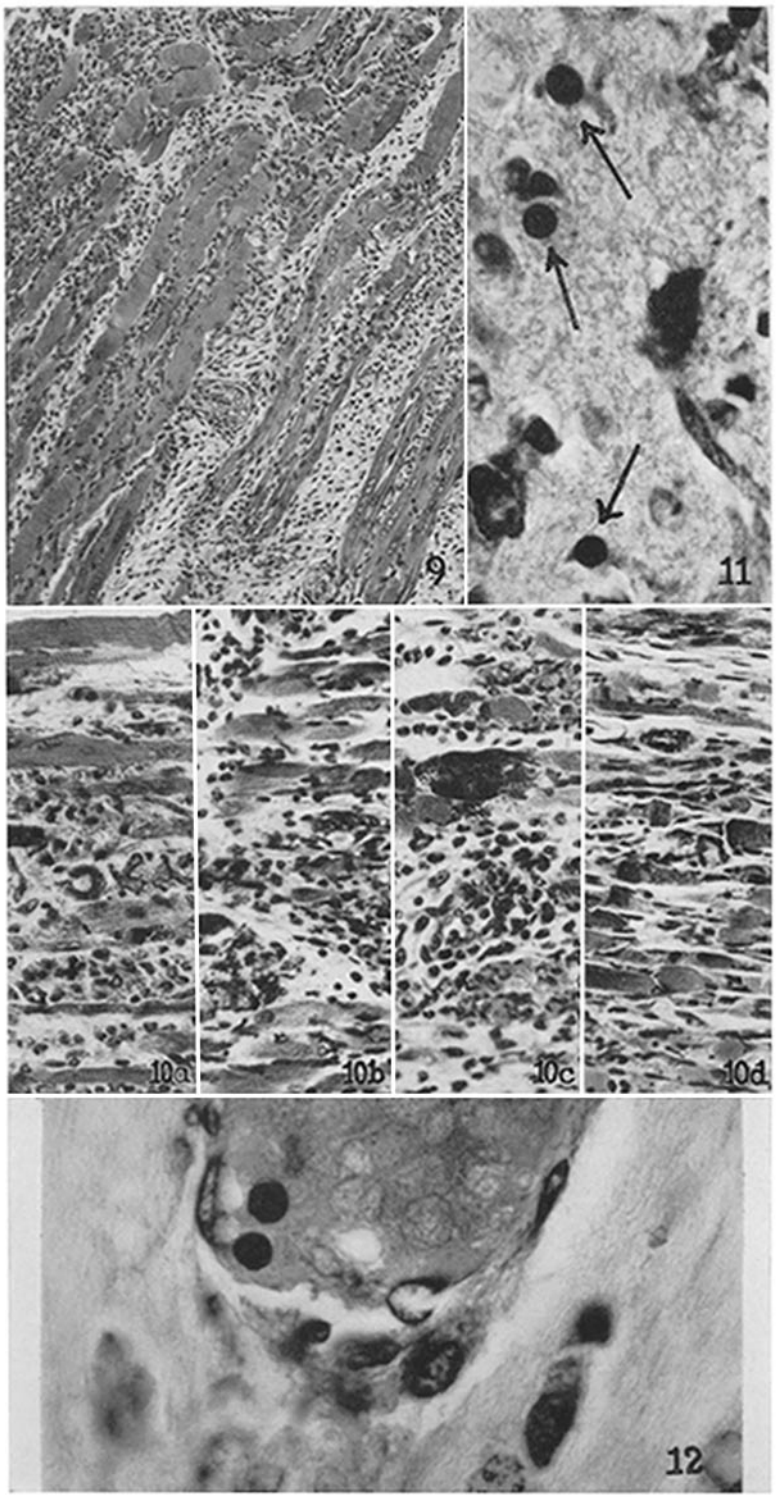
PLATE 24

FIG. 9. Mouse 5405. Injected i.c. at 3 days with 6 day blood from infected mouse. Paralyzed and hunched when killed 14 days later. Leg muscles show large areas of myositis in healing stage; fibers are widely separated by inflammatory tissue and there are many small regenerating fibers. Hemotoxylin and eosin. × 333.

FIG. 10. Myositis produced by various strains of Theiler and Coxsackie virus. (*a*) Mouse 5239. DA strain. 3 day mouse inoculated i.c. with infected amniotic fluid. Moribund on 13th day after injection. × 350. (*b*) Mouse 5487. FA strains. 3 day mouse, inoculated i.c. with 10^{-2} brain suspension. Sacrificed 6 days after inoculation. × 333. (*c*) Mouse 5454. 4727 strain. 3 day mouse inoculated i.c. with 10^{-1} brain suspension. Sacrificed 6 days after injection. × 375. (*d*) Mouse 3877. High-Point strain of Coxsackie virus. 0 day mouse inoculated i.p. with 10^{-3} brain suspension. Sacrificed on 3rd day. × 350.

FIG. 11. Mouse 3470. Injected i.c. with infected amniotic fluid; sacrificed 11 days later. Severe encephalomyelitis. Several "spherical bodies" ranging from 3.5 to 4 μ are shown. Giemsa. × 1184.

FIG. 12. Mouse 4982. Inoculated i.c. with 3rd passage material on day of birth. Killed 14 days later. Two "spherical bodies" free within lumen of capillary. Eosin-thionin × 1184.



(Daniels *et al.*: Encephalomyelitis of mice)