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CHAPTER 1 *Remarks on the Classification of Viruses*

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To know, is to classify.
Stuart Mill

The coining and application of any collective or generic term represents an act of classification.
Ernst Mayr

I. Introduction

Only a few years ago, the viral world was a chaos of "small infectious particles." Consequently, it embraced, at the same time, viruses and bacteria. Today, the viruses are well defined by the sum of the distinctive traits of the virion. These distinctive traits are as follows:

1. Presence of a single nucleic acid.
2. Incapacity to grow and to divide.
3. Reproduction from the genetic material only.
4. Absence of enzymes for energy metabolism.
5. Absence of ribosomes.
6. Absence of information for the production of enzymes in the energy cycle.
7. Absence of information for the synthesis of the ribosomal proteins.
8. Absence of information for the synthesis of ribosomal RNA and transfer RNA.

It would appear that a correlation exists among all these characteristics: only one of them is, in fact, sufficient to establish that an infectious particle

belongs to the viral world. In the group of viruses recognized as such by the virtue of a definition, it became evident that order was mandatory.

In fact, the remarkable homogeneity, attested to by the number and extent of the common characteristics, masks a no less remarkable diversity. The viral infectious particle presents, in fact, a great diversity in composition and structure. Order could be achieved only through a classification, which is a system of order. The goal of biological classification is to group together organisms presenting certain analogies and certain affinities and, if possible, to also bring out phylogenic relationships.

TABLE I

DISCRIMINATIVE CHARACTERS OF VIRUSES AND PROTISTS OR BACTERIA

	Virions	Protists
Nucleic acid	DNA *or* RNA	DNA *and* RNA
Reproduction from the sole genetic material	+	0
Growth	0	+
Division	0	+
Information for the synthesis of enzymes of the energetic metabolism	0	+
Presence of the enzymes	0	+
Information for the synthesis of transfer RNA	0	+
Information for the synthesis of ribosomal RNA	0	+
Presence of ribosomes	0	+

The conceptions relative to the methodology of taxonomy, which is the science of classification, are diverse. The divergence of the conceptions necessarily leads to discussions, the heuristic value of which is incontestable. Nevertheless, a good classification must allow predictions and must also pose problems.

A few years ago we (Lwoff and Tournier, 1966) presented a critical history of various classifications of viruses, so we will not go back to that topic again. However, the symposium devoted to the classification of microorganisms, held by the "Society of General Microbiology" in 1962, was overlooked. In the corresponding volume are to be found remarkable general articles and vast amount of information. Wildy (1962b) presented an article on the classification of viruses: his conclusions were a restatement of those of Horne and Wildy published the previous year and no new classification was proposed.

In the course of this meeting, Sneath (1962) discussed the structure of taxonomic groups. Sneath, as is known, is one of the principal champions of the numerical method. His study was devoted principally to bacteria, but one short paragraph dealt with viruses. There, it is seen that the numerical method has permitted recognition of three groups: the arboviruses, myxoviruses, and enteroviruses; that is not much. Pirie (1962), too, talked about the classification of viruses and discussed the criteria, but did not propose any system.

The general atmosphere of the symposium was pessimistic; it was held that the viruses could not be classified, owing to insufficient data. But if a certain caution is always necessary, an exaggeratedly critical attitude is sterilizing. It was necessary to go ahead and to act.

II. The LHT system

A. The System

Lwoff, Horne, and Tournier, from here on referred to as LHT, proposed a System of Viruses (Lwoff *et al.*, 1962a,b) (see Table II) and discussed the classification (Lwoff and Tournier, 1966).

Four characteristics were utilized: the nature of the genetic material, the symmetry of the capsid, the naked or enveloped nature of the nucleocapsid, and, finally, the number of capsomers for the virions with cubical symmetry, the diameter of the nucleocapsid for the virions with helical symmetry, the numerical data allowing for diversity. LHT had noted that other properties in addition to those adopted could be used for grouping viruses; among them were: the molecular weight of the nucleic acid, the proportion of nucleotides, the number of strands in the nucleic acid, the properties of the capsomers, the nature of the envelope, its origin, the antigenicity of the viral proteins, the host, and the virulence. Twenty-nine characteristics were thus enumerated.

In 1965, the provisional committee on nomenclature of viruses (Anonymous, 1965) proposed that the groups defined by the four characteristics of the LHT system be considered as families. Since 1962, the LHT system has become enlarged: some new families have found their place in the system without difficulty. Table III affords a general picture of the viral world as we understand it in 1969. Table IV shows the various properties of the virus families.

TABLE II

THE LHT SYSTEM IN ITS ORIGINAL 1962 FORM[a]

I Nucleic acid	II Capsid symmetry	III Naked (N) or enveloped (E)	IV Capsid diameter or no. of capsomeres	Viruses	Groups
RNA	H	N	100–130 Å	White clover mosaic, potato X, cactus potato F, pea stripe, wheat stripe	RHN 115
			170–200 Å	Tobacco mosaic, Vigna sinensis, cucumber 3	RHN 185
			250 Å	Barley stripe mosaic	RNH 250
		E	90–100 Å	Influenza, fowl plague	I myxo (1)
			170 Å	Parainfluenzae, mumps, sendai, Newcastle disease, rinderpest, Carré disease, measles	II myxo (1)
	C	N	32	Turnip yellow mosaic	RCN 32
			60	Bushystunt, polio (2)	—
			92	Wound tumor, REO III	REO
DNA	H	E	90–100 Å	Vaccinia, infectious pustular dermatitis	Pox (3)
	C	N	12	ϕX174	DCN 12
			42	Polyoma, warts, SV40	Schizo (4)
			252	Adeno, infectious canine hepatitis Gal (5)	Adeno
			812	Tipula	DCN 812
		E	162	Herpes, varicella, pseudorabies	Herpes
	B	N	Prism	Phage T2	T pairs
			Icosahedron	Phage of *B. megaterium*	—

[a] The table contains abbreviations which are those of the phanerogram.

TABLE III

THE LHT SYSTEM IN 1969

Nucleic acid	Capsid symmetry	Naked (N) or enveloped (E)	Helical diameter or no. of capsomeres	
DNA	H	N	50 Å	Inoviridae
		E	?	Poxviridae
	C	N	12	Microviridae
			32	Parvoviridae
			42	Densoviridae
			72	Papilloviridae
			252	Adenoviridae
			812	Iridoviridae
		E	162	Herpesviridae
	B	N Urovirales		Phages with tail
RNA	H	N Rhabdovirales		
			90 Å	Myxoviridae
			180 Å	Paramyxoviridae
		E Sagovirales		Stomatoviridae
			?	Thylaxoviridae
	C	N Gymnovirales	32	Napoviridae
			92	Reoviridae
			?	Blue tongue virus (sheep)
		E Togavirales	?	Encephaloviridae

Most of the elements of this table have been known for many years and can be traced back to the work of Luria and Darnell (1968), Davis, Dulbecco, Eisen, Ginsberg, and Wood (1968), and Fenner (1968). We have completed the table with the help of recent data. Now follows a sequence of comments on the various families (presented in the same order as Table IV).

1. Inoviridae—Type Genus: *Inovirus*

This family brings together the filamentous phages, the adsorption of which depends on the existence of sexual pili. It includes, specifically phages fd, fl, and M13. The fact that the single-stranded DNA is circular implies a different structure for the virion from that of the tobacco mosaic virus. Two patterns were proposed. One, roughly cylindrical, with the DNA located in a central core, the other with two parallel cylinders, each containing half of the DNA. In both cases, the units of the structure (protein B) join together in a helical formation.

TABLE IV

THE CHARACTERISTICS OF VARIOUS FAMILIES OF VIRUSES

Viridae	Nucleic acid	Symmetry	Naked (N) or enveloped (E) nucleocapsid	Virions with cubic symmetry			Virions with helical symmetry		Mol. wt. of nucleic acid ($\times 10^6$)	Number of nucleic acid strand (5)
				Number of capsomers	Diameter of the nucleocapsid (Å)	Diameter of the envelope (Å)	Diameter and length nucleocapsid of the (Å)	Dimensions of the enveloped virions (Å)		
Ino-	D	H	N				5–6 × 760–850		1,7–3	1
Pox-	D	H?	E				?	2500 × 1600 3000 × 2300	160–240	2
Micro-	D	C	N	12	250				1,7	1
Parvo-	D	C	N	32	220				1,8	1
Denso-	D	C	N	42	200				1.6–2.2	1
Papilloma- (papova)	D	C	N	72	450–550				3–5	2
Adeno-	D	C	N	252	700				20–25	2
Irido-	D	C	N	812	1300				126	2
Herpes-	D	C	E	162	775	1500–2000			54–92	2
Uro-	D	BC	N	—	—					2
Rhabdo-	R	H	N				20 × 130 10 × 1250			1
Myxo-	R	H	E				90 × ?	1000	2–3	1
Paramyxo-	R	H	E				180 × ?	1200	7,5	1
Stomato- (rhabdo)	R	H	E				180 × ?	1750 × 680	6	1
Thylaxo	R	H	E				?	1000	10	1
Napo	R	C	N	32	220–270				1,1–2	1
REO	R	C	N	92	700				10	2
Cyano	R	C	N	32 or 42	540				—	2
Encephalo	R	C	E	?	?		600–800		2–3	1

2. Poxviridae—Type Genus: *Poxvirus*

The helical structure of the nucleocapsid of the Poxviridae remains entirely hypothetical: the helical tubules seen at the periphery of the virion are part of the envelope system but are not associated with the nucleic acid. The main viruses or diseases include: vaccinia, small pox, mouse ectromelia, myxoma and fibroma of Shope, bovine papular stomatitis (dermatitis), orf, avian small pox (avipoxviruses), and *Molluscum contagiosum.*

3. Microviridae—Type Genus: *Microvirus*

The DNA of phage ϕX174 and related phages S13 and ϕR is circular.

