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VIRULENCE ALTERATIONS OF TACARIBE VIRUS INFECTION IN ADULT MICE--ETC(U)
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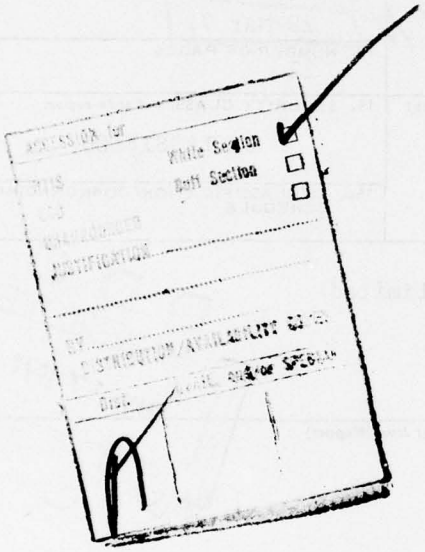

Virulence Alterations of Tacaribe Virus Infection in Adult Mice: Lethal Model for Fatal Encephalitis

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RUNNING HEAD: LETHAL TACARIBE INFECTION OF MICE

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Abstract

Selection of lethal substrains of Tacaribe virus strain 11573 was done by successive serial intracerebral (i.c.) passage of the virus in adult mice. Substrains have been partially characterized in suckling, weanling, and adult mice by determination of percent mortality and calculation of median intracerebral lethal dose values, and by histopathologic changes observed in brains of adult mice. Some of the derived virus substrains produced 80 - 90% mortality by second or third adult i.c. passage and maintained this virulence for 1 - 3 passages, after which the virulence rapidly declined with subsequent passages. Clinical signs of infection in adult mice were manifested by a rough hair-coat, ventriflexed posture, diminished activity, increased excitability, flaccid hind-limb extension with progressive paralysis and death. Histologic examination revealed meningoencephalitis.

Arenaviruses of the Tacaribe complex as originally isolated from nature are not lethal for adult rodents, although some have been shown to kill suckling mice and hamsters (6, 11, 13, 15). Two viruses, Tacaribe and Tamiami, have been adapted by successive suckling mouse brain passage to kill weanling mice with minimal increases in lethality for adult rodents (3, 5). Guinea pigs are marginally susceptible to unpassaged Junin and Machupo viruses. Junin, Machupo, and Lassa fever viruses are the only reported virulent human pathogens within the Tacaribe complex (2, 8, 10).

Controlled laboratory studies with the virulent human viruses of this complex have been hampered by the lack of suitable animal models. A guinea pig model was developed for Junin virus (12) and a monkey model for Machupo virus (6). Although such models exist, both viruses are extremely hazardous and laboratories with specialized containment facilities are required, thus limiting widespread utilization of the models. To date, adult animal models for assessment of lethality for the remaining members of the Tacaribe complex do not exist. In order to evaluate cross-protection between viruses of the Tacaribe complex not lethal for humans and to aid in elucidating the reported immunopathological nature of Tacaribe virus infection of mice it was necessary to develop a lethally infectious model using adult rodents. The present studies describe the selection of lethal subpopulations of Tacaribe virus, strain 11573, by successive passage in adult mouse brain.

MATERIAL AND METHODS

Mice: Flow DUB (ICR) (Flow Research Animals, Inc., Dublin, Va.) females received at 6 weeks of age or at various ages for age-dependent studies were used throughout with the exception of passage at the third level, which was in National (NLW) (National Laboratory Animals Co., O'Fallow, Mo.) specific-pathogen-free (SPF) female mice.

Viruses: Tacaribe virus strain TRVL 11573 obtained from American Type Culture Collection was designated VR-114. It had been passaged 21 times in normal baby mouse brains (nbM/21), cloned by three successive plaque isolations in rhesus monkey kidney cells and then passaged once again in nbM brains (nbM/22). Upon receipt at our laboratory it was further passaged once in suckling hamster brains (shb/1) passed again in nbM (nbM/23) and finally in shb. The final passage shb/2 became our working stock and was prepared as a 15-20% brain homogenate in TNE (Tris-HCl, 0.01 M, NaCl, 0.15 M, EDTA, 0.001 M) buffer pH 7.4 containing 20% heat-inactivated fetal calf serum (FCS). All virus-containing specimens were held at -70 C. Tacaribe virus strains 15007 and 12498 were received from Patricia Webb, Middle American Research Unit and had been passaged three times in suckling mouse brains (smb/3) and twice in diploid fetal rhesus lung cell culture (FRL/2). A single additional passage in FRL (FRL/3) was made upon receipt.

