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Cooperativity and Regulation Through Conformational Changes as Features of Phage Assembly

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DNA VIRUSES

Cooperativity and regulation through conformational changes as features of phage assembly

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[Plates 1 and 2]

The assembly of bacteriophages provides experimental model systems for the study of regulation at the level of gene products. We discuss the hypothesis of regulation through sequentially induced conformational changes by which precursor-assemblies become ready at a specific stage of maturation to interact with an additional gene product or nucleic acids. Phage mutants provide excellent experimental model systems for studying, for example, the role and fate of the core in the prehead assembly. The conservative maturation of the prehead to the final, stable head consists of several steps whose complexity reflect that of the virus. It includes packaging of DNA. The surface lattice of maturing preheads apparently undergoes several steps characterized by different conformational states as suggested by *in vitro* studies on a morphological variant of the prehead, the polyhead of phage T4 (Steven, Couture, Aebi & Showe 1976; Laemmli, Amos & Klug 1976). Addition of a partly purified, enriched proteolytic fraction – which is gene 21-dependent – to empty purified polyheads leads to different conformational states. These seem to go in a direction approaching the structure of the surface of finished capsids as studied by Aebi *et al.* on gene 24 related (Bijlenga, Aebi & Kellenberger 1976) and other genetically defined giant-variants of T4 phage (Doermann, Eiserling & Boehner 1973). We show some experiments which suggest that high cooperativity is responsible for the stabilization of capsids. The activation energy necessary for dissociation of capsids is very high, 247 kJ for T4 capsids, and 10% smaller for T2. Once the energy barrier has been overcome, the capsids are first structurally modified before they undergo partial and finally complete dissociation.

1. Introduction

Virus formation shows very particular characteristics: the products of the so-called late genes are not time-regulated at the level of transcription and translation but entirely at the level of particle formation. While the intracellular growth of particles is fully asynchronous, each individual particle is formed according to a sequence of definable neatly timed steps. These steps can be conveniently studied with the help of conditional lethal mutations (Epstein *et al.* 1963).

The regulatory processes involved in the sequential steps of virus formation are probably of general interest, because many observations suggest that a wide variety of mechanisms are used. Fortunately, this diversity of mechanisms does not affect the general line of the pathways of assembly and maturation which are found for many viruses:

(*a*) *Simplest viruses* undergo assembly in few steps, nucleic acid being immediately involved. In the simplest case, the information contained in the protein subunits is sufficient to build the particle together with nucleic acid and in some cases even without it.

(*b*) In more *complex viruses*, observations accumulate which show that two major phases can be distinguished (for references, see Showe & Kellenberger 1975; Casjens & King 1975).

In the first – *the assembly phase* – a precursor particle (provirion, prohead, prehead) is made, in which an internal protein core apparently plays a scaffolding role. This particle is then converted in the second – *the maturation phase* – into the final particle in steps, of which the number and the types of processes implicated varies widely for different species of viruses. The most dramatic step is certainly the packaging of nucleic acid into the head precursor, about which we will hear more in this symposium (Laemmli, Hohn). However, many other steps are no less interesting: the relatively labile precapsid (or capsoid) is transformed conservatively. i.e. with an invariant number of molecules of the major head protein (Bijlenga, van den Broek & Kellenberger 1974 for T4; Hohn *et al.* 1974 for λ) into the very stable capsid; core proteins are removed or processed, minor proteins are added. As indicated by many observations, an enlargement (10–20 % in linear dimensions) seems to occur. However the general determination of form, like the prolate shape of the head of phage T4, seems to be completed in the assembly phase.

In bacteriophage with tails, the tail assembly proceeds independently from the head. Wood *et al.* (1968) have demonstrated three independent converging assembly pathways for phage T4: head, tail and tail fibres. In each of these pathways, various regulatory mechanisms are used.

Consequently phages afford very good experimental systems for the study of such fundamental regulatory processes on the gene product level as:

Form and length determination

While in the simplest cases, the form or length of assemblies of identical protomers is determined by the geometry and bonding properties of the protomers themselves, in more complex aggregates other supplementary form-determining gene products are involved (Kellenberger 1966, 1969, 1972). They might do so in transient (or catalytic) ways (King, Lenk & Botstein 1973) as discussed by King in this symposium for a core protein contained in a prehead or they might be incorporated into the virus as minor constituents. A particularly challenging problem is the length determination of phage tails (see Kellenberger p. 27).

Regulation of sequential protein interactions

As already mentioned, all proteins involved are available simultaneously. They interact with their potential partners only at some precise step of particle formation and maturation. Not only are interactions newly created but certain bonded complexes can apparently change their interactions again at the right moment so as to free one of the partners which might leave the particle or undergo processing to yield a protein capable of some other function. This 'regulation through sequentially induced conformational changes' (Kellenberger 1972) is schematically represented in figure 1. For further discussion, the reader is referred to a recent review by Showe & Kellenberger (1975).

Packaging of nucleic acid

In all known cases, it appears that the replicating nucleic acid is in a relatively extended form, comparable to that of a bacterial nucleus, packaging into a particle requires that the

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local concentration has to increase at least 10- to 15-fold. This entropy loss has to be compensated by concomitant chemical reactions with entropy increase (Kellenberger 1961; Hohn, this symposium).

In contributions to this symposium, details will be given of how the above processes are at present being studied. To illustrate how facets of the molecular basis of the regulatory processes involved can be studied, I refer to a few experiments made in our department.

