ELECTRON MICROSCOPY OF KOONGOL GROUP ARBOVIRUSES

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Abstract

Koongol and Wongal virions are isometric, enveloped, and 90–100 nm in diameter. They develop by budding into smooth-surfaced vesicles in or near the Golgi region of the cytoplasm of infected cells. In morphology and morphogenesis they thus resemble other members of the Bunyamwera supergroup of arboviruses.

I. Introduction

The arboviruses comprise a very large and heterogeneous collection, consisting at present of more than 250 viruses (Taylor 1967). About 70% of these can be classified into antigenic groups by means of complement fixation and haemagglutination-inhibition or neutralization tests, but there are at least 28 such groups (W.H.O. Scientific Group 1967) so that classification of isolates is laborious.

Casals (1963) simplified this grouping when he found serological cross-reactions between members of the groups Bunyamwera, California, Bwamba, Simbu, and Koongol, and reported that Shope had found similar distant relationships between the groups C, Capim, and Guama. All these groups except Koongol were later included in the Bunyamwera supergroup (W.H.O. Scientific Group 1967) and the Patois group has since been added (Zarate *et al.* 1968). For practical purposes, however, the original separate groups have remained the units of classification.

Recent electron microscopic studies also show great promise for simplifying arbovirus classification, and bringing it into line with that of other types of virus. Several arboviruses in minor serological groups have been shown to be rhabdoviruses (Murphy *et al.* 1970; Holmes and Doherty 1970) and a number of others are morphologically similar to bluetongue (Murphy *et al.* 1968*b*; Schnagl, Holmes, and Doherty 1969; Verwoerd 1970; Borden *et al.*, unpublished data), and will form part of a new virus group.

The structure and morphogenesis of arboviruses of groups A and B have been well studied. While their basic structure appears similar, viruses from these two groups differ in the size and site of maturation of their particles (Acheson and Tamm 1967; Murphy *et al.* 1968*c*).

Despite the fact that the Bunyamwera supergroup is now the largest serologically defined group of arboviruses, and in fact includes more members than groups A and B together, until recently few morphological studies have been carried out on its members. Murphy *et al.* (1968*a*, 1968*d*) have shown that several viruses of the Bunyamwera and California groups resemble each other and their particles differ

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morphologically from those of viruses of groups A and B. A comparative study of viruses from the groups Bunyamwera, California, C, Simbu, and Guama (Holmes 1971) has confirmed and extended the findings of Murphy *et al.*; the particles of all the supergroup viruses examined have the same size and morphogenesis.

We therefore examined the morphology and development of virions of the Koongol group viruses in order to check whether they, too, resembled those of other members of the supergroup, since the serological relationship was equivocal.

II. Materials and Methods

(*a*) *Viruses*

Freeze-dried specimens of Koongol virus (strain MRM 97, seven passages in suckling mouse brain) and Wongal virus (strain MRM 168, five passages) were kindly provided by Dr. R. L. Doherty, Queensland Institute of Medical Research. After two or three further intracerebral passages in 1-day-old suckling mice, 20% suspensions of infected brains had infectivity titres of $10^{8\cdot5}$ and $10^{8\cdot7}$ mouse LD_{50} per millilitre respectively.

(*b*) *Experimental Animals*

Litters of 1-day-old Melbourne University albino strain mice were inoculated intracerebrally with 0·015 ml of one-tenth diluted stocks of Koongol or Wongal viruses. From 1 day after inoculation, brains were harvested at intervals of about 12 hr until the mice were moribund (about 76 hr). Control specimens of brain were taken from suckling mice $58\frac{1}{2}$ hr after intracerebral inoculation of 0·015 ml of diluent, which was 0·75% bovine serum albumin in normal saline.

