ENCEPHALOMYELITIS ACCOMPANIED BY MYELIN DESTRUCTION EXPERIMENTALLY PRODUCED IN MONKEYS

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Plates 32 to 34

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The etiology of the encephalomyelitis accompanied by demyelination that occasionally follows antirabic vaccination (1–4) and certain acute infections, *e.g.* vaccinia and measles, has been the source of many discussions, and the cause of the malady is still not definitely known. The fact that large amounts of heterologous brain material are injected into patients undergoing antirabic vaccination, has induced certain investigators (1, 5) to consider this procedure responsible in some manner for the changes found in the central nervous system of those who become paralyzed. Indeed, a few workers have been able to produce paralysis in rabbits by means of repeated injections either of heterologous brain tissue (5, 6) or of autolyzed homologous brain material (7). In spite of the fact that paralysis can be caused in rabbits in this manner, investigators (6, 7) have been unable to demonstrate lesions in the nervous system to account for its occurrence.

Rivers, Sprunt, and Berry (8) have reported that they observed an encephalomyelitis with myelin destruction in 2 of 8 monkeys that had received repeated intramuscular injections of aqueous emulsions and alcohol-ether extracts of normal rabbit brain. Inasmuch as demyelinating maladies occasionally occur spontaneously (9–15) in monkeys, these workers thought it best not to draw any definite conclusions concerning the relation of the injections to the production of the encephalomyelitis in the monkeys. The investigations have been continued, and at the present time we shall present the results of an experiment that seems to indicate that the repeated intramuscular

injections of aqueous emulsions and alcohol-ether extracts of rabbit brain into monkeys are in some way related to the occurrence of the lesions found in the central nervous system.

Methods and Materials

Monkeys.—All monkeys (*Macacus rhesus*) used in the experiment were healthy and approximately half-grown.

Fresh Aqueous Emulsions of Rabbit Brain.—The fresh aqueous emulsions of rabbit brain were prepared in the following manner: One normal rabbit brain was thoroughly ground with alundum in a mortar. 40 cc. of Locke's solution and 10 cc. of 95 per cent alcohol were added. Then the emulsion was centrifuged at speed 5 for 3 minutes. 3–5 cc. of the supernatant material were injected intramuscularly into each monkey. Fresh emulsions were made for each set of inoculations. The sterility of the materials employed was tested by means of cultures. It is fully realized that a certain percentage of the normal rabbits used may have had the spontaneous encephalitis that is found in rabbits of this country.

Alcohol-Ether Extracts of Rabbit Brain.—The brains of 4 rabbits that had been exsanguinated were removed and thoroughly ground without an abrasive in a mortar. This material was then placed in a large flask and extracted for 4 days at 37°C. with 300 cc. of 95 per cent alcohol. The alcohol was drawn off and saved. Then 300 cc. of ether were added to the brain tissue and extraction was allowed to take place at 37°C. for 6 days. The ether was removed and allowed to evaporate under the influence of heat and vacuum until only 20 cc. of a "soapy-looking" material remained. To this material were added the 300 cc. of alcohol with which the first extraction was made. The soapy-looking material went into solution. By means of heat (70°C.) and vacuum the volume of the mixed extracts was reduced to 150 cc. The concentrated alcohol-ether extract was stored in a cold room kept at 0°C. At this temperature a white waxy sediment appeared in the extract; but when the temperature of the extract was raised to 70° or 80°C. the sediment again went into solution. For the injection of each monkey 1 cc. of the alcohol-ether extract heated to 70–80°C. was added to 3 or 4 cc. of sterile distilled water. The resulting mixture consisted of a milky-looking fluid with the appearance of a Wassermann antigen.

Injections of Brain Emulsions and Extracts.—The brain emulsions and extracts were repeatedly injected intramuscularly in monkeys, the animals usually receiving 3 inoculations a week consisting either of 2 emulsions and 1 extract or of 2 extracts and 1 emulsion. The animals at times were allowed periods of rest during which no inoculations were given. An occasional animal developed an abscess at the site of inoculation and while the abscess was present the injections were discontinued. The schedule of inoculations for the group of monkeys is summarized in Table I.

Housing and Feeding of Monkeys.—2 monkeys were kept in each cage which was large enough for exercise. The cages were in a large well ventilated room.

The animals received bananas or oranges for breakfast, bread for lunch, and heated milk for supper. At times raw carrots and roasted peanuts were also included in the diet.

Control Monkeys.—8 monkeys received the repeated injections. 8 control monkeys were housed in the same room under conditions identical with those to which the test monkeys were subjected with the exception that they received no injections.

Autopsies.—Complete autopsies were performed on all 8 of the test monkeys and on 4 of the control animals. Cultures to test the sterility of the brains were made aerobically and anaerobically in meat-infusion broth, and aerobically on blood-agar, on Petroff's egg medium for acid-fast organisms, and on Sabouraud's medium for fungi.

Stains.—Sections from different parts of the cerebrum, cerebellum, pons, and cord were stained with hematoxylin and eosin, with Giemsa's stain, according to Marchi's method, and according to Kulschitzky-Wolter's modification of Weigert's myelin sheath stain.