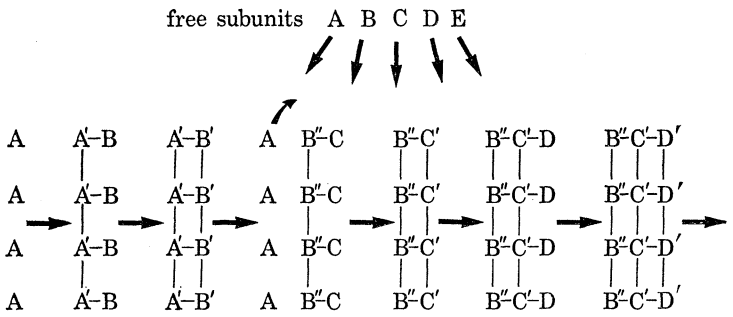

FIGURE 1. Schematic representation of the model of 'regulation of assembly through sequentially induced conformational changes'. Subunits of type A assemble and, by that, change conformation; the product $(A')_n$ is now able to interact with B. Through an induced conformational change B′ is made, which interacts with C and so on. By the same sort of induced conformational changes A might again be released from the structures explaining transient actions of gene products. By the same mechanism protein processing might be induced at the correct moment.

2. DIFFERENT CONFORMATIONAL STATES OF SUBUNITS IN A T4 SURFACE LATTICE

The transformation of the surface lattice of a capsoid during the maturation phase can be studied by image processing on polymorphic variants of either the prehead or the head. While differences in the crystalline quarternary structure are obvious, the proof for concomitant changes of tertiary structure of the involved proteins needs refinement. An approach to this study of sequentially induced conformational changes are the observations by A. Steven and M. Showe on the *in vitro* transformed empty polyheads of phage T4. During maturation of the prehead into the final head, this phage has the characteristic that the major capsid protein undergoes proteolytic cleavage (for general discussion and references see Showe & Kellenberger 1975, and appendix 1). The major capsid protein P23 with a relative molecular mass of 58300 is truncated by about 20% and becomes P23* with a mol. mass of 47500. A morphological variant of the prehead, the polyhead, allows crystallographic studies (Finch, Klug & Stretton 1964). M. Showe who is studying the role of P21 in the cleavage of several T4 proteins (Onorato & Showe 1976) has succeeded in our laboratory in isolating a highly active, proteolytic fraction from T4 am-mutant-infected cells (Showe, Isobe & Onorato 1976). After incubating purified empty polyheads in the presence of this partially purified, proteolytically active fraction, a modification of the lattice has been observed (Steven *et al.* 1976). By using quantitative, non-selective specimen preparation techniques for electron microscopy combined with protein analysis by SDS-polyacrylamide gel electrophoresis, these workers have shown that the surface lattices of 90% of the empty polyheads, initially composed of P23, have been transformed and the resulting particles are composed of P23*. Optical diffraction

and filtering (figure 2, plate 1) show clearly not only that the lattice constant has changed from 11.2 to 13.0 nm but also that a rearrangement of the mass-distribution within the unit cell has taken place. The orientation of the capsomer is 10° in native polyheads and 21° in transformed polyheads. The peak-to-peak distance between summits emerging from the negative stain (capsomer-diameter) does not greatly change for the major class of transformed polyheads. Other types of smooth particles, similar to those discussed below (table 1 and figure 3, plate 2) are also observed.

TABLE 1. COMPARISON OF THE SURFACE LATTICES OF GIANT HEAD T4 AND T4 + HEAD − RELATED PARTICLES

(Courtesy A. Steven & U. Aebi)

property of respective hexagonal surface lattice	coarse T4 polyheads	smooth T4 polyheads†		giant T4 phage
		type A	type B	
lattice constant/nm	11.2	13.0	13.0	13.0
type of capsomere (reasonable peaks in negatively stained preparations)	6	6	6+1	6+6+1
orientation angle (of capsomere relative to lattice lines)	10°	21°	20°	22°
capsomere diameter/nm (centre-to-centre distance between oppositely situated 'peaks' of promoters)	7.8	8.1	8.0	7.5
protein composition	P23	P23†	P23† (+ ?)	P23† + others

† In earlier studies by DeRosier & Klug (1972) and Yanagida, DeRosier & Klug (1972) of smooth T4 polyheads, surface lattices similar to those of types A and B above in all respects except that of capsomere diameter (8.5–9.5 nm) were reported.

Smooth polyheads occur 'spontaneously' (DeRosier & Klug 1972; Yanagida, DeRosier & Klug 1972; see also appendix 1) upon storage and, more reproducibly, when partially purified lysates containing polyheads are incubated at 37 °C (Laemmli, Amos & Klug 1976; Steven *et al.* 1976). At least three distinct structural states are observed by various authors for 'spontaneously' transformed polyheads (figure 3). From the experiments quoted above and from the morphological similarity it is reasonable to conclude now that these spontaneously occurring smooth polyheads are made of cleaved P23, a correlation which, at the time of their discovery, could not be established experimentally (see appendix 1).

DESCRIPTION OF PLATE 1

FIGURE 2. Coarse and *in vitro*-transformed T4 polyheads (courtesy of A. C. Steven, E. Couture & M. Showe). (*d*) Electron micrograph of 'coarse' T4 polyhead from mutant of gene 24 (tsB86), negatively stained with 2% NaPT. The optical diffractogram of the windowed area is shown in (*e*). A single-sided optical filtration is shown in (*f*). The morphology of the hexagonal surface layer, composed of molecules of uncleaved P23 (mol. mass = 58300) is common to polyheads produced by mutants in genes 20, 22, IPIII, 24 and 40 (Steven *et al.* 1976). When purified emptied coarse polyheads are incubated for a short time in the presence of a phage-derived proteolytically active enzyme they are transformed (aspects of transformation: increase in lattice constant from 11.2 to 12.8 nm, reorganization of capsomeric mass distribution, cleavage of P23 to P23* (mol. mass = 47700)). An example of the predominant species of transformed polyhead (type A smooth polyhead) appears in (*a*). NaPT 2% negative stain. In the optical diffraction pattern (*b*), the distribution of intensity on the reciprocal lattice is significantly different from that of coarse polyheads (*e*). An optical filtration revealing the mass distribution associated with the transformed polyhead is also given (*c*).