4. Parvoviridae—Type Genus: *Parvovirus*

The DNA of "minute virus of mice" (Crawford, 1966) and of the K virus of rats is single-stranded.

5. Densoviridae—Type Genus: *Densovirus*

This type is the densonucleosis virus of *Galleria mellonella* as described by Kurstak and Cote (1969). The structure of its nucleic acid is not yet strictly determined.* These authors postulated a capsid with 42 capsomers. The same figure was first proposed for the papilloma and polyoma viruses but agreement has now been reached on the figure of 72.

6. Papillomarividae (or Papovaviridae)—Type Genus: *Papillomavirus*

This family includes two genera: *Papillomavirus*, with a diameter of 55 mμ, whose circular DNA has a molecular weight of 5 million [main viruses: rabbit papilloma virus (Shope), the human wart, bovine, equine papilloma] and *Polyomavirus*, with a diameter of 45 mμ whose circular DNA has a molecular weight of 3 to 3.5 million (main viruses: polyoma virus, SV40 virus).

7. Adenoviridae—Type Genus: *Adenovirus*

The 31 human adenoviruses, the simian, bovine, avian, and canine adenoviruses (Rubarth hepatitis).

8. Iridoviridae—Type Genus: *Iridovirus*

Tipula iridescens virus, *Chilo*, and *Sericesthis* viruses.

* See Note Added in Proof, p. 42.

9. Herpesviridae—Type Genus: *Herpesvirus*

In man this genus includes: herpes simplex virus, varicella-zoster viruses, cytomegalic inclusion virus disease, infectious mononucleosis virus, viruses associated with Burkitt's disease, and rhinocarcinoma of the pharyn. (The last three viruses are, if not identical, at least closely related.)

In animals this genus includes: feline, bovine rhinotracheitis viruses, abortion in equidae, cytomegalic inclusion virus disease in guinea pigs, mice, etc.

10. Urovirales

The world of bacteriophages provided with a tail. They are grouped, not into a family, but into an order: the urovirales.

The structural diversity of these phages enables us to envision a classification that will take into consideration the following:

1. *The tail*:
    a. Whether or not it is contractile.
    b. The structure of the terminal plaque.
    c. The structure of the collar.
    d. The length.
    e. The existence of fibers.
2. *The head*: octahedral, icosahedral, or any other form.
3. *The nucleic acid*: its molecular weight, the sequence of its nucleotides, its homologies with the nucleic acid from other phages. Its eventual single- or double-stranded structure.

11. Rhabdovirales—Type Genus: *Rhabdovirus*

This order brings together at least fifty of the plant viruses, classified by Brandes and Berks into six different groups, the most extensively studied of which is the tobacco mosaic virus. The name *Rhabdovirus* was proposed in 1965 by the Provisional Committee for the Nomenclature of Veruses (PCNV) (see Anonymous, 1965).

12. Myxoviridae—Type Genus: *Myxovirus*

The 3 types of *Myxovirus influenzae* A, B, and C.

13. Paramyxoviridae—Type Genus: *Paramyxovirus*

Among the RNA viruses with helical symmetry, enveloped, and with a nucleocapsid 180 Å in diameter, the following forms have been separated:

subspherical virions, which are the Paramyxoviridae, and bullet-shaped virions, which are the Stomatoviridae. The genus type of Paramyxoviridae is *Paramyxovirus*.

Apart from the parainfluenzae viruses I, II, III, and IV, the group includes the viruses of mumps, canine distemper, and rinderpest.

14. Stomatoviridae—Type Genus: *Stomatovirus*

This family, as was previously stated, groups viruses of the bullet-shaped virions. The name *Stomatovirus* was proposed in 1965 by the PCNV. A few years later, a group of the International Committee for the Nomenclature of Veruses (ICNV) proposed the name Rhabdoviridae for the same group.

Now, as already mentioned, the name Rhabdoviridae was proposed by the PCNV for plant viruses. It is unfortunate, and even inadmissible, to see virologists appropriate a name already given to one group and to apply it to another. Such practices can only lead to confusion. They are forbidden by the codes of plant, animal, and bacterial nomenclature and it is to be hoped that this ruling will soon apply, also, to the code for the nomenclature of viruses.

The group comprises rabies, vesicular stomatitis viruses, and the *Drosophila* sigma virus.

15. Thylaxoviridae—Type Genus: *Thylaxovirus*

These represent the group of RNA oncogenic viruses. The structure of their nucleocapsid is still poorly elucidated. A few observations suggest that it could be of helical symmetry and of smaller diameter lower than the Myxoviridae. The name was proposed by a study group (Anonymous, 1966):

Virus of avian sarcomas and leukosis
Virus of murine sarcomas and leukosis
Virus of the mammary tumor of mice (Bittner).

In Fenner's treatise, the Thylaxoviridae are called leukovirus.

16. Napoviridae—Type Genus: *Napovirus*

This is an important family by virtue of the number of its representatives. It comprises:

1. The *Napoviruses*, of which the typical species is the yellow mosaic virus of the turnip and several plant viruses with cubical symmetry.

2. The small RNA bacteriophages: f2, MS2, R17, R23, Qβ.

3. The polioviruses, coxsackieviruses, echoviruses, rhinoviruses, and the foot-and-mouth virus. These viruses are often referred to under the name of "picornavirus"—but it is not known whether this name corresponds to a genus or to a subfamily (see Lwoff and Tournier, 1966).

17. Reoviridae—Type Genus: *Reovirus*

The characteristics of the *Reovirus* virions and those of the wound tumor virus are the same. Furthermore, the RNA is double-stranded. For this reason, these viruses are classified together (Lwoff and Tournier, 1966). The group comprises:

1. The three types of *Reoviruses* I, II, and III common in humans and other mammals.

2. The clover-wound tumor virus, the rice dwarf virus.

18. Cyanoviridae—Type Genus: *Cyanovirus*

The blue tongue virus in sheep has a different structure from that of the reovirus. It has 32 or 42 capsomers, and its ribonucleic acid is double-stranded. Possibly, it represents the type of a new family.

19. Encephaloviridae (and Arboviruses)—Type Genus: *Encephalovirus*

This is one of the families resulting from the breaking up of the arboviruses. These are defined, not by their structure, but by their mode of transmission. They represent, therefore, an ecological group. These viruses appear to belong to three distinct groups:

1. The *Encephalovirus* group. The icosahedral structure of the capsid has been demonstrated only for a few viruses of this extremely vast group, which includes several subgroups:

Group A: eastern equine encephalitis (EEE), western equine encephalitis (WEE), Venezuela equine encephalitis (VEE), and sindbis.

Group B: West Nile virus (WNV), Japanese B encephalitis virus (JBE), St. Louis encephalitis virus, yellow fever virus, tick-borne encephalitis virus.

Group C: Oriboca.

Outside of these three groups are phlebotomus fever virus and hemorragic fever viruses.

For reasons of structure, the following are not included in the "Encepha-

loviridae" although these are "arboviruses" in the ecological sense of the term: African horsesickness virus, blue tongue virus, and vesicular stomatitis virus.

2. The vesicular stomatitis group: RNA, helical symmetry, nucleocapsid, enveloped, bullet-shaped.

3. The blue tongue virus: RNA, cubical symmetry, no envelope, 32 or 42 capsomers.

Miscellaneous

A. It was proposed to group under the name *Coronaviruses* (Anonymous, 1968) avian infectious bronchitis viruses, mouse hepatitis, and certain strains isolated during respiratory tract infections in humans. These contain a ribonucleic acid and are surrounded by a lipid envelope, but the type of symmetry of the nucleocapsid is not yet known.

B. A virus present in a *Penicillium cyaneofulvum* strain has recently been described. It has a diameter of 32.5 mμ, appears to present a cubical symmetry, its nucleocapsid is naked, and contains a double-stranded RNA. The number of capsomers has not been determined.

C. The alphalpha mosaic virus is elongated. The rounded extremities are semiicosahedral cut perpendicularly to a ternary axis. The cylindrical part in the center is a flat hexagonal network. Although elongated, the virion therefore belongs to a cubical symmetry system (Hull *et al.*, 1969).

B. Discussion

Let us bear in mind that the viruses belonging to a given family in the LHT system have the following characteristics in common:

A. The four obvious characteristics of the LHT system:

1. Nature of the nucleic acid.
2. Symmetry of the capsid.
3. Presence or absence of an envelope.
4. Number of capsomeres (cubical symmetry) or diameter of the nucleocapsid (helical symmetry).

B. Other characteristics:

5. The molecular weight of the nucleic acid.
6. The proportion of nucleic acid in the nucleocapsid.
7. The single- or double-stranded structure of the nucleic acid.

8. The percentace of guanine + cytosine (within certain limits).
9. The pattern of the doublets.
10. The homologies of the genetic material (within certain limits).
11. The form of the nucleocapsid.
12. The dimensions of the nucleocapsid (within certain limits).
13. The form of the virion (with one exception).
14. The dimensions of the virion.

The correlation of characteristics 5–14 with the sum of characteristics 1–4 could not be accidental. The viruses belonging to the families of the LHT system present similarities and are probably biologically related. It has been said that the families of the LHT system were conceived "by intuition." Such is not the case. The principles of the classification used by LHT and the choice of the characteristics are derived from a rational analysis and numerous trials.

Be that as it may, a system must prove itself and it is not the greater or lesser role played by intuition that will determine its value.

The opponents of the LHT system should explain why the grouping is not satisfactory. It must be recognized that the opponents are in a privileged situation, since they have not proposed any general classification of viruses. It is certainly easier to criticize than construct.

