THE FINE STRUCTURE AND COMPOSITION OF TYPE 5 ADENOVIRUS; AN INTEGRATED ELECTRON MICROSCOPICAL AND CYTOCHEMICAL STUDY

M. A. EPSTEIN, S. J. HOLT AND A. K. POWELL

From the Bland-Sutton Institute of Pathology and the Courtauld Institute of Biochemistry, The Middlesex Hospital, London, W.1

Received for publication March 10, 1960

CURRENT interest in the morphology and composition of viruses has been much influenced recently by the fact that data regarding structure and composition lead directly to the consideration of such fundamental questions as the basic biological nature of viral agents, the manner in which they replicate, and the mechanisms whereby their effects are produced.

Viruses of the adeno group have received considerable attention in this connection following the demonstration by Kjellén, Lagermalm, Svedmyr and Thorsson (1955) of intranuclear virus-like particles in crystalloid array in sections of infected tissue culture cells examined with the electron microscope. This provided the first example of an animal virus so arranged *in vivo*, and the finding has subsequently been confirmed and amplified (Harford, Hamlin, Parker and van Ravenswaay, 1956; Morgan, Howe, Rose and Moore, 1956; Lagermalm, Kjellén, Thorsson and Svedmyr, 1957). The individual particles have been described as being round or ellipsoid when seen in sections and they are known to contain a dense inner body or nucleoid surrounded by a less dense outer zone or viroplasm (Harford, Hamlin, Parker and van Ravenswaay, 1956; Lagermalm *et al.*, 1957; Morgan and Rose, 1959). However, particles dried from suspension and examined as whole mounts have revealed a hexagonal outline (Tousimis and Hilleman, 1957; Valentine and Hopper, 1957) and this discrepancy has been ascribed by some workers to an artefact caused by drying (Morgan and Rose, 1959).

When the Feulgen reaction was applied to infected cell nuclei it was found that those regions shown by electron microscopy to be occupied by virus-like particles gave an intensely positive response for deoxyribonucleic acid (DNA) (Bloch, Morgan, Godman, Howe and Rose, 1957), and because of this it has been assumed that adenoviruses are of DNA type (Bloch *et al.*, 1957; Morgan and Rose, 1959). However, the positive Feulgen reaction could well have been due to host cell material lying between the particles rather than to a component of the particles themselves and it is in fact known that a great increase in cellular DNA does occur during adenovirus infection (Ginsberg and Dixon, 1959). There is also evidence that DNA can be present in large amounts without being detectable in sections with the electron microscope (Holt and Epstein, 1958), the Feulgen stain not being visible, of course, by this means. But whatever the nature of the nucleic acid in the adenovirus, it seems probable, as was suggested recently (Pereira, 1959), that it might be concentrated in the dense central nucleoid, presumably in the same manner as was found to be the case with the Rous virus (Epstein, 1958*a*).

Now in the course of earlier studies with vaccinia and the Rous virus, it was shown that when these agents were fixed with potassium permanganate for electron microscopy (Luft, 1956), excellent preparations were regularly obtained and important new structures were revealed (Epstein, 1958*b*, *c* and *d*). Furthermore, such fixation did not interfere with the subsequent digestion of nucleic acids by specific nucleases (Epstein, 1958*a* ; Holt and Epstein, 1958) and integrated electron microscopical and cytochemical investigations based on this yielded valuable information concerning the morphology and composition of the Rous virus (Epstein and Holt, 1958).

It was considered that similar methods might usefully be applied to the adenovirus particle and as a first step the isolation and identification of this agent in suitable pellets was undertaken. A fluorocarbon separation procedure was used and pellets were obtained in which a dense white zone consisting of virus particles was found (Epstein and Powell, 1960).

In the first part of the present investigation samples from the virus-containing zones of pellets (Epstein and Powell, 1960) were fixed with permanganate (Luft, 1956) and prepared for electron microscopy in order to study the fine structure of the adenovirus after such treatment ; a brief preliminary account of this has been given (Epstein, 1959). In further work, the effects of specific digestions on the morphology of the adenovirus have been assessed with the electron microscope and integrated with the results of parallel cytochemical studies. Both parts of this investigation are described in detail here.

MATERIALS AND METHODS

Preparation of pellets.—Suspensions of Type 5 adenovirus were made from infected HeLa cell cultures by 3 treatments with a fluorocarbon, and pellets were then prepared from them ; the methods used have been described elsewhere (Epstein, 1958*c* ; Epstein and Powell, 1960).

Electron microscopy.—Samples were taken from the virus-containing white zones of the pellets and were fixed, embedded, sectioned and examined with the electron microscope as in previous work (Epstein, 1958*c* ; Epstein and Powell, 1960). In addition, samples from the pellets were fixed for about 1 hr. as before, or for 15 min., taken through 30 per cent ethanol until they reached room temperature (Luft, 1956) and were then subjected to various digestive treatments before being prepared for electron microscopy. The methods of treatment and the controls used are described below.

Cytochemical tests

Taking of samples.—Samples from the virus-containing portions of the pellets, taken exactly as those used for electron microscopy, were smeared in streaks on to spirit-cleaned microscope slides with a lachrymal sac knife and were allowed to dry at room temperature.

Staining for nucleic acids.—Acridine orange staining for the fluorescence microscopy of nucleic acids was done on the dried smears of pellets fixed either in Carnoy's fluid or in 4 per cent acetic acid saturated with mercuric chloride.

In addition, this staining was done on dried smears which had been fixed and then subjected to digestive treatments. Where the latter included the use of deoxyribonuclease (DNase), Carnoy's fixative was applied, and where ribonuclease (RNase), mercury acetic. The digestive treatments and their controls are set out below, whilst the methods used for staining, examining and photographing the preparations by ultra-violet light (UVL) were those described earlier (Epstein and Holt, 1958).

