Physicochemical and Morphological Relationships of some Arthropod-borne Viruses to Bluetongue Virus – A new Taxonomic Group. Physicochemical and Serological Studies

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SUMMARY

Several arthropod-borne viruses were grouped on the basis of relative stability to lipid solvents and sodium deoxycholate, lability at pH 3.0, and lack of antigenic relationship to major arbovirus serologic groups A, B, and Bunyamwera. These viruses, previously ungrouped or in minor serogroups, include bluetongue, epizootic haemorrhagic disease of deer, Eubenangee, IbAr 22619, B 1327, Colorado tick fever, African horse sickness, Irituia, Changuinola, BeAr 35646, BeAr 41067, Kemerovo, Chenuda, Tribec, Wad Medani, Mono Lake, Huacho, Lebombo, Palyam, D’Aguilar, G8886, G15534, Corriparta, Acado, MP359, CH9935, and MRM 10434. In contrast to other arboviruses, the reduction in infectivity of these viruses caused by lipid solvents or sodium deoxycholate was less than 10^-1.5 suckling mouse intracerebral LD50. This degree of sensitivity was reproducible and was significantly different from the absolute resistance of reoviruses and picornaviruses. After exposure of each of these viruses to pH 3.0 for 3 hr, no residual infectivity was recovered. By complement-fixation testing no serological relationship to members of other virus taxonomic groups was found. The viruses themselves had no common group antigen but were placed into ten serologically distinct subgroups on the basis of individual cross-reactions. Although probably containing a double-stranded RNA genome, the relatively solvent resistant arboviruses were distinguished from reoviruses by acid lability, slight solvent sensitivity, and serology.

INTRODUCTION

Bluetongue virus, Colorado tick fever virus, African horse sickness virus, and about 50 other arboviruses have remained uncategorized or have been placed in minor serogroups because of their lack of antigenic relationship to the major arbovirus serological groups A, B, and Bunyamwera supergroup. The classification of these viruses as arboviruses has been based upon fulfillment of ecological criteria outlined by the WHO Scientific Group on Arboviruses (1967). This definition requires a principal maintenance of the virus in nature.

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via a biological transmission cycle between susceptible vertebrates and haematophagous arthropods. By its nature, such an ecological classification has allowed grouping of viruses of diverse physicochemical properties. Many arthropod-borne viruses have common physicochemical and morphological characteristics and thus remain together in more recent taxonomic schemata which utilize common virion properties rather than biological transmission as a basis for classification.

Other arthropod-borne viruses, such as bluetongue and African horse sickness, differ in that they are ether resistant (Studdert, 1965; Svehag, Leendertsen & Gorham, 1966; Ozawa, 1968) and have a double-stranded RNA genome (Verwoerd, 1969; Verwoerd, Louw & Oellerman, 1970; Oellerman, Els & Erasmus, 1970). As a solution to this ambiguity stemming from physical heterogeneity of the arbovirus ecological group, stricter taxonomic definitions are being proposed by the Vertebrate Virus Subcommittee of the International Committee on Nomenclature of Viruses. The term arbovirus is to be used in a strictly biological sense and not taxonomically. Togavirus is the name to be used taxonomically for those viruses with the physicochemical and morphological characters of the arbovirus groups A and B (Andrewes, 1970). We shall adhere to the proposed terminology in this paper.

To determine whether any other arboviruses, currently in minor serogroups or ungrouped, should be separated from the togaviruses, we have studied the physicochemical properties of a number of these viruses. We have examined their stability to sodium deoxycholate and lipid solvents, the effect of acid pH, and their sensitivity to heating to 37°. The serological relationships of these viruses have been re-examined. In a parallel study we examined the morphology and morphogenesis of the same viruses (Murphy et al. 1971).

