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K. W. Adolph and P. J. G. Butler

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Assembly of a spherical plant virus

By K. W. Adolph and P. J. G. Butler
Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH

[Plate 20]

The conditions previously reported as necessary for the reassembly of spherical viruses have been distinctly unphysiological and such reassembly cannot be related directly to the *in vivo* reaction. Mild conditions for the *in vitro* reassembly of cowpea chlorotic mottle virus (CCMV) from its isolated components have now been described (Adolph & Butler 1975) and the reassembled virus characterized. This reassembly involved the co-aggregation of the RNA and protein around neutrality and at ionic strength 0.2, giving yields of 70 % encapsidation at pH 6.0. The reaction was independent of temperature over the range 5–25 °C and did not require the presence of Mg^{2+} ions.

The reassembled virus shows a stability similar to that of native CCMV, with the same change in sedimentation coefficient around pH 6.5. The molecular mass and buoyant density in CsCl are also the same as those of native CCMV, while the electron microscope reveals a surface morphology on the reassembled particles like that on native CCMV.

Analysis of the number-average, mass-average, and *Z*-average molecular masses of the purified protein at both pH 6.0 and pH 7.5 suggests that the active unit for reassembly is a dimer of the protein subunit.

Introduction

The first demonstration of the self-assembly of a virus from its isolated protein and nucleic acid components was with the rod-shaped tobacco mosaic virus (Fraenkel-Conrat & Williams 1955), and subsequent exploration of the properties of the protein has led to conditions for the reproducible and rapid formation of complete virions at room temperature, neutral pH and a reasonable ionic strength (about 0.1) (Butler & Klug 1971). By contrast, the successful reassembly of a number of spherical viruses has previously only been accomplished under conditions which are clearly non-physiological. Thus the reassembly of infective bacteriophage (containing the A-protein) of either the R17 or Qβ families requires mixing the components under denaturing conditions and subsequent renaturation, either from 5.7 M urea (for R17; Roberts & Steitz 1967) or from 8 M guanidine hydrochloride (for Qβ; Hung & Overby 1969). Similarly the reassembly of viruses of the cowpea chlorotic mottle virus (CCMV) group (Bancroft & Hiebert 1967; Hiebert & Bancroft 1969) and of cucumber mosaic virus (Kaper & Geelen 1971) occurs at low temperature upon removal of the high salt concentration that is used to dissociate these viruses.

In this paper we discuss the reassembly of cowpea chlorotic mottle virus (CCMV) under mild conditions which are plausible for the *in vivo* assembly reaction (Adolph & Butler 1975). These physiologically plausible conditions include a pH around neutrality, ionic strength 0.1 or 0.2, and a temperature of 20–25 °C.

CCMV is a simple, spherical RNA virus. It has a diameter of 25 nm and a particle mass of 4.6×10^6. (The basic properties of CCMV have been included in reviews by Bancroft (1970) and Lane (1974).) The protein component of the virus consists of 180 copies of a single type of polypeptide chain of molecular mass 19400 (Bancroft, McLean, Rees & Short 1971). These subunits are arranged on the $T = 3$ surface lattice of Caspar & Klug (1962). RNA constitutes about 24% of the particle mass. Four species of RNA can be extracted from a preparation of CCMV, with molecular mass of 1.1, 1.0, 0.8 and 0.3×10^6 (Bancroft 1971). The two smallest species appear to be packaged together in a single virus particle.

Before describing the reassembly of infectious virus, some basic properties of CCMV particles and of the protein and RNA components will be discussed which further an understanding of the reassembly.

Structural transitions of the virus particles

CCMV was shown to differ in its sedimentation behaviour at low pH and at (relatively) high pH by Bancroft, Hills & Markham (1967), who found that while the virus was stable with a sedimentation coefficient of 88S at pH 5.0, the sedimentation coefficient had decreased to 78S at pH 7.0. At the same time the molecular mass appeared unchanged (Bancroft, Hiebert, Rees & Markham 1968). Thus the situation with CCMV seemed like that with the structurally similar brome mosaic virus (BMV), where the decrease in the sedimentation coefficient was related to an increase in the virus radius (Incardona & Kaesberg 1964). The details of the structural transitions have since been investigated in greater detail with the BMV system (Incardona, McKee & Flanegan 1973), where two contributions to the structural rearrangements that are responsible for the swelling were distinguished. These are a pH-induced transition and a thermal effect. While these studies were undertaken at low ionic strength, a recent paper (Pfeiffer & Hirth 1974*a*) has shown that an artificial top component can be produced from BMV in 1.5 M NaCl around pH 6.4.

As part of our study on the mechanism of assembly of a spherical virus, the structural changes in CCMV which resulted from varying the pH, ionic strength and temperature have been investigated in greater detail than previously and over a wider range of conditions (Adolph 1975*a*). At ionic strength 0.2 and 5 °C, the structural transition was detected as an abrupt decrease in the sedimentation coefficient at pH 6.5, followed by a more gradual decrease until a plateau was reached at pH 8.0. The overall decrease in the $s_{20,w}$ value was from 83S to 73S. The structural transition is even more dramatic at high ionic strength ($I = 1.0$), where the sedimentation coefficient drops from around 80S at pH 6.5 to less than 40S at pH 7.0. When the effect of ionic strength was examined more carefully, a critical ionic strength of 0.4 was found at which the CCMV particles disassembled to the 40S aggregates. The effect of adding Mg^{2+} to solutions of the virus was to modify the swelling somewhat, but not to change it fundamentally. Increasing the temperature from near 0 to 40 °C resulted in a gradual but substantial decline in the $s_{20,w}$ values at ionic strengths 0.2 and 1.0 for each representative pH that was studied (pH 4.5, 6.5 and 7.5).