EXPERIMENTAL

The experiment reported in this paper was planned to determine whether injections of aqueous emulsions and extracts of rabbit brain had any causal relation to the lesions described by Rivers, Sprunt, and Berry (8) in the nervous system of the monkeys used in their work. Eight monkeys (*Macacus rhesus*) received repeated intramuscular injections of aqueous emulsions and alcohol-ether extracts of normal rabbit brain made and administered according to the directions detailed above. Eight other monkeys (*Macacus rhesus*) were kept as controls in the same room under conditions identical with those to which the test animals were subjected with the exception that they received no injections. The results obtained in each animal appear below in detail under the animal's number, after which follows a description of the lesions observed in stained sections of the nervous system. It would be repetitious to describe in detail the lesions found in each monkey because the same type of pathological change occurred in every animal that became sick.

Monkey 8.—On Dec. 29, 1933, Monkey 8, having received 44 injections according to the schedule in Table I, exhibited slight ataxia, and held its head nearer the right than the left shoulder. The head could be rotated without apparent discomfort to the animal. The power in all the limbs was good. Temp. 102.4°F. On Jan. 2, 1934, the ataxia was more marked; the head was held more to the right,

and at times the chin rested on the shoulder. Temp. 102.2°F. The monkey received its 45th injection. Jan. 3, temp. 101.6°F. Jan. 4, the monkey although eating fairly well showed loss of weight. The ataxia had progressed to the extent that the animal was unable to move about without holding to the sides of the cage. Temp. 102.4°F. The animal received its 46th injection. Jan. 5, temp. 101.8°F. Jan. 6, the monkey was much weaker and more ataxic. A definite strabismus of the eyes was noted. Inasmuch as it appeared that the animal would not live much longer, it was sacrificed. While the monkey was

TABLE I

Schedule of Injections Received by Monkeys during 1933–34

Month	Day of month																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Aug.																						w		a		w		a		w	
Sept.	a				w		a		w		a		w		a			w		a		w			a		w		a		
Oct.			w		a		w		a		w		a			w		a			w									
Nov.													a		w					a		w		a			w		a		
Dec.		w			a		w		a			w		a		w		a		w		a								
Jan.		w		a		w		a		w		a				w		a												
Feb.					w			a		w				a			w		a		w		a			w		a		
Mar.												w		a		w			a			w		a							
Apr.		w			a				w		a		w											a		w		a		w	
May		a		w										a		w		a			w		a		w						

w indicates that the monkeys received aqueous emulsion of rabbit brain.

a indicates that the monkeys received alcohol-ether extract of rabbit brain.

Eight monkeys received injections. Some became paralyzed sooner than others: 4 received 85 injections; 1 received 80; 2 received 62; 1 received 46.

under ether anesthesia and before it was killed, a cisternal puncture was made. Clear fluid with 220 cells per c.mm. (40 per cent polymorphonuclear and 60 per cent mononuclear elements) giving a doubtfully positive Pandy reaction was obtained.

Necropsy.—No evidence of tuberculosis was found. All of the organs except the lungs, which showed a slight amount of consolidation at the edges of some of the lobes, appeared approximately normal. The brain and cord exhibited no lesions to the unaided eye. Anaerobic and aerobic cultures made from different parts of the cerebrum and cerebellum in meat infusion broth, and aerobic cultures on Sabouraud's medium remained sterile. Half of the brain was fixed in Zenker-

formol solution, the other half in 10 per cent formalin. Acid-fast and Gram-Weigert stains of sections of the central nervous system showed no fungi or bacteria. Hematoxylin and eosin, Giemsa, Marchi, and modified Weigert stains showed typical lesions accompanied by demyelination, which will be described in detail later. The meninges particularly in the neighborhood of the cerebellopontine angle were thickened and showed evidences of inflammation. Many lesions, most numerous in the neighborhood of the 4th ventricle, were found in the cerebellum and pons. In the cerebrum a slight amount of perivascular infiltration was seen in a number of sections, in one of which a large lesion also was found near the base of the occipital lobe.

Results of Animal Passages.—Each of 2 monkeys received intracerebrally 1 cc. of a 10 per cent emulsion of the brain of Monkey 8. Neither of them showed evidences of illness; one was sold 5 months later, the other is still under observation, 1 year, and is in perfect condition. Each of 2 rabbits received intracerebrally 0.25 cc. of the emulsion. Both remained well for 2 months. Then they were sacrificed, and, upon histological examination, their brains were found to be normal. Each of 6 Swiss albino mice received intracerebrally 0.03 cc. of the emulsion. All of them remained well for 2 months after which time they were discarded.

Monkey 1.—On Feb. 19, 1934, Monkey 1, that received 62 injections, showed ataxia and a right facial palsy. Temp. 101.6°F. Feb. 21, temp. 102.6°F. Feb. 23, the ataxia and right facial weakness were more pronounced. Drooling from mouth was noted for the first time. Feb. 28, the ataxia was so marked that the animal moved about with great difficulty. Mar. 1, the animal's condition was about the same. While the monkey was under ether anesthesia, a cisternal puncture was made which yielded a clear fluid with 21 cells per c.mm. (60 per cent polymorphonuclear and 40 per cent mononuclear elements) and a positive Pandy reaction. Then the animal was sacrificed.