Some virologists consider that a classification must comprise coefficients of similarity. Within the LHT system, assuming that the viruses have 4, 3, 2, or 1 characteristics in common, the coefficients will be 100, 75, 50, or 25%, respectively. If the coefficient is 100%, the viruses will belong to the same family. The fact that a computer is not needed to calculate the coefficients of similarity should not diminish their value.

Within the LHT system, the characteristics are placed in a hierarchical order. The justification of the hierarchy has been presented (Lwoff and Tournier, 1966) and it seems useless to go back on that point again.

In the absence of phylogenic data, any hierarchy, as noted by Lwoff and Tournier (1966), is necessarily arbitrary. However, a hierarchical system for viruses presents some advantage. It affords, at a glance, an overall picture of the viral world and provides, if not enlightenment, at least a certain order. And order, even though arbitrary, is better than confusion.

The unitary concept of the viral world evolved somewhat belatedly. For a long time, the virologists were only concerned with groups pertaining to their specific interest: plant viruses, animal viruses, and bacterial viruses. With regard to the animal viruses, specialization came more and more to the fore. The human viruses, vertebrate viruses, invertebrate viruses, and

insect viruses were considered separately. The situation was naturally complicated by the fact that certain plant viruses were evolving in insects, and certain vertebrate viruses in diverse arthropods.

The separatism manifested itself as classifications dealing exclusively with one group of viruses, followed by the observation that the virions of certain plant, bacteria, insect, and vertebrate viruses were similar. Little by little, the idea imposed itself that it was preferable to classify viruses according to the characteristics of the virion rather than according to the nature of the host or the vector. The unitary concept of the viral world is, today, universally accepted. It becomes more and more difficult to accept classifications that adopt as a discriminative character the systematic position of the host—or sometimes, even of the vector.

At present, the LHT system is the only one that embraces the entire viral world. It permits the definition of taxons having one, two, three, or four characteristics in common. It so happens that taxons exhibiting four common characteristics correspond to groups of genera, that is to say, to families that are today recognized by the majority of virologists. The LHT system also permits the classification of plant viruses. Nevertheless, plant virology is strongly handicapped by a group of factors that are more closely related to the mental confusion of some people than to the viruses themselves.

We would like to add a remark here. When total disorder reigns within a given domain, any system of order that emerges is resented by many as a constat of deficiency; all the more so, of course, as it affords more clarity.

C. Remarks on Viruses without Capsids

In their studies on the spindle tuber potato virus, T. O. Diener and Raymer (1969) were able to prove that the terminal form of the cycle is a double-stranded naked RNA, and that there are no nucleocapsids, in other words no virions. The molecular weight of the RNA is from 100 to 200,000; in other words, the nucleic acid can most likely "code" for one single protein. This protein, if it is unique, could be nothing more than replicase.

Naturally, we must ask ourselves if this naked nucleic acid can be considered a virus. It is a question of definition. The virus may be defined as an infectious particle that has only one type of nucleic acid and reproduces itself from the single genetic material, and the viral infection as the introduction into the cell of the genetic material of a virus. If these definitions are accepted, then the spindle tuber potato RNA is a virus.

Does this virus represent a primitive form or a degraded evolved form? For the moment, it is impossible to answer this question; the problem of the origin of RNA viruses was discussed recently (A. Lwoff, 1969). However, the very existence of this naked viral RNA obliges us to provisionally divide the RNA viruses into two groups: one with a capsid and the other without a capsid. When we shall know the frequency of the doublet pattern and the percentage of guanine cytosine, and when cross-breeding experiences have been performed, perhaps it will be possible to relate this RNA to the RNA of some "normal" virus.

III. Pros and Cons of the LHT System

It was evident that the LHT system would be accepted by some and rejected or even contested by others.

A. Pros

A. Cohen (1969) includes the LHT system in his "Textbook of Medical Virology"; M. Frobisher (1968) reproduces it in his "Fundamentals of Microbiology" and writes: "Neither perfection nor immutability are claimed for the system; but being the first of its kind, it marks a milestone in the science of virology."

The system is also reproduced in "General Virology" by S. Luria and J. Darnell (1968), who write: "A major advance came when a system was proposed that took as its basis the structure and composition of virions and could embrace all viruses... ."

In addition, we know that many virologists use the LHT system in their teaching.

B. Cons

The offensive first began at Cold Spring Harbor. During the discussion that followed the presentation of the system, Wildy (1962a) declared that the viruses form a heterogeneous collection of entities considered together by virtue of an arbitrary definition.

It is only necessary to refer to the title of the Marjory Stephenson Lecture "Concept of Viruses" (A. Lwoff, 1958) to realize that, for a long time,

viruses were held as a concept. In nature, one encounters individuals, not species, types, or families. Nature does not know categories constructed by the human mind; nevertheless, there is nothing to prevent taxonomists from operating logically and rationally.

Let us state, first of all, that the virus group is no more heterogeneous than any other group. The animal kingdom and vegetable kingdom are heterogeneous. The vertebrates constitute a heterogeneous group, as do the mammals. A taxon, whichever it may be, is a gathering of various organisms, that is to say, a heterogeneous group; and the higher the taxon in the hierarchy, the greater the heterogeneity. The herring and man belong to the same taxon, as do the paramecium and the elephant. Do the viruses differ more among themselves than the various representatives of certain categories of the animal kingdom? The judgment arrived at will depends upon the idiosyncrasy of the individual. Some people are inclined to retain what separates, others what unites. We belong to the latter group. This is why the group of viruses appears to us to be remarkably homogeneous. At any rate, if others consider it to be heterogeneous, it is up to them to put an end to the controversy by professing an alternative definition of viruses that will be less arbitrary.

Gibbs wrote, in 1969, that the LHT classification resembles, as much in its principles as in its defects, the first classifications of plants and animals. Gibbs adds: "see review by Adanson (1763)." The same author, in the same article, also writes (p. 309): "There are many ways to organize groups, but most seem quite arbitrary and of little value. The hierarchy based on four properties of the virions, as proposed by LHT in 1962, is of this type, for there is no evidence that any of the properties used in their proposed hierarchy will cluster related groups of viruses."

It should be noted that, within the LHT system, only one category is defined by a single character, the others are defined by two, three, or four characteristics. But Gibbs forgets this, no doubt unintentionally.

It is on the strength of these four characters, the RNA, a naked capsid with cubical symmetry, and the 92 capsomeres, that the LHT system has united, in one group, what is today the family Reoviridae, the reoviruses, and the plant rumor viruses. Gibbs (1969) also states that these viruses are related, but omits mention that LHT arrived at the same conclusion 7 years earlier.

The LHT system has been criticized as being arbitrary. As we have said repeatedly and will repeat again, all classifications are arbitrary in the sense that categories or taxons do not exist in nature. Categories and taxons are concepts, as are evolution, heredity, or allosteric interaction. And any

hierarchy shares the arbitrary characteristic of the classification. To blame a classification or a hierarchy for its arbitrary characteristic, is like blaming a cube for not being spherical.

There is more. Virologists have recognized that a certain number of species present some affinities. The species have been grouped into genera. Genera showing common characters have been grouped into families. The four characters of the LHT system make it possible to define families. The opponents of the LHT system make a point of ignoring that this system makes it possible to group viruses according to their natural affinities. To dispute the value of the LHT system under these conditions is curious. In a more general way, to dispute the principles of the classification of viruses without proposing a classification and to pretend to ignore that viruses are already distributed into families, to say the least, is somewhat singular. It is evident that the LHT system is very embarrassing to those who affirm that it is impossible to classify viruses.

Bellett (1967b) has stated that the LHT system is not scientific. He has in fact adopted Poper's ideas concerning classifications—which we will now summarize. A scientific classification is based on a scientific theory that attempts to consider the properties of the entities and the distribution of these properties within the population. That is alright. Starting with these premises, Bellett affirms that the LHT classification does not pretend to be scientific, that it belong to a kind of system constructed merely to solve the practical problems of nomenclature and identification, and that, in fact, LHT did not take into consideration the natural affinities of the viruses. It is perfectly true that we did not assign a scientific character to our system —that would have been pretentious. We did not intend to solve problems of nomenclature and identification: in our publication (Lwoff *et al.*, 1962b), there is no question of nomenclature or of identification. Finally, here is what we wrote: "In other terms, we feel that the various viruses, when their essential integrants are established, will find their "natural" place in the system. By natural place, we mean they will fall in the same group as biologically related entities."

Therefore, contrary to what Bellett affirms, LHT were concerned with the natural affinities of viruses. After all, it matters little whether a classification is based on one principle or another. What is important for a classification of viruses is that it groups the viruses according to their affinities and, also, that it presents a clear synoptical picture of the viral world. If it succeeds in so doing, the classification will be right. If it does not succeed, it will be wrong, even if it is based on a so-called scientific theory.

IV. Phanerogram, Cryptogram, and Gymnogram

A. Phanerogram*

Nothing prevents the name of a virus from being followed by an abbreviation corresponding to the four characteristics of the LHT system: D and R for DNA and RNA, C and H for cubical symmetry and helical, etc. —as was done by LHT in 1962. In this way, for each virus, we can establish a formula, which will be referred to as a phanerogram.

B. Cryptogram

In 1966, Gibbs, Harrison, Watson, and Wildy proposed the use of eight characteristics for identifying viruses: the type of nucleic acid, its molecular weight, the percentage of nucleic acid within the virion, the form of the particle, the form of the nucleocapsid, the host, and the vector. The various parameters are defined by abbreviations and the whole of the formula has been named cryptogram.