Passage Procedure: Serial 10-fold dilutions of virus strains in TNE buffer containing 20% inactivated (56 C for 30 min) FCS, horse, or rabbit serum were inoculated intracerebrally (i.c.) into 3-month-old mice. Brains of individual mice were removed when moribund, (8 to 12 days postinoculation), and stored at -70 C. It was necessary to inoculate mice i.c. with the maximum possible dose (10^5 pfu/0.03ml) in order to

obtain the infected brains to initate this study. Brains from individual mice were harvested separately. They were removed aseptically, ground in a chilled mortar and pestle as a 20% brain homogenate in TNE plus 20% serum, diluted 1:10 in the buffer with 20% serum, and repassaged in 3-month-old mice. A similar procedure was followed in the second passage level after which brains of infected mice from each passage were pooled to obtain large quantities of individual substrains. We measured virulence at selected passage levels for five substrains by inoculating serial 10 fold dilutions in suckling mice (1 to 3-days old), young mice (21 to 23-days old) and in adult mice (75 to 100-days old). Log median intracerebral lethal doses ($ICLD_{50}$) and mean time-to-death (MTD) were calculated. Brain tissue was taken for histopathological examination. Sera obtained from challenged survivors were tested for plaque reduction neutralization (PRN) antibody using parent strain 11573, complement-fixing (CF) antibody titers against strain 11573 and lymphocytic choriomenigitis (LCM) antigens, the presence of PPLO and bacterial contamination, and the presence of 11 adventitious mouse agents including LCM and GD VII viruses. Determinations for adventitious agents were done by Microbiological Associates, Walkersville, Md.

Histological Procedures: Tissue samples were fixed in 10% buffered neutral formalin, cut at 6 μm, and stained with hematoxylin and eoxin (H&E) for microscopic examination.

Plaque Assay: All virus-containing samples were assayed on Vero or BSC-1 cell monolayers grown in standard 6-well plates (10 cm^2/well). Serial 10-fold dilutions were added to the plates, incubated for 60 min at 37 C in 5% CO_2, and overlayed with basal medium (eagle) with Earle's salts, 25mM Hepes, 2% heat-inactivated FCS, 1% Agarose, and Penicillin (100

units/ml), and streptomycin sulfate (100 μg/ml). Plates were stained after 6 days incubation at 37 C with a solution of Puck's saline A containing 17.5 ml of 1:300 aqueous solution of neutral red/500 ml saline. Plaques (PFU) were counted 24 h later.

RESULTS

A preliminary study to correlate age with the i.c. virulence of parent Tacaribe virus indicated that, as the age of the mice increased, the percent mortality at a given dose level decreased, which was also indicated by an increasing PFU:$ICLD_{50}$ ratio (Table 1).

Data from a similar experiment using substrains of increased virulence at the third or fourth passage levels are given in Table 2. In contrast to the results obtained with parent virus, the $ICLD_{50}$ values remained constant for all the substrains in the various age groups, indicating that substrains had been selected with a much broader age range of lethality. This consistency of virulence among substrains is also reflected in the data for suckling mice (SM) (Table 2). The $SMICLD_{50}$ values remained at the indicated levels through passages 5 and 6 in adult mouse brain (data not shown). Thus, it appears that all substrains are similar, based on the mouse assays and could be used interchangeably in mouse studies.