**METHODS**

**Viruses.** The viruses used, the reference to their isolation, and their passage history (all passages in mouse brain) are as follows: Chenuda (Taylor 1967), AT1152 – 11 passages and AT1170 – 3 passages; Colorado tick fever (Florio, Stewart & Mugrage, 1944), CONDON strain – 37 passages, FLORIO strain – about 75 passages, and CDC patient isolate 67-1071 – 2 passages; Corriparta (Doherty et al. 1963) – 8 passages; epizootic haemorrhagic disease of deer (Shope, MacNamara & Mangold, 1960), N.J. strain – 6 passages; Eubenangee (Doherty et al. 1968) – 7 passages; Iritia (Taylor, 1967) – 8 passages; Lebombo (SA AT136)* – 22 passages; Palyam (Dandawate, Pavri & Work, 1969) – 3 passages; Tribec (Gresikova et al. 1965) – 14 passages; Wad Medani (Taylor, Hoogstraal & Hurlburt, 1966) – 5 passages; MP359 (Williams & Woodall, 1965), ibh13019 strain – 10 passages; yellow fever, French neurotropic strain – > 250 passages; reovirus, type 3 – 5 or 6 passages; mouse encephalomyelitis, GDVII strain – 187 to 190 passages (all kindly provided by Dr N. Karabatsos, World Health Organization International Reference Centre, Yale Arbovirus Research Unit, New Haven); Modoc – 6 passages; Semliki Forest – 11 passages; (both kindly provided by Dr Adrian Chappell, CDC Arbovirus Reference Laboratory, Atlanta). Additional viruses used only in the serological studies include bluetongue, strain BT8 – 15 tissue culture and 5 mouse passages; ibar22619 (Moore & Lee, 1971) – 7 mouse passages; dakarb1327 (Digoutte, manuscript in preparation, 1971) – 2 passages; Acado (Schmidt et al. 1966), strain ethar1846-64 – 12 passages; Changuinola (Taylor, 1967), strain BT436 – 3

* The staff of the South African Medical Research Institute graciously permitted us to study Lebombo virus even though details of its isolation have not yet been published. This report is not intended to constitute primary publication.
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passages; bear35646 and bear41067 (Woodall, 1967) – 4 passages each; Kemerovo, rio
(Chumakov et al. 1963) – 11 passages; Huacho, strain calar883 and mona lake, calar861
(Casals & Hoogstraal, 1971) – 7 and 6 passages respectively; 68886 (Virus Research Centre,
1970) – 2 passages; G15534 (Dandawate, Pavri & Work, 1969) – 2 passages; D’Aguilar
(Doherty et al. 1970) B8112 – 2 passages; ch9935 and mrm10434 (Doherty et al. 1970) – 2
and 3 passages respectively.

Virus titrations. Virus titrations for studies involving ether, sodium deoxycholate, chloro-
form, heat, and pH sensitivities were performed in litters of ICR Swiss mice 1 to 3 days of
age. Animals were inoculated intracereberally with 0.02 ml. of serial tenfold virus dilutions.
Mice were free of signs of intercurrent infections. Titration end-points were calculated by the
method of Kärber (1931) and expressed as LD50/ml.

