Studies on Lipid Metabolism in Cells Infected with Adenovirus

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SUMMARY

Lipid metabolism in cells infected with adenovirus type 5 was studied using [\(^{32}\)P]orthophosphate and [\(^{14}\)C]acetate precursors. Within the first 6 hr after infection, uptake of isotopes into cellular lipids was increased, although at this time no qualitative changes in the pattern of labelling were observed. At 12 to 18 hr after infection there was a markedly increased uptake of [\(^{14}\)C]acetate into cellular triglycerides. Increased lipid metabolism was demonstrated using ultraviolet-irradiated virus and purified penton base capsid subunits as well as whole infective virus. Highly purified adenovirus type 5 from cells labelled with [\(^{32}\)P]orthophosphate during infection contained small amounts of radioactive lipid; the data presented suggest that this represented cellular lipid which remained attached to the virus, rather than lipid intrinsic to the virus particle.

INTRODUCTION

The importance of lipids and lipid-containing membranes in the study of the replication and assembly of animal viruses is now better appreciated. Data concerning the effect of virus infection on the metabolism of lipids in tissue culture cells is, however, relatively scanty. Green (1959) reported the stimulation of phospholipid synthesis during the infection of KB cells with adenovirus type 2, and similar stimulations were found in HeLa cells in the first 6 hr of infection with vaccinia virus (Gausch & Youngner, 1963) and in 1 1/2 hr or more after infection with poliovirus (Cornatzer, Sandstrom & Fischer, 1961; Penman, 1965).

This investigation is part of a study into the role of membranes in adenovirus infection and describes the changes in lipid metabolism in a line of human cell (HEK) after infection with adenovirus type 5. An intensive examination has been made of purified adenovirus for the presence of significant but small amounts of lipid, although the virus particle, which does not possess an envelope, has been considered to contain only protein and DNA (Green & Piña, 1963). It was important to study the particle in this way, in view of the nature of the studies to be reported, and also in view of the possible existence of an internal ‘membrane’ enclosing the nucleic acid and protein core (Morgan, Rosenkranz & Mednis, 1969).

METHODS

Virus and cells. The virus (type 5, AD75) was propagated in HeLa cells or in KB cells (Russell et al. 1967a). For the studies on lipid synthesis HEK cells were employed. These cells, originally obtained from Dr T. Gotlieb-Stematsky, were reputedly derived from a human embryo kidney and are similar in appearance and other properties to HeLa cells.

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They were cultivated either in Eagle's medium or in Leibovitz medium (Leibovitz, 1963) containing 10% tryptose phosphate broth and 10% calf serum supplemented with penicillin and streptomycin. To attain a greater synchrony of infection, cells were infected in suspension at an added multiplicity of approximately 200 p.f.u./cell and plated out as monolayers (Russell et al. 1967a). The infected cells and control uninfected cells were maintained in medium containing 0.5% calf serum. In some experiments the cells were maintained in medium without calf serum. When arginine was withdrawn from the culture medium, the procedures described by Russell & Becker (1968) were used.

Purification of virus. The virus was purified by a sequence of steps involving homogenization in fluorocarbon, gel filtration on Sephadex G25 (Pharmacia), followed by a 'velocity' centrifugation and two equilibrium density-gradient centrifugations in cesium chloride (Russell, Valentine & Pereira, 1967b). Digestion by various enzymes and incubation with sodium desoxycholate were included in some purifications. Partially purified virus was used as infecting seed in all experiments. The virus was obtained after the first 'velocity' gradient, diluted in Eagle's medium containing 0.5% rabbit or calf serum and dialysed against Eagle's medium at 4°C for 2 hr with vigorous stirring. In one series of experiments the infecting virus was irradiated with ultraviolet (u.v.) light by diluting the standard seed fivefold in phosphate-buffered saline (PBS) and irradiating for 30 min. with a dose of $4 \times 10^8$ ergs/cm.$^2$/sec. The irradiated virus was then diluted twofold in Eagle's medium containing 0.5% rabbit serum.

Infectivity. Virus was titrated for infectivity by plaque assay. HEK cells were infected in suspension and plated out in the presence of carboxymethyl cellulose (Russell, 1962).