A general conclusion that emerges from these experiments is that the RNA of CCMV must play a structural role in maintaining the stability of the virus particles. This follows from the observation that while the isolated protein capsid of CCMV disaggregates above pH 5.5 (see the next section), the fundamental structural transitions of the virus particles take place at

pH 6.5 and above. The difference of one pH unit must be due to an involvement of the RNA in stabilizing the protein shell.

States of aggregation of the protein

Introduction

A detailed understanding of the mechanism of assembly of simple viruses requires a systematic survey of the aggregates formed by the purified protein. Such a survey is necessary to uncover any intermediate aggregates which may play a crucial role in assembly. An example of such a crucial intermediate aggregate is the disk of TMV protein, which is involved in the initiation of assembly with RNA and in the elongation of the helix (Butler & Klug 1971; Butler, this volume, p. 151). A survey of the states of aggregation of the isolated protein also points out the relationships of the forms to one another, and reveals the important regions of pH, ionic strength, temperature and protein concentration. In these ways the significance of the various forms for assembly is made clearer.

The favourability of the CCMV system for studies on the mechanism of assembly was suggested by previous work which had demonstrated a variety of aggregates. Most significantly, the isolated protein was shown to form a 'pseudo-top component' with a surface morphology similar to that of the virus (Finch & Bancroft 1968). But many other aggregates could also be formed. For example, treatment of swollen CCMV with ribonuclease without removal of the RNA fragments, generated several types of tube (Bancroft *et al.* 1967). A number of other structures were derived from CCMV protein, including double-shelled and rosette-like particles, and laminar and platelike forms (Bancroft, Bracker & Wagner 1969).

Recent work with BMV (Pfeiffer & Hirth 1974*b*) has mapped the pH region of the transition between the pseudo-top component and the dissociated protein rather more thoroughly. In a paper concerned with the preparation of CCMV protein, Verduin (1974) demonstrated a hysteresis in the proportions of dissociated and undissociated protein on changing the pH.

Effect of pH, ionic strength and temperature

To characterize the aggregates formed at equilibrium from purified CCMV protein more closely, we have studied the protein aggregation over a wide range of conditions of pH, ionic strength, temperature, and protein concentration with both the analytical ultracentrifuge and the electron microscope (Adolph & Butler 1974). Difficulties in interpretation due to the presence of contaminating RNA fragments were avoided by using ion exchange chromatography to rid the protein solutions of residual RNA.

When the pH of a protein solution was varied at fixed ionic strength, temperature and protein concentration, most of the protein was in three sedimentation classes: 3S, 50S and greater than 100S. (These names for the sedimentation species are given for convenience; the precise sedimentation coefficients, $s_{20,w}$, of the 3S and 50S species are 2.7S and 52S, respectively.) The situation was simplest at high ionic strength ($I = 1.0$), where only the 3S and 50S aggregates were found. (The results at 20 °C and at 5 °C were similar.) Below pH 5.5 the 50S aggregate predominated, while the 3S aggregate was most abundant (more than 95 %) above this pH. At extremely low pH (3.5–3.75) a precipitate and an 11S component were occasionally detected, but it is unlikely that this 11S aggregate is of any relevance to assembly since it was only infrequently found and then at an extreme of pH. As the ionic strength was lowered

to below $I = 0.3$, an additional category of aggregate appeared with sedimentation coefficients of greater than 100S. These components were most commonly found just below the pH (pH 5.5) where the switch from the 3S aggregate to the 50S aggregate is found. A discrete aggregate which belongs to this category had a sedimentation coefficient, $s_{20,w}$, of 103S which is consistent with this form being a double-shelled particle. This view was strengthened by electron microscopic observations of the protein aggregates (described in the following section).

All these experiments were performed with aggregates which represented equilibrium states of the protein. Potentiometric titration experiments have recently shown (Jacrot 1975) that there is a marked hysteresis in going from the disaggregated state to the capsid. Equilibrium does, however, appear to be established within 48 h, the time allowed in the experiments described above for equilibrium to be attained during the dialysis against the appropriate buffers.

