426 (1963); J. Carey and N. L. Warner, *ibid.* **203**, 198 (1964); L. G. Ortega and B. K. Der, *Fed. Proc.* **23**, 546 (1964).
7. M. D. Cooper, R. D. A. Peterson, R. A. Good, *Nature* **205**, 143 (1965).
8. O. C. Bruton, *Pediatrics* **9**, 722 (1952); R. A. Good, W. D. Kelly, J. Rötstein, R. L. Varco, *Progr. Allergy* **6**, 187 (1962); D. Gitlin, C. A. Janeway, L. Apt, J. M. Craig, in *Cellular and Humoral Aspects of the Hypersensitivity States*, H. S. Lawrence, Ed. (Hoeber-Harper, New York, 1959), p. 375.
9. D. Pressman, D. H. Campbell, L. Pauling, *J. Immunol.* **44**, 101 (1942); J. Gordon, B. Rose, A. E. Sehon, *J. Exp. Med.* **108**, 37 (1958).
10. J. J. Scheidegger, *Int. Arch. Allergy Appl. Immunol.* **7**, 103 (1955).
11. B. Glick, *Poultry Sci.* **39**, 1097 (1960).
12. R. L. St. Pierre and G. A. Ackerman, *Science* **147**, 1307 (1965); B. D. Janković and S. Leskowitz, *Proc. Soc. Exp. Biol. Med.* **118**, 1164 (1965).
13. J. Sterzl, J. Kostka, L. Mandel, I. Riha, M. Holub, in *Mechanisms of Antibody Formation* (Publishing House of the Czechoslovak Academy of Sciences, Prague, 1960), p. 130; G. J. Thorbecke, H. A. Gordon, B. Wostmann, M. Wagner, J. A. Reyniers, *J. Infect. Dis.* **101**, 237 (1957); D. L. Larson and L. J. Tomlinson, *J. Lab. Clin. Med.* **39**, 129 (1952); H. H. Zinneman and W. H. Hall, *Ann. Int. Med.* **41**, 1152 (1954); P. F. Layani, P. Grabar, A. Aschkenasy, A. Brussard, E. Lemétayer, *Sang* **21**, 62 (1950); P. B. Dent and R. A. Good, *Nature* **207**, 491 (1965); M. D. Cooper, R. D. A. Peterson, R. A. Good, in *Proc. Developmental Immunology Workshop*, 1st, Sanibel Island, Florida, in press.
14. S. Barandun, K. Stampfli, G. A. Spengler, G. Riva, *Helv. Med. Acta* **26**, 203 (1959).
15. Aided by grants from the National Foundation, American Cancer Society, U.S. Public Health Service (HE-02085, NB-02042, AI-00798), and American Heart Association. We thank Miss Ann Gabrielsen for criticism, Mrs. Ronee van Meter for technical assistance, and Miss Dolores Breen for the photomicrography.

9 November 1965

Autoimmune Encephalomyelitis and Ocular Lesions in Monkeys Sensitized during the Neonatal Period

Abstract. *The neonatal rhesus monkey is susceptible to the induction of autoimmune encephalomyelitis. The disease has been produced regularly by injection of neonatal animals with guinea pig spinal cord antigen in complete Freund's adjuvant. The onset of the disease, as compared with onset in adults, is delayed and is most often heralded by intrinsic eye lesions, notably widespread retinal hemorrhages.*

Autoimmune encephalomyelitis has been produced with regularity in mice, rats, guinea pigs, rabbits, dogs, cats, chickens, and monkeys (*1*) by injection of homologous or heterologous brain or spinal cord tissue emulsified in Freund's adjuvant containing mycobacteria. Study of this experimental syndrome, notably in the transfer of autoimmune encephalomyelitis by lymph-node cells in rats and guinea pigs (*2*), has proved that the disease is autoimmune in nature. The newborn animal, however, is refractory to this disease, as has been shown in rats and guinea pigs (*3*). This lack of susceptibility or increase of resistance has been ascribed variously to neonatal tolerance, lack of myelin, lack of access to the target organ, or a deficit in the "development" of committed cells at some stage between sensitization and response. Our own recent findings have indicated that, while some newborn guinea pigs are indeed completely or partially refractory to the disease, others are markedly susceptible; susceptibility depends on various genetic factors. For example, sensitization of newborn, inbred guinea pigs (strain 13) with homologous or isologous spinal cord, emulsified in Freund's adjuvant, results in a delayed appearance of a chronic form of autoimmune encephalomyelitis, characterized by severe damage to the spinal cord, chronic wasting, and clinical and pathological findings qualitatively different from those of the acute form in the adult animal (*4*).