Digestive treatments

All the treatments were applied for 2 hr. at 37° except in the case of Expt 5 (Table I) where one incubation in the sequence was continued for only 70 min. The incubation media were aqueous and contained ethanol to a final concentration of 30 per cent. Controls for the enzyme digestions were incubated in the appropriate enzyme-free medium.

RNase.—The enzyme was used at a concentration of 1 mg. per ml. and at pH 7·0.

DNase.—This was used under the same conditions as RNase except that the solution also contained magnesium chloride at a concentration of 0·003 M.

DNase II (Acid DNase).—In this case the enzyme was used at a concentration of 0·5 mg. per ml. in 0·1 M acetate buffer at pH 5·0, containing a suitable concentration of magnesium chloride (0·001 M) (Oth, Fredericq and Hacha, 1958); the enzyme was generously supplied to us by Professor E. Fredericq.

Pepsin.—This enzyme was applied at a concentration of 0·2 mg. per ml. in 0·01 N hydrochloric acid (pH 2·0).

Alkaline buffer.—Michaelis veronal buffer pH 9·0 was used.

Experimental procedure

In each experiment a fluorocarbon-treated suspension of adenovirus was subjected to high speed centrifugation and a pellet obtained from it. Samples were taken from the virus-containing white zone of the pellet and were prepared for electron microscopy or cytochemical testing; in both cases preparation was done either directly, or after the application of one or more digestive treatments, or after appropriate control treatments. The sequences of treatments and controls used in the various experiments are shown in the Tables.

RESULTS

Electron microscopy

Morphology of the virus particle examined directly.—When prepared directly and examined in the electron microscope the adenovirus was found to be about 60 mμ in diameter and hexagonal in profile (Fig. 1). Varying amounts of individual particles were of course present in a given section, and those cut centrally showed a central electron-dense nucleoid surrounded by a less dense viroplasm (Fig. 1); in addition, a fine outer limiting membrane seemed to be present. The nucleoids had a diameter of about 35 mμ whilst the surrounding viroplasm of low density was about 12 mμ wide, the whole particle thus being about 60 mμ across (Fig. 1).

The examination of very thin sections at high magnification showed that the nucleoid contained a threadlike component apparently rolled up into a ball in a haphazard manner (Fig. 2), that the limiting membrane around the particle was very insubstantial, and that the surface was smooth (Fig. 2 and 3); in such material the hexagonal outline of the virus particle was particularly striking. In favourably orientated sections of extreme thinness the threadlike element in the nucleoid could be seen arranged in parallel curving array and a second nucleoidal component consisting of diffuse dense material could be distinguished (Fig. 3).

Effects of digestive treatments on virus morphology.—All the digestive treatments, irrespective of any specific effects, made the particles look somewhat ragged and extracted by comparison with those prepared directly, and the greater the number of treatments the more this was noticeable. Nevertheless, even after 4–6 hr. incubation in watery medium the essential features of the virus remained intact, the density of the nucleoid being preserved and even appearing emphasized in contrast to the extracted viroplasm surrounding it (Fig. 4, 6 and 7). Another result of this non-specific extraction was to disclose a beaded appearance of the viroplasm accompanied by irregularity in the usually smooth surface of the virus (Fig. 4).

The effects on the morphology of the nucleoid of the digestive treatments applied in the various experiments are summarized in Table I. It can be seen that the nucleoid remained unaffected by RNase, DNase, pepsin and the acidic medium in which the pepsin was dissolved (Expt 1 and 2). Both RNase and DNase were likewise ineffective when applied after preliminary treatment with pepsin (Expt 3),

TABLE I.—*The Effects of Various Digestive Treatments on the Morphology of the Nucleoid of Permanganate-fixed Adenovirus as Observed with the Electron Microscope. Treatments applied for 2 hr. Except where Indicated*

Expt. No.	Fixation time (min.)	Treatments applied to pelleted virus			Effect on nucleoid
		1st	2nd	3rd	
1*a*	60	RNase	—	—	None
b	60	RNase control	—	—	,,
2*a*	60	Pepsin	—	—	,,
b	60	Pepsin control	—	—	,,
c	60	DNase	—	—	,,
d	60	DNase control	—	—	,,
3*a*	60	Pepsin	RNase	—	,,
b	60	,,	RNase control	—	,,
c	60	,,	DNase	—	,,
d	60	,,	DNase control	—	,,
4*a*	50	,,	DNase	Pepsin	,
b	50	,,	,,	Pepsin control	,,
c	50	,,	RNase	Pepsin	,,
d	50	,,	,,	Pepsin control	,,
5*a*	55	,,	DNase II*	Pepsin	,
b	55	,,	DNase II control*	,,	,,
c	55	,,	DNase II*	Pepsin control	,,
6*a*	15	,,	DNase	Pepsin	,,
b	15	,,	DNase control	,,	,,
7*a*	15	Buffer at pH 9	—	—	,,
8*a*	15	,, ,, ,,	RNase	—	,,
b	15	,, ,, ,,	DNase	—	Digested
c	15	,, ,, ,,	DNase control	—	None

* Treatment applied for 70 min.

or when employed in this way and followed by a further pepsin treatment (Expt 4). DNase II used in a similar sequence also failed to cause recognizable morphological changes (Expt 5). In all these experiments the virus had been fixed for about 1 hr.; the nucleoid of virus fixed for only 15 min. was equally resistant to the consecutive applications of pepsin, DNase and further pepsin (Expt 6).