Physicochemical treatments. A. Lipid solvent sensitivity. Ether (anaesthesia grade) and
chloroform (reagent grade) sensitivity were determined by the methods of Andrewes &
Horstmann (1949) and Feldman & Wang (1961), respectively. A 10^-2 dilution of virus
propagated in mouse brain was prepared in modified Eagle’s medium with 10 % newborn
calf serum. Virus preparations were held with solvent for 16 to 20 hr at 4°; tubes containing
preparations were sealed with tape and agitated occasionally during the period. Serial
dilutions for inoculation were made in the above diluent after removal of the solvent by
evaporation (ether) or centrifugation (chloroform). Sodium deoxycholate testing was per-
fomed according to a modification of the method of Theiler (1957). Infected mouse brain
suspensions were prepared in 0.75 % bovine serum albumin in phosphate buffered saline
at pH 7.4 (10 %, w/v), and centrifuged at 10,000 rev./min. for 1 hr. The resulting supernatant
fluids were mixed with equal volumes of 1 % sodium deoxycholate. The control was pre-
pared similarly but with bovine albumin diluent replacing the sodium deoxycholate. After
incubating these mixtures at 37° for 1 hr they were diluted in serial tenfold steps and inocu-
lated. Early in the study, a few serial dilutions of virus were mixed with 1500 sodium
deoxycholate before incubation, but this method was discontinued because of toxicity of
deoxycholate in this concentration to baby mice. B. Heat sensitivity. Virus, prepared as for
ether or chloroform treatments but with no solvent added, was incubated for 18 hr at 37°C ± 0.5°
and then titrated. Controls were kept at 4°. C. pH sensitivity. Virus in modified Eagle’s
medium with 10 % newborn calf serum at pH 7.5 was mixed 1/2 with Eagle’s medium
adjusted to pH 2.7 with a bicarbonate HC1 buffer; the resulting pH was 3.0. Controls were
diluted 1/2 with Eagle’s medium at pH 7.5. All specimens were held at 4° for 3 hr and then
diluted for titrations.

Serological Methods. Complement fixation (CF) and haemagglutination testing were per-
fomed as described by Casals (1967). Antigens for CF and haemagglutination tests were
prepared by the sucrose acetone technique (Clarke & Casals, 1958). Sera and ascitic fluids
were made in mice by hyperimmunization, usually with addition of Freund’s complete
adjuvant to the inoculum. Ascitic fluids for epizootic haemorrhagic disease of deer, Changui-
nola, and Colorado tick fever viruses were supplied by the Research Reference Reagents
Branch of the National Institute of Allergy and Infectious Disease of the National Institutes
of Health. For CF tests, fourfold dilutions of antigen in veronal buffer were titrated against
twofold dilutions of immune-mouse ascitic fluid or serum previously inactivated at 60° for
20 min. Standard arbovirus haemagglutination testing employed goose erythrocytes and a
pH range of 5.8 to 6.6.
RESULTS

Lipid solvents and sodium deoxycholate*

Certain ungrouped arboviruses were much more resistant to ether, chloroform, and sodium deoxycholate than togaviruses (Table 1). Togaviruses were unequivocally sensitive. In a random sampling from the data reported in the *Catalogue of Arthropod Borne Viruses of the World* (Taylor, 1967), on the solvent sensitivity of 25 togaviruses from the major antigenic groups, the mean infectivity reduction was \( > 10^{3.7} \) with ether, \( > 10^{3.8} \) with chloroform, and \( > 10^{4.0} \) with deoxycholate. No togavirus was inactivated less than \( 10^2 \) by any of these solvents. Testing of representative togaviruses in our laboratory confirmed this sensitivity. Reovirus-3 was clearly resistant to these solvents. Many of the ungrouped arboviruses proved almost as resistant as reoviruses or picornaviruses (Tables 1 and 2). Deoxycholate gave the most uniform results; the reduction of infectivity of the listed ungrouped arboviruses was less than \( 10^{1.5} \). Chloroform was least satisfactory for separation, but even with this solvent these viruses were evidently more resistant than togaviruses.

Although decidedly more resistant than togaviruses to lipid solvents and sodium deoxycholate there was a consistent but slight infectivity reduction after treatment of these ungrouped arboviruses. To confirm this, Lebombo virus was subjected to sodium deoxycholate testing on six occasions (Table 2). The degree of inactivation was not marked, but was significant when compared to mouse encephalomyelitis virus. Conversely, yellow fever virus was extremely sensitive.

There were only two exceptions to the relative solvent stability of these viruses; these occurred in a single strain of Colorado tick fever virus and a single strain of Chenunda virus.

* Lipid solvent sensitivity. Although sodium deoxycholate is chemically a detergent rather than a solvent, its effects will be discussed together with the effects of the true solvents, ether and chloroform. The term ‘solvent resistance’ refers to ether, chloroform, and deoxycholate.