In view of the differences among guinea pigs of various ages with respect to the disease, it was considered important to determine whether the newborn primate was susceptible to the induction of this disease, and, if this proved to be the case, to determine what pattern the disease would take.

We used six laboratory-bred monkeys of the species *Macaca mulatta* (rhesus), whose dates of conception and gestational age were known. The babies were delivered vaginally or by caesarean section and were 2 to 16 days of age when inoculated. From birth and throughout the experiment, the monkeys were individually housed, fed milk formula by bottle, and provided with intensive nursing care when required by their clinical status. Detailed and continuous clinical, neurological, and laboratory observations were made throughout the course of their disease.

Guinea pig spinal cord from inbred, immunologically homogeneous, strain 13 animals (*5*) was emulsified with complete Freund's adjuvant and injected intradermally into four sites in the scapular region. Each animal received a single dose of 0.5 ml of emulsion containing 0.25 ml of 50-percent suspension of spinal cord in phenol water, 0.25 ml of Arlacel-Bayol mixture, and 1 mg of killed *Mycobacterium tuberculosis* H37Rv (*2, 6*).

Neurological disorders were observed in all the monkeys, appearing 33 to 74 days after injection (Table 1). These were sometimes preceded or accompanied by conjunctivitis, retinal abnormalities, or both. The neurological signs were extremely variable, but those most commonly seen were visual disturbances with impaired pupillary reflexes, nystagmus, strabismus, ataxia, and severe motor impairment. In three cases the onset of symptoms

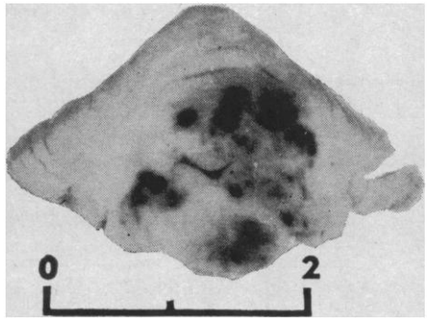

Fig. 1. Cerebellum and pons of an animal that died 1 day after symptoms appeared (58 days after injection). Note multiple large and small rounded areas of hemorrhagic infiltration and the distortion of landmarks. Scale in centimeters.

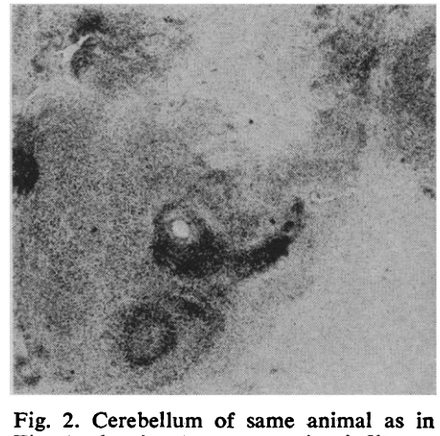

Fig. 2. Cerebellum of same animal as in Fig. 1, showing large, extensive infiltrates consisting of polymorphonuclear neutrophils, hemorrhage, and necrosis. Hematoxylin and eosin (× 23).

Table 1. Autoimmune encephalomyelitis in newborn monkeys. Symptoms: C, coma; M, motor impairment or paralysis; N, nystagmus; P, impaired pupillary reflexes; S, strabismus; T, torticollis; X, convulsions or myoclonic jerks. All animals were ataxic.