In marked contrast to this, virus particles which had been subjected to DNase digestion following preliminary incubation in buffer at pH 9·0 (Expt 8b) had their nucleoids removed and showed an empty space in the central area (Fig. 5). Control particles incubated in the alkaline medium and then subjected to RNase or enzyme-free DNase medium (Expt 8a and c), showed no change in the density of their nucleoids (Fig. 6 and 7). In addition the particles exposed to the alkaline buffer appeared slightly swollen (Fig. 6 and 7), those subsequently digested with DNase showing the greatest degree of enlargement (Fig. 5).

Cytochemical tests

After direct acridine orange staining the virus smears exhibited an orange-red fluorescence irrespective of whether they were fixed in Carnoy's fluid or in the mercury-containing fixative (Fig. 8). A similar orange-red fluorescence was

obtained with appropriately fixed smears that had been digested with RNase (Fig. 9) or DNase before staining with acridine orange. When however the smears were previously incubated with veronal buffer for 2 hr at pH 9·0, and then stained with acridine orange, the orange fluorescence was abolished and replaced by the green fluorescence illustrated in Fig. 10. When smears that had been incubated in the alkaline buffer for 2 hr. were subsequently incubated with DNase for a further 2 hr. and then stained with acridine orange, the fluorescence response was almost completely abolished ; certainly no trace of green fluorescence was seen, but only a barely perceptible orange colour (Fig. 11). Control preparations incubated in the alkaline buffer and then treated with enzyme-free medium alone before the staining showed the same green fluorescence as those in which staining was done directly after incubation at pH 9·0 (Fig. 10). These results are summarized in Table II which also shows the results of the parallel electron microscopy

TABLE II.—*The Effects of Various Treatments on the Fluorescence Response in UVL Given by Smears of Pelleted Adenovirus Stained with Acridine Orange. The State of the Viral Nucleoid Observed by Electron Microscopy After the Treatments, is Shown in Parallel. All Treatments Applied for 2 hr.*

Expt. No.	Treatments applied to smears of virus before staining		Fluorescence response (UVL)	State of nucleoid after treatment (electron-microscopy)
	1st	2nd		
9*a*	—	—	Red	Intact
b	RNase	—	,,	Intact (1*a*)*
c	DNase	—	,,	Intact (2*c*)*
d	Buffer at pH 9	—	Green	Intact (7*a*)*
e	,, ,, ,,	DNase	Abolished	Digested (8*b*)*
f	,, ,, ,,	DNase control	Green	Intact (8*c*)*

* Numbers in brackets refer to the experiments listed in Table I.

experiments, taken from those given in Table I, in order to bring out the correspondence between the two sets of findings. It should be noted that the true colours of the fluorescence responses are only approximately reproduced in Figs. 8–11.

DISCUSSION

Most of the considerations underlying the technical aspects of the present work have already been discussed (Epstein, 1958*c* ; Epstein and Holt, 1958 ; Epstein and Powell, 1960), but an important point arising from the use of permanganate fixation for electron microscopy calls for further comment. It concerns the fact that all the digestive treatments were applied in a medium of 30 per cent ethanol. Luft (1956), in his original description of the potassium permanganate fixative, recommended a specific series of alcohols to follow the fixation in order to ensure the best possible morphological preservation of specimens. It has been pointed out earlier (Epstein and Holt, 1958) that by making the digestions in 30 per cent ethanol, adherence to this recommended series has been closely maintained. It was of course, known from these earlier studies that both types of nuclease were fully active in alcoholic solutions (Epstein and Holt, 1958).

Turning to the results which have been obtained, certain points of interest regarding the fine structure of the adenovirus particle have been established.

Thus, after permanganate fixation extracellular adenovirus has been found to have a hexagonal profile when seen in methacrylate sections. This agrees well with a very recent report regarding the shape of the intracellular form of the agent prepared in a similar way (Peters, 1959), whilst findings resulting from newly introduced techniques (Brenner and Horne, 1959) have demonstrated the basis of the 6-sided outline by showing that the virus particle is an icosahedron (Horne, Brenner, Waterson and Wildy, 1959). Earlier observations regarding the possible shape of the virus made on air-dried specimens mounted whole (Tousimis and Hilleman, 1957 ; Valentine and Hopper, 1957) have therefore been confirmed, thus ruling out the criticism that in such material the shape might have been caused by distortion during the drying (Morgan and Rose, 1959).

All reports on sectioned adenovirus in the past have agreed in describing the particle as round or ellipsoid (Kjellén *et al.*, 1955 ; Morgan *et al.*, 1956 ; Harford *et al.*, 1956 ; Lagermalm *et al.*, 1957) but in none of this work was permanganate used as the fixative : where it has been used, here and in other recent studies (Epstein, 1959 ; Peters, 1959), the hexagonal shape has been clearly preserved, and the importance of this method of fixation for the electron microscopy of viruses demonstrated yet again.

As measured here, the size of the virus particle corresponds well with that noted in previous studies using methacrylate-embedded sectioned material (Kjellén *et al.*, 1955 ; Morgan *et al.*, 1956 ; Harford *et al.*, 1956 : Lagermalm *et al.*, 1957), and if allowance is made for flattening during drying, it also agrees with estimates made by examining whole mount preparations (Tousimis and Hilleman, 1957 ; Valentine and Hopper, 1957) ; in addition, the dimensions of the nucleoid show a similar conformity.

The finding of a threadlike element within the nucleoid (Fig. 2 and 3) is considered to be of some importance ; the fact that it has only been seen in the very thinnest sections is probably due to the obscuring effect of the amorphous material also observed in this region of the virus.