Necropsy.—To the unaided eye the liver, spleen, kidneys, brain, and cord were negative. The lungs showed a few very small areas of consolidation. No evidence of tuberculosis was found. Cultures of the brain and cord for ordinary bacteria remained sterile. Sections of the organs other than the brain and cord showed no lesions of importance. Sections of the brain and cord showed a few typical lesions in the cerebrum, and a number in the cerebellum and pons particularly around the 4th ventricle. The only changes seen in the cord were brought out by the Marchi stain, and consisted of a degeneration of the myelin in the anterior part of the cord near the fissure and in one of the lateral bundles. Acid-fast and Gram-Weigert stains revealed no bacteria or fungi in the tissues.

Results of Animal Passages.—Each of 2 monkeys received intracerebrally 1 cc. of a 10 per cent emulsion of the brain of Monkey 1. Both remained well for 2 months after which time they were discarded. Each of 2 rabbits received intracerebrally 0.25 cc. of the brain emulsion, remained well for 2 months, and were then discarded. Each of 6 Swiss albino mice received intracerebrally 0.03 cc. of the brain emulsion. While under observation for 2 months they remained well.

Monkey 7.—On Feb. 21, 1934, Monkey 7, that received a total of 62 injections,

showed a ptosis of both eyelids. On Feb. 28, the animal was found to be ataxic. Temp. 101.2°F. The ataxia increased gradually until Mar. 12 when the monkey was scarcely able to move about in the cage. No definite evidence of paralysis of the extremities was observed. The bilateral ptosis persisted and the animal appeared to be blind. While the monkey was under ether anesthesia a cisternal puncture was made which yielded a clear fluid containing 6 mononuclear cells per c.mm. and a slightly positive Pandy reaction. The animal was then sacrificed and all the organs were examined.

Necropsy.—To the unaided eye all the organs including the brain and cord seemed to be normal. Cultures of the brain and cord on ordinary media and on Sabouraud's medium remained sterile. Sections of the liver, spleen, kidneys, and lungs showed nothing of importance. Sections of the cerebrum, cerebellum, and pons stained with hematoxylin and eosin revealed typical lesions to be described later. Gram-Weigert and acid-fast stains showed no microorganisms. Weigert's stain showed myelin destruction, much of which was around blood vessels, in the cerebrum, cerebellum, and pons. Some degeneration of myelin in the spinal cord near the anterior fissure and in the lateral columns was made evident by means of Marchi's stain.

Results of Animal Passages.—Each of 2 rabbits received intracerebrally 0.25 cc. of an emulsion of the brain of Monkey 7. Both animals remained well for a period of 2 months and then were discarded. A guinea pig received 0.1 cc. of the brain emulsion intracerebrally, remained well for 2 months, and then was sacrificed. Stained sections of its central nervous system revealed no lesions. Each of 6 Swiss albino mice received intracerebrally 0.03 cc. of the brain emulsion, remained well for 2 months, and then was discarded.

Monkey 2.—Monkey 2 received 80 injections. On Oct. 10, 1933, a small abscess appeared in the muscles of the right leg. The injections were discontinued for a short time in order to allow the abscess to heal. On Apr. 24, 1934, the face of the animal had a mask-like appearance and the animal was slightly ataxic. May 14, during the past 3 weeks the animal has gradually become more ataxic. No definite paralysis of the extremities was observed, but a right facial paralysis was present at this time. The injections were discontinued. May 21, the head is held turned towards the left. June 6, no appreciable change has been noted in the animal's condition since the injections were discontinued. The animal was sacrificed for examination.

Necropsy.—Aerobic and anaerobic cultures of bits of the brain and cord in meat-infusion broth, and aerobic cultures on blood-agar, on Petroff's egg medium, and on Sabouraud's medium remained sterile. To the unaided eye all the organs including the brain and cord appeared normal. Sections of the lungs, kidneys, liver, spleen, testicles, and muscle stained with hematoxylin and eosin and Giemsa's stain showed no lesions of importance. Sections of the central nervous system stained with hematoxylin and eosin and Giemsa's stain showed typical lesions in the cerebrum, cerebellum, pons, and cord. The meninges in the region of the cerebellopontine angle and at certain levels of the cord were also involved. De-

myelination in the cerebrum, cerebellum, and pons was demonstrated by means of Weigert's stain. Marchi and Weigert stains showed changes or destruction of myelin in the cord near the anterior and posterior fissures and near the surface of the cord posteriorly and laterally.

Results of Animal Passages.—One monkey received intracerebrally 1.0 cc. of an emulsion from the brain of Monkey 2. It remained well for 6 months at which time it was used in another experiment. When the animal was sacrificed at the end of the experiment all the organs were found to be essentially normal.

Monkey 3.—Monkey 3 received 85 injections. On Apr. 4, 1934, a slight ataxia was observed for the first time. May 21, the ataxia has gradually progressed. June 7, the animal has become very ataxic. No definite paralyses were observed. The animal was sacrificed for histological examination after a cisternal puncture had been made. The fluid was clear, contained 16 cells per c.mm. of which one was polymorphonuclear, and yielded a positive Pandy reaction.