It is evident that phanerogram and cryptogram correspond exactly to the same approach, which is the selection of characters. It should be noted, however, that the International Committee for the Nomenclature of Viruses named only one "cryptogram commission." It is true that the characteristics chosen by LHT were not given Greek names.

C. Comparative Values of the Characteristics of the Phanerogram and the Cryptogram

1. *The Form*

It is now time to critically examine the value of the characters proposed by one or the other, and we will start with the form.

We can do no better than to cite the ideas expressed by Horne and Wildy (1961): "Size and shape of the virion have so far been the only morphological characters used to classify viruses. These attributes are both unreliable and misleading as criteria and we suggest that they be abandoned forthwith." The form of the virion is "misleading," add Horne and Wildy, because it results from the symmetry of the capsid and from the presence or absence of such structures as the envelope and tail.

* Phanerogram from phaneros, visible and from gramme, writing. The list of discriminative characters employed by LHT in their classification.

Our colleagues conclude by saying that we must abandon the form in favor of the symmetry of the capsid. The number of capsomeres will also be a useful characteristic for the classification of viruses with cubical symmetry. Finally, Horne and Wildy proposed a scheme of classification of viruses based on the symmetry of the nucleocapsid and on the nature of the genetic material. Nevertheless, when Horne, with Lwoff and Tournier, put into practice the principles he had formulated with Wildy himself, the latter, together with Gibbs, Harrison, and Watson abandoned his stand.

In fact, while LHT were in favor of the *symmetry* of the nucleocapsid, Gibbs *et al.* were utilizing its *form*. For purposes of definition, the virologist has the choice, in the cryptogram, between *essentially spherical*, on one hand, *elongated*, on the other hand (with some variations), and, finally, *complex*. In the example given by Gibbs *et al.*, they talk about a spherical nucleocapsid. However in 1969, Gibbs refers to isometrical nucleocapsids. Here, the reader is confused; since isometrical has two different meanings. In current language, isometrical means of equal dimensions: a sphere is isometrical. In the language of crystallography, isometrical is synonymous with cubical symmetry. When Gibbs talks of *isometrical* capsid, is he using current language or that of crystallography? Probably current language, since isometrical is opposed to anisometrical—but this is only an assumption. Anyhow, the cryptogram considers the form, not the symmetry.

2. *The Elongated Character*

Next to the spherical character of the nucleocapsid, we mention, in the cryptogram, the elongated nucleocapsid with parallel sides. It is likely that this category corresponds to the nucleocapsids with helical symmetry. Nevertheless, here again, the reader is confused. In fact, do we have the right to say that the nucleocapsid of the tobacco mosaic virus, for instance, has parallel sides? Gibbs *et al.* obviously thought of a cylinder since, in a cylinder, the hypothetical straight lines perpendicular to both terminal circles are in fact parallel; and while a nucleocapsid with helical symmetry is roughly cylindrical, it does not, in fact, have "parallel sides." Furthermore, "elongated" does not always correspond to helical symmetry. The elongated nucleocapsid of certain viruses has a cubical symmetry.

3. *The Envelope*

The phanerogram takes into consideration the naked or enveloped character of the nucleocapsid. In the cryptogram, one considers the form of the virion and that of the nucleocapsid, separately. But, the one and only

difference that the virion has to offer in relation to the nucleocapsid is the presence of an envelope.

Now, let us suppose that a "spherical" nucleocapsid is surrounded by an envelope, which too is "spherical." It will be impossible, according to the formula of the cryptogram, to know if the nucleocapsid is naked or enveloped. No clear distinction has been made in the cryptogram between naked and enveloped nucleocapsids (Gibbs, 1969).

This being said, it becomes evident that in certain very precise cases the form of the virion is a useful characteristic. In this way, in our classification, the Paramyxoviridae (family including the measles and the mumps viruses) are separated from the family of the Stomatoviridae = Rhabdoviridae (which includes the rabies and the *Drosophila* sigma viruses) by the characteristic, whether spherical or bullet-shaped, of the envelope.

D. Other Characters of the Cryptogram

1. *The Molecular Weight of the Nucleic Acid*

This is undoubtedly an excellent character.

2. *The Percentage of Nucleic Acid within the Virion*

This character can be maintained, even though it does not seem to be of great usefulness in a classification. The percentage of nucleic acid within the nucleocapsid should be more significant.

3. *Hosts*

It is known that several viruses are liable to evolve in a great number of, sometimes quite remote, organisms. The characteristic of the host could, nevertheless, be useful at times.

4. *Vectors*

The existence or lack of a vector, and the nature of the vector, should not be utilized for defining categories higher than the species.

E. General Remarks

Examination of the comparative table of the phanerogram and the cryptogram (Table V) enables us to observe the similarities and differences. It is seen that the "form" characteristic of the cryptogram will, sooner or

TABLE V

PHANEROGRAM AND CRYPTOGRAM

Virus		Phanerogram	Cryptogram
Nucleic acid	DNA or RNA	+	+
	Single- or double-stranded nucleic acid	0	+
	Molecular weight	0	+
	Percentage in the virion	0	+
Virions	Presence or absence of an envelope	+	0
	Shape	0	+[a]
Nucleocapsid	Symmetry	+	0
	Number of capsomers (cubical symmetry) or diameter (helical symmetry)	+	0
	Form	0	+[b]
Hosts		0	+
Vectors		0	+

[a] The difference between virion and nucleocapsid is necessarily related to the presence of an envelope (see text).

[b] The shape of the nucleocapsid will evolve rapidly toward symmetry (see text).

later, evolve toward the "symmetry" characteristic of the phanerogram. We also note that the cryptogram characteristic "form of the virion" is equivalent to the phanerogram characteristic "presence or absence of an envelope." In approaching the phanerogram, the cryptogram will no doubt gain in precision. Is it really justified to present the cryptogram as an original model at the expense of the phanerogram?

F. GYMNOGRAM*

Under the heading of gymnoviruses, we designate the viruses without capsid and whose infectious phase is a naked nucleic acid (see Diener and Raymer, 1969). The gymnoviruses could be classified according to a certain number of characteristics:

1. Nature of the genetic material.
2. Number of strands.
3. Molecular weight of the nucleic acid.
4. Percentage of guanine + cytosine.

* Gymnogram from gymnos, naked. The list of characters proposed for the classification of naked viruses, without capsid.

5. Affinities such as result from the hybridization test.
6. Sequences of bases.
7. Number and nature of the viral proteins.

Each one of these characteristics will be represented by abbreviations and the formula will be called *gymnogram.*

V. Evaluation of Characteristics

In order to define taxons and classify viruses, should we use the "totality" of characteristics or, on the contrary be selective?

As mentioned before, LHT chose four characters. Gibbs *et al.*, 1966, write that for them it is not possible to determine the relative importance of the different characters. They conclude that it is essential to follow the principles of Adanson, who suggested "that all the data should be used and that all the characteristics should be considered to be of equal importance."

However, Mayr (1965a) says in his excellent article "Numerical Phenetics and Taxonomy": "It is thus clear that Adanson... did not propose that the taxonomist abandon his prerogative to evaluate the characteristics. Indeed, Adanson can be considered the father of the method of character evaluation as practiced in classical taxonomy. It is a gross injustice to Adanson to label the nonweighing of characters: the Adansonian method."

Mayr adds that "the careful weighing of the few available characters is an absolute necessity.... To dilute these few useful characters by large numbers of useless ones in order to acquire a false sense of quantitative security is a procedure that, quite rightly, was already ridiculed by Adanson, who was not that naïve."

Mayr finally adds that "the sooner the myth that it is a sin to weigh characteristics disappears, the better for the numerical method. The numerical method will prove most successful that solves the problem of weighing most efficiently."

Thus, Adanson was a supporter of evaluation. It is of little importance since, any way, we do not accept the dogma of the infallibility of Adanson. It is preferable to ask, objectively, where is the refusal to evaluate and the aforementioned phenetic or numerical method leading to, and where is the computer leading to? Sokal and Senath, the principal protagonists of the nonevaluation method, admit that the only thing that the computer can do is to determine the similarity among taxons. "The rest of the taxonomical

task," Sokal and Sneath write (1963) "still needs the experience and the judgement of specialists in the field."

The evaluation, or the choice of a character, is, therefore, considered by Gibbs *et al.* to be arbitrary. What should be said, then, of the refusal to chose, of the arbitrary decision according to which all characters are of equal value?

As was rightly noted by Mayr (1965b) the pheneticist does not differentiate between the important characteristics and those without any value. The pheneticist ignores the existence of "marked discontinuities" among the groups. "The categories of the pheneticists are based on arbitrary levels of phenetic distances."

It is to be wondered, then, why the pheneticists do not want to attribute different values to the characteristics. Perhaps it is because they do not know how to assess their value. Perhaps, also, it is because the programming of information into a computer is easier if all the characters have the same value, that is, the same content of information. Whatever it may be, a method should be judged according to the results it affords.

Certain characters are, as we know, stable. One has never seen either the nature of the genetic material or the symmetry of the capsid modified by a mutation. One also knows perfectly well that other characteristics are unstable: the virulence is easily modified by mutations. All the characters, therefore, do not have the same value. To attribute the same value to all of them is to renounce reason and to sink willingly into error.

What can be expected of a machine that has been supplied with sixty characteristics of necessarily unequal values? We agree with Sokal and Sneath: "the computer will determine the similarities among taxons." However, since these similarities are founded on characters of unequal values, the numerical expression of similarities will not make much sense.

Therefore, Gibbs *et al.* consider that a classification must rely on at least sixty characteristics to which an equal value must be attributed. Let us see where the refusal to evaluate can lead to.

Virus A is an RNA virus with cubical symmetry.