Another important question regarding the structure of the adenovirus concerns its surface configuration. Particles prepared and sectioned directly have usually appeared to possess a smooth exterior contrasting markedly with the elaborate surface structure shown recently by new surface staining methods (Horne *et al.*, 1959). Now it could be that the techniques used in the present work failed to reveal existing surface structure ; but it could equally well be that the particle is in fact smooth and that some procedure used during the surface staining (Brenner and Horne, 1959) might have been responsible for bringing out an underlying structural pattern. That this could indeed have happened is suggested by the finding of a beaded appearance in the outer zone of particles incubated in watery medium before embedding. For in such particles favourably orientated to the plane of sectioning the beading appears to correspond to surface units (Fig. 4) and seems to have been made visible as a result of osmotic or extraction influences ; such factors, or a drying effect, could likewise have come into play in Brenner and Horne's surface staining procedure (Brenner and Horne, 1959 ; Horne *et al.*, 1959). But whether or not the virus is in actuality smooth, and whatever the mechanism permitting surface units to be seen, the present findings confirm those of Brenner and Horne (Horne *et al.*, 1959) in making it clear that the outer zone or viroplasm of the adenovirus has a basic geometrical substructure.

The presence of a definite limiting membrane around the adenovirus seems doubtful in view of the findings where high magnifications were used on very thin sections (Fig. 3 and 4). It is more probable that the viroplasm extends to the surface without a covering layer of different composition, the line of slight surface density discernible in the thicker sections (Fig. 1) representing perhaps traces of contaminating material deposited during extraction or minor changes in the surface of the particle caused during preparation.

The features of fine structure of the adenovirus discussed above are valid as far as they go, but they suffer from the limitation of being strictly morphological in nature. However, they provide a point of reference for the important question of integrating structure with composition which the digestion experiments of the present investigation were intended to elucidate. In the earliest of these experiments (Table I, Expt 1) the failure of RNase to affect the virus nucleoid directly was taken to indicate that this zone might contain DNA, since it was known that the relatively small RNase molecule could certainly penetrate and act upon permanganate-fixed Rous virus (Epstein and Holt, 1958). In view of the theoretical difficulty of getting the relatively larger DNase molecule into the virus, the negative results obtained when this enzyme was applied directly (Table I, Expt 2*c* and *d*) were followed by an experiment in which preliminary treatment with pepsin was undertaken before the nuclease digestions (Table I, Expt 3) ; this too left the nucleoid unaffected.

Three possible reasons for this further failure were considered and provided the rationale for subsequent experiments. Firstly, that although DNA in the nucleoid had been broken down by the DNase, some other associated material, loosened but not removed by the specific nuclease digestion, might have been responsible for the undiminished density in this case ; a final treatment with pepsin was therefore tried out to attack such material (Table I, Expt 4) but without success. Secondly, that fragmentation of the DNA had not been completed ; treatment with DNase II was accordingly applied, since this enzyme has been reported (Davidson, 1957) to cause very extensive breakdown of DNA. The procedure, however, was unsuccessful. Lastly, that after being fixed for 1 hr. with permanganate the nucleic acid could no longer be digested by DNase ; a 15 min. fixation time was substituted (Table I, Expt 6) but again without influencing the result.

All these negative findings made it seem possible that neither the pepsin nor the acidity of the medium in which it was applied had affected the viroplasm, so that the DNase might perhaps have been unable to penetrate the virus and act on the nucleoid.

As an alternative method for opening up the viroplasm, mildly alkaline buffer was tested, and when this was followed by appropriate nuclease digestion, clear cut results were obtained for the first time (Table I, Expt 8). Thus, particles treated with buffer at pH 9·0 and then incubated with either RNase or 30 per cent ethanol with magnesium showed no change in the electron density of their nucleoids (Fig. 6 and 7) whilst in marked contrast to this the nucleoids were digested from similarly treated particles incubated with DNase (Fig. 5). In view of the specificity of the nuclease digestion and the findings of the control incubations these results demonstrate unequivocally that the adenovirus contains a substantial amount of DNA localized in the nucleoid.

The results obtained by the application of acridine orange staining and

fluorescence microscopy support the findings of the electron microscopy experiments regarding the nature of the nucleic acid in the adenovirus and this additional support is of particular significance since it is of an unrelated kind. Although light microscopy cannot resolve individual virus particles, the overall fluorescence response obtained from the stained smears with and without the nuclease digestions reflects the collective response of such particles since it had previously been shown that the material of which the smears were made consisted of them (Epstein, 1959 ; Epstein and Powell, 1960). Now, when stained with acridine orange and examined in UVL, RNA-containing structures usually exhibit an orange-red fluorescence and DNA-containing structures a yellowish-green fluorescence (Bertalanffy and Bickis, 1956 ; Armstrong, 1956), these colour responses being specifically diagnostic if they are abolished by treatment with the appropriate nuclease before staining. Thus, the red fluorescence given by the smears when stained directly with acridine orange (Fig. 8) cannot be ascribed to the presence of RNA since incubation with RNase before staining did not affect it (Fig. 9). This type of non-specific fluorescence is not uncommon with acridine orange staining techniques and examples of it have already been encountered with material of the kind used here (Epstein and Holt, 1958).

When the virus was treated with alkaline buffer it was clearly significantly affected, since after subsequent staining with acridine orange it gave a green fluorescence for the first time (Fig. 10). This green fluorescence definitely confirms the presence of DNA in the adenovirus in view of the fact that it was abolished in alkaline-treated preparations incubated with DNase before staining (Fig. 11) yet remained totally unaffected by similar incubation in enzyme-free medium alone.

Whatever the change in the permanganate-fixed virus particles which incubation in an alkaline medium brought about, it both permitted penetration of the nucleoid by DNase (Table I, Expt 8*b*) and altered the fluorescence response after acridine orange staining from a non-specific red colour to the green characteristic of DNA. The most probable effect of the alkaline buffer would seem to have been a degradation of the outer protein coat or viroplasm and this fits well with the findings of those who have dissected viruses chemically by using alkali to remove the outer protein coat in the course of preparing infective nucleic acid (Fraenkel-Conrat and Williams, 1955 ; Schramm, Schumacher and Zillig, 1955).