Necropsy.—Cultures of bits of the brain made aerobically and anaerobically in meat infusion broth, and aerobically on blood-agar plates, on Sabouraud's medium, and on Petroff's egg medium remained sterile. All the organs including the central nervous system appeared normal to the unaided eye. Stained sections of the lungs, testicles, muscle, kidneys, pancreas, liver, adrenals, and spleen showed no lesions of importance. Sections of brain and cord stained with hematoxylin and eosin, Giemsa's stain, modified Weigert's stain, and Marchi's stain showed typical extensive lesions in the cerebellum and pons. The meninges in the region of the cerebellopontine angle were involved. The cerebrum and cord showed few if any noteworthy changes.

Results of Animal Passages.—One monkey received intracerebrally 1.0 cc. of an emulsion of bits of the brain of Monkey 3 and has remained well for a period of 7 months. The animal is still under observation.

Monkey 6.—Monkey 6 received 85 injections. On Oct. 10, 1933, an abscess occurred in the subcutaneous tissues of the right leg, and the injections were discontinued for 2 weeks in order to allow it to heal. On May 21, 1934, the animal was slightly unsteady in its movements. May 31, the monkey was definitely ataxic. June 6, the ataxia has progressed very rapidly and the animal has great difficulty in getting around in the cage. No definite paralyses were noted. The head was held twisted to the left. While the monkey was under ether anesthesia a cisternal puncture was made which yielded a clear fluid in which numerous small flocculi were seen. The fluid contained 280 cells per c.mm. (18 per cent polymorphonuclear and 82 per cent mononuclear elements) and gave a positive Pandy reaction. The animal was sacrificed for histological examination.

Necropsy.—Cultures of the brain made aerobically and anaerobically in meat infusion broth, and aerobically on blood-agar, on Sabouraud's medium, and Petroff's medium remained sterile. All the organs appeared normal to the unaided eye. Stained sections of the lungs, liver, spleen, pancreas, adrenals, testicles, kidneys, and muscle showed no significant changes. Sections of the brain and cord stained with hematoxylin and eosin and Giemsa's stain revealed typical

lesions in the cerebrum near the lateral ventricles, in the cerebellum and pons in the neighborhood of the 4th ventricle and cerebellopontine angle. The meninges in this neighborhood were also involved. Weigert's stain demonstrated destruction of myelin much of which had occurred around blood vessels. Marchi's stain showed degenerating myelin in the cord near the anterior fissure and in the lateral columns near the surface.

Results of Animal Passages.—A monkey received intracerebrally 1.0 cc. of an emulsion of bits of the brain of Monkey 6. The animal has remained well for a period of 7 months and is still under observation.

Monkey 4.—Monkey 4 received 85 injections. The animal never showed definite signs of involvement of the central nervous system. Nevertheless, during the last 3 months of the experiment it was docile and refused to run around very much. On June 7, 1934, while the animal was under ether anesthesia a cisternal puncture was made which yielded a clear fluid containing 42 cells per c.mm. (54 per cent polymorphonuclear and 46 per cent mononuclear elements) and gave a negative Pandy reaction. The monkey was then sacrificed for histological examination.

Necropsy.—Aerobic and anaerobic cultures of bits of the brain in meat infusion broth, and aerobic cultures on blood-agar, on Sabouraud's medium, and on Petroff's medium remained sterile. All the organs including the brain and cord appeared normal to the unaided eye. Stained sections of the lungs, kidneys, spleen, pancreas, adrenals, liver, testicles, and muscle showed no lesions of significance. Section of the brain and cord stained with hematoxylin and eosin, and modified Weigert's stain revealed a few typical lesions with destruction of myelin in the cerebrum, near the ventricles, and in the cerebellum and pons. Marchi's stain showed a few degenerating myelin sheaths near the anterior fissure of the cord.

Monkey 5.—Monkey 5 received 85 injections. At no time during the experiment did the animal exhibit signs of involvement of the central nervous system, and on June 6, 1934, it was sacrificed for histological examination after a cisternal puncture had been made. The fluid obtained was clear, contained 8 mononuclear cells per c.mm., and yielded a negative Pandy reaction.

Necropsy.—All cultures of the brain remained sterile. All of the organs including the brain and cord appeared normal to the unaided eye, and stained sections of them showed no significant lesions.

Control Monkeys.—Monkeys 9–16 were kept under conditions identical with those to which Monkeys 1–8 were subjected except that they received no injections. At no time did any of the control animals exhibit signs of involvement of the central nervous system. When the experiment was terminated 4 of the control animals, Monkeys 9–12, were sacrificed for complete histological examination. None of the organs including the brain and cord revealed lesions of significance.

The results of the experiment detailed above are summarized in Table II, an examination of which reveals that 6 of the 8 monkeys that received repeated intramuscular injections of aqueous emulsions

and alcohol-ether extracts of normal rabbit brain tissue developed signs of involvement of the central nervous system and that in the brains of 7 of the 8 animals lesions, extensive in many instances, were found by means of histological examinations. None of the control monkeys became sick and in the brains of 4 of them no lesions were found when histological examinations were made. The other 4 control monkeys were not sacrificed.