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Table 1. Lipid solvent resistance of selected arboviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Ether</th>
<th>CCl₂</th>
<th>DCA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluetongue</td>
<td>0.2</td>
<td>2.0</td>
<td>+0.3</td>
</tr>
<tr>
<td>Epizootic haemorrhagic disease of deer</td>
<td>0.5</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>tbar 22619</td>
<td>1.4</td>
<td>1.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Colorado tick fever (CONDON strain)</td>
<td>1.4</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Changuinola subgroup</td>
<td>1.4</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Iriutia</td>
<td>0.4</td>
<td>2.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Kemerovo subgroup</td>
<td>0.1</td>
<td>+0.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Chenunda (egg 1152)</td>
<td>2.3</td>
<td>3.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Tribec</td>
<td>2.3</td>
<td>3.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Wad Medani</td>
<td>0.3</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Lebombo</td>
<td>0.9</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Palyam</td>
<td>0.9</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>UgMP 359</td>
<td>0.9</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Semliki Forest (group A, togavirus)</td>
<td>4.6</td>
<td>3.5</td>
<td>4.7</td>
</tr>
<tr>
<td>Modoc (group B, togavirus)</td>
<td>&gt;4.7</td>
<td>&gt;4.7</td>
<td>&gt;6.3</td>
</tr>
<tr>
<td>Mean, arbovirus catalogue‡</td>
<td>&gt;3.7</td>
<td>&gt;3.8</td>
<td>&gt;4.0</td>
</tr>
<tr>
<td>Reovirus-3</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

* DCA, sodium deoxycholate.
† +, infectivity increase.
‡ mean of solvent reduction from data reported on 25 randomly selected togaviruses in the Catalogue of Arthropod Borne Viruses of the World (Taylor, 1967).
The FLORIO strain of Colorado tick fever virus was much more sensitive than either the CONDON strain or a low-passage strain of this virus isolated from a patient. On repeated testing the Florio strain was inactivated with ether, chloroform and sodium deoxycholate. In contrast, the low-passage patient isolate was reduced only $10^{0.8}$ by ether, $10^{1.6}$ by chloroform, and $10^{0.8}$ by sodium deoxycholate. CONDON strain was inactivated only $10^{1.4}$, $10^{1.7}$, and $10^{0.8}$, respectively. The sensitivity of FLORIO strain may have been related to its high-passage level. Chenuda virus, strain egARI170, was inactivated $>10^{6.1}$ on repeated testing with sodium deoxycholate in contrast to the relative solvent resistance of Chenuda virus strain AF1152 (Table 1). The serological identification of these atypical strains was reconfirmed by complement-fixation tests with reference sera prepared against the solvent stable strains.

The relative solvent resistance of the arboviruses, bluetongue (Studdert, 1965; Svehag et al. 1966), African horse sickness (Ozawa, 1968), Corriparta (Carley & Standfast, 1969) and Eubenangee (Doherty et al. 1968) has been previously reported.

**Heating at 37°**

Reoviruses are considered relatively heat stable and togaviruses relatively heat labile. As expected, reovirus infectivity decreased only $10^{0.2}$ in 24 hr at 37°. A togavirus, Semliki Forest

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**Table 2. Repetitive sodium deoxycholate testing of a representative solvent resistant virus, a togavirus, a picornavirus and a reovirus**

<table>
<thead>
<tr>
<th>Virus</th>
<th>No. of determinations</th>
<th>Log LD50 infectivity reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Lebombo</td>
<td>6</td>
<td>0.3 to 2.0</td>
</tr>
<tr>
<td>Yellow fever (togavirus)</td>
<td>9</td>
<td>&gt;4.5 to &gt;6.7</td>
</tr>
<tr>
<td>Mouse encephalo-myelitis (picornavirus)</td>
<td>9</td>
<td>0.1 to +0.6*</td>
</tr>
<tr>
<td>Reovirus-3</td>
<td>2</td>
<td>+0.3 to +0.5</td>
</tr>
</tbody>
</table>

* Infectivity increase.

