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NOTES

Two African Viruses Serologically and Morphologically Related to Rabies Virus

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Lagos bat virus and an isolate from shrews (IbAn 27377), both from Nigeria, were found to be bullet-shaped and to mature intracytoplasmically in association with a distinct matrix. They were related to, but readily distinguishable from, rabies virus and each other by complement fixation and neutralization tests. The three viruses, including rabies, form a subgrouping within the rhabdoviruses.

Despite repeated testing by various serological techniques, an antigenic relationship between rabies and other viruses, including recognized rhabdoviruses, has heretofore never been found. The serological studies reported here in preliminary form, which relate Lagos bat virus and another African isolate, IbAn 27377, to rabies virus, were initially prompted by electron microscopic observations.

Lagos bat virus was isolated from the brain of a Nigerian fruit bat (*Eidolon helvum*) in 1956 at Lagos Island, Nigeria, by Boulger and Porterfield (1). They found that it was pathogenic for 5- to 6-week-old mice by the intracerebral (ic) but not by the intraperitoneal (ip) route. The virus did not kill guinea pigs, rabbits, or *Cercocebus torquatus* monkeys by peripheral inoculation. Pathological effects of infection in mouse brain included perivascular inflammatory infiltration and neuronal degeneration, but inclusion bodies were not seen.

Three strains of another virus were isolated in 1968 from pooled lung, liver, spleen, kidney, and heart of shrews (*Crocidura* sp.) captured in and near Ibadan, Nigeria. IbAn 27377 was selected as the reference strain. This virus, used as second to fifth mouse brain passage material, killed 3-day-old Swiss mice with an average survival time of 4 days after ic inoculation. Ten-day-old mice survived ic inoculation about 2 days longer and usually resisted ip inoculation completely. The virus had an infectivity titer in 3-day-old mice of 10^5 to 10^6 LD_{50}/0.02 ml. Pretreatment with chloroform reduced the titer by 10^4 LD_{50}. Inclusion bodies compatible with Negri bodies were not observed in fourth-passage infected mouse brain by light microscopy.

In brain tissues from hamsters and newborn and weanling mice infected with Lagos bat virus and examined by electron microscopy, bullet-shaped virus particles were found in many neurons (Fig. 1). Virus particles were approximately 180 nm in length and 65 nm in diameter. Maturation occurred via budding through intracytoplasmic membranes; viral matrix or inclusion material, similar to the massed nucleoprotein material described in rabies virus-infected cells by Hummeler et al. (3), accumulated within cytoplasm beneath budding virus. Viral budding from neuronal plasma membranes was observed rarely. In all aspects, Lagos bat virus resembled street rabies virus ultrastructurally. IbAn 27377 virus, in hamster and mouse brain, was also indistinguishable in morphology and mode of replication from rabies virus.

Serological testing involved complement fixation (CF) and two different types of serum neutralization tests. A relationship of Lagos bat and IbAn 27377 viruses to each other and to rabies was evident by CF test (Table 1). Antigens of all three reacted with rabies virus and IbAn 27377 mouse ascitic fluids; the ascitic fluid for Lagos bat virus appeared specific by CF but was less potent than the other preparations. The three viruses were equally distinguishable in repeated CF tests. In addition, the same reagents were tested by CF reciprocally with other animal viruses presently included within the rhabdovirus group: vesicular stomatitis (Indiana and New