Ptosis of the eyelids, mask-like expression of the face, facial paralysis, abnormal position of the head (held to the right or to the left), blindness, and ataxia were the usual clinical signs and symptoms noted. Little or no paralysis of the extremities was detected. The animals did not have fever. There was a pleocytosis and an increased amount of globulin in the majority of the spinal fluids of the sick monkeys.

The injections were continued even after the monkeys evidenced signs of involvement of the central nervous system. This was done in order to obtain pronounced lesions for histological investigations. In one instance (Monkey 2), however, the injections were discontinued after the ataxia had developed. The animal was then observed for 3 weeks during which time no appreciable change in its condition was noted. At least there was no evidence that the animal would recover.

The localization of the lesions in the central nervous system is interesting. The cerebellum and pons, particularly in the neighborhood of the 4th ventricle, seemed to be the portions of the brain most frequently and most severely attacked. The cerebrum exhibited lesions which appeared most often near the ventricles. Nevertheless, some lesions were seen in the cortex. Except for tract degenerations which were seen in many instances, the cord of only 1 monkey showed pathological changes. The meninges, especially in the vicinity of the cerebellopontine angle, were usually, but not always, involved.

Description of the Pathological Changes in the Central Nervous System

Inasmuch as the pathological changes in the brains and cords of the different monkeys were almost identical in character, a general description of them will be made instead of giving in detail the findings in individual animals.

For the most part, the pathological changes seemed to have some

TABLE II

Summary of Results Obtained in Monkeys by Means of Repeated Intramuscular Injections of Aqueous Emulsions and Alcohol-Ether Extracts of Rabbit Brain

Monkey No.	No. of injections	Date of first signs	Date killed	Spinal fluid					Typical lesions with demyelination	Intracerebral passage to other animals			
				Fluid	Cell count	Differential: Polymorphonuclears	Differential: Mononuclears	Pandy		Monkeys	Rabbits	Guinea pigs	Mice
Injected monkeys													
		1933	*1934*										
8	46	Dec. 29	Jan. 6	Clear	220	40	60	±	+	2 neg.	2 neg.		6 neg.
		1934											
1	62	Feb. 19	Mar. 1	Clear	21	60	40	1+	+	2 neg.	2 neg.		6 neg.
7	62	Feb. 21	Mar. 12	Clear	6	0	100	±	+		2 neg.	1 neg.	6 neg.
2	80	Apr. 24	June 6						+	1 neg.			
3	85	Apr. 24	June 7	Clear	16	6	94	1+	+	1 neg.			
6	85	May 21	June 8	Contained flocculi	280	18	82	1+	+	1 neg.			
4	85	None ?	June 7	Clear	42	54	46	−	+				
5	85	None	June 6	Clear	8	0	100	−	−				
Uninoculated controls													
9	None	None	June 6						−				
10	None	None	June 6						−				
11	None	None	June 7	Clear	7	0	100	−	−				
12	None	None	June 8	Clear	18	5	95	−	−				
13	None	None											
14	None	None											
15	None	None											
16	None	None											

relation to the blood vessels. This was particularly true of the small lesions. When the changes became extensive, however, the perivascular distribution was not always easily discernible. Alterations in the vessels themselves, such as thrombosis, were not found. Both the gray and white matter were involved, the latter being more severely implicated.

The first stage in the development of the lesions was apparently a proliferation of cells giving rise to elements larger than those usually found in the supporting tissues of the brain (Figs. 1, 6, 7). Perivascular changes (Figs. 1, 5) made their appearance early and consisted of proliferation of glial elements and an infiltration of mononuclear cells and polymorphonuclear eosinophiles. As the pathological changes progressed compound granular corpuscles became exceedingly numerous, and in some of the large lesions hemorrhages occurred (Figs. 3, 4). One of the most striking features of the pathological picture was the presence of large numbers of polymorphonuclear eosinophiles. In many sections indefinite crystal-like structures were seen some of which were intracellularly situated.

Foreign body giant cells (Figs. 1, 2, 6) containing ingested cells and debris were not infrequently found in early as well as in advanced lesions. In addition to the giant cells there were multinucleated elements (Fig. 2) somewhat similar to the "globoid" cells seen in sections of the brain of a case of Schilder's disease and described by Collier and Greenfield (16) in the following manner:

> "In all regions where myelin destruction was active, particularly in those regions where it appeared to be of more recent date, there were large 'globoid' cells of peculiar character. Their nuclei were always multiple and sometimes were numerous, and were arranged as a chain of thin flattened nuclei under the capsule of the cell."

By means of a modified Weigert stain myelin destruction was noted in all parts of the brain. It was most extensive, however, around the ventricles and in the pons and cerebellum (Figs. 9, 10). Much of the demyelination was perivascularly situated (Figs. 11, 12). Often bits of the broken myelin sheaths had the appearance of large bacteria (Fig. 12). At other times they appeared as globules within phagocytic cells (Fig. 13).

DISCUSSION

The results of the experiment reported at this time are similar to those described by Rivers, Sprunt, and Berry (8). The pathological changes are identical in both sets of animals and in some respects do not resemble the spontaneous lesions of monkeys that have been described on previous occasions by other workers (9–15).