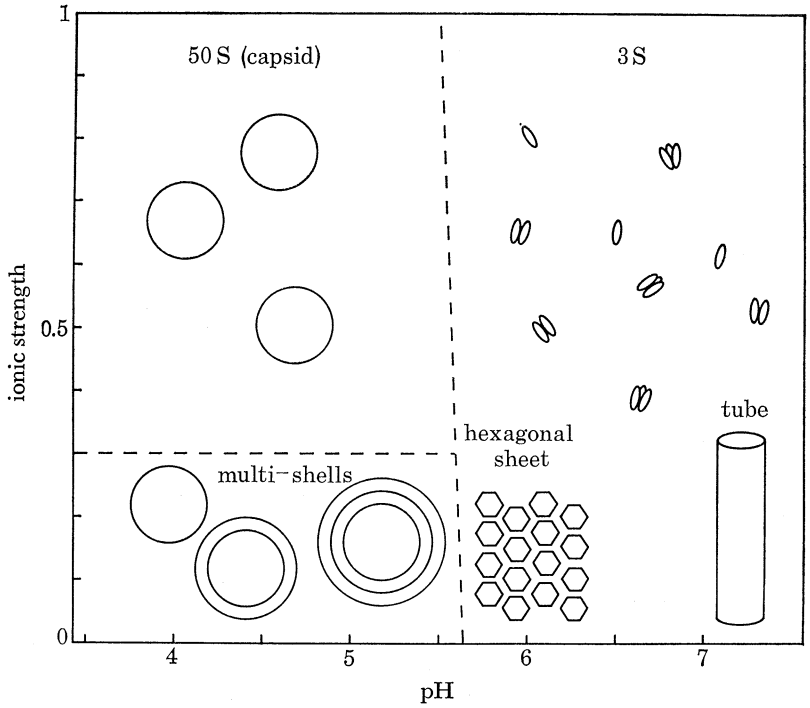

FIGURE 2. Map of the occurrence of important protein aggregates with pH and ionic strength. This is for about 0.5–1.0 mg protein/ml at 5 °C, but the map at 20 °C is very similar.

Electron microscopy of the aggregates

Examination of the protein samples with the electron microscope confirmed the dramatic shift in the sizes of the aggregates which occurred around pH 5.5 (figure 1, plate 20). At low ionic strength ($I = 0.1$), single- and multi-shelled particles were frequently observed below pH 6.0 on grids stained with uranyl acetate. The single-shelled particles had the same diameter as native CCMV of 25 nm, appeared empty, and (from electron microscope observations of high ionic strength protein solutions) could be identified as the material sedimenting at 50S. Calculations of the sedimentation coefficient to be expected for the protein capsids of CCMV suggested that the 25 nm spherical particles which sedimented at 50S were indeed reassembled CCMV capsids. Above the critical pH of 5.5, the frequency of large, shell-like aggregates declined sharply, with most of the protein lacking a definite structure but producing a coarse

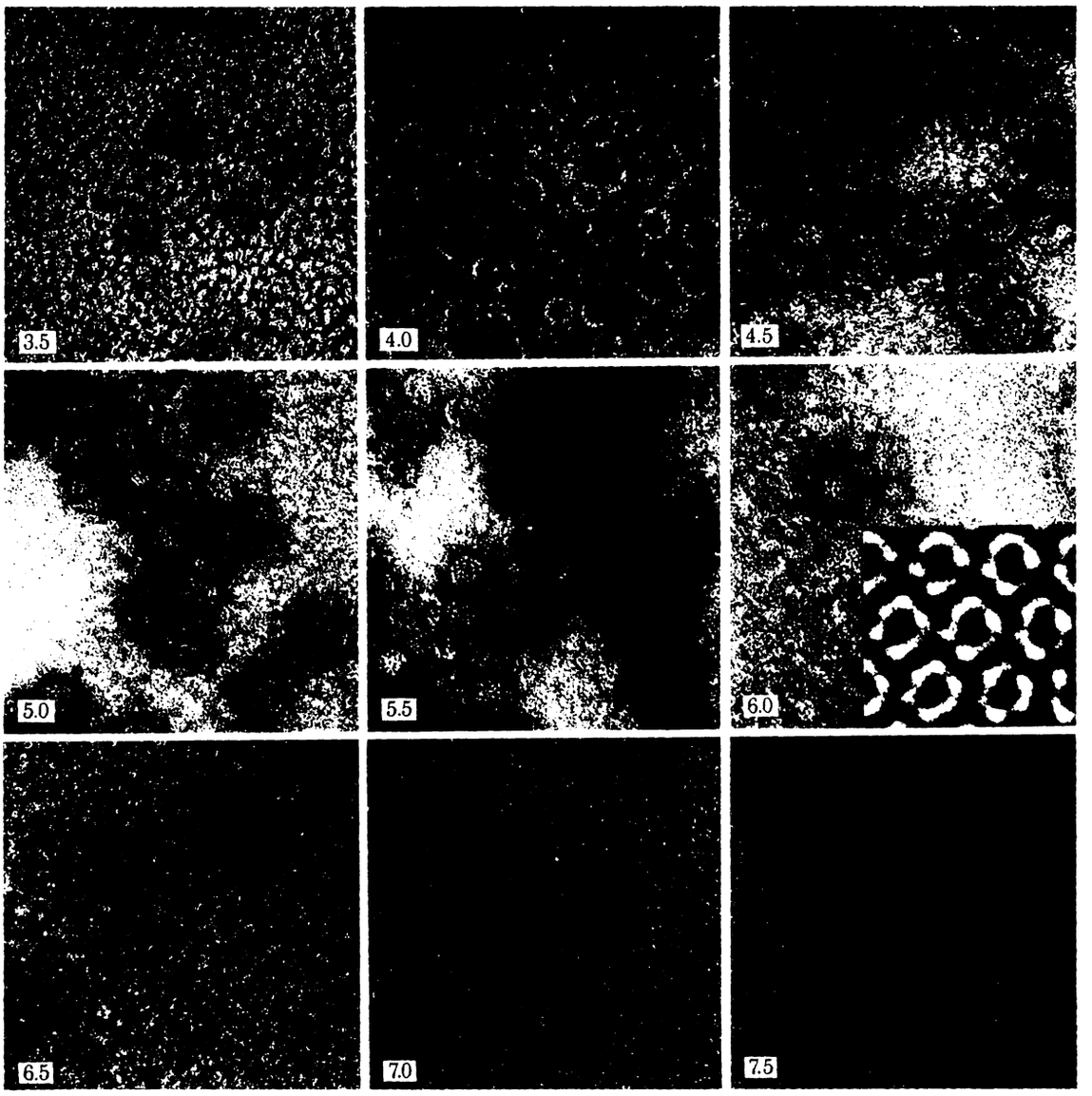

FIGURE 1. Electron micrographs of CCMV protein aggregates found at ionic strength 0.1 and 5 °C, at various pH values. Inset at middle right shows enlarged reconstructed image, after noise filtering from the transform using the computer filtering technique of Amos & Klug (1972), from area showing protein net which sometimes occurs at grids at pH 6.0, ionic strength 0.1 and 5 °C. (Magnification approximately × 200 000 and × 2 000 000.)