**Table 3. Acid lability of relatively solvent resistant arboviruses, togaviruses and reovirus**

<table>
<thead>
<tr>
<th>Virus</th>
<th>pH 7.5</th>
<th>pH 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorado tick fever</td>
<td>5.6</td>
<td>0†</td>
</tr>
<tr>
<td>Changuinola subgroup Iritua</td>
<td>5.2</td>
<td>0</td>
</tr>
<tr>
<td>Bluetongue subgroup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epizootic haemorrhagic disease of deer</td>
<td>3.7</td>
<td>0</td>
</tr>
<tr>
<td>Eubenangee</td>
<td>4.1</td>
<td>0</td>
</tr>
<tr>
<td>Kemerovo subgroup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chenuda</td>
<td>6.9</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Tribec</td>
<td>7.5</td>
<td>0</td>
</tr>
<tr>
<td>Wad Medani</td>
<td>5.3</td>
<td>0</td>
</tr>
<tr>
<td>Lebombo</td>
<td>4.9</td>
<td>0</td>
</tr>
<tr>
<td>Palyam</td>
<td>5.6</td>
<td>0</td>
</tr>
<tr>
<td>Semliki Forest (group A, togavirus)</td>
<td>6.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Modoc (group B, togavirus)</td>
<td>7.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Reovirus-3</td>
<td>6.6</td>
<td>6.6</td>
</tr>
</tbody>
</table>

* For 3 hr at 4°.
† No infectivity in undiluted material.
<table>
<thead>
<tr>
<th>Initial dilution</th>
<th>Bluetongue</th>
<th>EHD, NJ</th>
<th>Ib Ar 22619</th>
<th>Eubenangee</th>
<th>B 1327</th>
<th>Corriparta</th>
<th>Acado</th>
<th>Changuinola</th>
<th>Irituia</th>
<th>Be Ar 35636</th>
<th>Be Ar 41067</th>
<th>CTF</th>
<th>Kemerovo</th>
<th>Tribec</th>
<th>Chenuda</th>
<th>Mono Lake</th>
<th>Huacho</th>
<th>Wad Medani</th>
<th>Palyam</th>
<th>Palam</th>
<th>G 15534</th>
<th>D'Aguilar</th>
<th>MP 359</th>
<th>Lebombo</th>
<th>CH9935</th>
<th>MRM 10434</th>
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<tr>
<td>4</td>
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<td>4</td>
<td>4</td>
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<td>4</td>
<td>32</td>
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<td>4</td>
<td>4</td>
<td>16</td>
<td>4</td>
<td></td>
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</tbody>
</table>

* Reciprocal of dilution giving 50% or greater fixation.
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virus, was also inactivated only slightly $10^{6.4}$. Representative viruses of the relatively solvent resistant group were variable in their response. Colorado tick fever and Tribec viruses were inactivated $>10^{6.0}$, whereas Chenuda, Irituia and the virus of epizootic haemorrhagic disease of deer were inactivated only $10^{4.9}$ by this treatment. Bluetongue (Svehag et al. 1966) and Corriparta viruses (Carley & Standfast, 1969) have been reported as relatively resistant. Thus, treatment at 37° for 24 hr was not a reliable way of distinguishing these viruses from togaviruses or reoviruses.

Acid pH

Reoviruses were stable at pH 3.0 for 3 h at 4°, as has been shown by other investigators (Stanley, 1967). Representative togaviruses were labile (Table 3), but the solvent resistant arboviruses were even more strikingly labile than the togaviruses tested. A similar marked pH lability of bluetongue (Svehag et al. 1966), African horse sickness (Ozawa, 1968) and Corriparta (Carley & Standfast, 1969) viruses has been described previously.