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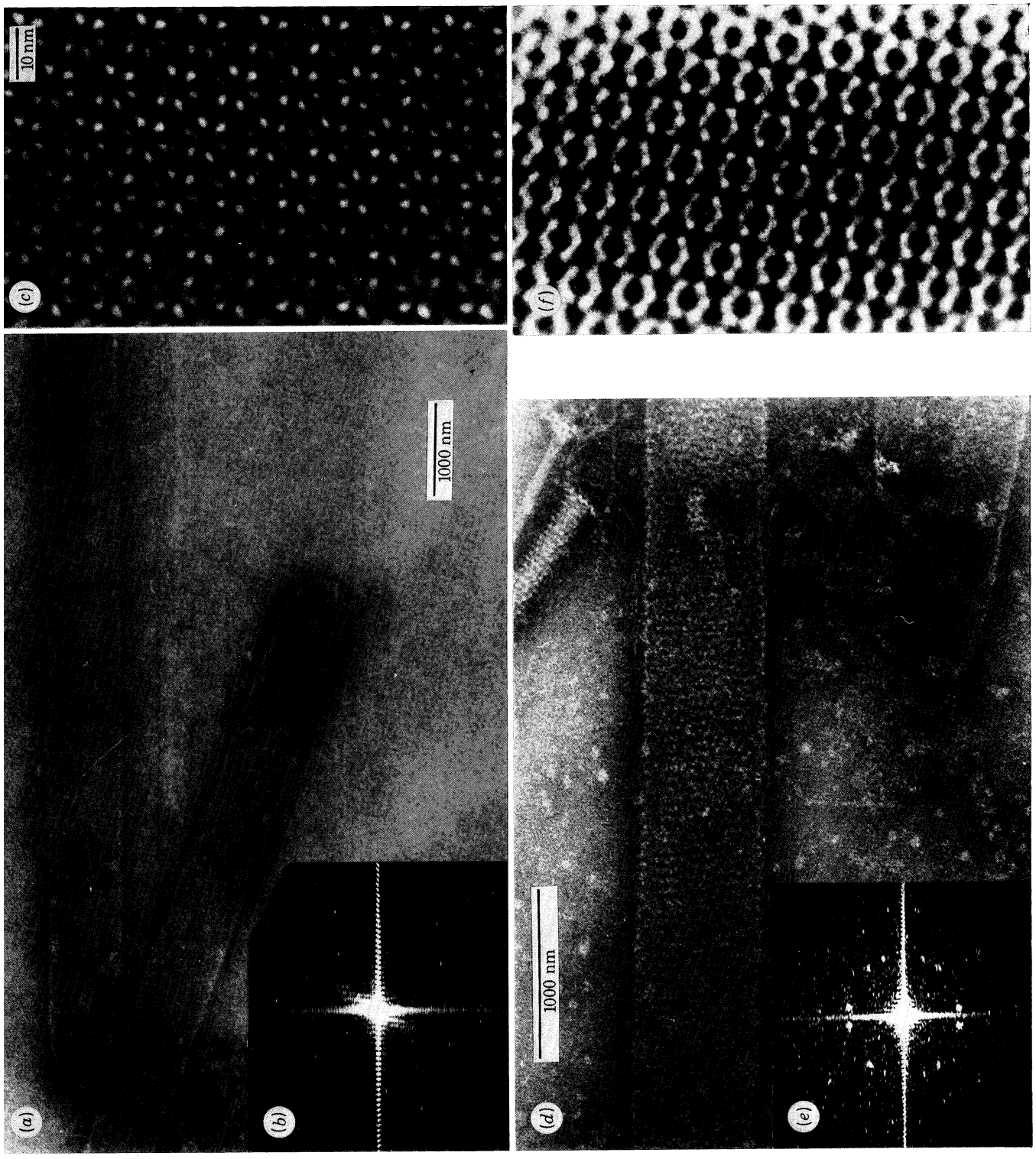

FIGURES 2(*a–f*). For description see opposite.

(*Facing p.* 6)