Mean time to death and percent mortality were determined for five of the virus substrains obtained at each passage level of Tacaribe virus 11573 in adult mouse brain (Table 3). Virulence increased with successive, initial passages reaching a maximum of 80-90% by second or third passage, remained at 70-80% for one or two additional passages and then declined with subsequent passage. Since virulence decreased to 30-60%

by the seventh passage, additional loss of virulence beyond this level was not determined. Mean time to death values could not be correlated with virulence and were variable both between and within passage levels. Plaque forming unit determinations of live virus contained in aliquots used in the determination of mortality and $ICLD_{50}$ values were generally low, and resulted in $ICLD_{50}$:PFU ratios of 10:1 or greater. As passage levels increased, the PFU titers decreased and in some cases were undetectable in samples that were lethal by mouse assay.

The identity of both parent and derived substrains of Tacaribe virus were verified by several methods. Early in these studies, i.e. second and third passage levels, substrains were verified as Tacaribe virus by both CF and PRN tests using Tacaribe virus antibody prepared in rhesus monkeys. Virus strains were also shown to be free of PPLO and LCM virus, the latter by CF test. Sera from mice surviving i.c. challenge with second and third passage strains were free of both LCM and GD VII virus antibodies, the only common adventitious, murine viruses which elicit clinical and pathological signs which could be confused with those of Tacaribe virus infection. To verify further the identities of substrains, parent virus strain 11573 and strains 15007 and 12498 were used to immunize both 21-23 day-old and 77-day-old mice prior to i.c. challenge with 11573 and two second-passage strains of increased lethality for older mice.

A single subcutaneous dose of any of the three Tacaribe virus strains protected 21 to 23-day-old mice 20-93% survival compared to control mice depending on the challenge virus used (Table 4). Strain 15007 afforded greater protection than strain 12498 at the dosage levels

used. Second passage level substrains A and #4 killed 100% of the control mice, whereas parent 11573 resulted in only 80% mortality in 21 to 23-day-old mice. It should be noted that the developed substrains were used at challenge doses 2 to 3 logs lower than the parent virus. In the 77-day-old mice protection varied from 18 to 77% when compared to control mice (Table 5). The previously mentioned observation that parent virus has a decreased virulence for older mice was further confirmed in that 80% of the 21 to 23-day-old control mice and only 31% of the 77-day-old control mice succumbed to challenge. Substrain A showed a decreased virulence with increasing age (100% and 77% mortality in control groups). Parent virus strain 11573 and isolated substrains obtained by passage in adult mouse brains have therefore been identified as Tacaribe virus by the protection afforded against them in mice by Tacaribe virus strains 15007 and 12498. The variation in the degree of protection afforded by 15007 and 12498 against 11573 and the substrains with increased virulence is unexplained at this time, but may be a reflection of the variation in both protective and challenge dosages or may, in fact, represent actual antigenic strain variation.

Both the initial Tacaribe virus stock and substrains with increased virulence were inoculated i.c. into adult mice for observation of clinical signs and histopathological examination. In contrast to a previous report (1), in which 60-day-old mice did not become ill when inoculated i.c. with 1000 newborn mouse LD_{50}, infected adult mice exhibited rough haircoats, ventriflexed posture, and increased excitability in which CNS involvement was evident and progressed by days 6-8 postinoculation to include rear-limb extension, paralysis and eventual death by days 10-14. Histological examination of brains from these mice revealed

meningoencephalitis (mulifocal, perivascular, nonsuppurative, with neuronal necrosis most prominent in hippocampal gyri) and gliosis. Figures 1 and 2 compare the nature and extent of the pathological changes to normal uninfected mouse brains. When the normal hippocampus shown in 1a, is compared to infected hippocampus, segmental necrosis of neurons in the hippocampal gyrus and the presence of perivascular infiltrates were seen (Fig. 1b). Figure 1c is at higher magnification (400x) and shows a perivascular lymphoid cuff in the meninges in which necrosis is present in underlying cerebral cortex as indicated by the presence of pyknotic nuclei. When compared to the normal cerebr[illegible] the same area in the infected mouse (2b) reveals necrosis of [illegible] neuronal layer. The pia mater is artifactually torn but a mild increase in lymphocytes is evident in that portion remaining attached. The normal cerebellar folium is shown in Fig. 2c and in Fig. 2d, that of an infected mouse showing necrosis in the granular layer with many clear spaces containing cellular debris. The most consistent findings are the perivascular and meningeal lymphocytic infiltrates with extensive necrosis of the hippocampal neurons; small scattered foci of cerebral cortical necrosis are also present. Unpublished studies conducted in our laboratory in which 7 to 8-week-old female, Dunning-Fisher rats were infected i.c. with virulent substrains and sacrificed at the time of maximal CNS involvement (moribund, day 14) indicated similar histopathologic abnormalities. Examination of other organs of either rats or mice did not indicate any additional changes that could be attributed to the Tacaribe virus infection.