Virus B differs from A by its virulence, a characteristic that is apt to vary under the influence of mutations.

Virus C differs from A by the nature of the nucleic acid, a stable characteristic.

Virus D differs from A by the symmetry of the capsid, an equally stable characteristic.

Therefore, B, C, and D differ from A, each of them by a single characteristic. Since we decided that all the characteristics have the same value, B,

C, and D are, all three of them, at the same phenetic distance from A. And since A, B, C, and D differ by only one of the sixty characteristics, they will in a *numerical* classification, be placed into the same group. In a *normal* classification, A and B will belong to the same family, C to a different family from A and D, and D to a different family from A and C.

We can go further and pose a few questions to the virologists.

A. It is reasonable to place within the same species, the same genus, or the same family, two viruses differing by:

1. The nature of the genetic material.
2. The symmetry of the capsid.
3. The number of capsomeres.
4. The naked or enveloped nature of the nucleocapsid?

B. Is it reasonable to bring together within the same species, the same genus or the same family, viruses differing by two, three, or four of the cited characteristics?

Once again, species, genus, and family are arbitrary categories, or concepts, in the same way as the taxon virus is a concept. To use the word group is an evasion and it does not alter in any way the problem. This being said, we are now awaiting an answer from the followers of nonevaluation.

It should be noted, however, that Gibbs, along with Harrison, Watson, and Wildy, having asserted categorically that all characteristics have an equal value and, no less categorically, that we do not have the right to assign a different value to them, suddenly discovered, in 1969, that certain of the characteristics are stable while others are not, and that the value of one and the other is different. This is a reversal of their previous stand.

Perhaps it would serve some purpose here, to examine the very concept of viruses as it presents itself in regard to the nonevaluation and the numerical method.

The virus concept was formulated with the help of a comparative analysis of the characteristics of the small infectious particles. It was recognized that some of them had a group of characteristics in common, that were absent in others. In view of these discriminative characters, the small infectious particles were separated into *viruses* and *nonviruses*. The virus concept was, therefore, based on a rational analysis and on the evidence of discriminative characters. The supporters of the numerical method will obviously say that the choice is arbitrary. Nevertheless, it does not prevent them from using the term virus in the sence given to it by a method we consider rational.

That is what Bellett (1967c) expressed: "I shall use the term 'viruses' to refer to a class of entities defined by the sum of the properties listed under 'viruses' in Table 4 of Lwoff 1968, that is, to infectious agents which contain one type of nucleic acid and do not grow and divide (as defined by Lwoff) or contain a Lipmann system."

It is obvious that one should define what one is talking about. However, some virologists discuss viral nomenclature and classification without saying what they mean by viruses: they are the irreducible followers of the numerical and the nonevaluation methods.

Mayr (1965b) insisted very strongly on the fact that the numerical taxonomist ignores the sharpness of discontinuities among taxons and that his categories are based on arbitrary levels of phenetic distances.

Let us assume that a supporter of the numerical method decides to classify the small infectious particles. Since he refuses to make a choice, he will use all the characteristics among which, of course, some are not discriminatory, as, for instance, dimensions, form, virulence, symptoms. He will thus arrive at the conclusion that, among viruses and nonviruses, there is a certain phenetic distance, but he will not be able to say in what way viruses differ from nonviruses. In order to classify viruses, therefore, the followers of the numerical method make use of principles that do not permit to define viruses. This is the death sentence of the numerical method and of the nonevaluation.

We can only define viruses by discriminatory characters. What is true for the taxon virus is true for hierarchically inferior viral taxons.

VI. Categories and Taxons, Nomenclature

A. Names, Categories, Taxons

For certain primitive peoples, the name designating an individual possesses a magical value. To know the name of an individual, is to know him, himself. The name, insofar as it denotes one individual to the exclusion of all others, is a specific element combining itself to the body to constitute the total being. This is the *nama-rupa*, the "name and body" of the Buddhists.

Doubtlessly these propositions will not be wholly accepted, but it is quite obvious that a name can and must express a virus, without being necessarily "combined" to the virion. However, it is the properties of the virion that define its personality and make it possible to assign a name to it. We are not too far from the *nama-rupa*.

Names are symbols that make it possible to recognize objects and organisms. A nomenclature is a system of names. A biological classification is a distribution of organisms into categories of various hierarchical value. Phyla, classes, orders, families, genera, and species are categories. A category corresponds to a rank within the hierarchy.

When a classification is applied to clearly defined organisms, a category embraces a definite group of organisms united by reason of certain similarities: the category thus becomes a *taxon*. Bacteria, mollusks, mammals, and viruses are taxons.

Finally, let us add that taxonomy, which is etymologically the distribution of taxons, is the science of classification.

B. Remarks on the Term "Group"

A few virologists reject species, genus, and family, and use the word "group" exclusively. While species, genus, and family are categories of a definite hierarchical value, "group," or its English equivalent *cluster*, have no definite hierarchical value.

In fact, at times, the *group* unites strains and corresponds to the species; in other instances, it unites species and conforms to the genus; and at other times, it unites genera and is the equivalent of a family.

In any case, nature knows nothing but individuals, and any grouping, as we said before, and are repeating, and shall repeat again, is arbitrary and the group is no less arbitrary than the species, the genus, and the family. Its sole interest is the lack of precision.

C. Rules of Nomenclature

Since it is necessary to know of what we speak, there is no classification without nomenclature. It is necessary, here, to recall certain rules.

We will give the essential points of the present international rules, as were established by the International Committee for Nomenclature of Viruses (INCV). First, it should be noted that, since these rules apply to species and to genera, it was necessary to define the species and genus.

1. The species groups identical viruses.
2. The genus is a group of species having common characteristics.
3. The name of the genus must terminate in the suffix *virus*.

The INCV decided that an effort should be made toward binomial Latin nomenclature. By adding the suffix *virus*, the names of the genus are auto-

matically Latinized. The recommendation of the Committee is lawful as regards the genus. It is noted that the termination corresponds to usage. Such names as *Adenovirus*, *Myxovirus*, and *Poliovirus* have long been in use.

4. Each genus must have a type species. This is an indispensable measure in order to assure the stability of the name of the genus and in order to avoid interminable and irrelevant discussions. The name of the genus will always remain linked to the type species, whatever classification is adopted.

5. The Committee did not define families that are and can only be groups of genera.

The names of families must terminate in *idae*.

For Bellett, a nomenclature does not need to be based on a classification. That is correct as regards the nomenclature of individual viruses. However, the uniting of strains into species and of species into genera are acts of classification. The function of binomial nomenclature is to give a name to genera and species, and no scientific nomenclature is possible in the absence of this classification, which is elementary but essential.

Gibbs *et al.* (1966) consider that "a Latin binomial system is based rigidly on chosen characteristics in order to determine the form and the hierarchy of taxons: division, order, family, etc."

Divisions, orders, and families, as said before, are categories and not taxons. Now, the name of the genus and that of the species is not based on characters selected in order to determine the form or the hierarchy of categories above the genus. *Herpesvirus simplex* is the name of one of the viruses in the "herpes group." This name designates a specific organism and it will be the same, whichever classification of viruses is chosen. The names of the genus and species, are, once again, independent, totally independent, of characteristics used to define categories of a hierarchical order above the genus. We see no foundation for the statement of Gibbs *et al.*

D. Classification and Nomenclature

Not only do virologists often confuse category and taxon, but they also confuse classification and nomenclature. For example, Gibbs *et al.* (1966) attributed to Lwoff *et al.* (1962b) the authorship of a nomenclature code. Here is what LHT had said: "...that the binary nomenclature might be applied to viruses is subject to controversy. The problem will not be discussed here." This is the only reference to nomenclature in this paper.

The persistent confusion between nomenclature and classification is really curious.

E. Opposition to Binomial Nomenclature

Among the opponents of binomial nomenclature are Gibbs *et al.*, 1966, who write that the nomenclature of viruses must be based on a general classification of viruses established with the use of Adanson's principles. A nomenclature can only be international. Now, the committees on nomenclature are not competent in the matter of taxonomy. An international classification of viruses does not, will not, and cannot exist. To aspire to found an international nomenclature of viruses on a classification that will never exist is to hinder, forever, any international nomenclature. Furthermore, who has the right to decide if Adanson's principles have the force of law.

Gibbs (1969) once again joined the battle against Latinized binomial nomenclature, declaring it to be retrograde. One of the arguments is that not one international periodical publishes the articles on viruses in Latin. Nevertheless, plant virus specialists use Latinized binomial nomenclature for the designation of hosts and vectors. And does there exist one international periodical devoted to plants and arthropods that is printed in Latin?

The other argument is that binomial nomenclature is based on the species; that is true. However, if the viral species are refused, the nomenclature of viruses will become a catalog of strains.

Let us add that the International Committee on the Nomenclature of Viruses has accepted the genus and the species, that the name of the genus is already Latinized, and that the virologist must comply with the international rules in force.

Fenner (1966) also joins the war against latinized binomial nomenclature. "The use of Latinized binomial names," he writes, "is not successful and should be discontinued." There are two different targets for Fenner's condemnation: binomial nomenclature, on the one hand, and Latinization of the other. Since Fenner accepts neither species nor genus, binomial nomenclature cannot have any sense for him, naturally. We note, however, that Fenner has used Latinized names for "groups": myxovirus, adenovirus, reovirus, etc. Since in each one of these groups there are subgroups, and since in each of these subgroups there are infra subgroups, it can be presumed that Fenner's groups are families, subgroups genera, and infra subgroups species.

Fenner obviously forgets that Burnet (1967) furnished a remarkable review of the Poxviridae group and used a Latinized binomial nomenclature: *Poxvirus variolae*, *P. officinalae*, *P. bovis*, *P. avis*, and *P. myxomatis*. These are excellent names. One cannot see in what way they are inferior to the

nomenclature used by Fenner in 1968: group of poxviruses, subgroups of vaccinia, infra subgroups variolae."