The appearance of foreign body giant cells in the brains of the affected animals is extremely interesting, and the character of the lesions observed is such that one would suspect that an infectious agent caused them. Nevertheless, we have been unable to demonstrate the presence of such an agent by means of stains and cultures. Furthermore, inoculation of monkeys, rabbits, guinea pigs, and mice with emulsions of the brains involved failed to disclose a transmissible agent. However, failure on our part to find an infectious agent does not necessarily mean that one was not present.

The fact that the control animals remained well seems to indicate clearly that the pathological changes which occurred in the brains of the treated monkeys were in some manner, either directly or indirectly, brought about as a result of the repeated intramuscular injections of aqueous emulsions and alcohol-ether extracts of sterile normal rabbit brain. Whether the aqueous emulsions or the extracts were responsible remains to be determined. Furthermore, it is possible that some obscure infectious agent was activated by the injections. If that be the case, then 7 of the 8 monkeys carried the agent and at least 2 of the 8 monkeys treated by Rivers, Sprunt, and Berry (8) also carried it.

The relation of our results to the paralysis accompanied by destruction of myelin that is known to follow the repeated injections of emulsified rabbit brain containing fixed rabic virus used in the vaccination of human beings against rabies, is not clear. In the Pasteur treatment of human beings 14 to 21 injections are made, and only about 1 of every 4000 vaccinated individuals becomes paralyzed. Each of our animals received more injections than are used in the Pasteur treatment, the smallest number being 46, the largest 85. It was not possible to buy and house several thousand monkeys. Consequently, we decided to use a large number of injections in a small group of animals. In any event we have experimentally produced lesions accompanied by myelin destruction in the brain of monkeys.

Further work is under way to determine the nature and the mechanism of the production of such lesions.

SUMMARY

The repeated intramuscular injections of aqueous emulsions and alcohol-ether extracts of sterile normal rabbit brains in some manner produced pathological changes accompanied by myelin destruction in the brains of 7 of 8 monkeys (*Macacus rhesus*). Eight control monkeys remained well. Cultures from the involved brains remained sterile, and no transmissible agent was demonstrated by means of intracerebral inoculations of emulsions of bits of the brains into monkeys, rabbits, guinea pigs, and white mice.

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EXPLANATION OF PLATES

PLATE 32

FIG. 1. Perivascular infiltration and giant cells. Hematoxylin and eosin. × 100.

FIG. 2. Two definite foreign body giant cells and a cell somewhat similar to the "globoid" cell described by Collier and Greenfield. Hematoxylin and eosin. × 400.

FIGS. 3 and 4. Typical advanced lesions. In Fig. 4 a hemorrhage has occurred in one of the lesions. Hematoxylin and eosin. × 100.

PLATE 33

FIG. 5. Type of perivascular infiltration observed; proliferation of glial elements, infiltration of mononuclear elements and polymorphonuclear eosinophiles. Hematoxylin and eosin. × 400.

FIGS. 6 and 7. Early lesions showing proliferative changes without the presence of polymorphonuclear eosinophiles. Hematoxylin and eosin. × 400.

FIG. 8. Edge of an advanced lesion showing proliferative changes and infiltration of polymorphonuclear elements. Hematoxylin and eosin. × 400.

PLATE 34

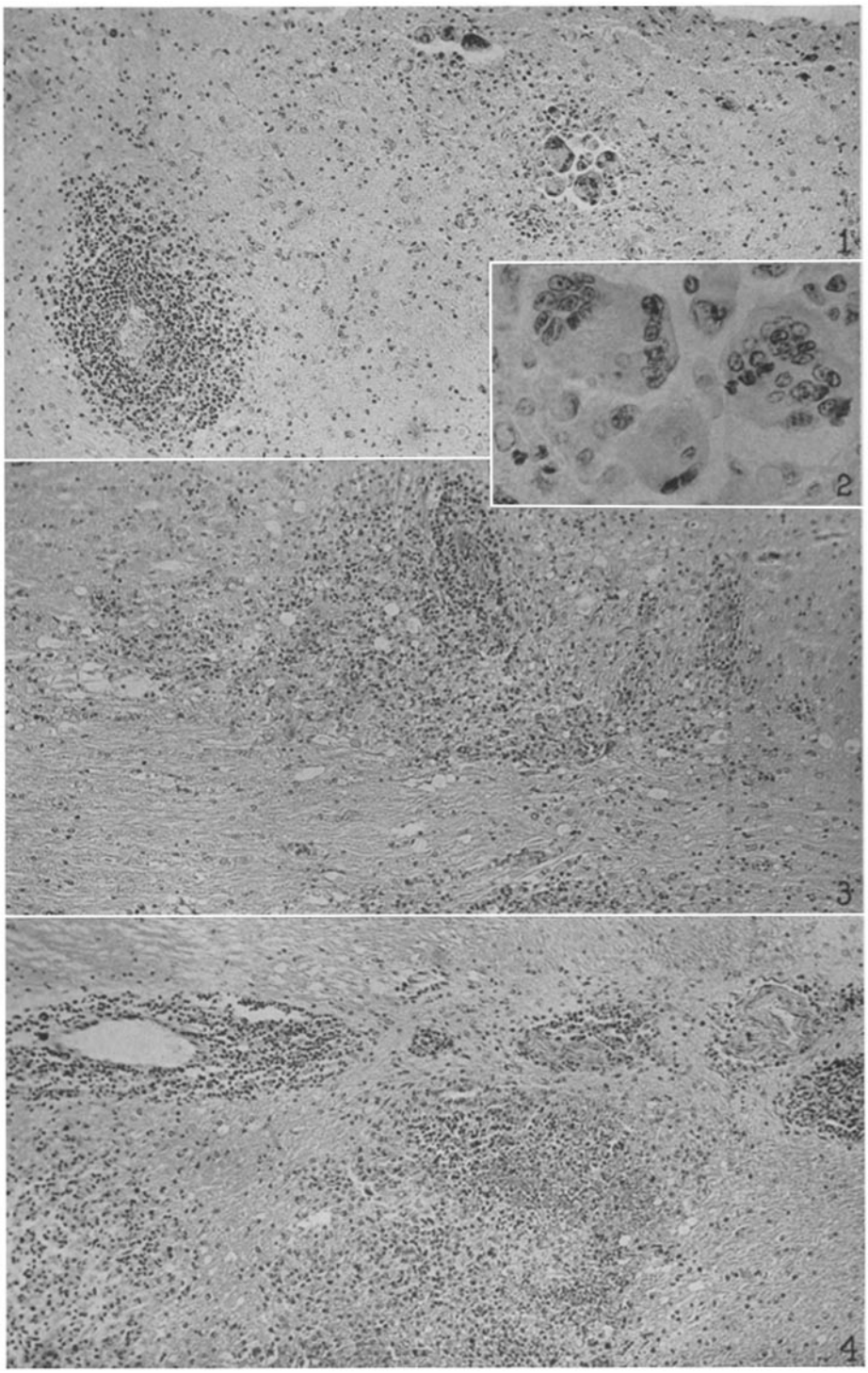
FIG. 9. Marked destruction of myelin in the pons, particularly around the central canal. Modification of Weigert's stain. × 3.5.