DISCUSSION

We have developed a rodent model of lethal infection for one of the viruses of the Tacaribe complex not pathogenic for humans. Lethal substrains were easily obtainable and were potentially usable in a variety of studies. With the exception of the Junin (12) and Machupo virus (6) models, the described system is the only adult model which allows investigations in which survival or death of the adult host animal is the basis for efficacy. The uniformity of strains as assayed in suckling, weanling, and adult mice of various ages (20 to 100-days old), as well as the uniformity of pathological lesions, indicate the isolation of a single entity of increased lethality rather than a number of substrains. The inability to sustain a lethal Tacaribe virus infection in adult mice was first reported by Downs, et al. (5) who also reported that the infection was ultimately lost on repeated passage. However, the number of passages required was not reported. Their observation that suckling mice inoculated with higher dilutions often died before those inoculated with lower dilutions and with increased mortality was also confirmed.

Verification of the isolated strains as Tacaribe virus by CF and PRN tests and by the protection elicited by Tacaribe virus strains 12498 and 15007 against the isolated strains conclusively identitfy the isolated substrains as Tacaribe virus, as do the negative tests for the presence of murine adventitious agents, specifically LCM and GD VII.

LCM, typically characterized by a nonsuppurative choriomeningitis, has been reported to occasionally produce lesions within the parenchyma of the brain. Changes are described as perivascular cuffing and gliosis within the caudate and lenticulate nuclei (4,9). Necrosis occurred

only when mice were chemically immunosuppressed and given immune spleen cells following infection with LCM virus (7).

Tacaribe-infected adult mice developed meningitis and, in one instance, choroiditis compatible with LCM virus-induced lesion. However, the two infections are distinguished from one another by the widespread foci of necrosis, gliosis, and perivascular cuffing within the brain parenchyma, particularly in the hippocampus, in Tacaribe-infected mice. The segmental necrosis of the hippocampal gyri that is consistently produced with Tacaribe virus is not a feature described in histopathological studies of LCM virus-infected mice.

LCM virus infection is often manifested as a persistant lifelong infection in newborn mice, while adult mice develop a fatal immunopathologically mediated disease of the central nervous system. Conversely, unpassaged Tacaribe virus is lethal in newborn mice, but avirulent in adults. The described substrains of Tacaribe virus are lethal by i.c. inoculation for mice up to and including 100 days of age. We are currently using the substrains in several studies to be reported seperately and anticipate their usefulness in elucidating the pathogenesis, and immunological responses in adult mice by viruses of the Tacaribe complex.

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LITERATURE CITED

1. Borden, E. C., and N. Nathanson. 1974. Tacaribe virus infection of the mouse: an immunopathologic disease model. Lab. Invest. 30: 465-473.

2. Buckley, S. M., and J. Casals. 1970. Lassa fever, a new virus disease of man from West Africa. III. Isolation and characterization of the virus. Am. J. Trop. Med. Hyg. 19:680-691.

3. Calisher, C. H., T. Tzianabos, R. D. Lord, and P. H. Coleman. 1970. Tamiami virus, a new member of the Tacaribe group. Am. J. Trop. Med. Hyg. 19:520-526.

4. Collins, D. N., H. Weigand, and J. Hotchin. 1961. The effects of pretreatment with x-rays on the pathogenesis of lymphocytic choriomeningitis in mice. II. The pathological histology. J. Immunol. 87:682-687.

5. Downs, W. G., C. R. Anderson, L. Spence, T. H. G. Aitken, and A. H. Greenhall. 1963. Tacaribe virus, a new agent isolated from Artibeus bats and mosquitoes in Trinidad, West Indies. Am. J. Trop. Med. Hyg. 12:640-646.