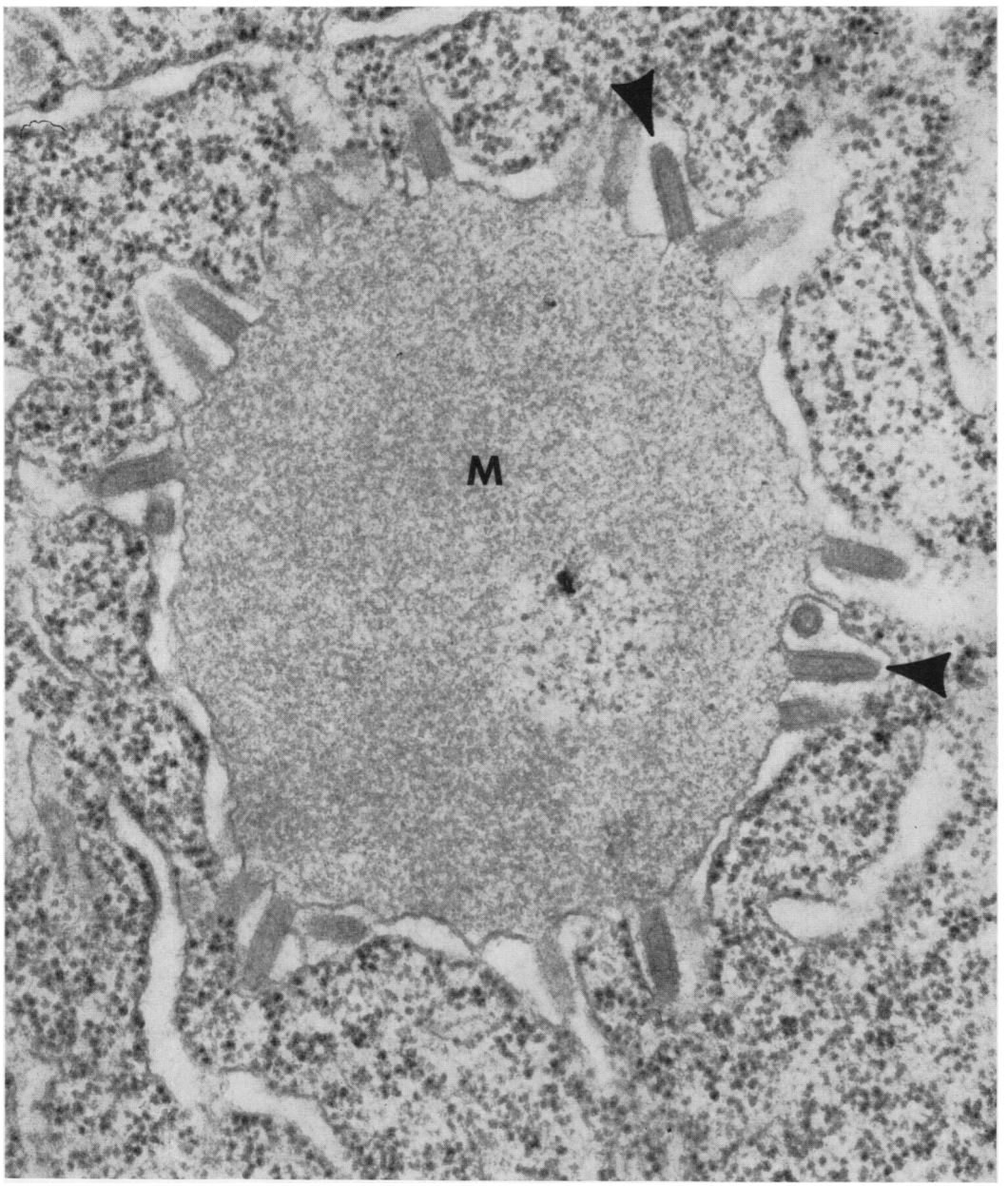

FIG. 1. *Lagos bat virus (arrows) budding from intracytoplasmic membranes in a hippocampal neuron of a hamster. Viral matrix (M) formed inclusions too small to be seen by light microscopy. × 64,000.*

Jersey serotypes), Cocal, Chandipura, Piry, Hart Park, Flanders, M-1056, Mount Elgon bat, and Kern Canyon viruses. (M-1056 is a bullet-shaped virus from *Microtus*, supplied by Harald N. Johnson. Mention here is not intended to constitute formal publication of this new agent.) These antigen and antibody preparations, which yielded homologous antibody titers of 1:64 to 1:256, showed no cross-reactivity with Lagos bat virus and IbAn 27377 virus.

Neutralization tests, carried out at the Yale Arbovirus Research Unit (YARU), in newborn mice by ic inoculation of undiluted hyperimmune mouse ascitic fluid versus 10-fold dilutions of

TABLE 1. *Complement fixation reactions of rabies-related viruses*

Antigen	Hyperimmune mouse ascitic fluid			
	Rabies	Lagos bat	IbAn 27377	Control
Rabies, Pasteur	512/512[a]	0/0	128/512	0/0
Lagos bat	32/128	64/128	128/512	0/0
IbAn 27377	32/32	0/0	512/512	0/0
Control	0/0	0/0	0/0	0/0

[a] Titer expressed as reciprocal of dilution: ascitic fluid/antigen; 0 = <4.

TABLE 2. *Serum-dilution neutralization test of rabies-related viruses*

Virus	Inoculum: mouse LD_{50}	Serum (S) or mouse ascitic fluid (MAF)			
		Rabies burro S	Lagos bat MAF	IbAn 27377 MAF	Control MAF (M-1056)
Rabies, strain CVS	160	15,625[a]	11	0[b]	0
Rabies, vampire bat	200	15,625	11	1	0
Lagos bat	50	5	280	18	0
IbAn 27377	100	3	15	320	0
Control (M-1056)	125	0	0	0	25

[a] Reciprocal of 50% neutralization end point of serum or ascitic fluid; fivefold serum dilutions versus constant virus dilution.

[b] No survivors.

Pasteur strain rabies virus (mouse passage 202) gave the following log neutralization indexes (LNI): 3.1 with IbAn 27377 (homologous LNI >3.2), 0.4 with Lagos bat, and >5.3 with two rabies virus ascitic fluids prepared against Pasteur and TRVL 5843 strains. At the University of Ibadan, a horse antirabies serum with a homologous LNI of >2.9 failed to neutralize IbAn 27377 virus.

To study the neutralization phenomenon further, serum dilution versus constant virus neutralization tests were performed at the National Communicable Disease Center (NCDC) in baby mice by ic inoculation. These tests employed reagents different from above (except for the IbAn 27377 ascitic fluid) and included street rabies strains. There was neutralization of all three viruses by at least one serum or ascitic fluid of each virus (Table 2). The three agents were, however, readily distinguishable. The results of constant serum-varying virus dilution neutralization tests showed a degree of cross-reactivity comparable to that seen in the CF tests and greater than that seen in the serum dilution neutralization tests in which differences were marked. Potent antisera preparations used in low dilution appeared to be required for clear demonstration of the serological relationships.

Minor antigenic differences among rabies strains have been reported (2, 4) and have been noted in repeated CF and neutralization testing of isolates at YARU and NCDC; however, the magnitude of these differences has not corresponded to the relationship found here between Lagos bat virus, IbAn 27377 virus, and rabies virus. The degree of cross-reactivity among the three substantiates a distinct subgrouping within the rhabdovirus group.

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