Serological studies

Complement-fixation tests were made to determine relationships between the relatively solvent resistant arboviruses and the togaviruses as well as representative rhabdo-, pox-, herpes-, myxo-, and arenaviruses. Antigens of bluetongue, epizootic haemorrhagic disease of deer, Eubenangee, Corriparta, Acado, Changuinola, Irituia, Colorado tick fever, Kemerovo, Tribec, Chenuda, Wad Medani, Palyam, Lebombo, Aus ch9935, and Aus MRM 10434 viruses were tested against polyvalent grouping and/or specific antisera for arbovirus groups A, B, Bunyamwera, C, Simbu, California, Guama, Quaranfil, Bandia, Kaisodi, Anopheles A, Anopheles B, Turlock, phlebotomus fever, and Capim; rhabdoviruses vesicular stomatitis (three serotypes) and rabies; arenaviruses Tacaribe group-lymphocytic choriomeningitis; herpes simplex; vaccinia; reovirus 3; and Newcastle disease viruses. No cross-reactivity was observed. Study of CF relationships of 26 of the relatively solvent resistant viruses defined nine antigenic subgroups (Table 4); bluetongue, Corriparta, Changuinola, Colorado tick fever, Kemerovo, Palyam, Lebombo, Aus ch9935 and M 359. Viruses were grouped antigenically if they reacted with at least one other virus of a subgroup. The nine viruses of the African horse sickness complex (Verwoerd, 1970) form a tenth serological subgroup, which was not studied because of quarantine regulations.

No haemagglutinin for goose cells was found with sucrose-acetone extracted antigens of Colorado tick fever, Changuinola, Irituia, Corriparta, Eubenangee, epizootic haemorrhagic disease of deer, Kemerovo, Chenuda, Tribec, Wad Medani, Lebombo, or Palyam.

DISCUSSION

A group of arthropod-borne viruses has been distinguished from the togaviruses on the basis of resistance to lipid solvents and antigenic independence. We show them in the following paper (Murphy et al. 1971) to be morphologically distinct from the togaviruses, but similar to each other in size, morphology, and morphogenesis. Although their morphogenesis and genome were reovirus-like, these relatively solvent resistant arboviruses were separated from reoviruses by lability at acid pH, serology, and capsid structure.

The viruses studied were only slightly sensitive to sodium deoxycholate, ether and chloroform, and were decidedly more stable to these reagents than the togaviruses. Resistance to sodium deoxycholate, ether, and chloroform has often been correlated with an unenveloped virus particle; electron microscopic studies of the relatively solvent resistant arboviruses
have shown them to be naked (Murphy et al. 1971). The slight but reproducible solvent sensitivity of these viruses may represent a fundamental virion property or laboratory artifact. Protein may be necessary for virus stabilization, since bluetongue virus became more sensitive to lipid solvents when purified and was stabilized by albumin (Verwoerd, 1969). Our studies with Colorado tick fever virus suggested that increased lability may be related to repeated laboratory passage of the virus. However, it may be that the slight solvent sensitivity of these viruses is related to lipid in the virus itself. Verwoerd (1969) reported that purified bluetongue virus contained 2% lipid. Whether this lipid was host-cell contaminant or an integral part of the virus particle remains to be determined.

Antigenic characterization is important for virus identification and, when a relationship is found, for taxonomic characterization. By complement-fixation test, the listed solvent resistant arboviruses were unrelated to other known viruses or virus groups. Restudy of the serological inter-relationships of these viruses was indicated after the common solvent resistance was noted. Ten CF subgroups were distinguished and the CF inter-relationships were reproducibly observed. Neutralization tests have substantiated the distinctness of serotypes within the subgroups bluetongue and African horse sickness (Verwoerd, 1970; Moore & Lee, 1971), Corriparta (R. E. Shope, unpublished observations), Changuinola (Woodall, 1967), Kemerovo (Casals & Hoogstraal, 1971), and Palyam (Dandawate et al. 1969, R. E. Shope, unpublished observations). Haemagglutination of goose cells is characteristic of togaviruses (Casals, 1967), and human group O cells of reoviruses (Stanley, 1967). The relatively solvent resistant arboviruses did not agglutinate goose erythrocytes and bluetongue (Verwoerd, 1969), Corriparta (Carley & Standfast, 1969), and African horse sickness (Verwoerd, 1970) viruses did not agglutinate human group O cells.