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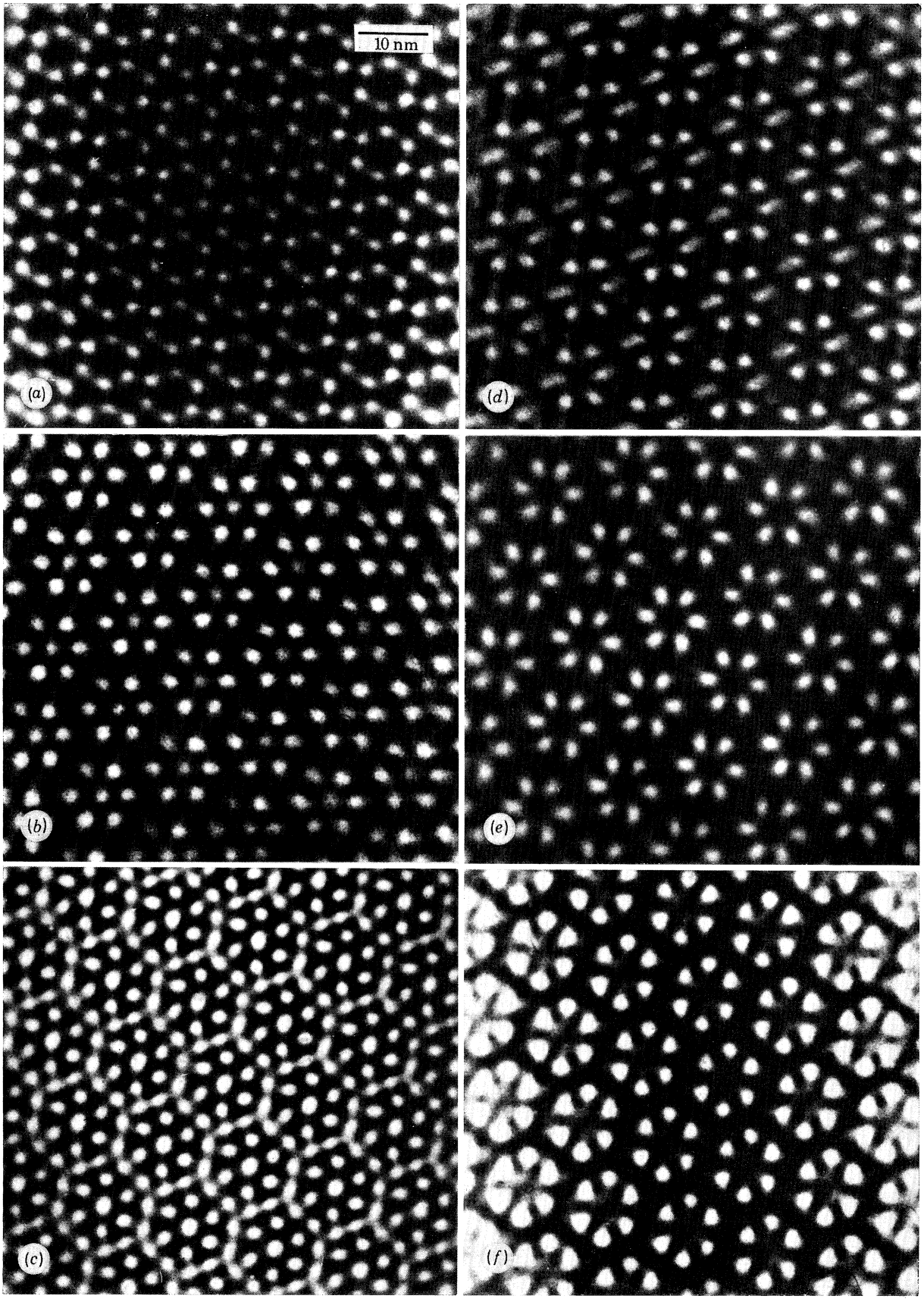

FIGURES 3(*a–f*). For description see opposite.

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Very comparable changes are observed in the capsid maturation of phage λ (Hohn, T., this volume, p. 51). Apparently the surface crystal of the precapsid, made of protein pE, undergoes a structural change and 'swelling' before it is able to bind protein pD in equimolar amounts.

It is highly important now to establish if the differences in the morphology of the unit cell – like the orientation angle and the capsomer diameter (table 1) – are reflecting different conformational states of the protein subunits.

Elsewhere we have discussed experiments of Dubochet *et al.* which suggest that the micro-dose technique of Unwin & Henderson (1975) with the beautiful results on the tertiary structure of the purple layer of halobacterium (Henderson & Unwin 1976) is not successful on all two-dimensional arrays of proteins (Kellenberger 1975). Apparently not all proteins have the same degree of intrinsic rigidity. Possibly for this and also other reasons polyheads range among the 'refractory' ones and therefore the hope of establishing the 'absolute' tertiary structure of the subunits by Unwin'stechnique are small. If, however, differences between conformational states can already be detected reproducibly by conventional methods of specimen preparation and image processing, then we possess a powerful tool for studies on the regulation through 'sequentially induced conformational changes' as discussed above. Theoretical reasons and some experimental indications give unexpected hopes in this respect (Kellenberger 1975; Aebi *et al.* in preparation).

3. Cooperativity phenomena

One of the most intriguing features of virus assemblies and subassemblies is the fact that they become stabilized as soon as the polymerization has progressed to the correctly sized substructure. After that, the completed structure may no longer dissociate into subunits (King, this symposium). Only by employing very non-physiological conditions, like denaturing agents (urea, guanidine hydrochloride, pH, heated detergents etc.) are we able to redissociate the proteins involved. It is obvious that we deal here with phenomena of high cooperativity. We will certainly learn more about these phenomena for the case of tails in the contribution of King & Kukichi in this symposium. In this case minor proteins play a role in terminating a structure and thereby stabilizing it, or initiate the stabilizing step. The molecular mechanism of this process is still not yet understood (for discussion, cf. Showe & Kellenberger 1975).

We had observed previously that the dissociation of different merids (appendix 2) of phage T4 (e.g. tail tubes, contracted tail-sheaths, polyheads, capsids) showed sharp transition points

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Description of plate 2

Figure 3. Optical filtration of structures associated with the precursor and mature forms of the surface lattices of T-even phage head (courtesy A. C. Steven, U. Aebi, E. Couture & M. Showe). A gallery of optical filtrations made of negatively stained electron micrographs of surface-shell morphologies of different types of particles associated with the T4 phage head. Further specification of the structural properties and composition of these surface lattices are given in table 1 (from Steven *et al.* 1976). (*a*) Coarse T4 polyhead from mutant 24 (tsB86). (*b*) B-type smooth T4 polyhead isolated from fresh crude 24 (tsB86) lysate obtained at nonpermissive conditions at 37° for 30′ post lysis. (*c*) Giant T4 phage produced by growing 24 (tsB86) mutant at intermediate temperature of 36° (Bijlenga, Aebi & Kellenberger 1976). (*d*) A-type smooth T4 polyhead produced by incubating purified emptied (22^-, 21^-) coarse polyhead with proteolytic phage-derived enzyme. (*e*) A-type smooth T4 polyhead isolated from fresh crude 24 (tsB86) lysate as described under (*b*). (*f*) T2L giant phage ('lollipop') produced by growing phage in presence of L-canavanine, and chasing with L-arginine (of which canavanine is an analogue) as described by Cummings, Chapman, DeLong & Couse (1973). (A detailed structural analysis is in preparation by Aebi *et al.*)

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in respect of the potency (e.g. concentration, temperature, or pH) of the dissociating agent (To, Kellenberger & Eisenstark 1969). These transition points are specific for each merid.