Gestational age (days)	Age at injection (days)	Birth weight (g)	Onset (days after injection)	Symptoms		Time of death (days after injection)
				Early	Late	
			Males			
162–163	16	555	74	X	C,M,N,P, S,T,X	173
167–168	10	533	33	C,N,S	N,P,S,T	Survives
			Females			
172–173	8		45	M,P,C		46
160–161	5	443	46	P,N,X		47
164–165	5	453	33–37	P,M,N	M,N,P,S,X	58
157–158*	2	580	57	M,N,P,S,C		58

* Delivered by caesarean section.

was followed rapidly by death; in the others a more prolonged course of neurological disorder with remissions and exacerbations of signs was seen, with death occurring as late as 6 months after the first appearance of symptoms; one of the animals still (12 months after onset) survives.

In addition to the oculomotor disturbances noted above, the pupillary reflexes to light were markedly depressed and sometimes completely absent. Two animals showed severe bulbar conjunctivitis, and one of these also showed marked pupillary irregularity. Ophthalmoscopic examination of the fundi revealed a hemorrhagic retinopathy in four of the animals. The retinal hemorrhages were multiple and flame-shaped, of sizes ranging from small to medium, and predominantly perivascular in distribution. Two of the animals developed a homogeneous red reflex in one eye suggesting unilateral, diffuse, intraretinal hemorrhage. Detailed clinical and pathological descriptions of the ocular disease will be reported elsewhere (*7*).

At autopsy almost all pathologic changes found other than those in the eyes were in the central nervous system. The gross lesions of the nervous system appeared hemorrhagic and consisted of multiple areas of reddish-gray discoloration associated with focal swelling and ranging in size from small to large. Other areas were grayish, translucent, and appeared softened. The lesions occurred in both the white and gray matter, particularly in the regions of the brainstem, including pons, medulla, and cerebellum (Fig. 1). Microscopically the lesions were primarily acute, perivascular, and inflammatory, and they consisted predominantly of infiltrates of polymorphonuclear neutrophils with varying amounts of hemorrhage, smaller numbers of lymphocytes and monocytes, and occasional plasma cells.

The perivascular distribution was most distinct in the smaller lesions. In many cases, the infiltrates were so numerous as to be completely coalescent, forming extensive areas of acute necrosis (Fig. 2). Fibrin occasionally accompanied the acute inflammatory exudate, and it was especially noted in the areas of acute necrosis. Such areas appeared in the medulla, pons, cerebellum, and, in one case, in the thalamus. Occasionally, lesions of a more chronic type accompanied the acute form; these consisted predominantly of lymphocytes and macrophages. In one animal there was severe vascular necrosis involving the media and intima of small- and medium-sized vessels. In addition, there were multiple foci of perivascular inflammation in the cerebral hemispheres and in the spinal cord in most animals, with less frequent acute necrotic lesions in these areas. The meninges showed varying amounts of chronic inflammatory reaction (Table 2).

Thus the infant rhesus monkey is immunologically responsive to injection of spinal cord antigens. In the infant monkey the disease may be clinically and pathologically different from that in the adult. The onset in the infant monkeys (*8*) usually is more delayed than in adult monkeys (*8, 9*). This longer period may be ascribed to: (i) a condition of partial immune tolerance; (ii) a state of immunological immaturity; (iii) immaturity of the route between sensitized lymphoid tissue and target organ (blood-brain barrier); or (iv) immaturity of the target organ itself (lack of myelin components). Antigen retained in a local adjuvant "depot" remains available until conditions for response become favorable. On the one hand, the disease in half of our animals had a rapid, fulminating course of approximately 24 hours, resulting in death. The abrupt clinical course was associated with extremely acute, severe, and frequently necrotizing lesions found in the central nervous system at autopsy; the features resemble some of those described by Levine and Wenk (*10*) for a hyperacute form of encephalomyelitis in the rat. On the other hand, the course of the disease in two animals was considerably prolonged and was accompanied by remissions and exacerbations; this clinical picture is suggestive of some human demyelinating diseases. The eye lesions seen in our animals included extensive retinal hemorrhages, exudates, fibrin deposition, and others (*7*). The eye lesions might be induced by injection of complete Freund's adjuvant alone, as Waksman and Bullington report for rats (*11*). However, these authors did not observe eye lesions in rabbits injected with complete Freund's adjuvant alone (*12*). The hemorrhagic retinopathy observed in our monkeys was not a feature of the ocular disease induced by these authors in rabbits (*12*), or by Kabat, Wolf, and Bezer in monkeys (*1*) with antigens from the central nervous system in complete Freund's adjuvant; and the mechanisms by which hemorrhagic retinopathy was induced in our monkeys may be different.

