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Structure of Isometric Viruses Containing Nonidentical Polypeptide Chains

A. KEITH DUNKER

Memorial Sloan-Kettering Cancer Center, New York, New York 10021

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The theory of Caspar and Klug (1962) for the structure of isometric viruses has been generalized to the case in which the identical repeating unit is composed of **n** nonidentical polypeptide chains. This modified theory accounts for the structure of picornaviruses, the lambda phage head, cowpea mosaic virus, and ϕX174, while at the same time conserving the principle of having identical subunits in identical environments. Furthermore, the modified theory suggests amending the triangulation number to $T[n]$ for capsids with **n** nonidentical polypeptide chains as the repeating unit.

Identical subunits located in identical or at least quasiequivalent bonding domains provide the foundation for the theory of the capsid architecture of spherical viruses (5, 6, 20), which have been shown to have icosahedral symmetry (5-7, 11, 20). Icosahedral symmetry requires $60n$ (n = 1, 2, 3, . . .) subunits; however, it is impossible to arrange more than 60 identical subunits on the surface of a sphere such that they are in identical bonding environments (6, 7, 20). Therefore, a severe dilemma arose when chemical data suggested about 180 rather than 60 identical polypeptide chains (14) for one small spherical virus.

The resolution to this dilemma was provided by the recognition that certain arrangements allowed the identical subunits to be in nearly identical (quasiequivalent) bonding environments (6, 20). The allowed arrangements could be classified by a triangulation number, T, where a capsid contains $60T$ identical units. The rules governing T were shown (6) to be $T = Pf^2$ where f = 1, 2, 3 and $P = h^2 + hk + k^2$ for all pairs of integers, h,k having no common factor.

T provides a selection rule. For example, 60 identical subunits (T = 1; f = 1; h,k = 1,0) and 180 identical subunits (T = 3; f = 1; h,k = 1,1) can be arranged in identical and nearly identical environments, respectively, but 120 cannot.

These constraints formed the basis for models which could be compared with the observed morphology of the small spherical viruses. This approach accounted beautifully for the surface morphology of turnip yellow mosaic virus (6, 16, 20, 29). Subsequently, determination of the triangulation number, T, has become the basis for structural classification of the spherical viruses.

By now several spherical viruses and empty capsids have been found to contain nonidentical polypeptide chains (1, 12, 17, 18, 21, 24, 25, 27, 28, 30, 32, 34) apparently in equimolar ratios. Picornaviruses of various mammalian hosts are heavily represented on this list (17, 18, 24, 25, 27, 30, 32, 34), but the list also includes an RNA insect virus (21), as well as two DNA bacteriophages (1, 28) and an RNA plant virus (12). The insect virus may be structurally equivalent to the mammalian picornaviruses.

It is proposed that the structure of these viruses can be explained by amending the structure theories of Caspar and Klug (6) for the more general case in which the identical subunit is formed from **n** nonidentical polypeptide chains.

In terms of their theory of virus construction, Caspar and Klug (6) discuss: (i) the possible surface morphologies of spherical viruses, (ii) the possible bonding contact patterns of the polypeptide chains, and (iii) the possible capsid fragments. In the present communication, each of these aspects of virus structure is considered for capsids with nonidentical polypeptide chains.

EXPERIMENTAL

(i) Possible surface morphologies for capsids with n nonidentical polypeptide chains. A hexagonal net of protein subunits contains sixfold, threefold, and twofold rotation axes. Conversion of selected sixfold axes to fivefold axes generates an icosahedral shell. The set of sixfold axes that are converted to fivefold determine the triangulation number (6).

Hexagonal nets with **n** nonidentical polypeptide chains as the basic unit can be used to generate icosahedral shells by exactly the same procedure as described above. An example is shown in Fig. 1.

For a net with identical polypeptide chains, the polypeptides can cluster about the twofold axis, the threefold axis, or the sixfold axis, depending on the relative strengths of the different bonding interfaces. Thus, the final capsid can have $60T$ monomers (no clustering), $30T$ dimers, $20T$ trimers, or $10(T-1)$ hexamers plus 12 pentamers (6).

With **n** type of subunits, it is possible for the different types of polypeptides to cluster about **n** different axes simultaneously.

Symmetry requires multiples of two chains about the twofold axis, multiples of three about the threefold axis, and multiples of six about the sixfold axis. Thus, there can be 0, 2, 4, . . . r chains clustered about the twofold; 0, 3, 6, . . . s about the threefold; and 0, 6, 12, 18, . . . t about the sixfold. Quasiequivalence permits a maximum of two identical chains to be clustered about the twofold axes, three about the threefold, and six about the sixfold. Therefore, the number of nonidentical polypeptide chains about the twofold is given by $r/2$, the number about the threefold is given by $s/3$, and the number about the sixfold is given by $t/6$. With **n** nonidentical chains, $r/2 \times s/3 \times t/6 = \mathbf{n}$.