Poxviridae and *Poxvirus variolae* appear to be much simpler and also easier to understand. Furthermore, with the suffix, one knows immediately which hierarchical category is concerned. Besides, binomial nomenclature has been used for a long time. When one says *Poliovirus I*, one makes use of binomial nomenclature whether one wishes to or not. *Poliovirus* designates the genus and I the species, that is to say "groups" of different hierarchical value.

F. Competence of International Committees for Nomenclature

In order to settle the problems of nomenclature, zoologists, botanists, microbiologists, and virologists created international committees for nomenclature. The competence of the latter is nomenclature. Taxonomy, grouping of species into genera and genera into families, grouping families into orders, etc., is left to the decision of each. Biologists have recognized that a classification is necessarily arbitrary and that each and everyone of us must be free to propose a system of his own choosing. No international body of nomenclature has the power to legislate in matters of taxonomy. This rule applies to the International Committee for the Nomenclature of Viruses.

However, in his excellent book: "The Biology of Animal Viruses," Fenner writes that the International Committee for the Nomenclature of Viruses rejected the classification suggested by the Provisional Committee for Nomenclature. Note: (1) that the classification suggested by this Committee is the LHT system; (2) that the International Committee does not have to accept or reject the LHT classification; (3) that if it had rejected it, the decision would have been null and void, because it is not competent in matters of taxonomy.

It is advisable that virologists wishing to discuss taxonomy and nomenclature should first assimilate a few elementary principles with regard to these disciplines. This would avoid considerable confusion.

VII. Lanni's System

Since viruses are reproduced from their sole genetic material, the whole virus is determined by its nucleic acid. The genotype of the virus is its genetic material. However, what is important in the nucleic acid, is not

its nature, its molecular weight, the percentage of purine and pyrimidine bases, or the patterns of doublet frequency. The major element is the information in the biological sense, that is, the sequence of codons responsible for the structure of the proteins.

Therefore, Lanni proposed creating a system based on the sequence of the codons. He called his system: molecular. Here is what we had to say about this system (Lwoff and Tournier, 1966, p. 61).

> Let us now examine Lanni's "molecular" system. Since viruses are reproduced from their genetic material, it follows that the whole virus, including the virion, is determined by the base sequence of its nucleic acid. Provided the code is entirely deciphered,—as today it is—knowing the base sequence of the structural genes means knowing the amino acid sequence of the proteins. Yet, for the time being, it is impossible to deduce the tertiary and quaternary structures of a protein from its amino acid sequence. Moreover, the architecture of a protein may be modified by various ligands. Of course, we all hope that in a not-too-distant future, the knowledge of the primary structure of the viral proteins will permit the deduction of the symmetry, size, and organization of the virion. This is not yet the case.
>
> Finally, let us assume that we are able to reconstruct the phylogeny of a given virus. Mutations have lead to substitution of amino acids, and to antigenic alterations. The question is immediately raised: how many amino acid substitutions will be needed in order to consider that we are dealing with a new species or a new genus? Not only the number of substitutions will have to be taken into account, but also their nature. If a few hundred amino acids belonging to twenty species are involved, one can foresee great battles.
>
> In Lanni's system, the base sequence of the nucleic acid is selected because it determines the properties of the virus and of the virion itself. Yet, if it is admitted that the most convenient classification of viruses is based on the virion, is it not simpler to consider the virion than the base sequence? We are afraid that, at least for a few years, it will be easier to state that a virion possesses DNA, a cubical symmetry, that to consider a list of 10,000 nucleotides. Lanni's system is a statement of inapplicable principles, and what virologists need is a real system.

In 1969 Gibbs also adopted the idea of virus classification based on the nucleic acid and arrived at a conclusion similar to ours, although neither Lanni nor Lwoff *et al.* were mentioned in this report (Gibbs, 1969).

VIII. Bellett's System

A. The System

Bellett (1967a,b,c, 1969) proposed classifying the viruses on the basis of the molecular weight of the nucleic acid and the percentage of guanine + cytosine. Since it is impossible, in this system, to compare usefully the

single- and double-stranded viruses, the viral world has been separated into two parts, according to whether the nucleic acid had one or two strands. Bellett rightly insisted on the fact that his classification should be considered a preliminary guide.

The data are fed into the computer. The results of the calculations are, on the whole, remarkable (see Figs. 1 and 2). However, it should be noted that isolated data provided by the computer will sometimes give rise to conclusions judged unacceptable by Bellett himself. For instance, RNA viruses, like the reoviruses, fall within the same group as the DNA viruses, like the group of papilloma viruses. In fact, Bellett accepted the results furnished by the computer only when they were in harmony with the data relative to the phenotypic characteristics of the virion.

That is why the Shope papilloma virus and coxsackievirus 10, for instance, which the computer had grouped together, were separated. Bellett considers, and quite rightly, that viruses that differ in their phenotypic characteristics cannot be grouped together. Let us add finally that, in certain cases, Bellett

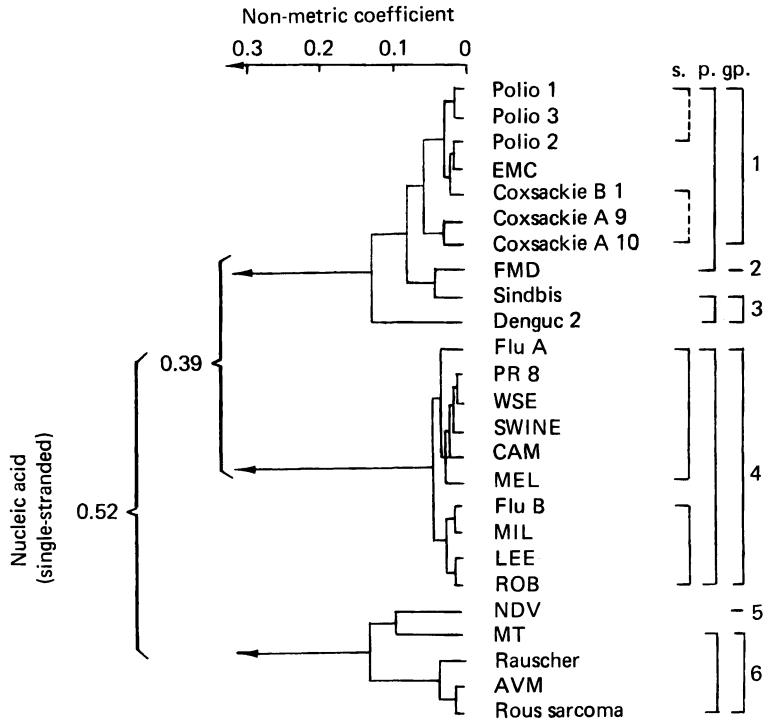

FIG. 1. Bellett's classification. Single-stranded viruses. S, Cross-breeding serological reactions; P, similar phenotypic properties. [With the author's permission, Bellett (1967c).]

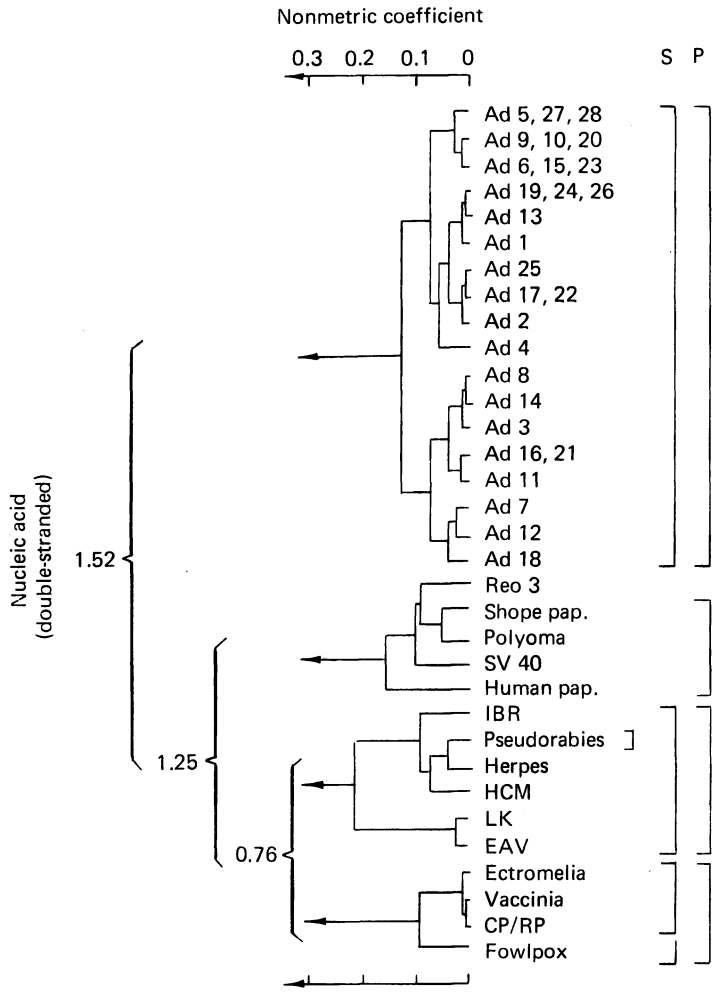

FIG. 2. Bellett's classification. Double-stranded viruses. S, Cross-breeding serological reactions; P, similar phenotypic properties. [With the author's permission, Bellett (1967c).]

used cross-breeding, which makes it possible to estimate the degree of homology of the nucleic acids.