Although earlier work has shown DNA in regions of infected cell nuclei containing much virus (Bloch *et al.*, 1957), the basis of this demonstration was such that it could not establish whether DNA was present in the adenovirus or whether the DNA demonstrated was merely host cell DNA accumulated around and between the virus particles. The experiments reported here have not only established that DNA is present in the adenovirus, but also the site of this material within the particle. It has not however been possible to determine whether the nucleic acid was associated with the amorphous component of the viral nucleoid or the filamentous, but on structural grounds the latter seems possible.

Besides leading to the above findings the present work has illustrated again the special usefulness in virus research of the electron microscopy of thin sections when coupled with ancillary techniques. It is true, of course, that exact information regarding the composition of a virus is most reliably obtained by chemical analysis, but for this complete purification is a pre-requisite which can in itself be extremely difficult or often impossible to attain. The present approach has

the advantage not only of being able to furnish information on composition where only partially purified material is available, but also of being able to relate composition to morphology. Analysis can show what a given virus contains as a whole ; by examining structures *in situ* the methods used here can investigate the nature of its various parts.

SUMMARY

Experiments designed to investigate the fine structure and composition of type 5 adenovirus are described.

Pellets were prepared from fluorocarbon-treated suspensions of the virus and samples from their virus-containing zones were taken for examination both in thin sections with the electron microscope, and by fluorescence microscopy after acridine orange staining. Specific nucleases, other digestive treatments and appropriate control media were applied to the virus material in many of the experiments.

The results show that the adenovirus particle has a hexagonal outline and contains both an amorphous and a filamentous component in its nucleoid. The surface of particles prepared and examined directly appeared smooth, but surface structure was detected when the virus was exposed to osmotic and extraction influences.

It has also been shown that the adenovirus contains a substantial amount of deoxyribonucleic acid and observations made with the electron microscope have demonstrated its localization in the nucleoid.

These findings are discussed and the general significance of the methods used are considered.

The expenses of this investigation were borne by the British Empire Cancer Campaign, and the Central Research Fund of the University of London.

REFERENCES

ARMSTRONG, J. A.—(1956) *Exp. Cell Res.*, **11**, 60.

BERTALANFFY, L. VON AND BICKIS, I.—(1956) *J. Histochem. Cytochem.*, **4**, 481.

BLOCH, D. P., MORGAN, C., GODMAN, G. C., HOWE, C. AND ROSE, H. M.—(1957) *J. biophys. biochem. Cytol.*, **3**, 1.

BRENNER, S. AND HORNE, R. W.—(1959) *Biochim. biophys. Acta*, **34**, 103.

DAVIDSON, J. N.—(1957) ' The Biochemistry of the Nucleic Acids '. London (Methuen), 3rd ed., p. 60.

EPSTEIN, M. A.—(1958*a*) *Nature, Lond.*, **181**, 1807.—(1958*b*) *Ibid.*, **181**, 784.—(1958*c*) *Brit. J. exp. Path.*, **39**, 436.—(1958*d*) *Brit. J. Cancer*, **12**, 248.—(1959) *J. biophys. biochem. Cytol.*, **6**, 523.

Idem AND HOLT, S. J.—(1958) *Brit. J. Cancer*, **12**, 363.

Idem AND POWELL, A. K.—(1960) *Brit. J. exp. Path.*, **41**, 559.

FRAENKEL-CONRAT, H. AND WILLIAMS, R. C.—(1955) *Proc. nat. Acad. Sci., Wash.*, **41**, 690.

GINSBERG, H. S. AND DIXON, M. K.—(1959) *J. exp. Med.*, **109**, 407.

HARFORD, C. G., HAMLIN, A., PARKER, E. AND VAN RAVENSWAAY, T.—(1956) *Ibid.*, **104**, 443.

HOLT, S. J. AND EPSTEIN, M. A.—(1958) *Brit. J. exp. Path.*, **39**, 472.

HORNE, R. W., BRENNER, S., WATERSON, A. P. AND WILDY, P.—(1959) *J. molecular Biol.*, **1**, 84.

KJELLÉN, L., LAGERMALM, G., SVEDMYR, A. AND THORSSON, K.-G.—(1955) *Nature, Lond.*, **175**, 505.
LAGERMALM, G., KJELLÉN, L., THORSSON, K.-G. AND SVEDMYR, A.—(1957) *Arch. ges. Virusforsch.*, **7**, 221.
LUFT, J. H.—(1956) *J. biophys. biochem. Cytol.*, **2**, 799.
MORGAN, C., HOWE, C., ROSE, H. M. AND MOORE, D. H.—(1956) *Ibid.*, **2**, 351.
Idem AND ROSE, H. M.—(1959) *Symp. Soc. gen. Microbiol.*, **9**, 256.
OTH, A., FREDERICQ, E. AND HACHA, R.—(1958) *Biochim. biophys. Acta*, **29**, 287.
PEREIRA, H. G.—(1959) *Brit. med. Bull.*, **15**, 225.
PETERS, D.—(1959) *Zbl. Bakt.* (I Abt. Orig.), **176**, 259.
SCRAMM, G., SCHUMACHER, G. AND ZILLIG, W.—(1955) *Z. Naturf.* **10b**, 481.
TOUSIMIS, A. J. AND HILLEMAN, M. R.—(1957) *Virology*, **4**, 499.
VALENTINE, R. C. AND HOPPER, P. K.—(1957) *Nature, Lond.*, **180**, 928.

EXPLANATION OF PLATES

FIG. 1–7.—Electron micrographs of sectioned adenovirus particles from the virus-containing regions of pellets made from fluorocarbon-treated suspensions of the virus.