The number of morphological subunits for such a capsid would be $30T \times (1-\delta[r]) + 20T \times (1-\delta[s]) + [10(T-1)+12] \times (1-\delta[t])$; where $\delta(r)$, $\delta(s)$, and $\delta(t)$ are the usual delta functions; that is $\delta(0) = 1$, $\delta(x \neq 0) = 0$. The various possibilities have been determined for $\mathbf{n} = 1, 2, 3, 4$ (Tables 1-4). Included in the tables are representative examples of viruses whose morphology can be accounted for by these considerations.

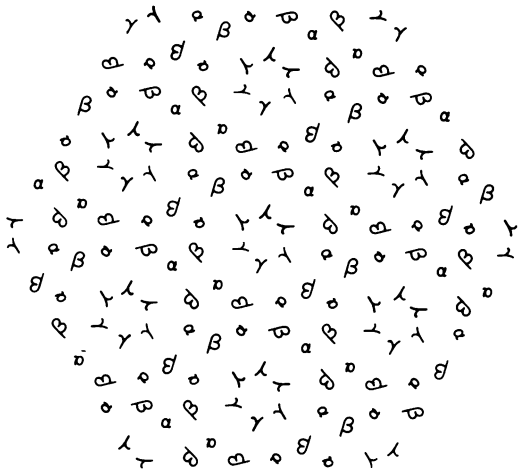

FIG. 1A. *Folding hexagonal nets containing nonidentical chains. An arbitrary choice of a hexagonal net with three nonidentical units is shown. Such a net can be folded by the methods of Caspar and Klug (6) to yield $T = 1[3]$, $T = 3[3]$, etc., shells (see Fig. 1B). Similar operations are possible for nets of* **n** *nonidentical subunits.*

TABLE 1. *Possible clustering patterns for $T[1]$*

No. of chains clustered about each axis			Name of pattern	No. of morphological subunits
Two-fold	Three-fold	Six-fold		
2	0	0	Dimer clustering[a]	$30T$
0	3	0	Trimer clustering	$20T$
0	0	6	Hexamer-pentamer clustering[b]	$10(T-1)+12$

[a] Turnip crinkle virus probably displays dimer clustering (10).

[b] Turnip yellow mosaic virus clusters into hexamers and pentamers (6, 10, 16, 20, 29).

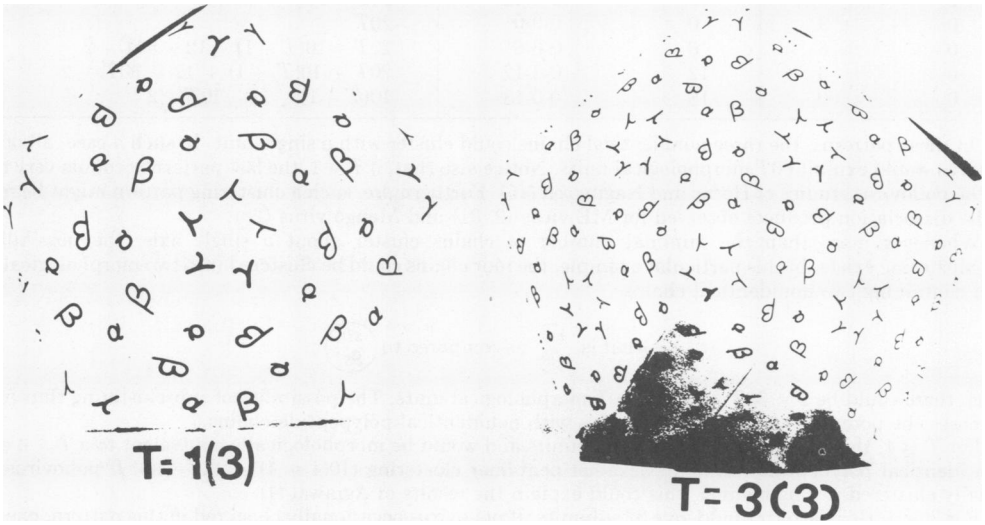

FIG. 1B. *See legend for Fig. 1A.*

TABLE 2. *Possible clustering patterns for T[2]*

No. of chains clustered about each axis			Name of pattern	No. of morphological subunits
Two-fold	Three-fold	Six-fold		
4	0	0	4-0-0[a]	$30T$
2	3	0	2-3-0	$30T + 20T = 50T$
2	0	6	2-0-6	$30T + 10(T + 1) + 12 = 40T + 2$
0	6	0	0-6-0[a]	$20T$
0	3	6	0-3-6[b]	$20T + 10(T - 1) + 12 = 30T + 2$
0	0	12	0-0-12[a]	$10(T - 1) + 12 = 10T + 2$

[a] In these patterns the two nonidentical chains could sub-cluster into a single unit. If this occurs, there would be $60T$ morphological units for each of these patterns.

[b] If $T = 1$, this is probably the clustering pattern observed for cowpea mosaic virus (8); if $T = 7$, this is the pattern observed in lambda phage heads (Williams and Richards, J. Mol. Biol., in press). Notice that for $T = 7$, there are $30 \times 7 + 2 = 212$ morphological subunits. For hexamer-pentamer clustering with only one type of chain, a triangulation number of $T = 21$ also gives 212 morphological subunits ($10(21 - 1) + 12 = 212$). Thus, it is not surprising that the lambda head was mistaken for $T = 21$ by freeze etching (2).