Acid lability has been utilized as an aid in virus classification (Hamparian, Hilleman & Kettler, 1963). Although not a major taxonomic character, it has been useful in separating human rhinoviruses from enteroviruses, a separation which was subsequently confirmed by more fundamental physical differences (Tyrrell, 1968). Reoviruses are stable over a wide range of pH values (Stanley, 1967). In contrast, the relatively solvent resistant arboviruses studied here were strikingly labile at pH 3.0; no infectivity was found in undiluted specimens after treatment. This character clearly distinguished these viruses from reoviruses.

The bluetongue virus genome, like that of reoviruses (Gomatos & Tamm, 1963a; Gomatos & Tamm 1963b; Langridge & Gomatos, 1963), has been shown to be double-stranded RNA by analysis of its sedimentation and thermal denaturation (Verwoerd, 1969; Verwoerd et al. 1970). Green (1970) analysed by sedimentation the RNA of actinomycin-treated cells infected with Colorado tick fever virus cells and confirmed the double-stranded nature of the virus genome. The nucleic acid of African horse sickness virus has similarly been demonstrated to be a duplex ribonucleotide (Oellerman et al. 1970). Characterization of the genomes of three serological subgroups have thus been completed; all have been double-stranded RNA.

We are aware of other animal viruses with properties similar to the viruses discussed here, including a number of unclassified viruses with similar physical or morphological properties and 5 to 10 more unnamed arthropod isolates serologically related to the viruses studied here. Characterization of these is incomplete but Ibaraki virus (Inaba et al. 1970), rabbit syncytium virus (Brown et al. 1970), simian virus SA-11 (Malherbe & Strickland-Chomley, 1967), equine encephalitis and x virus (Erasmus & Lecatsas, quoted in Verwoerd, 1970) have many properties suggestive of the relatively solvent resistant arboviruses. The virus of epizootic diarrhoeal disease of infant mice, for which nucleic acid characterization is incomplete, has a reovirus-like morphology and morphogenesis (Banfield, Kasnic & Blackwell, 1968), but
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is absolutely resistant to lipid solvents and is stable at pH 3.0 (J. Blackwell, Agricultural Research Service, Beltsville, Maryland, personal communication, 1969).

These physicochemical studies suggest that the relatively solvent resistant arboviruses should be considered a new virus group, a suggestion also advanced by Verwoerd (1969). Their resistance to lipid solvents is a prime basis for distinction from togaviruses. The relatively solvent resistant arboviruses probably all contain a genome of double-stranded RNA, but their decided sensitivity to low pH sharply differentiates them from reoviruses. Their antigenic independence from both toga- and reoviruses is also compatible with a separate grouping. Morphology and morphogenesis are consistent with the grouping suggested by these physicochemical studies (Murphy et al. 1971). While recognizing the unique features of these viruses, placement of such a group in a taxonomic schema must also consider those fundamental properties shared with reoviruses.

The findings and continued interest of Dr M. Theiler have contributed greatly to the completion of this work and are deeply appreciated.

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ADDENDUM

ORBIVIRUSES – proposed name for the bluetongue-like virus group.

The authors suggest as a name for the distinctive group of viruses described, ORBIVIRUSES (from ORBIS, (L.), ring or circle). This name reflects the especially large, doughnut-shaped capsomeres seen on the surface of virus particles in negative contrast preparations. It is proposed as a ‘genus’ name, equal in hierarchy to REOVIRUSES. This suggestion is not meant to prejudice consideration of the term DIPLORNAVIRUSES, which if otherwise acceptable to the International Committee on Nomenclature of Viruses, might best be reserved for a higher taxon to include REOVIRUSES, ORBIVIRUSES and other double-stranded RNA viruses with cubic symmetry.

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