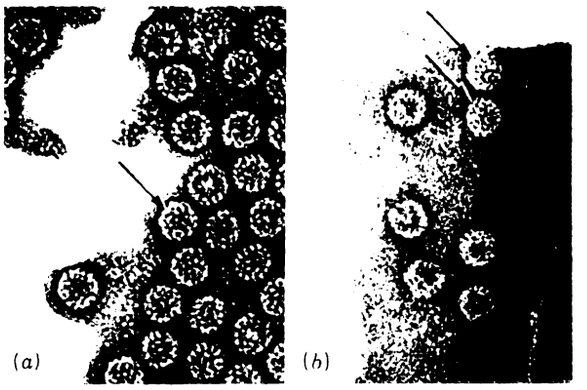

FIGURE 5. Electron micrographs of reassembled CCMV. Samples were negatively stained with 1 g/100 ml uranyl acetate and photographed over holes in the carbon substrate. *a*, control virus; *b*, reassembled virus. Arrows mark twofold views. (Magnification × 225 000.)

(*Facing p.* 116)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

granularity on the grid surface. The effect of increasing the ionic strength at pH values less than pH 5.5 was largely to eliminate the multi-shelled particles, a result consistent with the sedimentation experiments.

In addition to the major protein species, minor forms were also seen on the electron microscope grids. Tubes are one type of aggregate that were occasionally found (most frequently at low ionic strength around pH 5.0). Another interesting minor form was observed as an ordered array both on the carbon surface of an electron microscope grid and in films of uranyl acetate stain over holes. By applying the image processing technique described by Amos & Klug (1972) it was possible to resolve these ordered arrays into hexagonal nets of hexagons of the protein subunits (figure 1). A similar net was seen by Bancroft (1972) with a coat protein mutant of CCMV.

The results of characterizing the aggregates of purified CCMV protein on the basis of their sedimentation behaviour and appearance in the electron microscope are combined in figure 2 into a map of the occurrence of the aggregates with pH and ionic strength. This phase diagram includes the interesting minor forms – the hexagonal sheets of hexagons and the tubes – and is for a protein concentration of about 0.5–1.0 mg/ml.

Effect of protein concentration: capsid formation is a quasi-crystallization

The effect of protein concentration on the equilibrium demonstrates that capsid formation is a quasi-crystallization. That is, beyond a critical total protein concentration, all further protein goes into the 50S capsid while the concentration of the 3S aggregate remains constant. This quasi-crystallization behaviour was shown by measuring the concentrations of the 3S and 50S aggregates at 20 °C and ionic strength 1.0 (to avoid the complicating presence of the greater than 100S aggregates), and with three representative pH values: pH 5.0, pH 5.5 and pH 6.0. At pH 5.0 and pH 5.5, concentrations were reached beyond which a constant concentration of 3S aggregate was found; at pH 6.0 a constant concentration appeared to be approached. The behaviour of the CCMV protein capsid as a quasi-crystal resembles that of the disk of tobacco mosaic virus protein (Durham 1972; Durham & Klug 1972), which contains even fewer protein subunits, 34 for the disk compared to 180 for the CCMV capsid. In both cases, a high activation energy for the breakdown of the completed, closed structures makes release of subunits a rare event which is not dependent on the concentration of the 'quasi-crystal'. But a particular concentration of free subunits, determined by the solution conditions, is required since the resealing does depend upon the concentration of aggregating material. Both the CCMV capsid and the TMV disk would therefore show a critical protein concentration beyond which all further protein goes into the 'quasi-crystal'.

Subunit composition of the 3S *protein*

The nature of the 3S protein component has now been defined more precisely by performing high speed equilibrium sedimentation experiments to obtain the number-average, mass-average, and *Z*-average molecular mass. These averages were calculated from experiments at pH 6.0, ionic strength 0.2, 5 °C, where appreciable assembly of protein into capsid is beginning to occur, and at pH 7.5, ionic strength 0.2, 5 °C, where 95 % of the protein is found as the 3S aggregate. The curves showing the dependence of the number-average ($\bar{M}_n$) and mass-average ($\bar{M}_m$) molecular mass are given in figure 3. The *Z*-average molecular mass have been omitted since they are less reliable as data for any numerical analysis, but their general values are

consistent with the interpretation given below. Since at any given protein concentration the number-, mass- and Z-averages went in descending order – the opposite of the normal behaviour for an aggregating system – an attempt was made to fit $1/\bar{M}$ against concentration curves with the non-ideality equations:

$$1/\bar{M}_{\mathrm{n,app}} = 1/\bar{M}_{\mathrm{n},0} + (B/2)c,$$
$$1/\bar{M}_{\mathrm{m,app}} = 1/\bar{M}_{\mathrm{m},0} + Bc,$$