The symmetry axes are the same for capsids with identical and nonidentical polypeptide chains. Capsids with nonidentical chains can display capsomer arrangements that are not possible for capsids with identical chains only. However, when capsids with nonidentical chains have the same number of morphological subunits as capsids with identical chains, the symmetry of the particle requires that the positions of the morphological subunits be the same in both cases. Thus, capsids with identical and nonidentical polypeptide chains can be morphologically equivalent. The footnotes to Tables 2 and 3 contain particular examples of morphological equivalence.

For capsids with identical polypeptides, a single type of capsid can have all dimers, all trimers, or hexamers and pentamers. Thus, the capsomers must necessarily be of equal or of nearly equal size. Obviously, for capsids with nonidentical polypeptides, the capsomers may be of equal or very unequal sizes.

(ii) Possible bonding networks. In their original paper, Caspar and Klug (6) showed that the bonds between the polypeptide chains must circumscribe the two-, three-, and sixfold axes of the hexagonal net. Furthermore, they suggest that only two of the three bonds are necessary. Thus, for capsids with identical units, the set of possible bonding contacts is simple and well-defined; i.e., the net must contain dimer bonds + trimer bonds, dimer bonds + hexamer

TABLE 3. *Possible clustering patterns for T[3]*

No. of polypeptide chins clustered about each axis			Name of pattern	No. of morphological subunits
Twofold	Threefold	Sixfold		
6	0	0	6-0-0[a]	$30T$
4	3	0	4-3-0[b]	$30T + 20T = 50T$
4	0	6	4-0-6[c]	$30T + 10(T - 1) + 12 = 40T + 2$
2	6	0	2-6-0	$30T + 20T = 50T$
2	3	6	2-3-6	$30T + 20T + 10(T - 1) + 12 = 60T + 2$
2	0	12	2-0-12	$30T + 10(T - 1) + 2 = 40T + 2$
0	9	0	0-9-0[a]	$20T$
0	6	6	0-6-6[d]	$20T + 10(T - 1) + 12 = 30T + 2$
0	3	12	0-3-12	$20T + 10(T - 1) + 12 = 30T + 2$
0	0	18	0-0-18[a]	$10(T - 1) + 12 = 10T + 2$

[a] In these patterns, the three nonidentical chains could cluster with a single unit. In such a case, all of these patterns would exhibit $60T$ morphological units. Notice also that, if $T = 1$, the last pattern accounts very neatly for the poliovirus studies of Horne and Nagington (15). Furthermore, such a clustering pattern might be related to the dissociation products observed for ME virus (9, 31) and Mengo virus (25).

[b] Whenever more than the minimal number of chains cluster about a single axis, the possibility of sub-clustering exists. In this particular example, the four chains could be clustered into two morphological units each containing two nonidentical chains

that is,  as compared to  .

Thus, there could be $2 \times 30T + 60T = 80T$ morphological units. The possibility of sub-clustering thus further increases the potential complexity of capsids with nonidentical polypeptide chains.

[c] For $T = 1$, this pattern would give 42 subunits and would be morphologically equivalent to a $T = 4$ capsid with identical polypeptide chains in hexamer-pentamer clustering ($10(4 - 1) + 12 = 42$). If poliovirus occasionally clustered in this manner, this could explain the results of Agrawal (1).

[d] For $T = 1$, this pattern would give 32 subunits. If poliovirus occasionally clustered in this pattern, one could account for the results of Mayor (26). Note that for hexamer-pentamer clustering, a capsid with $T = 3$ ($10(3-1) + 12 = 32$) would display equivalent morphology.

TABLE 4. *Possible clustering patterns for T[4]*

No. of polypeptide chains clustered about each axis			Name of pattern	No. of morphological subunits
Twofold	Threefold	Sixfold		
8	0	0	8-0-0	$30T$
6	3	0	6-3-0	$30T + 20T = 50T$
6	0	6	6-0-6	$30T + 10(T- 1) + 12 = 40T + 2$
4	6	0	4-6-0	$30T + 20T = 50T$
4	3	6	4-3-6	$30T + 20T + 10(T - 1) + 12 = 60T + 2$
4	0	12	4-0-12	$30T + 10(T - 1) + 12 = 40T + 2$
2	9	0	2-9-0	$30T + 20T = 50T$
2	6	6	2-6-6	$30T + 20T + 10(T - 1) + 12 = 60T + 2$
2	3	12	2-3-12	$30T + 20T + 10(T - 1) + 12 = 60T + 2$
2	0	18	2-0-18	$30T + 10(T - 1) + 12 = 40T + 2$
0	12	0	0-12-0	$20T$
0	9	6	0-9-6	$20T + 10(T - 1) + 12 = 30T + 2$
0	6	12	0-6-12	$20T + 10(T - 1) + 12 = 30T + 2$
0	3	18	0-3-18	$20T + 10(T - 1) + 12 = 30T + 2$
0	0	24	0-0-24	$10(T - 1) + 12 = 10T + 2$

bonds, trimer bonds + hexamer bonds, or all three types of bond.