Table 2. Incidence of lesions in the central nervous system and the retina in fatal autoimmune encephalomyelitis of newborn monkeys.

Region affected	No. with lesions/ No. examined	
	Gross	Microscopic
Brainstem (pons, medulla, cerebellum, midbrain)	4/5	5/5
Cerebral hemispheres (cortex, white matter, thalamus, basal ganglia)	1/5	4/4
Spinal cord	1/4	2/3
Total central nervous system	5/5	5/5
Eyes (retina)	4/5	4/5

S. H. Stone, E. M. Lerner, II
R. E. Myers, W. H. Niemann
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, and *Laboratory of Perinatal Physiology, National Institute of Neurological Diseases and Blindness, San Juan, Puerto Rico*

References

1. E. A. Kabat, A. Wolf, A. E. Bezer, *Science* **104**, 632 (1946); *J. Exper. Med.* **85**, 117 (1947); I. M. Morgan, *J. Bacteriol.* **51**, 614 (1946); J. Freund, E. R. Stern, T. M. Pasani, *J. Immunol.* **57**, 179 (1947); M. M. Lipton and J. Freund, *ibid.* **71**, 98 (1953); P. K. Olitsky and R. H. Yager, *J. Exper. Med.* **90**, 65 (1949); P. Y. Paterson, E. D. Brand, *Fed. Proc.* **16**, No. 1839, 428 (1957), abstr.; W. G. Seller, *J. Pathol. Bacteriol.* **80**, 43 (1960); M. M. Lipton and A. J. Steigman, *J. Immunol.* **86**, 445 (1961).
2. P. Y. Paterson, *J. Exper. Med.* **111**, 119 (1960); H. Koprowski, G. A. Jervis, D. Schoeniger, *Fed. Proc.* **20**, 38 (1961), abstr.; S. H. Stone, *Science* **134**, 619 (1961).
3. M. W. Kies, *Ann. N.Y. Acad. Sci.* **122**, 239 (1965); H. A. Kaprowski, A. Paraf, R. Billingham, G. Jervis, *Comptes Rend.* **250**, 2956 (1960).
4. S. H. Stone and E. M. Lerner, II, *Ann. N.Y. Acad. Sci.* **122**, 227 (1965).
5. S. Wright, *U.S. Dept. Agric. Bull. No. 1090* (1922); J. A. Bauer, Jr., *Ann. N.Y. Acad. Sci.* **73**, 663 (1958).
6. J. Freund, *Adv. Tuberc. Res.* **7**, 130 (1956).
7. L. von Sallman, R. E. Myers, E. M. Lerner, II, S. H. Stone, in preparation.
8. A. Wolf, E. A. Kabat, A. E. Bezer, *Trans. Am. Neurol. Assoc.* **1948**, 92 (1948); A. Ferraro and C. L. Cazzulo, *J. Neuropathol. Exper. Neurol.* **7**, 235 (1948).
9. E. M. Lerner II, S. H. Stone, R. E. Myers, in preparation.
10. S. Levine and E. J. Wenk, *Science* **146**, 1681 (1964).
11. B. H. Waksman and S. J. Bullington, *Arch. Ophthalmol.* **64**, 75 (1960).
12. S. J. Bullington and B. H. Waksman, *ibid.* **59**, 435 (1958).

22 November 1965

Weaning of Young Rats: Effect of Time on Behavior

Abstract. *Male rats were weaned on the 15th, 16th, 17th, 25th, and 30th day of postnatal life. The rate of elaboration of the conditioned reflex at 8 months of age and the stability of the memory trace at 12 months were studied. Compared with rats weaned at 30 days of age, those weaned on day 15 elaborated the conditioned reflex much more slowly and the memory trace was less firm. Rats weaned on days 16 and 17 elaborated the conditioned reflex more quickly than rats weaned on day 15 but more slowly than those weaned on day 30. In all groups the memory trace showed the same stability and was significantly firmer than in rats weaned on day 15. The higher nervous activity of rats weaned at the age of 25 days more nearly resembles that of rats weaned at 15 days than of those weaned later.*

The time of weaning is considered to be the change from the diet of breast milk to that of the adult mammal. This change is usually gradual, and it is difficult to find out when the young animal has partaken of breast milk for the last time. We depend mostly on indirect findings (*1*) or experiences of breeders. Kon (*2*) and Leschi (*3*) cite the 21st day as the limit; Denenberg *et al.* (*4*, *5*) suggest that it is days 21 and 25. According to breeders this period starts between the 3rd and 4th weeks of life.