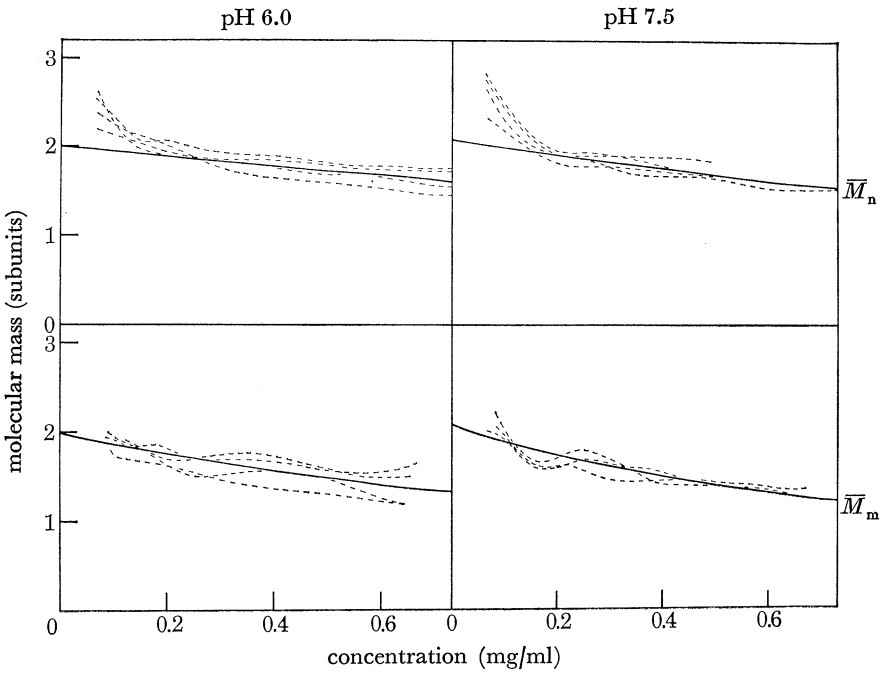

FIGURE 3. Dependence of molecular mass of 3S protein aggregate upon concentration. Solid lines represent calculated curves while broken lines show measured values for individual centrifuge cells.

where B is the same second virial coefficient in each case and c is the concentration. The fit, which is quite impressive, is presented in figure 3. The same values of $\bar{M}_0$ and of B were obtained for both the number and mass averages, showing that the material is essentially monodisperse (with 2 subunits) and that the spread of the molecular masses is due to non-ideality. The actual values are:

$$\text{pH } 6.0,\ M_0 = 2.00\ (\pm 0.04) \text{ subunits},\ B = 0.34\ (\pm 0.03) \text{ ml mg}^{-1} \text{ subunit}^{-1},$$
$$\text{pH } 7.5,\ M_0 = 2.08\ (\pm 0.03) \text{ subunits},\ B = 0.48\ (\pm 0.01) \text{ ml mg}^{-1} \text{ subunit}^{-1}.$$

Since the free protein remains as a dimer when the pH is decreased from pH 7.5 to pH 6.0, where assembly into the shell is starting to be appreciable, the low molecular mass protein aggregate which assembles into the capsid is identified as the dimer.

CONFORMATIONAL FEATURES OF THE RNA

The results described in the preceding two sections indicate that the RNA component of CCMV interacts with the protein shell to stabilize the capsid. This is apparent from the fact that while the shell of isolated CCMV protein disaggregates above pH 5.5, the virus particles

are quite stable at low ionic strength, although swelling does occur at pH 6.5. Several questions immediately come to mind concerning the structural role of the RNA in CCMV. Is the role of the RNA merely passive, or are changes in RNA conformation responsible for the swelling of the virus particles? Does the RNA have a well-defined structure inside the virus particles with secondary structure to the RNA in the virus and a regular arrangement of the RNA in its interactions with the protein capsid? In order to obtain some answers to these questions, certain properties of CCMV RNA were investigated (Adolph 1975*b*, *c*).

To determine whether studies on the structure of the extracted RNA were of any relevance to the structure in the virus particles, the hypochromicities of the RNA in the particles and after extraction were compared and found to be similar in value. This suggests that the amount of secondary structure is preserved even in purified RNA.

The question of whether changes in RNA conformation are responsible for the major structural transitions of the virus particles was approached by measuring the sedimentation coefficients ($s_{20,w}$) and hypochromicities of the RNA at pH values through the region of the important structural transitions.

The sedimentation coefficients of the separated RNA species of the multicomponent CCMV genome (Bancroft 1971) were found to remain essentially constant throughout the pH region where the virus swells. This implies that no large scale changes in RNA conformation have taken place. To confirm this, the hypochromicities of unfractionated CCMV RNA, which are a measure of the amount of secondary structure, were obtained from the 'melting' profiles of the RNA at pH 4.5 and pH 7.5, with ionic strengths of both 0.2 and 1.0. These experiments again indicated that the amount of secondary structure was similar at both pH values.

Although it seems clear that large changes in RNA structure are not responsible for the swelling of CCMV above pH 6.5, the precise nature of the interaction of the RNA with the protein capsid remains unclear. While considerable progress has recently been made with BMV by using neutron diffraction (B. Jacrot, this volume, p. 109), a combination of approaches will be required to uncover the details of the structure of the RNA in the virus particles. In addition to the measurements of the hypochromicity of the RNA in the virus particles which were discussed above, some information about the structure of the RNA in the virus has recently been obtained from experiments on the binding of the dye acridine orange to CCMV (Adolph 1975*b*). The results of this study suggested that extensive regions of double helix exist in the virus, and that the structures of the RNA both in the virus and the following extraction are similar.