For capsids with **n** identical polypeptide chains, the possibilities for bonding contact geometries become considerably more complex. In addition to the two bonds between the identical units to define the hexagonal net (these are the same as the bonds for the single polypeptide chain case), there must be at least $n - 1$ bonding interfaces within any repeating unit of **n** nonidentical chains. Furthermore, the locations of the bonding interfaces within the repeating unit are not restricted by the symmetry axes, which adds a further complicating feature.

In a hexagonal network with **n** distinct polypeptide chains, it is evident that there must be $n + 1$ bonding connections. For the particular case of **n** = 3, this is illustrated in Fig. 2.

The number of distinguishable ways of forming $n + 1$ bonding connections among **n** nonidentical chains arranged in a hexagonal net is given approximately by

$$mx(n_2 \times n_3 + n_2 \times n_6 + n_3 \times n_6)$$

where m is the number of ways of forming bonding interfaces within the identical unit, n_2 is the number of ways of forming connections between units to specify the twofold axis, and so on for n_3 and n_6. The definitions of m, n_2, n_3, and n_6 are clarified by a few examples for the particular case of **n** = 3 (Fig. 3). The above expression does not provide the exact number of distinct bonding nets (see below). Nevertheless, this expression does yield a method for listing the possibilities, which can then be checked for redundancies.

Case I, n = 1

m = 1 (The identical unit is a single polypeptide chain.)

n_2, n_3, n_6 = 1 (The bonds are between identical units.)

Thus, there are $1 \times (1 \times 1 + 1 \times 1 + 1 \times 1)$ = three distinct 2-bonded nets.

Case II, n = 2

$m = 1\ (a - b)$

n_2, n_3, $n_6 = 3\ (a - a, a - b, b - b)$

Thus, there are $1 \times (3 \times 3 + 3 \times 3 + 3 \times 3) = 27$ distinct 3-bonded nets.

Models show that nets with n_3, $n_2 = (a - b)$, n_6, $n_2 = (a - b)$, and n_6, $n_3 = (a - b)$ are actually the same. Thus, one net is counted three times, and so there are actually 25 rather than 27 distinct 3-bonded nets.

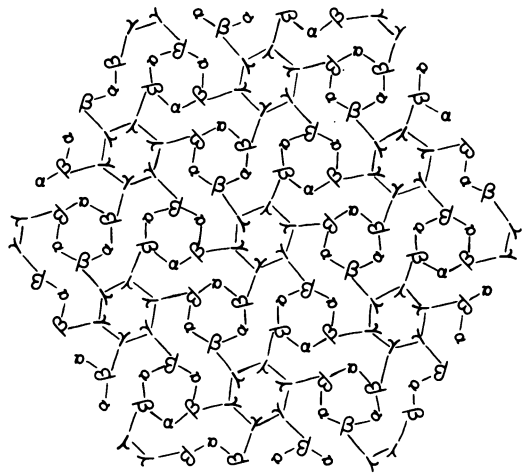

FIG. 2A. *Bonding connections for an* **n** *= 3 net. One of the 240 distinct bonding nets is shown. The four bonding interfaces (see Fig. 2B) can be divided into two bonds within an* ($\alpha\beta\gamma$) *unit (see Fig. 2C) and two bonds between* ($\alpha\beta\gamma$) *units (see Fig. 2D). It is apparent that for* **n** *nonidentical chains within a unit, there must be at least n−1 connections to hold the unit together. Thus, there are at least* $(n - 1) + 2 = n + 1$ *connections in a hexagonal net having* **n** *nonidentical units.*