6. Eddy, G. A., S. K. Scott, F. S. Wagner, and O. M. Brand. 1975. Pathogenesis of Machupo virus infection in primates. Bull. WHO 52:517-521.

7. Gilden, D. H., G. A. Cole, and N. Nathanson. 1972. Immunopathogenesis of acute central nervous system disease produced by lymphocytic choriomeningitis virus. II. Adoptive immunization of virus carriers. J. Exp. Med. 135:874-889.

8. Johnson, K. M., N. H. Wiebenga, R. B. Mackenzie, M. L. Kuns, N. M. Tauraso, A. Shelokov, P. A. Webb, G. Justines, and H. K. Beye. 1965. Virus isolations from human cases of hemorrhagic fever in Bolivia. Proc. Soc. Exp. Biol. Med. 118:113-118.

9. Lillie, R. D., and C. Armstrong. 1945. Pathology of lymphocytic choriomeningitis in mice. Arch. Pathol. 40:141-152.

10. Parodi, A. S., D. J. Greenway, H. R. Rugiero, M. Frigerio, J. M. de la Barrera, N. Mettler, F. Garzon, M. Boxaca, L. Guerrero, and N. Nota. 1958. Sobre la etiologia del brote epidemico de Junin. Dia Med. 30:2300-2301.

11. Pinheiro, F. P., R. E. Shope, A. H. Paes de Andrade, G. Bensabath, G. V. Cacios, and J. Casals. 1966. Amapari, a new virus of the Tacaribe group from rodents and mites of Amapa Territory, Brazil. Proc. Soc. Exp. Biol. Med. 122:531-535.

12. Tauraso, N., and A. Shelokov. 1965. Protection against Junin virus by immunization with live Tacaribe virus. Proc. Soc. Exp. Biol. Med. 119:608-611.

13. Trapido, H., and C. Sanmartin. 1971. Pichinde virus, a new virus of the Tacaribe group from Colombia. Am. J. Trop. Med. Hyg. 20: 631-641.

14. Webb, P. A., K. M. Johnson, J. B. Hibbs, and M. L. Kuns. 1970. Parana, a new Tacaribe complex virus from Paraguay. Arch. Gesamte Virusforsch. 32:379-388.

15. Webb. P. A., K. M. Johnson, C. J. Peters, and G. Justines. 1973. Behavior of Machupo and Latino viruses in Calomys callosus from two geographic areas of Bolivia. p. 313-322. In F. Lehmann-Grube (ed.), Lymphocytic choriomeningitis virus and other arenaviruses. Springer-Verlag, Berlin.

Table 1. Effect of age on resistance of mice (n = 20) to i.c. challengenge with parent Tacaribe virus strain 11573

Age (Days)	Challenge dose Log_{10} PFU/0.03 ml	Mortality %	MTD (Days)	Log_{10} PFU:$ICLD_{50}$
21	5	100	7.1	
	4	80	9.6	
	3	55	10.8	2.7
	2	20	10.5	
	1	20	9.8	
31	5	100	10.2	
	4	50	10.2	
	3	26	10.8	3.4
	2	20	12.3	
	1	5	15.0	
41	5	90	10.2	
	4	35	11.4	
	3	15	11.7	3.9
	2	10	15.5	
	1	0	-	
51	5	90	11.2	
	4	45	12.2	
	3	20	11.5	3.8
	2	10	11.5	
	1	0	-	
61	5	65	9.2	
	4	40	11.0	
	3	25	10.6	4.8
	2	15	9.7	
	1	15	20.0	

Table 2. Determination of $ICLD_{50}$ values/0.03 ml of substrains of Tacaribe virus in mice of different ages (N = 8)

Strain (Passage level)	$ICLD_{50}$ by age of mice in days					
	1 to 3	30	40	50	60	70
A_3		2.88	2.77	2.77	2.87	2.00
B_4		2.35	2.71	2.32	2.32	2.42
5_4	3.51,3.17	2.50	2.76	2.25	2.47	2.46
6_4	3.61,3.50	2.87	2.84	2.51	2.85	2.35
7_4	3.11,3.40	2.39	2.85	2.50	2.57	2.50