Bellett's method, *supplemented by the utilization of phenotypic characters*, enables to rediscover fifteen of the "groups" of the LHT system. It should be recalled that the Provisional Committee for Nomenclature of Viruses assigned the value of families to these groups.

Within Bellett's classification, groups are defined by coefficients of similarity; that is the major defect of classification arising from the use of a

computer. The computer is able to recognize that some viruses are related. But that will not suffice for the virologist, who will want to know the phenotypic characters of the virions. A mammal is not defined according to the molecular weight of its nucleic acid and according to the pattern of the doublets. It is defined by its phenotypic characteristics, which are and continue to be the basis of any classification.

Bellett then suggested that the theoretical basis for the classification of viruses could be furnished by the fact that its properties are determined by the base sequence of its nucleic acid. It is quite obvious, in fact, since viruses are reproduced from their genetic material, that the phenotype of the virion is determined by the genotype. It is noted, incidentally, that Bellett does not seem to be acquainted with Lanni's paper or with our remarks concerning this subject, which are cited here.

It is obvious that the similarity of the sequence of the codons will be important for establishing the phylogenic relationships of viruses. However, what is important in the genotype, is its expression. It is to be wondered whether the phenotype of the virion does not also furnish a major document on the functional value of the genetic information. These remarks in no way minimize the importance of Bellett's method or the results that he has obtained—and which confirm the validity of the LHT system.

B. Nucleic acids versus Capsids: Uroviruses

The viruses provided with a "tail," whatever the structure and the dimensions of this appendage, are called uroviruses. Bellett's method, leads to classify uroviruses into three groups, that include, respectively:

1. Phages T3 and T7.
2. Phages lambda P22, T1 and phi 80.
3. Phages T2, T4, and T6.

In Bellett's index (1967c), groups 1 and 2 are related, but groups 2 and 3 are separated by a whole series of viruses: pseudorabies virus, herpesvirus, etc. This would mean that phage T3 is more closely related to herpesvirus than phage T2. The nucleic acids of these phages, as noted by Bellett, differ more by their molecular weight than by the percentage of their nucleic bases. Bellett, quite rightly, considered the grouping as temporary. Nevertheless, the molecular weight of DNA of two uroviruses can present great differences. At this point a discussion on the origin of bacteriophages would be appropriate.

Most virologists think that uroviruses have originated from bacterial

DNA. In fact, there are some common sequences in the genetic material of certain bacteriophages and in the bacterial chromosome. These are responsible for the coupling of temperate bacteriophages with the bacterial chromosome. It can be accepted that the procaryotic protista represent a monophyletic group. The Schizomycetes, however, have undergone considerable diversification, as much from the physiological as from the morphological point of view. The genetic material itself, has also evolved as evidenced by the variations of the percentage of guanine + cytosine.

Let us consider the hypothesis that uroviruses came into being late during the evolution of procaryotes. It is not difficult to accept that the tails of the uroviruses, in spite of their morphological and functional diversity, are homologous organelles. This tail represents a remarkable and unique structure. It is, therefore, possible to think that uroviruses probably originated from homologous sectors of DNA in bacteria. Homologous, but nevertheless, potentially capable of being diversified during evolution. Therefore, it is not surprising that the genetic material of phages exhibits some important differences. However, we repeat, the tail of uroviruses is an original organelle.

Within LHT's classification, all uroviruses are brought together in a group to which the Provisional Committee of Nomenclature of Viruses has assigned the value of an order, called Urovirales.

Should the need arise, it would be permissible to include in this order all the families whose creation is judged to be useful. It is not that, however, that is important, but the fact that all the uroviruses are grouped together into a single taxon.

It is not normal to see two groups of uroviruses separated by a series of animal viruses. Here, the evaluation of distances by numerical methods must yield to common sense: the tail transcends the nucleic acid.

A general remark is necessary here. The tail of urophages is a constituent of variable complexity. If one were to give a general definition of it, one could say that it is an organelle that is fixed to the nucleocapsid itself and enables the transfer of the nucleic acid through the bacterial wall. Naturally, it is to be wondered, how the viruses, other than urophages, can possibly inject their nucleic acid into the cytoplasm of the bacteria.

In the phage FD, the capsid contains two different proteins. One is represented in numerous examples and corresponds to capsomeres. The other one is represented by a single molecule that is responsible for the specific attachment on the *pili F* and assures penetration of the nucleic acid into their core from where it will reach the cytoplasm.

In addition to capsomeric proteins, there exists in certain RNA phages

of cubical symmetry a single protein molecule which could play an analogous role. It is possible to envision a hypothesis, according to which the cycle of parasitic viruses of bacteria always require the presence of a specific structure responsible for the penetration of the nucleic acid into the cytoplasm. It is clear, however, that the single molecule of the phage FD can, under no circumstances, be considered as homologous to the tail or urophages.

IX. Miscellaneous Remarks

A. Symmetry

Certain capsids have a helical symmetry, others a cubical symmetry. For the sake of convenience, here, the corresponding capsomeres will be designated by "H" capsomeres and "C" capsomeres, respectively.

The structure of certain capsomeres thus involves a helical capsid architecture. Nothing is known concerning the tertiary structure that affects this property, except that it is controlled by the primary structure of the protein. Let us consider the facts.

We are now able to say that, within the primary structure of various "H" proteins, there are such sequences where the tertiary structure of capsomeres will force them to be arranged in helical sequences. The problem is to know whether or not the determining tertiary structures of all "H" capsomeres are bound to a common sequence of amino acids, that is, if the "H" symmetry of the capsomeres is bound to sequences of isosemantic codons on the structural gene of the capsomeric protein.

The same problem can arise as regards capsomeres with cubical symmetry or their subunits. The problem of the determinism of the specific properties of "H" and "C" capsomeres is not solved but merits consideration, since the hypothetical common sequences will not be found unless they are looked for.

B. RNA and DNA Viruses

A virus may have evolved from a given sector of DNA within the host cell. It could also very well have derived from the corresponding RNA messenger that contains the same information, both qualitatively and quantitatively.

Therefore, theoretically, different viruses could have originated from

nucleic acids of different but complementary nature. If this argument has any validity, then there should exist viruses whose phenotype would differ solely by the nature of the genetic material. Therefore, it would not be absurd to investigate whether the various nucleic acids of the viruses possessing an identical type of capsid may or may not be hybridized.

The Parvoviridae and the Napoviridae, as they are defined in our system, have a naked nucleocapsid with cubical symmetry and 32 capsomeres. Some are DNA and others are RNA. Hybridization merits consideration.

Should it evolve that viruses with an identical capsid and a different nucleic acid present complementary nucleotide sequences, it would be advisable to review the classifications and, in particular, to reconsider within the LHT system the hierarchical value of the nature of the nucleic acid.

C. Single- and Double-Stranded Viruses

The nucleic acid of viruses is either single- or double-stranded. It is instructive to discuss the problem of the origin of these two types.

Viruses, as is generally accepted, derive from the nucleic acid of their host. RNA viruses could, thus, possibly have their origin in the RNA messenger. As a rule, the latter is double-stranded. It so happens that the RNA viruses which have been investigated, with the exception of the *Reoviridae* and the *blue tongue virus*, are single-stranded and thus correspond to the characteristics of the messenger. It is permissible to assume that the double-stranded RNA viruses originated as a consequence of an alteration of the replication system of the RNA.

The DNA viruses could derive from a segment of the DNA of their host. The great majority of DNA viruses are double-stranded, which corresponds to the characteristics of the genome. Thus, only the Inoviridae (filamentous phages), the Microviridae (ϕX174 group), and the Parvoviridae (Kilham virus and "minute virus of mice" group) are single-stranded and each one of these groups includes a very small number of species. It is permissible to assume that the single-stranded DNA viruses originate as a consequence of an alteration of the replication system of the DNA.

Although completely justified by practical considerations, the division of viruses, as practiced by Bellett, into two groups, one single-stranded and the other double-stranded, does not seem to correspond to the phylogeny of viruses. We do not think that single-stranded DNA viruses are more closely related to single-stranded RNA viruses than to double-stranded DNA viruses.

D. The Frequency of the Doublet Patterns

It is possible to characterize a nucleic acid in terms of the average frequency of the nucleic base doublets. This method was applied to the viruses (Subak-Scharpe *et al.*, 1966; Subak-Scharpe, 1967; Hay and Subak-Scharpe, 1968).

Nine mammalian viruses were studied. It happens that the doublet pattern of four small oncogenic DNA viruses, the SV40, polyoma virus, Shope papilloma virus, and human papilloma virus, bears a marked resemblance to the DNA patterns of mammals. Five large nononcogenic viruses, the herpesvirus, pseudorabies, equine abortion, vaccinia, and adenoviruses do not show this resemblance.

Bellett (1967b) studied the frequency of the doublet patterns. In this respect, the SV40 virus is closely related to the hamster. In the same group as the polyoma viruses, we find: man, rabbit, chicken, ox, mouse, and salmon. Bellett concludes that the similarity of the frequency of doublet patterns does not prove that two entities are phylogenetically closely related. In fact, it appears that the results of the pattern study should be interpreted with caution.

The identity of the code has given rise to the conclusion that the living kingdom is monophyletic. The vertebrates appear to descend from a relatively homogeneous group. But, if man and the salmon present closely related patterns, one can reach only to one conclusion: similar patterns are compatible with considerable phenotypic differences, that is, with marked functional differences of the genetic information.

In conclusion, therefore, it can be said that the doublet frequency may reveal a phylogenic relationship. It does not give any information on the possible structural similarities of viruses. But this should not, in any way, prevent us from provisionally accepting the conclusions of Subak-Scharpe and Bellett: viruses of the papilloma group probably originated in animals closely related to their present mammalian host, whereas the herpesviruses and the poxviruses could have originated from bacteria.