Reassembly of infectious virus under mild conditions

Solution conditions for reassembly

The most efficient reassembly was found with freshly isolated protein. Such a protein was obtained from virus that was grown as previously described (Adolph & Butler 1974) by dissociating the virus at high pH and ionic strength and then removing the RNA by calcium precipitation and ion exchange chromatography (Adolph & Butler 1974) or by sedimentation of the RNA (Bancroft & Hiebert 1967). Use of the latter procedure allowed the protein to be purified and reassembled with other RNA within a few hours, and was therefore the procedure of choice for these experiments.

Reassembly of infectious virus was first obtained by mixing, at 25 °C, a weakly buffered protein solution (pH 8.0, $I = 0.2$) with strongly buffered RNA solution (pH 6, $I = 0.2$) to

give a final pH of 6.2, or by mixing the protein and RNA at about pH 7.5 and then dialysing to pH 6.0 (Adolph & Butler 1975). The final solution conditions of the reassembly have now been varied in order to determine the optimum conditions for reassembly. The effect of varying the final pH at ionic strength 0.2 is shown in figure 4. (The virus was reassembled with a 5:1 ratio of CCMV protein to RNA by dialysis for 16 h at 5 °C.) This figure shows the percentage of the optical density at 260 nm for the three sedimentation classes which were found: (1) reassembled virus sedimenting at about 80S, (2) non-encapsidated RNA, and (3) some heterogeneous material which sediments much faster than reassembled virus. The percentage of the optical density which sedimented at the rate for virus declined from a plateau value approaching 90 % at pH 5.5 and below to a new plateau value of almost 30 % at pH 7.0 and pH 7.5. At the same time the percentage of free RNA increased from less than 5 % at low pH to nearly 60 % at pH 7.0 and above. Throughout the range of pH, the amount of rapidly sedimenting material remained constant at 13 %.

The effect of changing the ionic strength was also studied (table 1). The percentage of the optical density sedimenting as reassembled virus was determined at pH 6.0, 5 °C and ionic strengths 0.1, 0.5 and 1.0. The amount of reassembled virus was similar in each case and was

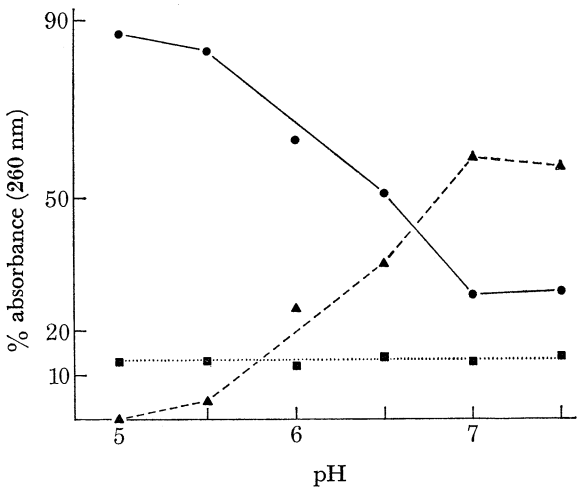

FIGURE 4. Effect of varying the final pH of the reassembly solution upon the percentage of reassembled virus. The ionic strength is 0.2 and the temperature is 5 °C. ●, reassembled virus (80S); ▲, free RNA (≪ 80S); ■, rapidly sedimenting material (≫ 80S).

TABLE 1. EFFECT OF SOLUTION CONDITIONS FOR REASSEMBLY; EXCEPT FOR THE FOURTH EXPERIMENT, REASSEMBLY WAS AT 5 °C

	reassembly with CCMV RNA 20 h	
	$s_{20,w}$(S)	% encapsidation
pH 6, I = 0.1	81	53
pH 6, I = 0.5	76	58
pH 6, I = 1.0	72	57
pH 6, I = 0.2, 25 °C	76	60
pH 6, I = 0.2+0.01 M Mg^{2+}	79	56
pH 5.75, I = 0.2+0.01 M spermine	82	51

close to the value at ionic strength 0.2. Increasing the temperature of the reassembly solution at pH 6.0, $I = 0.2$ to 25 °C produced results that were qualitatively the same as at 5 °C. This reassembly therefore differs from that of Bancroft and co-workers (reviewed by Bancroft 1970), where a low temperature was found necessary to produce spherical nucleoprotein particles. Since these workers also found that the presence of 5×10^{-3} M $MgCl_2$ was required for their reassembly, the effect of adding Mg^{2+} to the reassembly solutions was studied. (It was already clear that Mg^{2+} was not absolutely required.) With 0.01 M $MgCl_2$ added to a reassembly solution, the yield of virus (table 1) was similar to the value obtained in the absence of the ion figure 4). The addition of 0.01 M spermine produced about the same result.

Properties of the reassembled virus

The reassembled material had a sedimentation coefficient, $s_{20,w}$, which was the same, within experimental error, as that of the native virus under identical conditions. Values were obtained of 75–78S for the reassembled virus and 77–79S for native CCMV. The molecular mass of reassembled CCMV was determined by equilibrium sedimentation to be 4.5×10^6, a value similar to that of the native virus.

The buoyant density of reassembled CCMV in CsCl was determined with respect to a marker of native virus centrifuged to equilibrium under identical conditions. In this manner the buoyant densities of reassembled and native CCMV were found to be the same to within 0.004 g/cm^3. This result confirms the high quality of the reassembled virus by suggesting that the proportions of protein and RNA are the same as in the native virus. In earlier, preliminary experiments the reassembled virus was not as stable as the native virus in CsCl. This problem was overcome by using freshly isolated protein and reassembling the virus within 2–4 h of disassembly.