In the following I will summarize the results of some experiments on capsids made with R. van den Broek. While for completely isometric shells the cooperativity is easily explainable by the particular situation of the last set of subunits to be added in order to 'close' the shell, this is not as simple in the case of capsids of tailed bacteriophages. Here the shell has a unique polarity: normally only one single particular site of the shell will become fitted with some minor protein(s), which then are acting as receptors for the tail. For instance, in phage T4 the

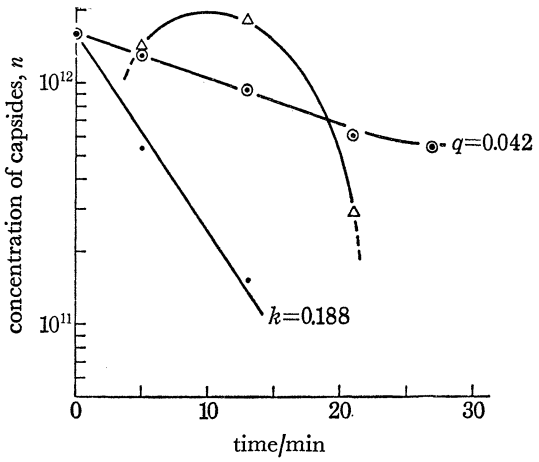

FIGURE 4. Kinetics of the dissociation and the morphological modification of capsids. A suspension containing 1.6×10^{12}/ml of capsids in water and 1% SDS was quickly heated up to 74 °C in a cuvet with heating mantle. On the one hand disintegration of capsids was followed by the decrease of optical density at 450 nm (⊙). The optical density is expressed in phage equivalents; indeed the optical density is proportional to the concentration n of the capsids. On the other hand samples were taken at various times for electron microscopy. In these samples the quantity of unmodified (•) and modified (△) capsids was determined by means of particle counts in the electron microscope on specimens prepared by agar filtration. (Kellenberger & Arber 1957; modified for negative stain, Kellenberger & Bitterli 1976.)

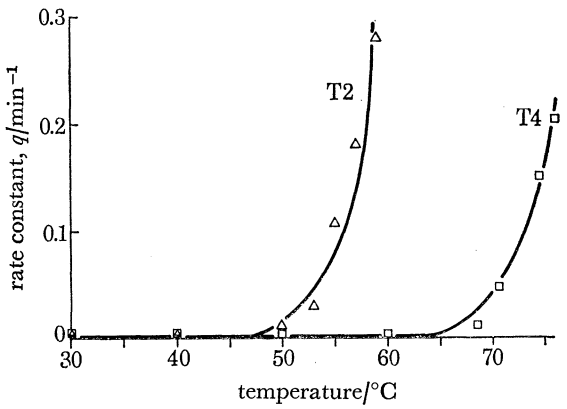

FIGURE 5. Temperature dependence of the rate constant of the dissociation of T2 and T4 capsids in SDS. For T2 and T4 capsids we determined the values of the rate constants q (min^{-1}) by measuring the slope of the decrease $d/d_0 = e^{-qt}$ of optical density at 450 nm at several temperatures as for figure 4. Below 45 °C for T2 and 60 °C for T4 the optical density stays constant over the 30–60 min observed. This difference between the transition points of T2 and T4 has been known for a long time (van Vunakis, personal communication, see Kellenberger 1968).

proteins P13 and P14 have to interact with heads before these can combine with tails (Edgar & Lielausis 1968). When tails are removed mechanically from finished phages, P13 now remains with the tails (Coombs & Eiserling, personal communication, publication in preparation).

We used T-even capsids and followed the dissociation by 1 % SDS with temperature as the variable parameter. Results with other agents show essentially the same basic behaviour. The SDS system has the advantage that it can be kept irreversible in contrast to those with other dissociating agents. On the one hand we monitored optical density at 450 nm as an index of dissociation and on the other hand observed and counted particles in the electron microscope. In figure 4, we see that optical density (d) decreases exponentially with time.

TABLE 2. RATE CONSTANT (k) OF THE TRANSFORMATION OF T4-CAPSIDS

The rate constant was determined from the plots of kinetics – such as those given in figure 4 – for the electron microscopy counts of non-modified capsids, $n/n_0 = e^{-kt}$.