E. Doublet Frequency and Selection

The idea has been defended (Subak-Scharpe *et al.*, 1966) that the transfer RNA population belonging to an organism is adapted in an optimal way to the translation from the codon sequences of the RNA messenger.

It appears to be perfectly clear, in fact, that maximum economy must be achieved when the proportions of each of the transfer RNA's correspond

to the frequency of incorporation of the corresponding amino acid into the proteins.

A virus, whose "pattern" is similar to that of a given organism, would therefore have its origin in an organism closely related to that organism. Viruses in which the pattern is different from the host's would be extrinsic. The pressure of selection would favor the viruses whose pattern is closely related to the host. Here, Bellett (1967c) has adopted Subak-Scharpe's conclusions. However, it should be noted that the vaccinia virus, despite the marked difference of pattern, multiplies quite well in mammals. It does not appear to have been selected against; quite the contrary.

X. Gibbs' Classification

Gibbs and Harrison (1968) reported that their classification method rediscovers several "intuitively constituted" groups of viruses.

The figures on the left-hand side of Fig. 3 correspond to the clusters in Table IV of Gibbs (1969). He took into consideration the shape, isometric or anisometric, of the capsid, the mode of transmission, the type of vector, the symptoms, and the accessory particles. The isometric particles of 25 to 30 mμ are divided into "rounded, angular, and squashy." In groups 1, 3, 4, and 5 we find viruses of both cubical and helical symmetry. It is impossible to know from the table whether the given virus is an RNA or a DNA virus, whether the symmetry of the capsid is cubical or helical, what is the number of capsomeres of the viruses with cubical symmetry or the dimensions of the viruses with helical symmetry. Gibbs' classification, could be cited as an example of nonfigurative virology.

XI. Classification of the Classifications

> *Moreover, some sort of arrangements is inescapable for organizing our facts about the bewildering diversity of living things; and a nomenclature is equally essential as a quick and accurate method of reference and communication.*
>
> A. J. Cain (1962)

In discussing the taxonomy of viruses, Pirie (1962) came to the conclusion that the first and essential step of a classification pertains to aes-

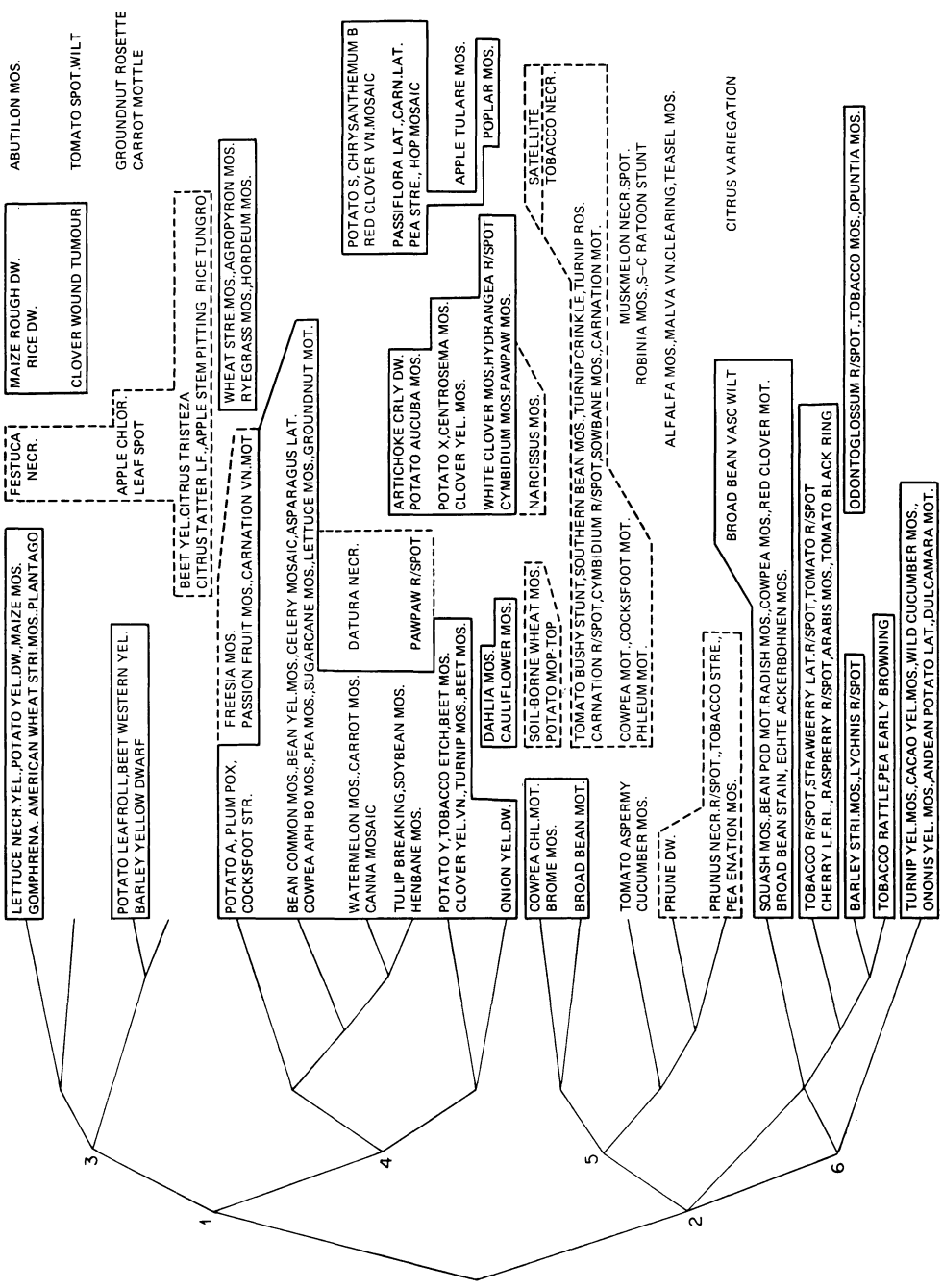

FIG. 3. Classification of plant viruses (modified from Gibbs, 1969).

thetics. It is a fact that the logic, symmetry, equilibrium, and harmony of a system pertain to aesthetics.

Now, let us try to fit the LHT system into the classification—necessarily arbitrary—of the classifications. For this purpose, we will follow closely the excellent article of Heslop-Harrison (1962). An artificial classification uses a small number of discriminatory criteria for subdivisions. A natural classification is based on the general resemblance and the maximum correlation of attributes. At first sight, since only the number of criteria is at stake, there is merely a difference of degree between artificial and natural classification.

In an artificial classification, when we use a small number of characteristics, each and everyone of the classes, regardless of its hierarchical level, is defined by the possession or absence of a single attribute. Each class, at each level, will be defined by attributes common to these elements. No individual will be "tolerated" in a category if it does not possess all of the diagnostic attributes of this category. "This," says Heslop-Harrison, "is an essential property of all logically constructed artificial classifications." The LHT system conforms to the definition of artificial classifications.

Within a natural classification, two individuals belonging to a group have more characteristics in common than an individual from this group has with an individual from another group. If a category was defined by the common possession of one or several characters, the category could be defined by these properties and the classification would then be artificial.

Within a numerical system of classification of viruses, since all of the characteristics have the same value, one may find within one family, or within the same group, two viruses that differ only by a single and unique characteristic, although it could be the nature of the genetic material or the symmetry of the nucleocapsid.

Naturally, the objection could be raised that, in a well-constructed numerical taxonomy, all of the viruses having the same nucleic acid, the same capsidal symmetry, etc., will be included in the same "group," species type, or family. If such were the case, this character could be used for the classification, which would then become "artificial."

As regards the taxonomic hierarchy of an artificial system, as well as the natural system, it has no bearing on phylogeny. As Heslop-Harrison writes, nobody would be so naïve as to think that taxons can be identified directly with the successive order of branching of a phylogenic tree. Heslop-Harrison adds that a state of chronic confusion exists between taxonomic tables and family tree. A classification, even a natural one, is in no way synonymous with phylogenic classification.

An artificial classification is the only one that can impart to the viral world an overall view that would enable us to visualize the structural and chemical characteristics of viruses. Since the architecture of the virion is controlled by the genetic information, the LHT system provides a picture of the functional value of the genetic information, that is, of the genotype.

XII. Conclusions

To reject the concept of viruses on the ground that the group is heterogeneous, to refuse any category and any hierarchy on the ground that there is no such thing in nature, to refuse a binomial nomenclature on the pretext that nature does not discriminate between species or genus, to systematically use the word "group," which does not correspond to any hierarchically defined category, to refuse discriminative characters on the ground that the value of characters cannot be ascertained, to accept, in spite of the evidence to the contrary, that all of the characters are of equal value, to feed these characteristics into a machine that will determine arbitrary distances, to claim that a table of coefficients of similarity is a classification, to cultivate imprecision and disorder, to call arbitrary any attempt at a universal, logical, and coherent system, to impose a Middle-Aged confusion on virology, this is what we are reproaching to the followers of the numerical method. A category exists only by the virtue of definition. A hierarchy implies a choice, a nomenclature demands a convention, a concept is the fruit of reasoning.

Science is a system of relations among facts and a body of concepts. To call arbitrary the logical procedures that are the very foundation of science is an antiscientific attitude.

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Note Added in Proof: We have learned that densonucleosis virus has a complementary single-stranded DNA, separately encapsidated. The MW of this DNA is 1.6×10^6 daltons (Kurstak *et al.*, *C. R. Acad. Sci., Paris* **272**, 762, 1970), or 2.2×10^6 daltons (Barwise and Walker, *FEBS Letters* **6**, 13, 1970).