FIG. 1.—The 60 mμ virus particles are hexagonal in profile, contain a central dense nucleoid surrounded by a less dense viroplasm, and appear to have a very fine outer limiting membrane. ×160,000.

FIG. 2.—An individual virus particle in greater detail. The hexagonal profile is well seen and the nucleoid seems to contain 2 elements, a diffuse dense material and a threadlike component rolled in a haphazard manner into a ball. A definite outer limiting membrane is barely discernible. ×375,000.

FIG. 3.—Very thin section showing the structure of the nucleoid. The threadlike component is here arranged in curving parallel array. ×375,000.

FIG. 4.—Particles showing some extraction effects after incubation in watery medium at 37° for 6 hr. (Table I, Expt. 5*a*). A regular beading of the viroplasm can be seen in those particles favourably orientated to the plane of sectioning (arrows). The nucleoids have retained their density. ×150,000.

FIG. 5.—Group of virus particles prepared after incubation in buffer at pH 9·0 for 2 hr. at 37° followed by a further 2 hr. incubation in DNase. The nucleoids have been removed from the particles which appear considerably swollen. ×100,000.

FIG. 6.—Virus particles incubated as those shown in Fig. 5 except that RNase was used in place of DNase. Although some swelling of the particles has occurred, their nucleoids have not been digested. ×100,000.

FIG. 7.—Control particles incubated as those shown in Fig. 5 and 6 but treated in the second period of incubation with enzyme-free medium alone. The nucleoids are intact and only slight swelling can be seen. ×100,000.

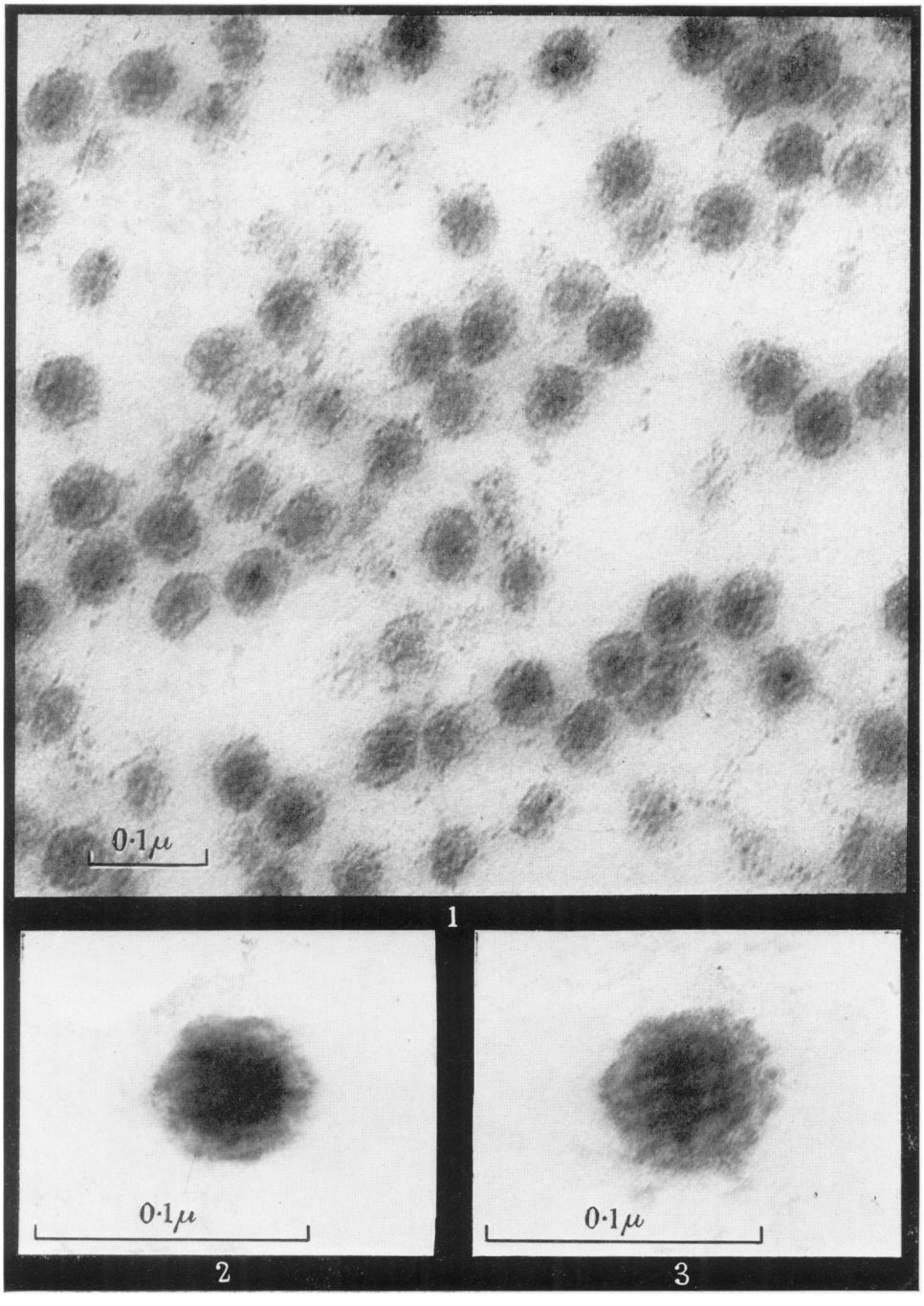
FIG. 8–11.—Fluorescence photomicrographs of acridine orange-stained smears from the virus-containing zones of pellets. ×160.

FIG. 8.—Orange red fluorescence of directly stained material.