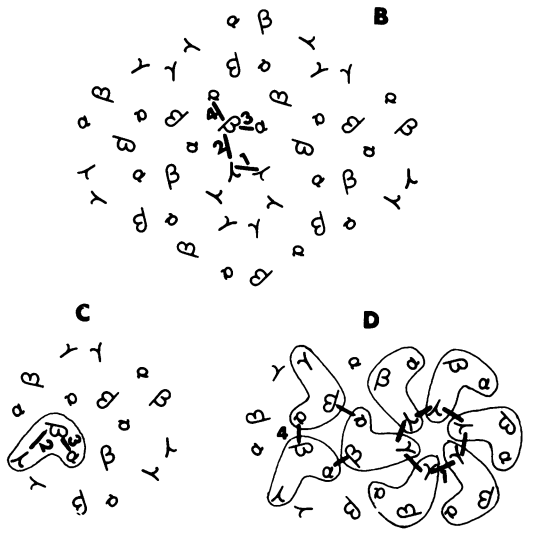

FIG. 2B, C, D, *See legend for Fig. 2A.*

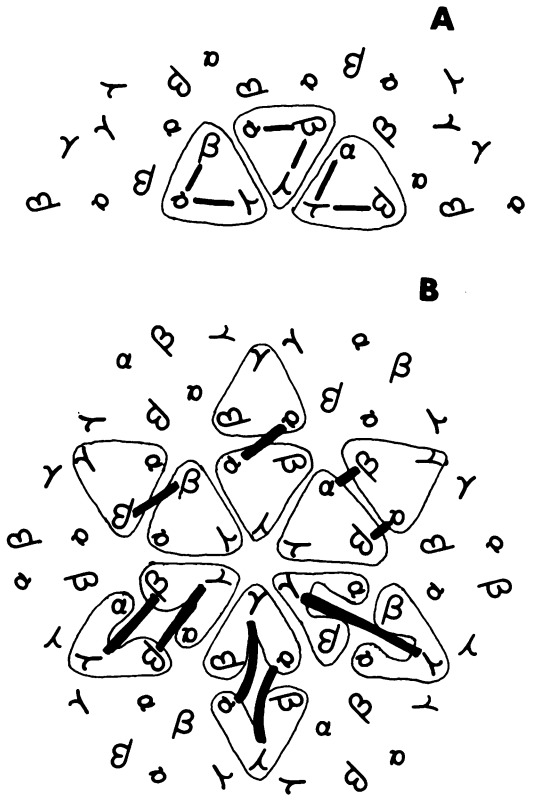

FIG. 3. *Counting the distinct bonding nets for* **n** = *3. For an* **n** = *3 hexagonal net, the number of ways of specifying intra-subunit bonds, m, is equal to 3 (A). The number of ways of specifying the twofold axis, n_2, is equal to 6 (B). Likewise, it is apparent than n_3 and n_6 also equal 6.*

Case III, n = 3

$m = 3\ (a - b - c;\ a - b - c;\ a - b - c)$

n_2, n_3, n_6
$= 6\ (a - a,\ a - b,\ a - c,\ b - b,\ b - c,\ c - c)$

Thus, there are $3 \times (6 \times 6 + 6 \times 6 + 6 \times 6) = 324$ networks.

Many nets were found to be redundantly counted; the total number of distinguishable nets was actually found to be 240.

Case IV, n = 4

$m = 16\ (a - b - c - d, a - c - b - d, a - b - d - c, a - d - b - c, a - c - d - b, a - d - c - b, b - a - c - d, b - c - a - d, b - d - a - c, b - a - d - c, c - a - b - d, c - b - a - d, a - d - c, b - c - d, a - b - d, a - b - c)$

$n_2, n_3, n_6 = 10\ (a - a, a - b, a - c, a - d, b - b, b - c, b - d, c - c, c - d, d - d)$

Thus, there are approximately $16 \times [10 \times 10 + 10 \times 10 + 10 \times 10] = 4{,}800$ networks.

The bonding nets described above (summarized in Table 5) contain the minimal number of bonding contacts. It is straightforward to work out the possibilities for capsids containing more than the minimal number of bonding interfaces.

TABLE 5. *Minimal bonding nets*

n	m	n_2, n_3, n_6	$mx(n_2n_3 + n_2n_6 + n_3n_6)$	Distinguishable nets
1	1	1	3	3
2	1	3	27	25
3	3	6	324	240
4	16	10	4,800	nd[a]

[a] nd, Not determined.

(iii) Possible capsid fragments. Caspar and Klug (6) indicated possible aggregates of polypeptide chains that might occur as intermediates of capsid assembly or dissociation. Although not considered in detail in their paper, the particular case of stepwise dissociation (or assembly) intermediates is of special interest. Stepwise dissociation intermediates are those intermediates that result from complete nonassociation of one or more types of bonding interfaces.

For capsids with identical polypeptide chains, the only possible stepwise intermediates are dimers, trimers, and hexamers/pentamers.

Characterization of dissociation fragments has provided the most useful approach to date for determining the structure of picnornaviruses (9, 18, 25, 30, 32). In the particular case of ME virus, essential clues for determining a structural model were provided by characterizing 14 and 5S subunits derived by controlled (stepwise) dissociation of the virus (9, 32). The same general approach should prove useful for other viruses containing nonidentical polypeptide chains. Controlled dissociation has an advantage as a potential means for shedding light on the identities of the polypeptide chains constituting particular morpho-

logical units. The main difficulty would be in finding conditions to specifically dissociate one type of bonding interface.

For purposes of illustration, the stepwise intermediates of a $T[3]$ capsid are presented.

For a capsid with three nonidentical polypeptide chains, consideration of the symmetry axes in turn suggests that there are 31 possible stepwise intermediates (Table 6). Conservation of mass can be used to determine the 45 ways of combining these fragments by the addition of one bond to yield a complete capsid (Table 7). Picornavirus maturation and assembly have added features that encourage a more detailed analysis than the one presented here (A. K. Dunker, manuscript in preparation).

It should be kept in mind that partial association of a particular capsid bonding interface could lead to still other intermediates. As an example, $(\alpha\gamma)_3$ units could associate to form $[(\alpha\gamma)_3]_2$, $[(\alpha\gamma)_3]_3$, etc. But complete association of the same bonding interface would lead to a spherical shell, $[(\alpha\gamma)_3]_{20T} = (\alpha\gamma)_{60T}$.

