VIROLOGY

Infectious Ribonucleic Acids derived from Mouse Brains infected with Two Kinds of Arbor Virus Group *B*

Infectious ribonucleic acid (RNA) has been extracted by phenol treatment[1] from cells infected with animal viruses. In Group *B* of the arbor viruses, it has been recently reported that the RNA extracted from mouse brain infected with Murrary Valley encephalitis virus[2] or Japanese *B* virus[3] and from Ehrlich ascites tumour cells infected with West Nile encephalitis[4] are infectious. The purpose of this present communication is to add a datum concerning the extraction of infectious RNA from mouse brains infected with dengue fever virus to the reports of viral RNA already published, and to compare the effects of cold- and hot-phenol extractions on the yields of infectious RNA from the cells infected with Japanese *B* encephalitis virus and dengue fever virus, respectively.

Five-day-old mouse brains infected intracerebrally with the Hawaiian strain of dengue fever virus (*M*-184) and three-week-old mouse brains infected with the Nakayama strain of Japanese *B* encephalitis virus (*M*-55) were employed as the sources of the materials for extraction of RNA. The brains were removed from exsanguinated mice shortly after the appearance of signs of involvement of the central nervous system, ground in a chilled mortar, and a 10 per cent suspension was made with sterile distilled water. Coarse particles were removed by low centrifugation, and the supernatant fluid was used for the experiment. The supernatant fluids were extracted three times with an equal volume of cold 80 per cent phenol at 4° C. for 3 min. following the procedure of Gierer and Schramm[1], or with an equal volume of molten phenol (50° C.) and shaken at 50° C. for 3 min. by Wecker's method[5]. After three such extractions the aqueous phase was shaken five times with peroxide-free ether and the excess ether was removed by bubbling with nitrogen. The aqueous phase obtained by each extraction was reconstituted to the original volume with sterile distilled water. Infectivity titres of the samples, expressed as LD_{50}, were determined by the intracerebral inoculation of serial 10-fold dilutions in mice, 0·02 ml. for three- to five-day-old mice and 0·03 ml. for three-week-old mice. Clarified 10 per cent infected brain suspension and the RNA preparation were regarded as 10^{-1} dilution. The results obtained are shown in Table 1.

Both infectious RNA of Japanese *B* encephalitis virus and dengue fever virus could be easily extracted by phenol treatment, and the yields of infectious RNA of these viruses were the same as that of poliovirus, that is, they amounted usually to about 0·1–0·01 per cent of the crude virus preparation. There were no differences between the effects of cold- and hot-phenol extraction on the yields of RNA derived from the two viruses.

The ultra-violet absorption spectra of these preparations of RNA were typical for nucleic acid. If such an extracted preparation was treated with 50 μgm./ml. of ribonuclease for 10 min. at room temperature prior to inoculation, its infectivity was completely abolished, whereas identical treatment of the virus suspension had no such effect. The preparations of RNA of these viruses had no hæmagglutinating activity under the conditions which were used for these viruses.

Table 1. Infectivity of Two Kinds of Arbor Virus Group *B* and Ribonucleic Acid Preparations

Material	Animal for titration (−log LD_{50}/ml.)	
	Suckling mouse	Adult mouse
Japanese *B* encephalitis virus:		
Crude virus	8·9	8·5
RNA extracted by cold phenol	4·7	4·2
RNA extracted by hot phenol	5·5	3·8
Virus + RNase 50 μgm./ml., 22° C.; 10 min.	9·2	7·5
RNA* + RNase 50 μgm./ml., 22° C.; 10 min.	—†	—
Dengue fever virus:		
Crude virus	8·1	6·0
RNA extracted by cold phenol	4·7	4·5
RNA extracted by hot phenol	4·9	5·0
Virus + RNase 50 μgm./ml., 14° C.; 10 min.	8·0	*N.T.*
RNA* + RNase 50 μgm./ml., 14° C.,; 10 min.	—	*N.T.*

* RNA was extracted by hot-phenol treatment.
† No mice died when given undiluted inoculum.
N.T. = Not tested.

It might be noted, as indicated in Table 1, that, in the case of dengue fever virus, there were no differences between the infectivity titres of RNA for suckling mice and that for adult mice; whereas if adult mice were used for titration, the titres of the crude virus were approximately 100 times lower than that in suckling mice. Therefore, the ratio of virus to RNA in adult mice was much nearer unity than that in suckling mice.

Experiments are now being carried out in the extraction of RNA from adult mouse brains infected with dengue fever virus and suckling mouse brains infected with adult mouse-adapted dengue fever virus.

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Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the Rate of Lesion Production on Pinto Bean

In experiments which required the daily counting of TMV lesions on pinto bean leaves (*Phaseolus vulgaris* L.) for the purpose of determining specific rates of lesion-appearance, it was noted that those leaves which had the greatest final number of lesions produced a greater percentage of their lesions earlier. Since comparisons of rates of lesion development could be seriously affected by differences arising from this, an attempt was made to determine its cause and extent.

Bald[1] had observed the same phenomenon when potato virus *X* was inoculated into *Nicotiana tabacum* L. (var. White Burley), but could not obtain these results when this virus or TMV was inoculated into *N. glutinosa* L. He suggested that either adjacent incipient lesions enhanced production of early lesions or else the greater the number of infective particles entering an infection site, the more rapid the rate of virus multiplication and hence lesion appearance. The first suggestion is not compatible with our results and the second seems unlikely, since it is generally held that only one virus particle can infect one site[2,3].