FIG. 10. Extensive destruction of myelin in the pons and cerebellum. Modification of Weigert's stain. × 3.5.

FIG. 11. Photograph to illustrate the perivascular distribution of the myelin destruction. Modification of Weigert's stain. × 37.

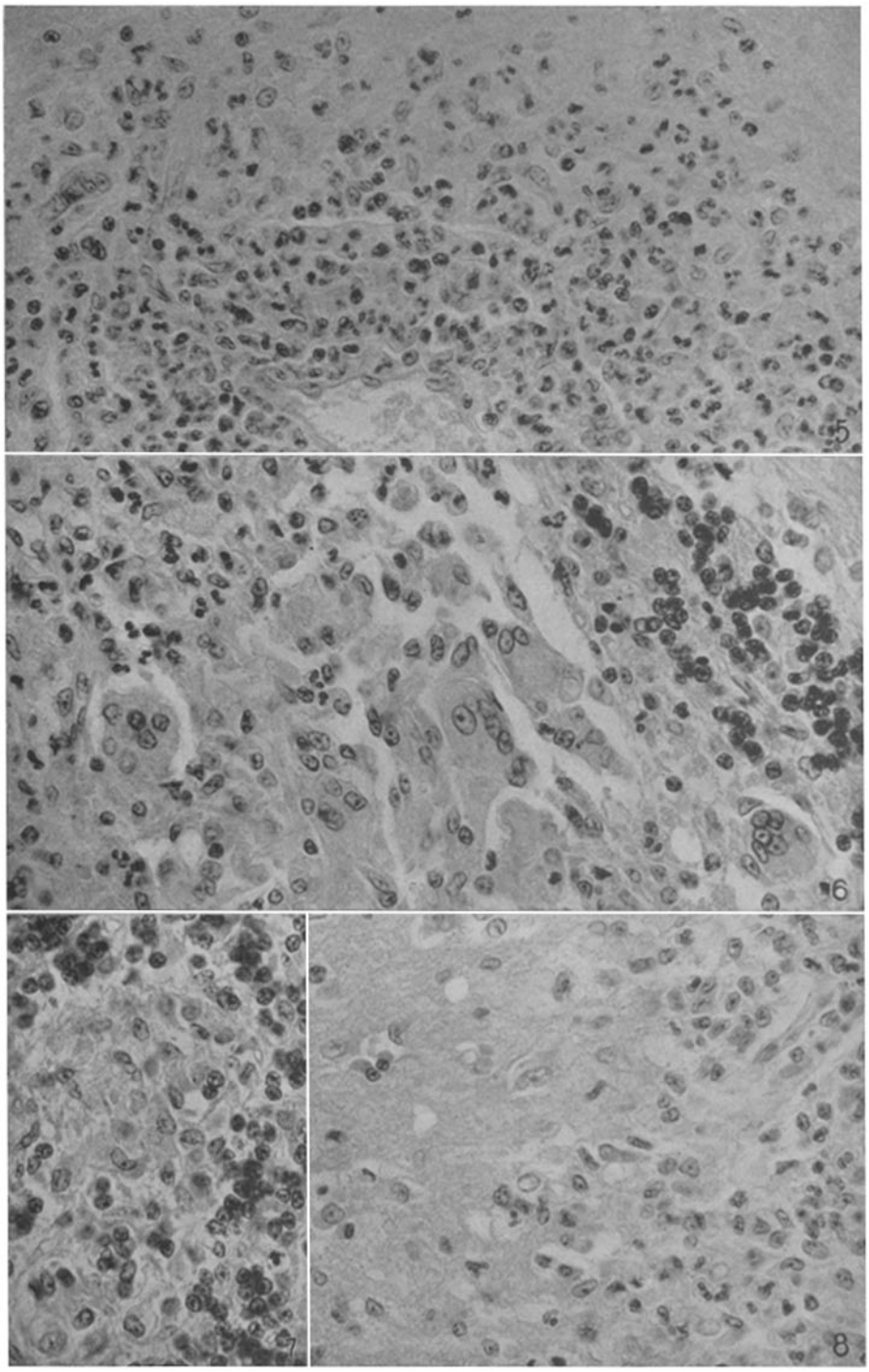
FIG. 12. Perivascular demyelination in the cerebral cortex. Note the broken myelin sheaths, bits of which have the appearance of large bacteria. Modification of Weigert's stain. × 400.