We have studied the relation of the higher nervous activity (the speed of the elaboration of the conditioned reflex and the stability of the memory trace) in adult rats to the length of the suckling period. We found that premature weaning on day 15 significantly influences the higher nervous activity throughout life (*6*, *7*).

We used male Wistar rats. The first group were those weaned on day 15, that is, at the time when the young ones can survive without the mother. The second group was weaned on day 16, the third on day 17, the fourth on day 25, and the fifth on day 30; in our laboratories we call day 30 the time of normal weaning. Each experimental group, consisting of male littermates, had 10 subjects. At the age of 8 months the conditioned reflex was elaborated. The rats were adapted in advance to the experimental situation until they learned to take in the whole daily dose of water only, in the conditioning box, within a few minutes. The conditioning stimulus was the sound of an electric bell; the unconditioned stimulus, the offering of water. During one experiment conditioned and unconditioned stimuli were administered ten times at intervals of 2 minutes. All animals were trained until they reached the acquisition criterion. A conditioned reflex was considered fully elaborated when the rat always (that is, in all ten trials of one experiment) showed the right reaction—drinking water from a tube under electrical registration. Between the conditioned stimuli alternating current was passed through the water which prevented the rats from drinking. At the age of 12 months the stability of the memory trace was tested by determining the percentage of positive responses in single groups of rats in the first session after a 4-month interval. We then studied how many conditioned and unconditioned stimuli are required for a complete reelaboration of the old conditioned reflex by comparing the rate of the first elaboration of the conditioned reflex and its reelaboration.

We tested the rate of the elaboration of the conditioned reflex in 8-month-old rats. Conditioned reflex was elaborated most quickly in rats weaned at 30 days of age. Rats weaned earlier than this elaborated the conditioned reflex more slowly. The slowest elaboration of the conditioned reflex occurred in rats weaned on day 15. The difference between day 15 and day 30 is statistically significant ($P \leq .001$). Rats weaned on days 16 and 17 elaborated the conditioned reflex at an intermediate time, and those weaned on day 25 can be placed between the groups

Table. 1 Rate of elaboration of conditioned reflex determined by the average number of connections of the conditioned and unconditioned stimuli (± the mean error). The statistical significance between the different groups of animals is also given; n.s., not significant.

Day of weaning	Number of connections	Significance ($P \leq$) between groups weaned on day			
		15	16	17	25
15	98.2±8.6				
16	74.6±5.8	.05			
17	62.0±8.3	.01	n.s.		
25	77.3±9.3	n.s.	n.s.	n.s.	
30	43.3±4.1	.001	.001	.05	.01

Table 2. Stability of the memory trace determined by the average of positive responses to the conditioned reflex (± the mean error). The statistical significance between the different groups is given; n.s., not significant.

Day of weaning	Responses (%)	Significance ($P \leq$) between groups weaned on day			
		15	16	17	25
15	15.0 ± 5.2				
16	60.0 ± 9.8	.001			
17	74.0 ± 8.8	.001	n.s.		
25	40.9 ± 10.1	.05	n.s.	.05	
30	76.7 ± 9.0	.001	n.s.	n.s.	.05

Table 3. Reelaboration of the conditioned reflex. The number of connections of the conditioned reflex is expressed as 100 percent (I); number of connections during reelaboration (II) is expressed as a percentage, calculated on the basis of the difference between I and II; n.s., not significant.

Day of weaning	I (%)	II (%)	$P \leq$
15	100	72.7	n.s.
16	100	28.5	.003
17	100	28.5	.003
25	100	30.0	.003
30	100	40.0	.01