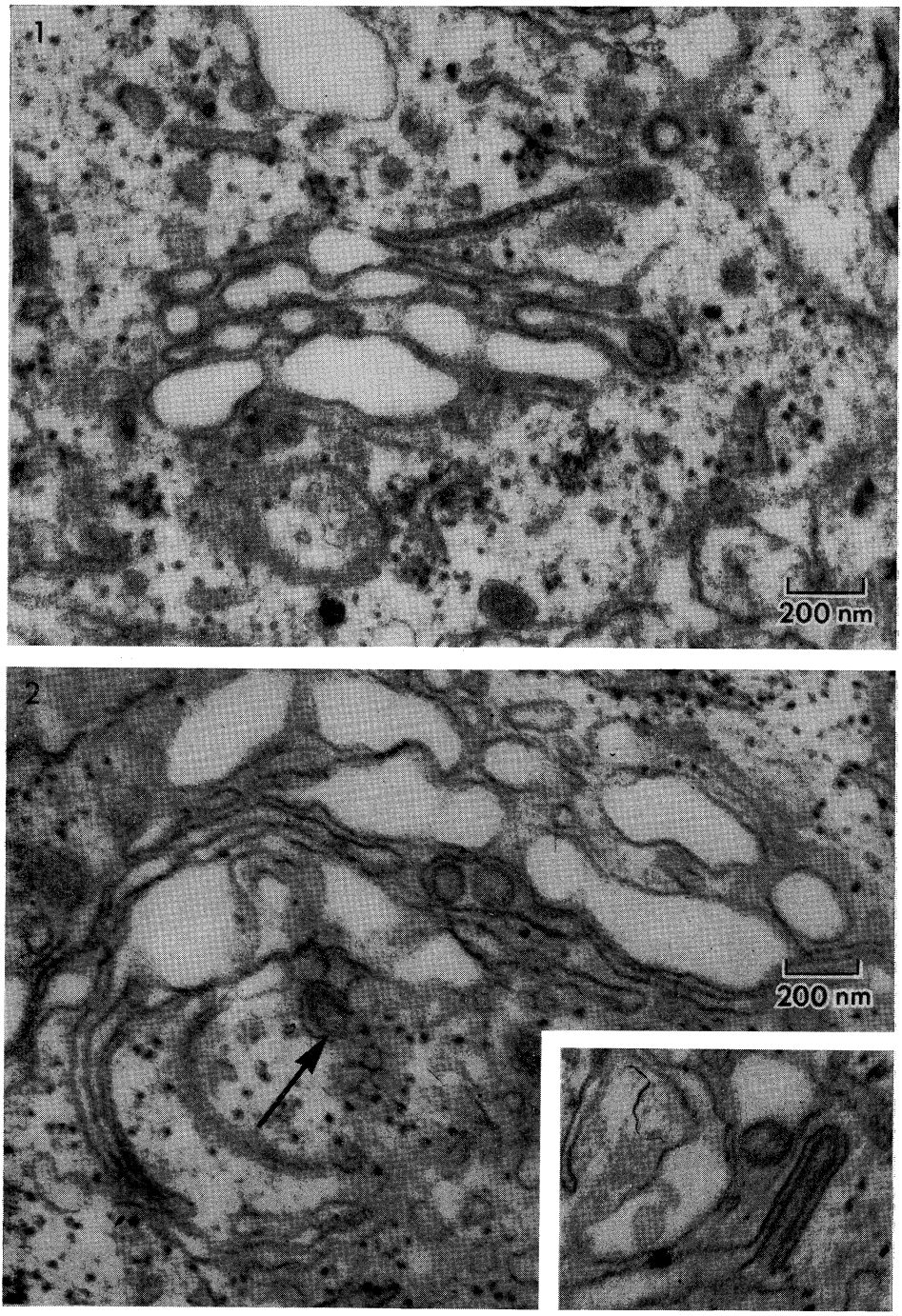
(*c*) *Electron Microscopy*

Immediately after removal, whole brains were fixed in 4% glutaraldehyde (Pease 1964) for 1 hr at 4°C. Portions of the forebrain were then sliced into blocks of approximately 1 mm^3, post-fixed in Millonig buffered 1% osmium tetroxide for 1 hr at 4°C, dehydrated in acetone, and embedded in Araldite. Thin sections were stained with lead citrate, and examined in a Hitachi HU-11A microscope operating at 50 kV. Magnification was calibrated using catalase crystals (Luftig 1967).