Table 3. Determination of percent lethality and mean time to death values of 75 to 100-day old mice[a] inoculated intracerebrally[2b] with Tacaribe viruses

Strain	Passage Level 1		2		3		4		5		6		7	
	%	MTD[c]	%	MTD	%	MTD	%	MTD	%	MTD	%	MTD	%	MTD
Parent 11573	20	8.7												
Substrain A			29	12.2	75	10.6	63	9.9	55	10.0	28	10.0		
					75	12.4	80	12.9	56	14.0	20	13.4		
					77	12.4	75	11.6						
					57	12.4								
Substrain B			36	12.3	93	10.9	75	10.6	58	10.3	42	11.4	35	14.6
					84	12.3	75	10.9			13	8.5		
					71	13.2	40	9.5						
					67	12.1								
Substrain 5			87	11.2	87	11.2	85	9.7	74	9.3	55	13.6	53	11.2
					80	13.5	67	13.4	80	14.0	42	10.6		
					80	14.1	85	10.7						
					90	10.8								
					60	18.1								
Substrain 6			90	11.4	100	11.5	60	9.7	70	9.8	60	11.3	28	12.2
			100	11.5			67	12.1	70	12.2				
Substrain 7			90	11.4	87	8.5	75	9.6	60	9.8	60	12.3	61	12.2
			87	8.5			73	12.4	95	12.5				
							75	10.6						

[a] n= 10-20 per point.

[b] 0.03 ml containing 10^5 PFU (parent) or $<10^2$-10^3 PFU (substrains).

[c] Mean time to death in days.

Table 4. Protection of 20 to 23-day-old mice given a single 0.1 ml subcutaneous dose of three strains of Tacaribe virus against subsequent 0.03 ml i.c. challenge with parent and second passage substrains. (n = 15)

Immunizing[a] virus	Challenge dose (PFU)	Survival %	MTD (Days)
11573	11573	93	8.0
15007	(1×10^5)	100	-
12498		80	9.7
Control		20	8.3
11573	Substrain A	93	13.0
15007	(8×10^2)	73	13.5
12498		20	12.4
Control		0	10.7
11573	Substrain 4	93	12.0
15007	(1×10^3)	80	11.3
12498		53	9.7
Control		0	10.7

[a] Immunizing doses (PFU):
11573 = 1×10^3,
15007 = 2×10^4,
12498 = 8×10^3.

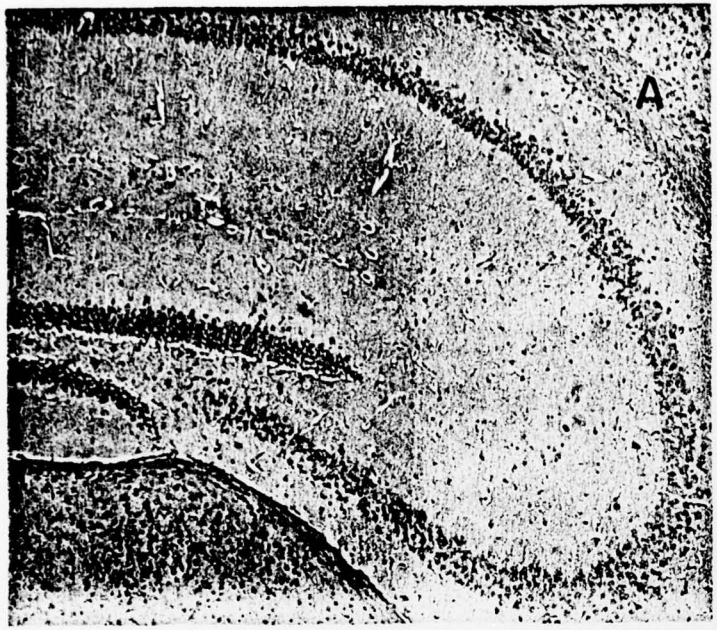
Table 5. Protection of 77-day-old-mice given a single 0.1 ml subcutaneous dose of three strains of Tacaribe virus against subsequent 0.03 ml i.c. challenge with parent and second passage substrains (N = 15)