FIG. 13. Phagocytic cells filled with globules of myelin. Modification of Weigert's stain. × 400.



Photographed by Louis Schmidt

(Rivers and Schwentker: Experimental encephalomyelitis)



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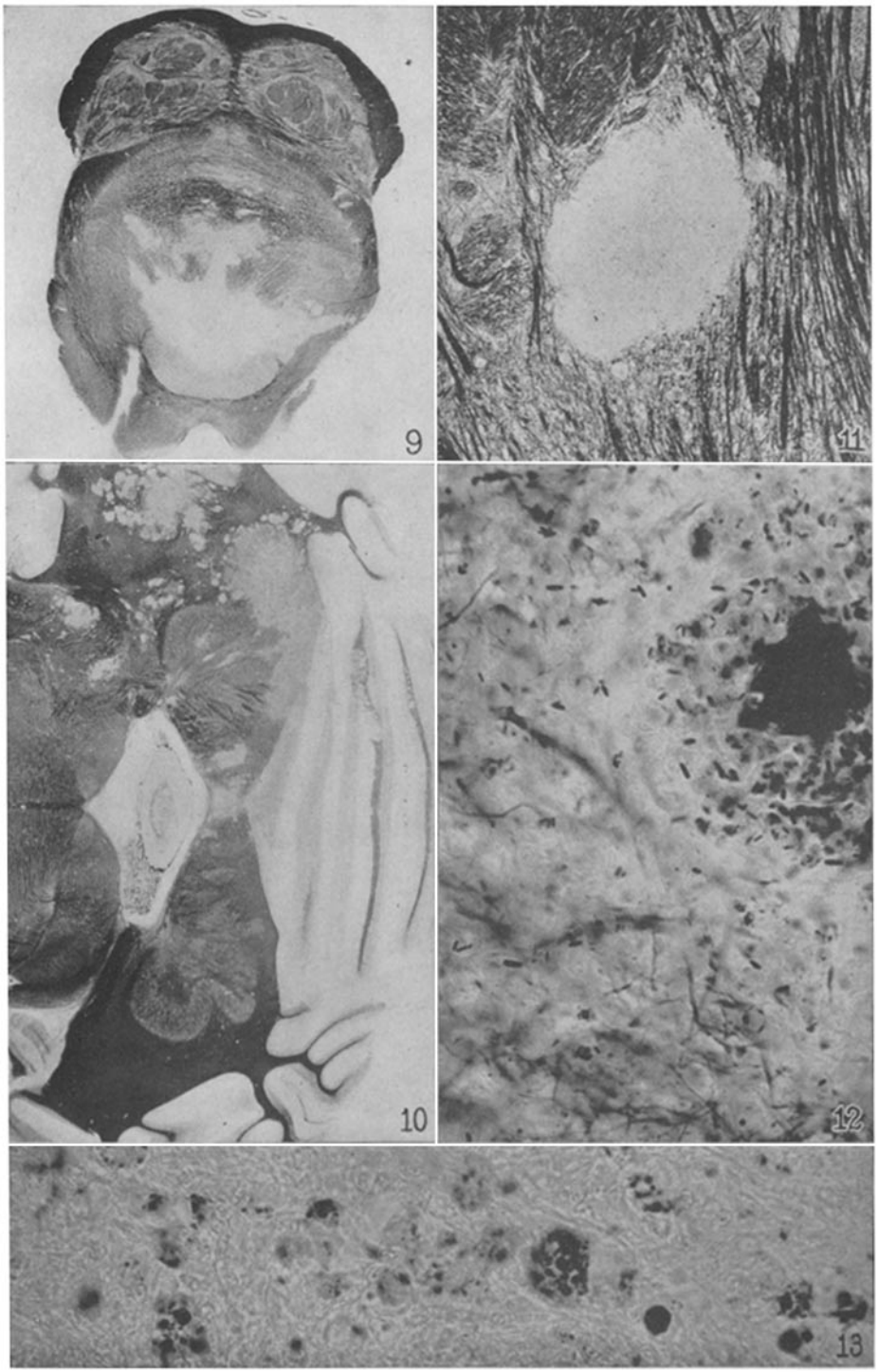

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