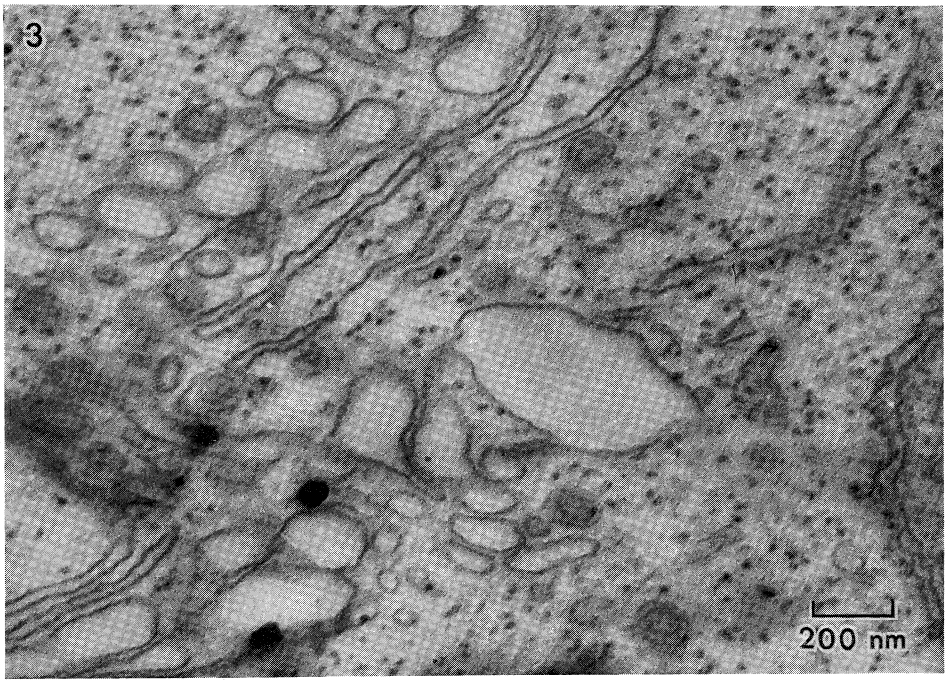
III. Results

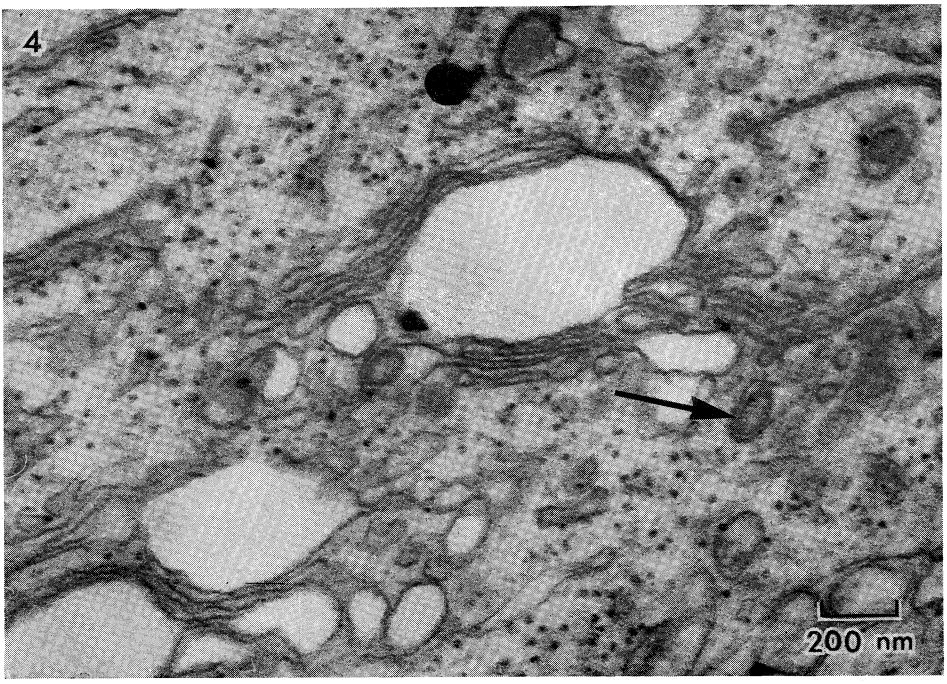
In the morphology and mode of development of their particles, and in the intracellular changes accompanying their muliplication, Koongol and Wongal viruses were indistinguishable. During the first 36 hr post-inoculation, neurons showed no remarkable changes and no virus particles were seen. By 48 hr some neurons contained small numbers of virions. At $58\frac{1}{2}$ hr greater numbers of virions were observed within vesicles in a significant proportion of neurons, and virions were also found (although rarely) in the intercellular spaces.