TABLE 6. *Possible stepwise intermediates for $T[3]$ capsids $(\alpha\beta\gamma)_{60T}$*

$(\alpha\beta)_{60T}$	$(\alpha\beta\gamma)_5$	$(\alpha\beta)_3$	$(\alpha\beta\gamma)_1$	α_3
$(\alpha\gamma)_{60T}$		$(\alpha\gamma)_3$		β_3
$(\beta\gamma)_{60T}$	$(\alpha\beta)_5$	$(\beta\gamma)_3$	$(\alpha\beta)_1$	γ_3
	$(\alpha\gamma)_5$		$(\alpha\gamma)_1$	
$(\alpha)_{60T}$	$(\beta\gamma)_5$	$(\alpha\beta\gamma)_2$	$(\beta\gamma)_1$	α_2
$(\beta)_{60T}$				β_2
$(\gamma)_{60T}$	$(\alpha\beta\gamma)_3$	$(\alpha\beta)_2$	α_5	γ_2
		$(\alpha\gamma)_2$	β_5	
		$(\beta\gamma)_2$	γ_5	

DISCUSSION

Due to the lack of a theory for capsids with nonidentical polypeptide chains, there has been some uncertainty about the proper triangulation number assignment for these viruses. For example, certain picornaviruses have three size similar but nonidentical polypeptide chains in equimolar ratios. Virtually identical structural models have been proposed for several such picornaviruses: ME virus (32), poliovirus (18), Coxsackie virus (30), foot and mouth disease virus (35), rhinovirus 1A (27), bovine enterovirus (17), and Mengo virus (25). Yet, when viewing the same model for the capsid structure, some workers have suggested that the picornavirus capsid should be classified as $T = 1$ (32), whereas others have suggested $T = 3$ (30). As pointed out previously (31), the ambiguity arises because there are 180 polypeptide chains of about equal size (which favors $T = 3$), but there are only 60 groups of equivalent sets of polypeptide chains (which favors $T = 1$).

In an effort to resolve this ambiguity we wish to point out that the derivation of the formula for T requires of the subunits only that they be identical. There is no requirement that these identical subunits be a single polypeptide chain; on the contrary, as we have shown, an identical structure unit composed of nonidentical polypeptide chains serves just as well.

Furthermore, note that T is independent of

TABLE 7. *Possible stepwise dissociation modes of a $T[3]$ capsid $(\alpha\beta\gamma)_{60T}$*

$(\alpha\beta\gamma)_{60T} \rightarrow (\alpha\beta)_{60T} + 12\gamma_5 + 10(T-1)\gamma_6$
$\rightarrow (\alpha\beta)_{60T} + 20T\gamma_3$
$\rightarrow (\alpha\beta)_{60T} + 30T\gamma_2$
$\rightarrow (\alpha\beta)_{60T} + 60T\gamma_1$
$\rightarrow (\alpha\gamma)_{60T} + 12\beta_5 + 10(T-1)\beta_6$
$\rightarrow (\alpha\gamma)_{60T} + 20T\beta_3$
$\rightarrow (\alpha\gamma)_{60T} + 30T\beta_2$
$\rightarrow (\alpha\gamma)_{60T} + 60T\beta_1$
$\rightarrow (\beta\gamma)_{60T} + 12\alpha_5 + 10(T-1)\alpha_6$
$\rightarrow (\beta\gamma)_{60T} + 20T\alpha_3$
$\rightarrow (\beta\gamma)_{60T} + 30T\alpha_2$
$\rightarrow (\beta\gamma)_{60T} + 60T\alpha_1$
$\rightarrow \alpha_{60T} + 12(\beta\gamma)_5 + 10(T-1)(\beta\gamma)_6$
$\rightarrow \alpha_{60T} + 20T(\beta\gamma)_3$
$\rightarrow \alpha_{60T} + 30T(\beta\gamma)_2$
$\rightarrow \alpha_{60T} + 60T(\beta\gamma)_1$
$\rightarrow \beta_{60T} + 12(\alpha\gamma)_5 + 10(T-1)(\alpha\gamma)_6$
$\rightarrow \beta_{60T} + 20T(\alpha\gamma)_3$
$\rightarrow \beta_{60T} + 30T(\alpha\gamma)_2$
$\rightarrow \beta_{60T} + 60T(\alpha\gamma)_1$
$\rightarrow \gamma_{60T} + 12(\alpha\beta)_5 + 10(T-1)(\alpha\beta)_6$
$\rightarrow \gamma_{60T} + 20T(\alpha\beta)_3$
$\rightarrow \gamma_{60T} + 30T(\alpha\beta)_2$
$\rightarrow \gamma_{60T} + 60T(\alpha\beta)_1$