temp./°C	k/min^{-1}
68.5	0.09
70.5	0.15
72.5	0.20
74.5	0.73
76.0	1.0

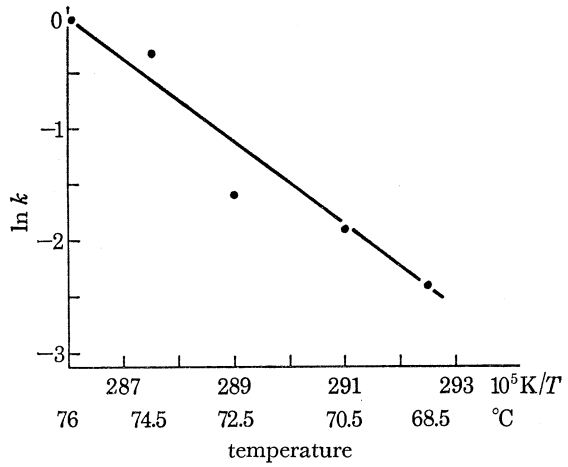

FIGURE 6. Arrhenius-plot of the rate constant of the morphological modification of T4 capsids in SDS. The rate constant k (min^{-1}) of the modification of T4 capsids was determined from their kinetics for each of five temperatures, by plotting the logarithm of the electron microscopy counts of unmodified capsids as shown for 72 °C in figure 4. The numerical values of k are given in table 2. According to Arrhenius' equation, $\ln k/k_0 = E_a/RT$, the activation energy E_a, is calculated as the slope of the linear part of the curve of $\ln k$ plotted against $1/T$. For T4 capsids we find $E_a = 247$ kJ/mol.

When determining the rate constant q for $d/d_0 = e^{-qt}$ (t, time) for different temperatures, we find that q rises abruptly above 65 °C for T4 and above 50 °C for T2 (figure 5). The exponential nature of the kinetics could have been due to an all-or-none effect. However, electron microscopy showed a more complicated picture. The number of normal, typically 'wrinkled' capsids also decreases in time as an exponential function ($n/n_0 = e^{-kt}$, (figure 4)). They are progressively replaced by a newly appearing form of altered capsids, of which an increasing proportion disintegrates into visible pieces, later apparently into non-observable subunits or small aggregates. A single event seems to occur, after which the modified capsids break down

progressively until they are completely dissociated. I. Katsura suggested that this behaviour be considered as analogous to the activation of enzyme reactions. By electron microscopy counts we have determined the kinetics of the unmodified capsid at different temperatures and calculated the values of k. When presented in an Arrhenius plot (figure 6), the data are linear over the range observed. From this preliminary data we have calculated the activation energy as 247 kJ/mole. The q values obtained from optical density measurements also showed a linear behaviour over a similar range in the Arrhenius plots; for higher values of $1/T$ the curves bend downward, indicating a still higher activation energy for this lower temperature range. When comparing T2 and T4 capsids by this procedure we find for T4 a 10 % higher activation energy than for T2.

In preliminary results obtained with the approach outlined above (figure 5), M. Showe (in our laboratory) found that the transition point for the dissociation of 'uncleaved' polyheads – if there is a real one – is certainly lower than that of empty polyheads transformed by P23-cleavage. First estimates of the transition point of transformed polyheads place it very near to that of normal capsids. This supports the previous hypothesis that smooth polyheads are more stable than coarse ones, as discussed in appendix 1.

Previous results (Kellenberger 1968, and unpublished results) strongly suggested that minor capsid proteins can be extracted differentially from capsids before the residual capsid loses its physical integrity. Up to now we have not succeeded in bringing under control all the parameters involved in this differential extraction. Therefore we cannot yet conclude to what extent some or all of the extracted minor capsid proteins are implicated in the break-down of cooperativity prior to complete capsid dissociation.

These were two examples of how one may try to approach the study of regulation through sequentially induced conformational changes and concomitant cooperativity in the maturation pathway of phage.

In the following contributions, illustrative examples by other workers will give you a more complete view of the different approaches used in this field.

I am most grateful to A. Steven, U. Aebi and M. Showe for communicating their results prior to publication and for providing experimental data. I am particularly indebted to A. Steven and A. Klug for critical reading and linguistic editing of the manuscript. The research work from our laboratory was supported by the Swiss National Foundation for Scientific Research and by an EMBO-fellowship to M. Showe. Elisabeth Leuthardt diligently and patiently typed the manuscript.

Appendix 1

Since publications in the field of polyhead-lattice transformations have been very much delayed relative to the actual work they do not necessarily convey the correct history of events, and a short narrative seems to be justified. Before the discovery of P23 cleavage, morphological variant polyheads ('smooth' polyheads) were found by Klug's group in a 'stored' polyhead suspension (DeRosier & Klug 1972). Yanagida, in Kellenberger's group reproduced this finding by artificial ageing and incubation under various conditions, and showed that smooth polyheads are frequently observed in increased relative amounts, but he did not succeed in defining conditions for reproducible results. At this time, quantitative studies had not yet been

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done, although it was known (Favre, Boy de la Tour, Segrè & Kellenberger 1965) that the total amount of unfixed polyheads in a suspension decreases rapidly upon storage. It was therefore not clear, whether a small proportion of smooth polyheads existed together with a large excess of coarse polyheads in a natural lysate, from which the coarse polyheads disappeared preferentially during storage, or if a transformation of native, coarse polyheads occurred 'spontaneously' in the test tube. Hence, nothing could be said about the origin of smooth polyheads. In collaboration between Klug's and Kellenberger's groups (Yanagida *et al.* 1972), optical filtration of the smooth polyheads again showed several different types of fine structure. As already mentioned, this work was essentially terminated before the discovery of cleavage in T4. In a Symposium contribution, protein cleavage, which was already known to occur in some animal viruses was proposed for investigation as a possible regulatory mechanism in T4 (Kellenberger 1969). The discovery of cleavage was then made independently in four groups (Laemmli 1970; Dickson *et al.* 1970; Hosoda & Cone 1970; Kellenberger & Kellenberger-van der Kamp 1970). Among these, the investigation of Laemmli (1970) was doubtless the most extensive; it not only described cleavage of P23 but also of other proteins in the head (P24, IPIII, P22). Laemmli started to investigate polyhead cleavage and its relation to smooth polyheads about 1969 when in Klug's group (Klug, personal communication and quoted in Yanagida *et al.* 1972). He communicated an important observation to us and others, namely that chloroform apparently inhibited spontaneous cleavage in crude lysates. Because his studies with mutants showed that the products of genes 21 and 22 were required for the *in vitro* cleavage, he postulated that the action of chloroform is through removal of the core (personal communication and Laemmli & Quittner 1974). Since chloroform is currently used in T4 research to induce lysis, Laemmli's observations explained some of the irreproducible results involving spontaneous cleavage obtained in several laboratories. M. Showe *et al.* in Haverford college started to purify and characterize the proteolytic cleavage system related to P21, applied first to P22 (Onorato & Showe 1975), which he continued in Kellenberger's laboratory with great success. Among other things (to be published), he found that many weakly polar, organic solvents inhibited reversibly the *in vitro* proteolytic activity of the P21 related system on its substrates. As discussed in the text, his highly enriched proteolytic fraction cleaves P23 of completely empty, i.e. core-free capsoids of polyheads, which were released from infected cells by chloroform-induced lysis. The polyacrylamide gels demonstrate, that these polyhead-capsoids were initially constituted of P23 in very high purity (Steven *et al.* 1976)