Examination of preliminary electron micrographs of reassembled CCMV revealed particles with characteristic twofold, threefold, fivefold and local twofold views (Adolph & Butler 1975). A more extensive electron microscopic study has now confirmed these earlier results. A representative field of reassembled CCMV is given in figure 5, plate 20, where particles displaying some characteristic views may be seen.

A preliminary comparison of the infectivities of reassembled and native CCMV by a dilution assay on cowpea plants established that their infectivities were similar. When inoculated at concentrations of 0.1 mg/ml, both produced systemic infections, but not when the concentrations were reduced to 0.02 mg/ml. At the same time, the purified RNA that was used in the reassembly was unable to produce an infection at 0.02 mg/ml (equivalent to 0.09 mg/ml virus).

Stability of the reassembled virus at high pH

A sensitive test of the quality of the reassembled virus was to examine the sedimentation behaviour of the material at high pH. Native CCMV undergoes an abrupt structural transition above pH 6.5, which was revealed as a decrease in the sedimentation coefficient by about 10S (Bancroft 1970; Adolph 1975*a*). This behaviour has been interpreted as a swelling of the virus particles and, since the capsid itself disaggregates above pH 5.5 (Adolph & Butler 1974), requires the stabilizing interaction of the RNA with the protein subunits of the capsid. Reassembled virus was prepared by dialysing a mixture of a 5:1 ratio of CCMV protein and RNA at pH 7.5 against buffer at pH 6.0, $I = 0.2$, 5 °C, and then samples were dialysed against various buffers in the range of pH from 5 to 8, $I = 0.2$, 5 °C for 3 h. Most of the material

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

sedimented at the rate for virus over this range of pH; thus the reassembled virus, like the native virus, is stable in the pH range where the capsid would disaggregate. Another important result is that a decrease in the sedimentation coefficient of the reassembled virus is found, suggesting that the reassembled virus swells similarly to the native virus. The decrease of 15S is centred at pH 6.5. It may be concluded that in this reassembly the RNA is not simply trapped inside the protein shell, but interacts with the subunits as does the RNA in the native virus.

Conclusion

Any reassembly requiring unphysiological changes of ionic strength or the presence of denaturing agents clearly departs from the normal assembly of the virus and such a reassembly may not even follow the same pathway. The mild conditions for the *in vitro* reassembly of CCMV which are described in this paper may be more directly related to the *in vivo* reaction, since the more relevant processes are likely to be found with a pH around neutrality, ionic strengths of 0.1 to 0.2, and temperatures from 20 to 37 °C. The opportunity is now presented to study significant aspects of the assembly of a spherical virus.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