Within neurons, Koongol and Wongal virions were located predominantly in the Golgi complex (Figs. 1–4). Some particles were within Golgi cisternae (Fig. 1) but more frequently they were seen in smooth-surfaced vesicles nearby. Virus particles seeming to bud into such vesicles (Fig. 2) were found, but rarely. The buds did not contain an electron-dense core or "precursor particle", and intravesicular particles had a moderately electron-dense periphery and, usually, a lighter central region (Figs. 1–5). Since the virions were often among many small vesicles, and were not very electron-dense (Fig. 3), careful scrutiny of the Golgi region was necessary for detection of infected cells.

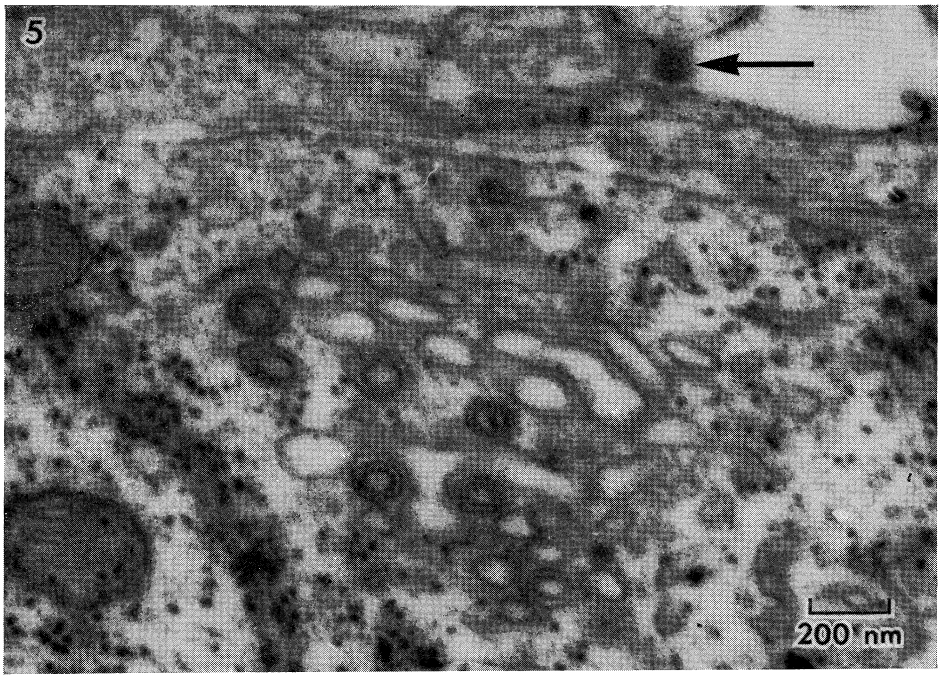


Figs. 1 and 2.—Koongol virions in vesicles within the Golgi zone of mouse neurons. Specimens from forebrain, 58½ hr post-inoculation. Note the budding particle (arrow) in Figure 2, and the abnormal budding form in the inset. ×50,000.





Figs. 3 and 4.—Wongal virus-infected mouse brain, showing characteristic virions in vesicles near the Golgi regions of neurons. The profile (arrow) in Figure 4 is of an abnormally elongated particle. $\times 50{,}000$.