Appendix 2

A *merid* is defined as an assembly based on a fixed number of identical protomers arranged in a specific form. Minor proteins involved in cooperativity are part of the merid.

We use the term *protomer* as synonymous with *structure unit* which was defined as the smallest asymetric unit positioned in a lattice (Caspar & Klug 1962). It can be composed of only one peptide chain, or of several different ones. For example: in phage λ the *protomer of the capsid* is (pE+pD), the *protomer of the prehead* pE.

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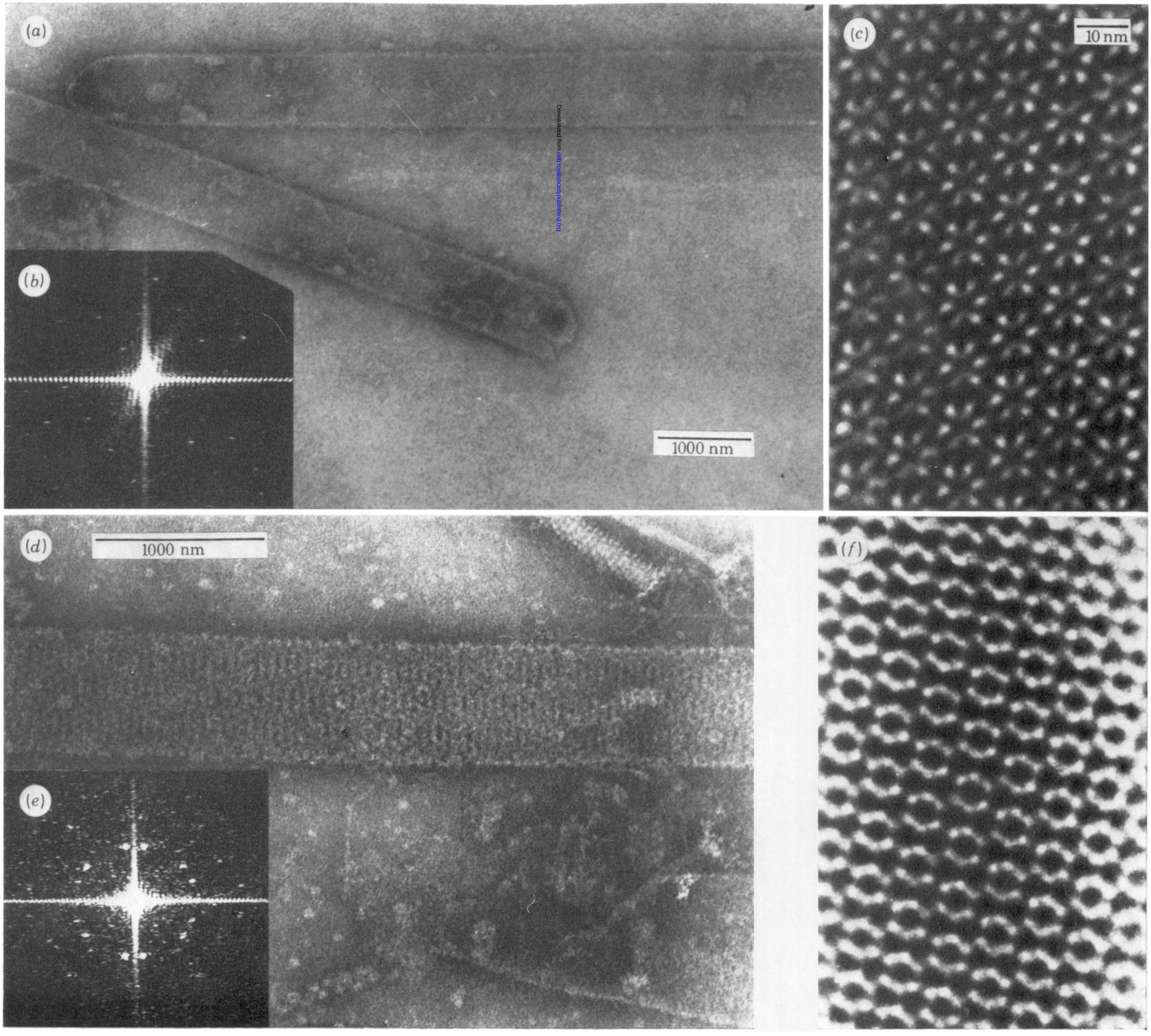
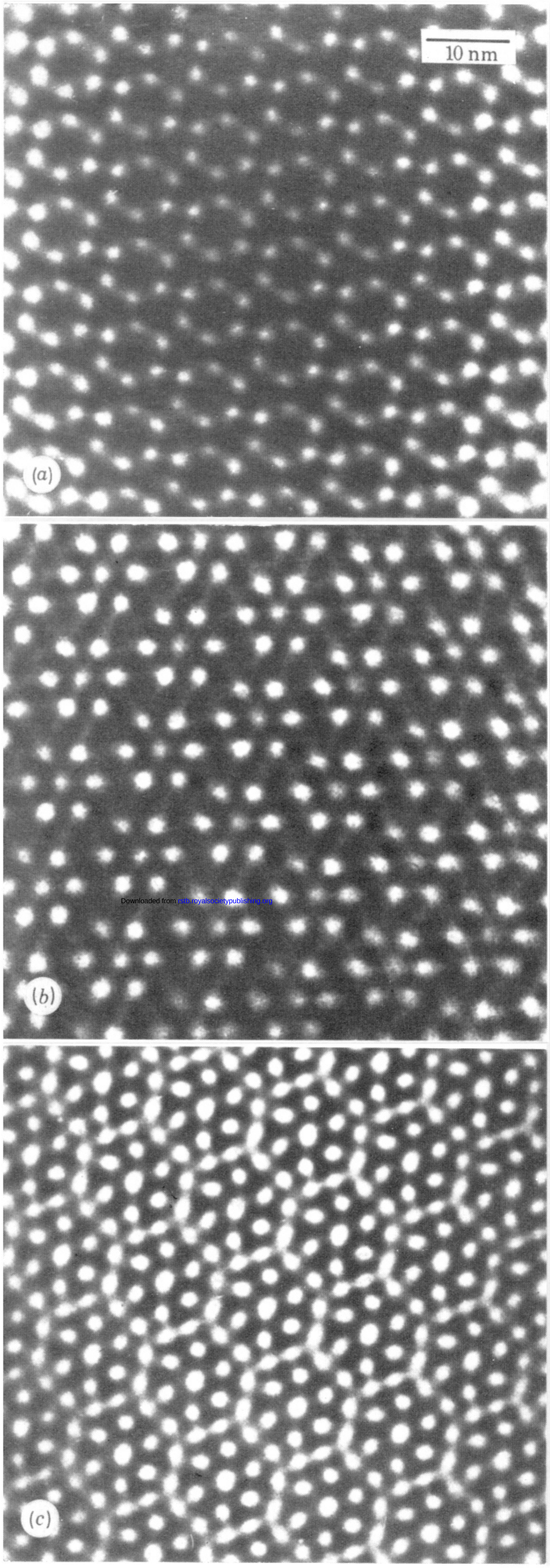
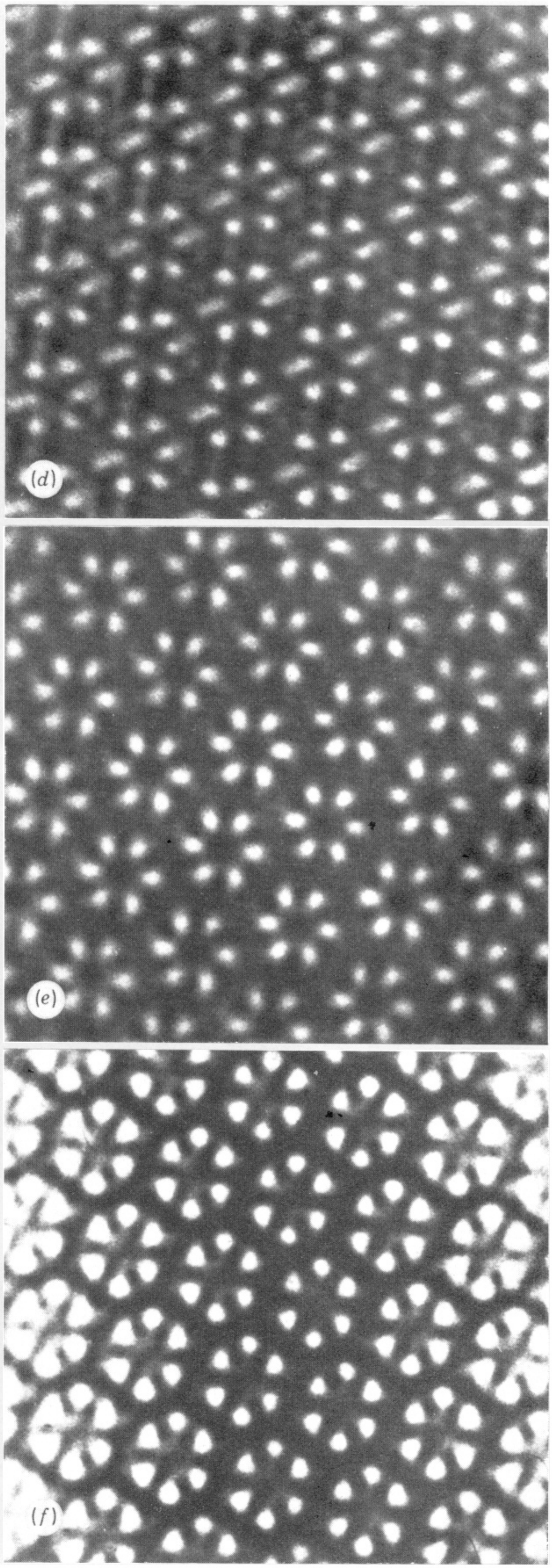


FIGURES 2 (*a–f*). For description see opposite.





FIGURES 3 (*a–f*). For description see opposite.