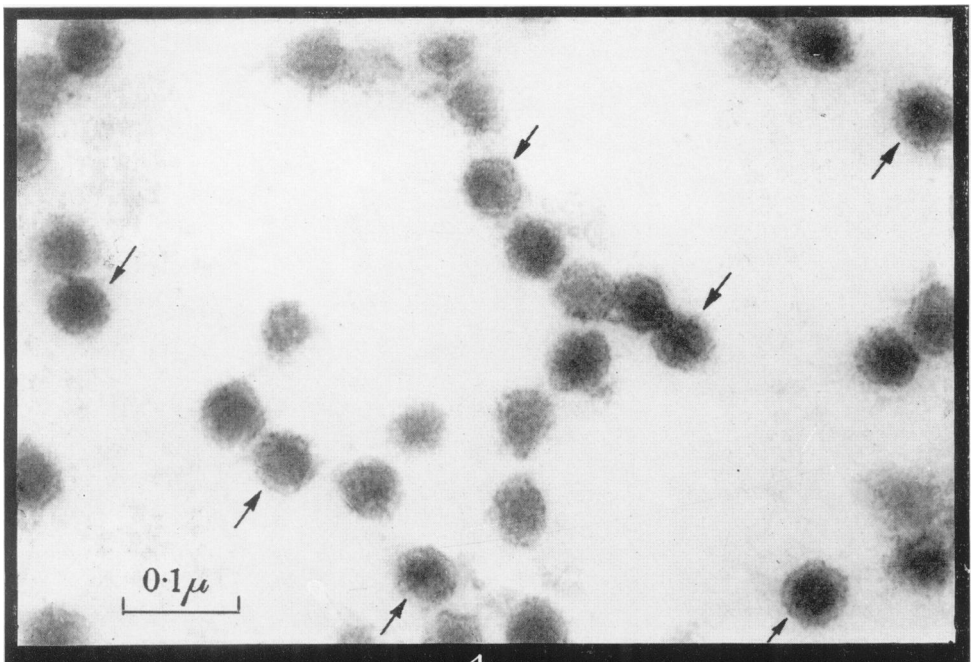
FIG. 9.—As Fig. 8 but stained after RNase digestion. Failure of the enzyme to change the colour of the fluorescence indicates that the latter is a nonspecific effect and not due to the presence of RNA.

FIG. 10.—Green fluorescence exhibited by material incubated in pH 9·0 buffer for 2 hr. and then stained with acridine orange. A few small crystals of the dye contaminate the lower right hand region of the background.

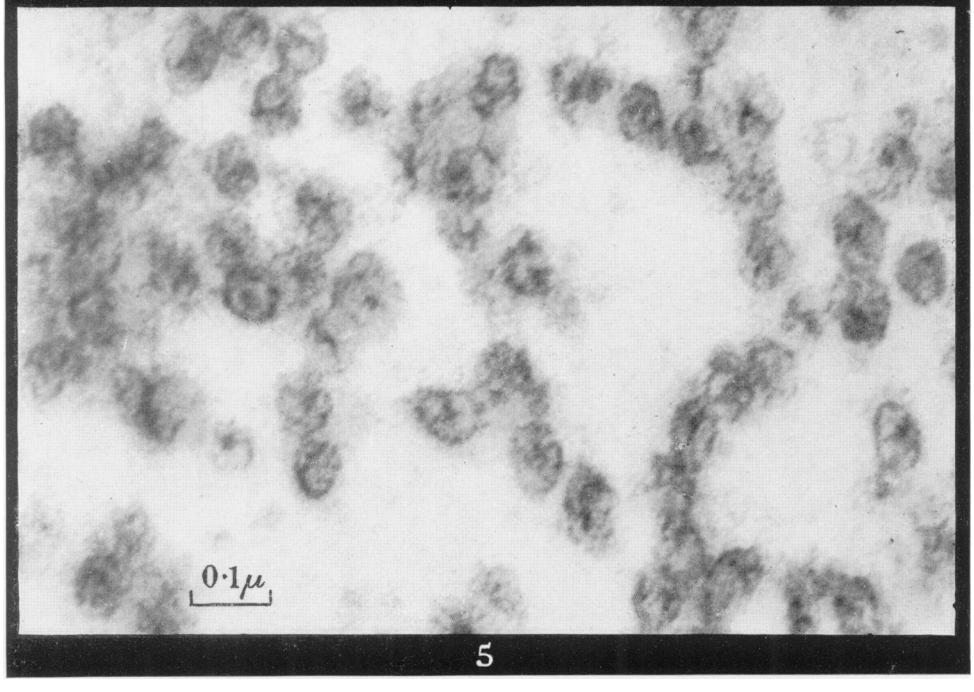
FIG. 11.—Feeble fluorescence given by a smear which had been incubated in pH 9·0 buffer for 2 hr., followed by a further 2 hr. incubation with DNase and then staining with acridine orange.