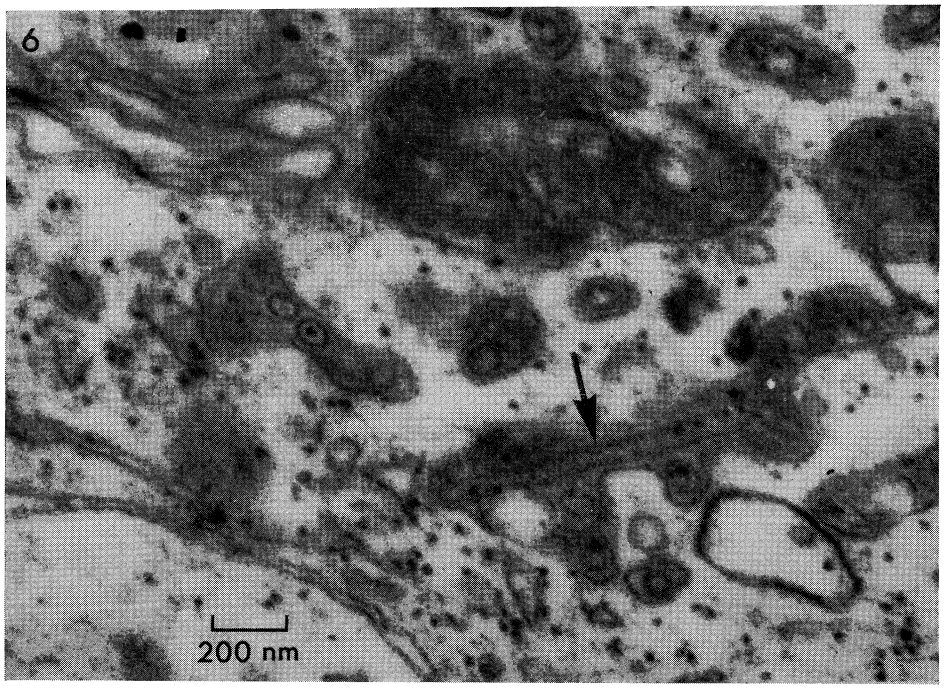


Fig. 5.—Koongol virus-infected neuron showing several virions within small vesicles and an extracellular virion (arrow) nearby. Note the electron-dense central region in the extracellular virion. ×50,000.

Fig. 6.—Atypical membrane-bound inclusions containing Koongol virions surrounded by electron-dense material. A long tubular aberrant particle is present (arrow). These inclusions may be lysosomal rather than sites of virus assembly. ×50,000.

Aberrant tubular forms [Figs. 2 (inset), 4, and 6] were uncommon but resemble those found in neurons infected with Bunyamwera and California group viruses (Murphy *et al.* 1968*a*, 1968*d*). The vesicles containing virions and a tubular form in Figure 6 were also atypical in that they contained electron-dense background material and may have been lysosomal in nature.

Average dimensions of particles are summarized in Table 1. Particles of Bunyamwera virus in sections of infected mouse brain were also measured for comparative purposes, and it can be seen that the three viruses are indistinguishable in size. Extracellular virions were not significantly different in size from intracellular ones, but their internal appearance was different (Fig. 5). Intravesicular particles were usually oval in section, with an electron-dense periphery, whereas extracellular particles were more circular in section and had an electron-dense central region, superficially resembling the particles of group A togaviruses although larger.

It was interesting to compare the ultrastructural changes in mice infected with Koongol or Wongal viruses with those produced by Bunyamwera virus. Morphologically, in dimensions (Table 1), and in mode of development all are more or less

TABLE 1

DIMENSIONS OF KOONGOL AND WONGAL VIRIONS, COMPARED WITH THOSE OF BUNYAMWERA

Virus	Mean diameter (nm)		No. of particles measured
	Intracellular	Extracellular	
Koongol	91±9	94±4	76, 24
Wongal	92±10	96±4	78, 22
Bunyamwera	90±6	93±4	50, 30

indistinguishable, but Bunyamwera virus was obviously far more prolific and produced more striking damage in the brain. Severe ultrastructural damage (lobulated nuclei in parenchymal cells, distended nuclear envelopes and rough endoplasmic reticulum, distorted mitochondria) was also observed in the liver of Bunyamwera-infected mice, but no virus particles were observed within liver cells. Koongol virus had no apparent effect on the livers of infected mice.

No cytopathic effects or electron-microscopic evidence of virus growth were observed in VERO or PS (porcine) cells 7 days after inoculation with Koongol virus, nor in PS cells inoculated with Wongal virus. Culture supernatants contained no infectious virus.

IV. DISCUSSION

Relatively little is known about the ecology of the Koongol group viruses. Both were originally isolated from *Culex annulirostris* mosquitoes near the Mitchell River Mission in northern Queensland (Doherty *et al.* 1963) but neither is known to cause disease. Antibodies to both viruses have been detected in cattle in several

areas of Queensland as well as in kangaroos and other animal species (Doherty *et al.* 1968, 1970).

On the bases of the size and morphology of their particles, and their maturation in the Golgi region of infected cells, Koongol and Wongal thus resemble other viruses of the Bunyamwera supergroup (Holmes 1971). Ironically, this does not really clarify their status in the serological classification, since there appear to be a considerable number of arboviruses of similar morphology, which are not known to be serologically related. Examples are *Anopheles* A virus (Holmes 1971), Rift Valley fever virus (Lecatsas and Weiss 1968; F. A. Murphy, personal communication), and Uukuniemi virus (von Bonsdorff, Saikku, and Oker-Blom 1970). At least it can be said that on morphological grounds, the Koongol group could be included with all the Bunyamwera supergroup viruses and various other arboviruses in a new, major, morphological group which will comprise almost half the known arboviruses.

V. Acknowledgments

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