Immunized[a] virus	Challenge dose (PFU)	Survival %	MID (Days)
11573	11573	87	10.0
15007	(2×10^5)	93	9.0
12498		93	9.0
Control		69	4.1
11573	Substrain A	100	-
15007	(2×10^3)	69	13.8
12498		69	13.7
Control		23	10.8
11573	Substrain B	92	4.0
15007	(6×10^3)	86	8.0
12498		87	12.0
Control		33	12.1

[a] Immunizing doses (PFU):
11573 = 1×10^6,
15007 = 3×10^4,
12498 = 7×10^3.

Fig. 1. Comparison of control and Tacaribe virus infected adult mouse brains. A. Normal hippocampus, B. Infected hippocampus showing segmental necrosis of neurons and perivascular lymphocytic infiltrates, C. Perivascular infiltrate in meninges of the cerebrum (400X).

Fig. 2. Comparison of control and Tacaribe virus infected adult mouse brains. A. Normal cerebrum, B. Infected cerebrum with evidence of necrosis of the neuronal layer and a mild increase in lymphocytes, C. Normal cerebellar folium, D. Infected cerebellar folium, showing necrosis of granular layer, presence of cellular debris, and almost total loss of Purkinje cell layer.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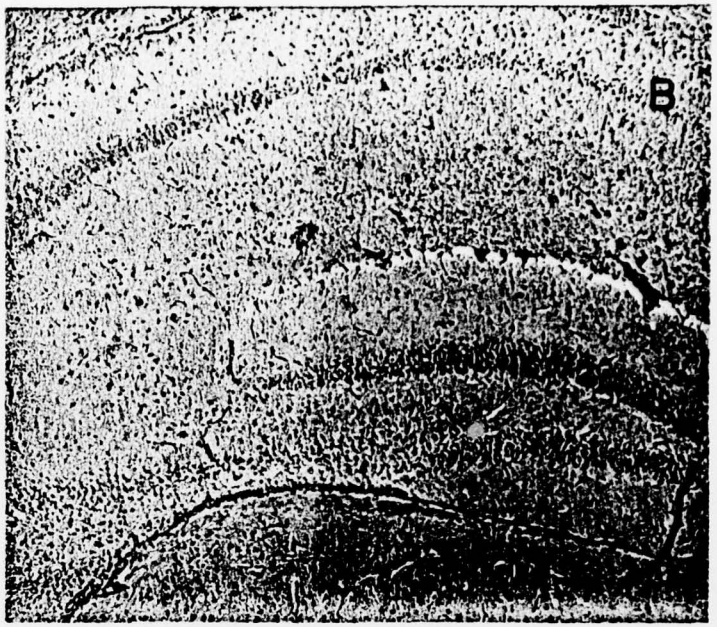
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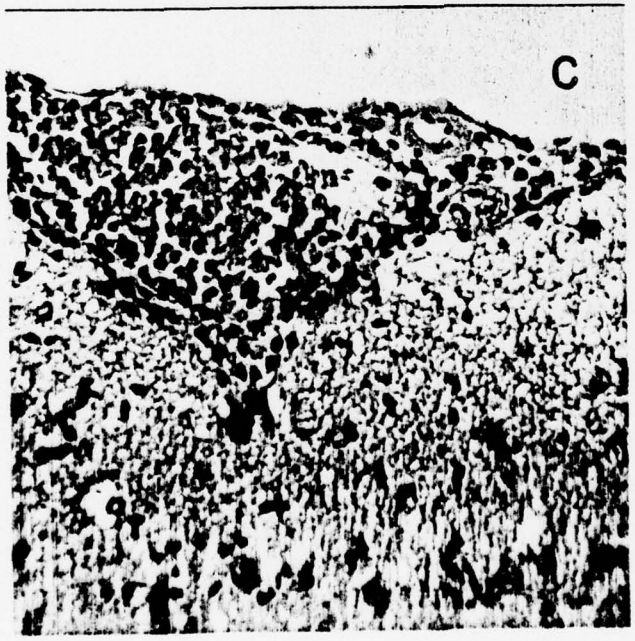
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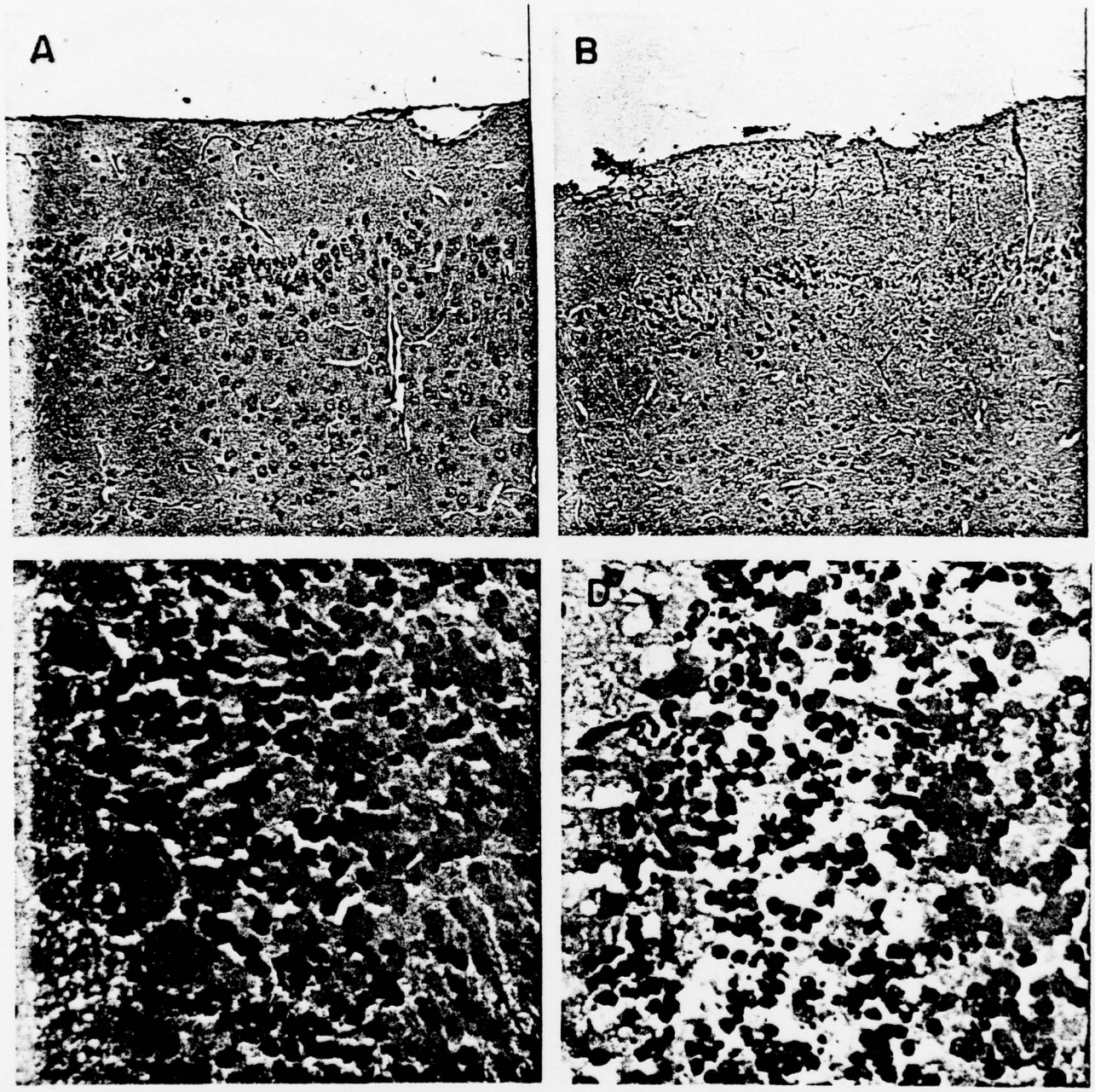
A
B
D