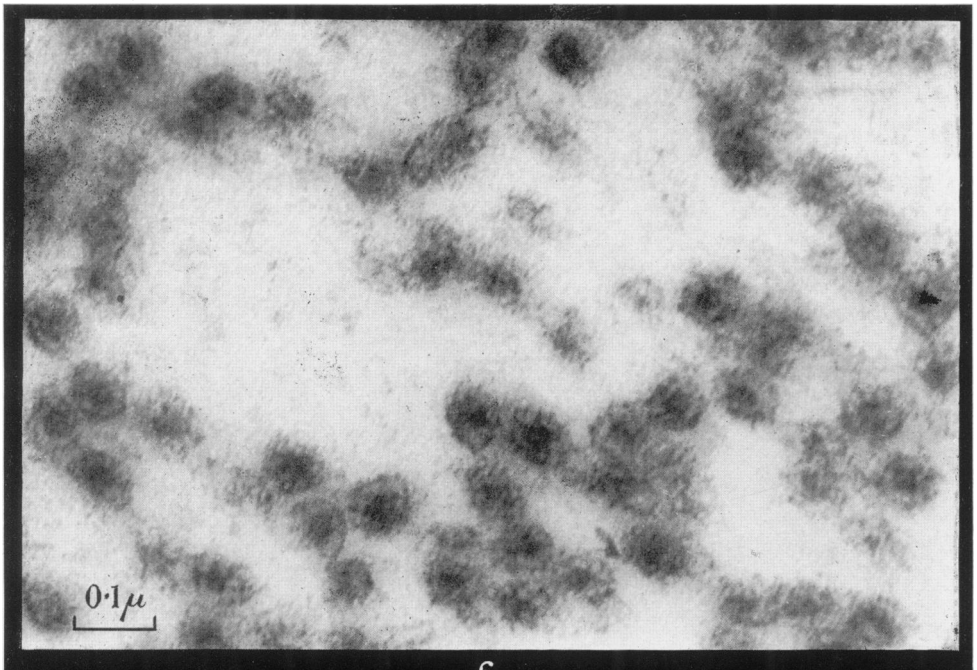
Epstein, Holt and Powell.



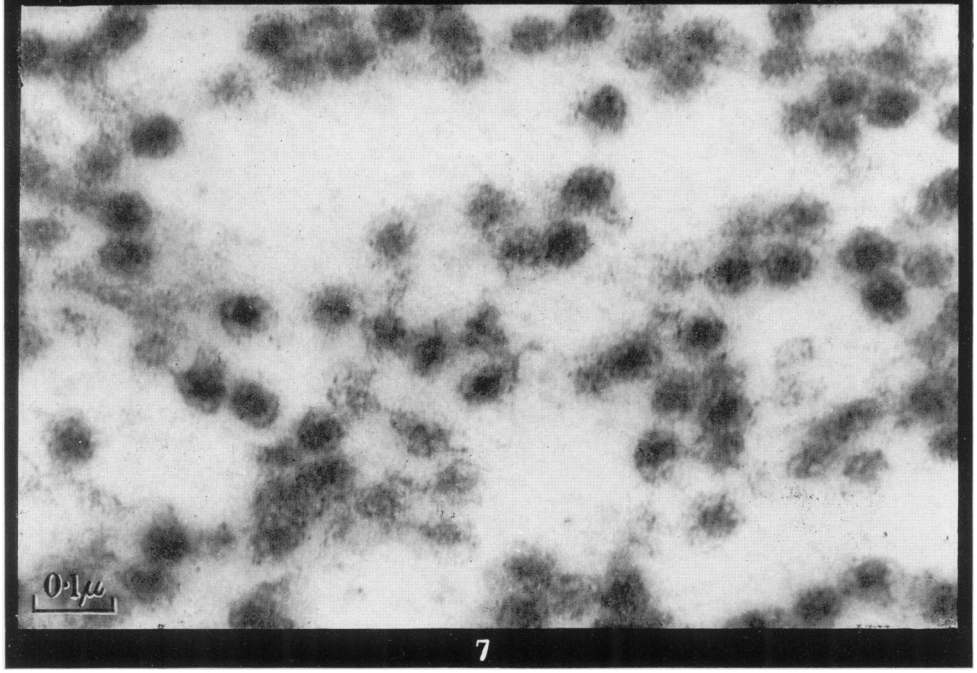
4



5



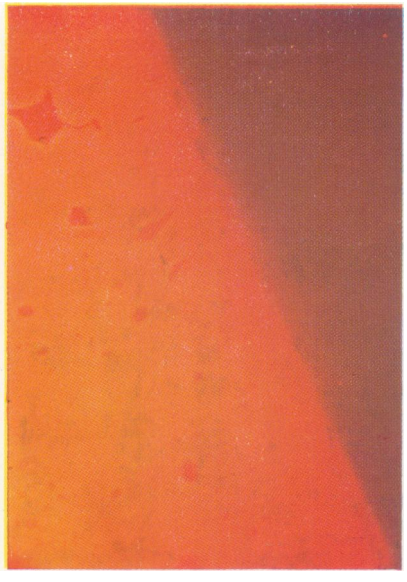
6



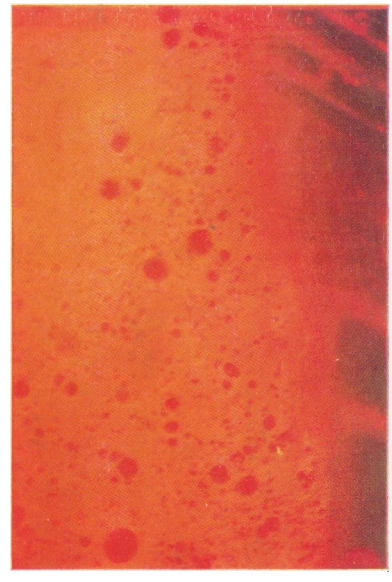
7

Epstein, Holt and Powell.

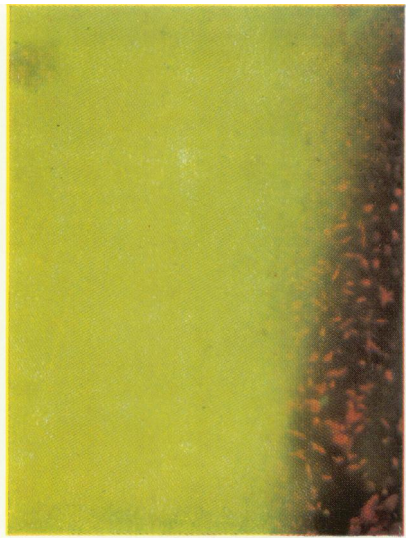
8



9



10



11