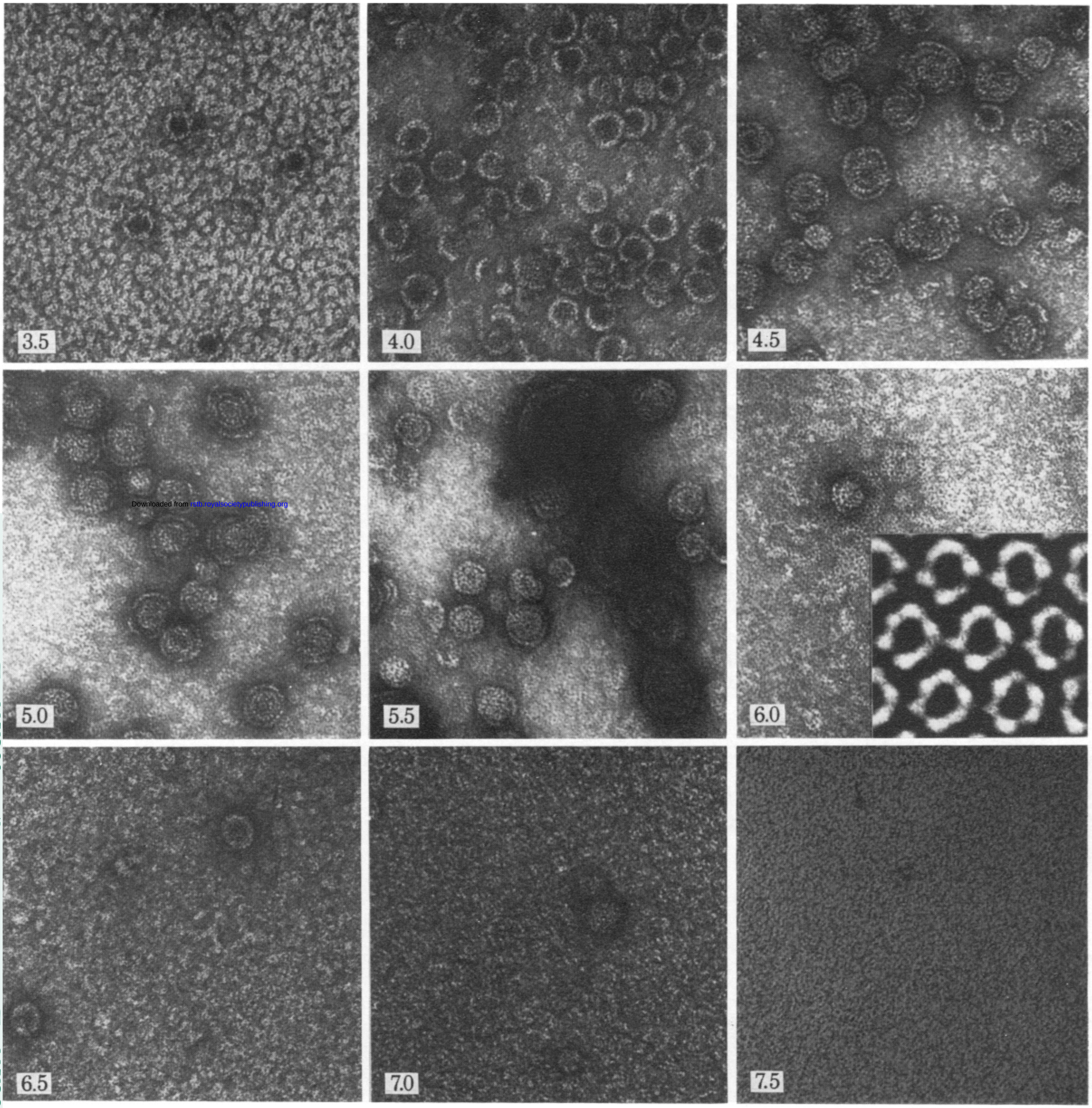
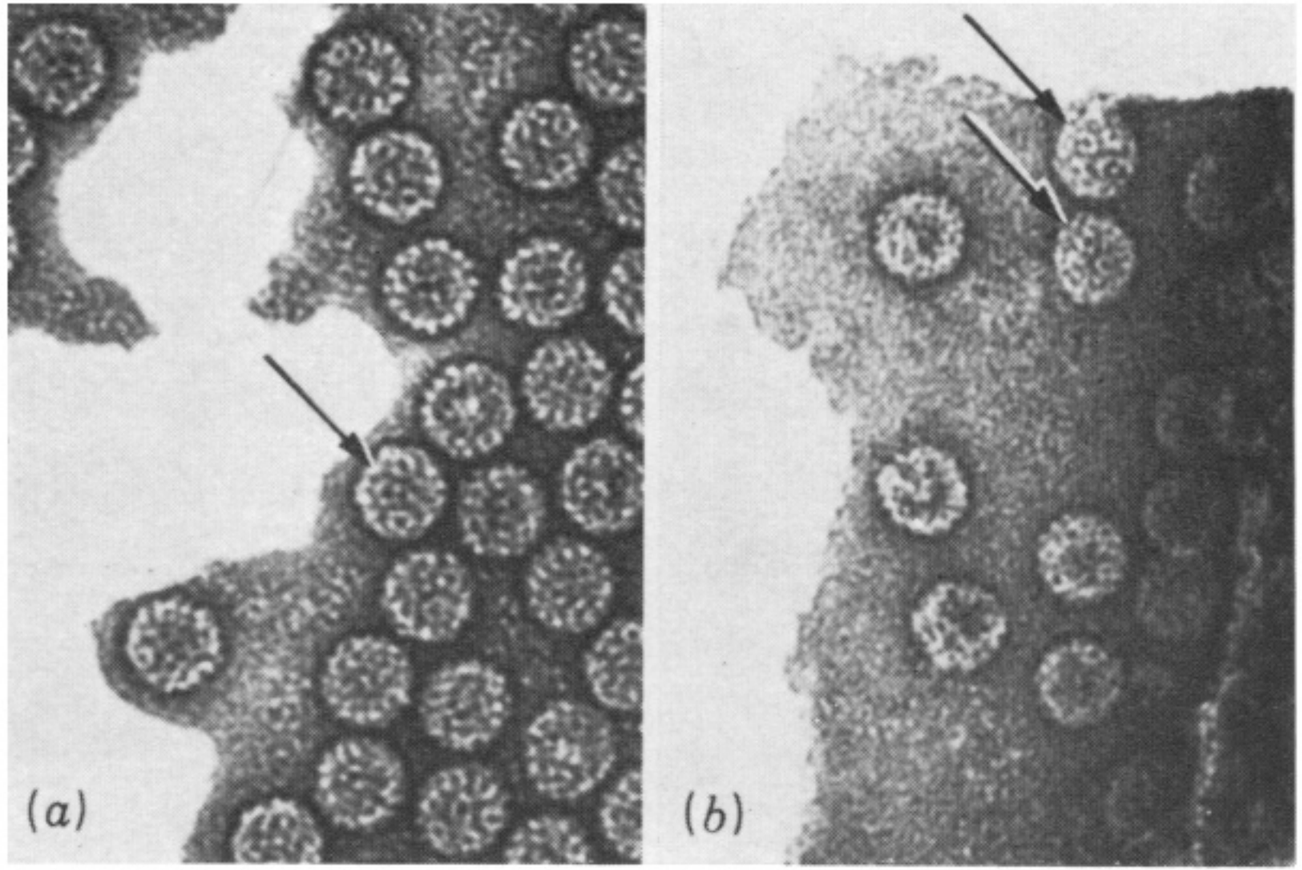


FIGURE 1. Electron micrographs of CCMV protein aggregates found at ionic strength 0.1 and 5 °C, at various pH values. Inset at middle right shows enlarged reconstructed image, after noise filtering from the transform using the computer filtering technique of Amos & Klug (1972), from area showing protein net which sometimes occurs at grids at pH 6.0, ionic strength 0.1 and 5 °C. (Magnification approximately ×200000 and ×2000000.)



GURE 5. Electron micrographs of reassembled CCMV. Samples were negatively stained with 1 g/100 ml uranyl acetate and photographed over holes in the carbon substrate. *a*, control virus; *b*, reassembled virus. Arrows mark twofold views. (Magnification × 225 000.)