$(\alpha\beta\gamma)_{60T} \rightarrow 12(\alpha\beta\gamma)_5 + 10(T-1)(\alpha\beta\gamma)_6$
$\rightarrow 20T(\alpha\beta\gamma)_3$
$\rightarrow 30T(\alpha\beta\gamma)_2$
$\rightarrow 12(\alpha\beta)_5 + 10(T-1)(\alpha\beta)_6 + 20T\gamma_3$
$\rightarrow 12(\alpha\beta)_5 + 10(T-1)(\alpha\beta)_6 + 30T\gamma_2$
$\rightarrow 12(\alpha\gamma)_5 + 10(T-1)(\alpha\gamma)_6 + 20T\beta_3$
$\rightarrow 12(\alpha\gamma)_5 + 10(T-1)(\alpha\gamma)_6 + 30T\beta_2$
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$\rightarrow 30T(\alpha\gamma)_2 + 20T\beta_3$
$\rightarrow 30T(\beta\gamma)_2 + 12\alpha_5 + 10(T-1)\alpha_6$
$\rightarrow 30T(\beta\gamma)_2 + 30T\alpha_6$

how the subunits are packed or clustered. For example, icosahedral capsids composed of 180 subunits are classified as $T = 3$ regardless of whether they are packed as 180 monomers, 90 dimers, 60 trimers, or 20 hexamers plus 12 pentamers. By the same token, assignment of T to a capsid containing nonidentical chains should be likewise independent of the packing details. As an example, a capsid that can be resolved into 60 symmetrically equivalent sets of three nonidentical chains should be classified as $T = 1$ regardless of whether the capsid is organized as (i) 60 identical three-chain clusters, e.g., $(a, b, c)_{60}$, (ii) 20 six-chain clusters plus 12 five-chain clusters, e.g., $(a_3b_3)_{20}\ (c_5)_{12}$, or (iii) any of the many additional clustering possibilities.

Finally, it should be kept in mind that the triangulation number is a means for structural classification; T is not dependent upon knowledge about the function of polypeptides, nor about the morphopoetic pathway of capsid assembly, nor about the stability of the different subunit bonding contacts against dissociation by various denaturing agents.

These three considerations suggest a simple way of clarifying the aforestated ambiguity; namely, include with the triangulation number the number of nonidentical polypeptides comprising the repeating subunit. Our proposal is to follow the triangulation number with brackets enclosing a description of the identical unit, that is $T[n]$. The number **n** is the number of nonidentical polypeptide chains within the repeating unit of the surface lattice of the virion.

This proposal has two very positive features. First, it refocuses attention away from the nonidentical polypeptide chains and back to the identical subunit as required by the foundations of triangulation theory. Second, it increases the information content of the triangulation number.

By this proposal the 60 subunit (protomer) model for picornaviruses (9, 25, 30, 32), in which each protomer (19) consists of four nonidentical chains (30, 34), would be assigned $T = 1[4]$. Similarly, the capsid composition (12) and three-dimensional image reconstruction (8) suggest $T = 1[2]$ for cowpea mosaic virus; the capsid composition (28) and morphology (R. C. Williams and K. E. Richards, J. Mol. Biol., in press) suggest $T = 7[2]$ for the lambda head.

The capsid of ϕX174 apparently contains 60 each of three nonidentical chains (1) and so should be classified as $T = 1[3]$, even though two of the proteins are commonly referred to as "spike" proteins rather than as capsid proteins.

Appendages, such as tails in lambda and the 12 copies of a spike protein in ϕX174 (1), are not part of the repeating unit in the capsid and so do not affect the current proposal. The maturation protein of the bacteriophages R_{17} and Q_β (33) and the proteins present in a single copy in ϕX174 (13) can also be logically relegated to the appendage category, whereas rare uncleaved polypeptides such as VP0 or the episilon chain in the capsid of mature picornaviruses (9, 22, 24, 27, 30) would more properly be regarded as "lattice defects" (R. R. Rueckert, personal communication).

In general, the electron microscope cannot distinguish nonidentical polypeptide chains. Thus, other methods are needed to determine which polypeptide chains lie adjacent to which other polypeptide chains. Chemical cross-linking, which is being pioneered by workers in the ribosome field (2, 23), probably offers the best hope for determining these bonding contacts. Cross-linking subviral fragments (A. K. Dunker, Ph.D. thesis, University of Wisconsin, Madison, 1969; 25, 30) should provide an extremely useful source of additional information.

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LITERATURE CITED

1. Agrawal, H. O. 1966. Studies on the structure of poliovirus. Arch. Gesamte Virusforsch. **19**:365-367.
2. Bayer, M. E., and A. F. Bocharov. 1973. The capsid structure of bacteriophage lambda. Virology **54**:465-475.
3. Bickle, T. A., J. W. B. Hershey, and R. R. Traut. 1973. Spatial arrangement of ribosomal proteins: reaction of the *Escherichia coli* 30 S subunit with bis-imidoesters. Proc. Nat. Acad. Sci. U.S.A. **69**:1327-1331.
4. Burgess, A. B. 1969. Studies on the proteins of ϕX coat. Proc. Nat. Acad. Sci. U.S.A. **64**:613-617.
5. Caspar, D. L. D. 1956. Structure of tomato bushy stunt virus. Nature (London) **177**:476-477.
6. Caspar, D., and A. Klug. 1962. Physical principles in the construction of regular viruses. Cold Spring Harbor Symp. Quant. Biol. **27**:1-24.
7. Crick, F. H. C., and J. D. Watson. 1956. The structure of small viruses. Nature (London) **177**:473-475.
8. Crowther, R. A., J. L. M. C. Geelen, and J. E. Mellema. 1974. A three-dimensional image reconstruction of cowpea mosaic virus. Virology **57**:20-27.
9. Dunker, A. K., and R. R. Rueckert. 1971. Fragments generated by pH dissociation of ME-virus and their relation to the structure of the virion. J. Mol. Biol. **58**:217-235.
10. Finch, J. T., and K. C. Holmes. 1967. Structural studies of viruses, p. 352-474. *In* K. Maramorosch and H. Koprowski (ed.), Methods in virology, vol III. Academic Press Inc., New York.

11. Finch, J. T., and A. Klug. 1959. Structure of poliomyelitis virus. Nature (London) **183**:1709-1714.
12. Geelen, J. L. M. C., A. van Kammen, and B. J. M. Verduin. 1972. Structure of the cowpea mosiaic virus. The chemical subunit: molecular weight and number of subunits per particle. Virology **49**:205-213.
13. Godson, G. N. 1971. Characterization and synthesis of ϕX174 protins in ultraviolet-irradiated and unirradiated cells. J. Mol. Biol. **57**:541-553.
14. Harris, J. I., and J. Hindley. 1961. The protein subunit of turnip yellow mosaic virus. J. Mol. Biol. **13**:894.
15. Horne, R. W., and J. Nagington. 1959. Electron microscopy studies of the development and structure of poliomyelitis virus. J. Mol. Biol. **1**:333-338.
16. Huxley, H. E., and G. Zubay. 1960. The structure of the protein shell of turnip yellow mosaic virus. J. Mol. Biol. **2**:189-196.
17. Johston, M. D., and S. J. Martin. 1971. Capsid and procapsid proteins of a bovine enterovirus. J. Gen. Virol. **11**:71-79.
18. Katagiri, S., S. Aikawa, and Y. Hinuma. 1971. Stepwise degradation of poliovirus by alkaline treatment. J. Gen. Virol. **13**:101-109.
19. Kellenberger, E. 1969. Polymorphic assemblies of the same major virus protein subunit. p. 349-366. *In* A. Engstrom and B. Strandber (ed.). Symmetry and function of biological systems at the macromolecular level. Almgvist and Wiksell, Stockholm.
20. Klug, A., and J. T. Finch. 1960. The symmetries of the protein and nucleic acid in turnip yellow mosaic virus. X-ray diffraction studies. J. Mol. Biol. **2**:201-205.
21. Korant, B. D., K. K. Longberg-Holm, and S. Halpern. 1970. Structural polypeptides of the rhinoviruses. Biochem. Biophys. Res. Commun. **41**:477-481.
22. Longworth, J. F., C. C. Payne, and R. MacLeod. 1973. Studies on a virus isolated from *Gonometa podocarpi* (Lepidoptera:Lasiocampidae). J. Gen. Virol. **18**:119-125.
23. Lutter, L. C., H. Zeichhardt, C. G. Kurland, and G. Stoffler. 1972. Ribosomal protein neighborhoods. I. S 18 and S 21 as well as S 5 and S 8 are neighbors. Mol. Gen. Genet. **119**:367-372.
24. Maizel, J. V., B. A. Phillips, and D. F. Summers. 1967. Evidence of differences between artificially produced and naturally occurring empty capsids of poliovirus type I. Virology **32**:629-699.
25. Mak, T. W., J. S. Colter, and D. G. Scraba. 1974. Structure of the Mengo virion. II. Physico-chemical and electron microscopic analysis of degraded virus. Virology **57**:543-553.
26. Mayor, H. 1964. Picornavirus symmetry. Virology **22**:156-160.
27. Medappa, K. C., C. McLean, and R. R. Rueckert. 1971. On the structure of rhinovirus 1A. Virology **44**:259-265.
28. Murialdo, H., and L. Siminovitch. 1971. The morphogenesis of the bacteriophage lambda. III. Identification of genes specifying morphogenetic proteins, p. 711-724. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, N.Y.
29. Nixon, H. L., and A. J. Gibbs. 1960. Electron microscope observations on the structure of turnip yellow mosaic virus. J. Mol. Biol. **2**:197-200.
30. Philipson, L., S. T. Beatrice, and R. L. Crowell. 1973. A structural model for picornaviruses as suggested from an analysis of urea degraded virions and procapsids of Coxsackie virus B3. Virology **54**:69-79.
31. Rueckert, R. R. 1971. Picornaviral architecture, p. 255-306. *In* K. Maramorosch and E. Kurstak (ed.), Comparative virology. Academic Press Inc., New York.
32. Rueckert, R. R., A. K. Dunker, and C. M. Stoltzfus. 1969. The structure of mouse Elberfeld virus: a model Proc. Nat. Acad. Sci. U.S.A. **62**:912-919.
33. Steitz, J. A. 1968. Isolation of the A protein from bacteriophage R_{17}. J. Mol. Biol. **33**:937-945.
34. Stoltzfus, C. M., and R. R. Rueckert. 1972. Capsid polypeptides of Maus-Elberfeld virus. I. Amino acid composition and molecular ratios in the virion. J. Virol. **10**:347-355.
35. Talbot, P., and F. Brown. 1972. A model for foot-and-mouth disease virus. J. Gen. Virol. **15**:163-170.