Virus antigens. Hexon, penton and fibre antigens (Ginsberg et al. 1966) were separated and purified by ion-exchange chromatography (Russell et al. 1967a) and the hexon antigen was further purified by crystallization (Pereira, Valentine & Russell, 1968). The quantity of virus antigens used in experiments can be expressed in terms of virus particle equivalents derived from published molecular weights for these antigens (Valentine & Pereira 1965). Thus, 1 µg. of hexon represents that amount present in $8.3 \times 10^9$ particles, 1 µg. of penton represents $1.3 \times 10^{10}$ particles, 1 µg. of fibre represents $6.3 \times 10^{11}$ particles, and 1 µg. of penton base represents $1.7 \times 10^{12}$ particles. The penton base component was prepared by preparative acrylamide gel electrophoresis (Winters, Brownstone & Pereira, 1970) and was kindly supplied by Dr W. D. Winters.

Preparation of radioactively labelled virus and cells. Virus labelled with [$^{32}$P]orthophosphate was prepared as described by Russell, Laver & Sanderson (1968). In these experiments the maintenance medium after infection contained no tryptose phosphate broth and no serum. Virus labelled with [$^{14}$C]acetate was prepared by adding [2-$^{14}$C]acetate ($2 \mu$c/ml., 50 mCi/mM) to the medium when virus was added to the cells. In experiments analysing the fate of label in infected cells the label was added either at the beginning of the infection or at various times afterwards, as indicated in the text. Radioisotopes were obtained from the Radiochemical Centre, Amersham, Buckinghamshire.

Enzymes. Deoxyribonuclease I (Sigma crystalline grade, 50 µg./ml., Mg$^{2+}$ 20 mM) and ribonuclease (Armour Pharmaceutical Co. Ltd, crystalline grade, 50 µg./ml.) were incubated together with virus preparations in PBS at 37°C for 30 min. Trypsin (Crystalline grade from Armour Pharmaceutical Co. Ltd) was used at a concentration of 50 µg./ml. in PBS, incubating at 37°C for 10 min.

Sodium desoxycholate (DOC). Virus suspensions were incubated in 0.2% DOC at 4°C for 30 min.

Lipid Extractions. Cells for lipid extraction were scraped or shaken off the glass and washed three times with PBS. The washed pellet of cells was then suspended in a small
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volume of demineralized water, treated in an ultrasonic water bath (Burndept Ultrasonic Cleaner) and lyophilized. The lyophilized cells were then extracted by shaking in a stoppered tube at room temperature with 2 ml. of chloroform + methanol (2:1) for 4 to 12 hr. After centrifugation (500 g, 10 min.) the chloroform + methanol solution containing the dissolved lipids was removed. The pellet was again extracted with 1 ml. of the solvent, and after centrifugation the second supernatant fluid was combined with the first. The solvent from the combined supernatant fluids was then evaporated at 56° in a stream of nitrogen and the residue redissolved in a small volume of chloroform + methanol (2:1).

**Lipid analysis.** The chloroform + methanol extracts were analysed by thin-layer chromatography by methods previously described (Payne, 1964). In most experiments, commercially prepared plates of silica gel G on aluminium (Merck) were employed. In some experiments, however, silica gels on glass plates were prepared in the standard manner. A three-solvent system to separate the major classes of lipids was used in preliminary experiments. In this technique (procedure I) chloroform + methanol + water (14:6:1) (solvent A) was allowed to proceed about two-thirds of the way up the plate, which was then removed and dried by evaporation at room temperature for 30 min. The second solvent system (B) contained n-propanol and 12.8% aqueous ammonia (4:1) and was run until the solvent level had proceeded about one-third of the way up the plate. After removal and drying as before, the plate was then placed in the third solvent (C), which consisted of petroleum ether (60 to 80°), diethyl ether and glacial acetic acid (73:25:2); the solvent was run to the top and the plate was removed and dried by evaporation. A solution of rat-brain lipid (Payne, 1964) was used as a standard in these experiments. The lipids were visualized by spraying the plate with concentrated sulphuric acid and charring at 130° for 15 min. To separate the polar lipids more satisfactorily, a simpler technique (procedure II) was adopted in which the lipids were chromatographed in solvent A almost to the top of the plate, the plate was dried, and then solvent B was run to a level almost half-way up the plate. To separate the neutral lipids (procedure III) only solvent C was employed. In all of these experiments the lipids were characterized with reference to their positions on the chromatograms using standard rat-brain lipids, and no attempt was made to identify the lipids by further chemical or physical methods.

The glycolipids from cells were examined in more detail by submitting the lipid extracts to alkaline hydrolysis (Gray, 1967) to remove the alkali-labile phospholipids. To 1 ml. of the chloroform + methanol extract 0.1 ml. of 2 M-methanolic sodium hydroxide was added, and the mixture was incubated with shaking at room temperature for 2 hr. This alcoholic solution was then neutralized by slowly adding drops of 0.5 N-hydrochloric acid (in an ice bath). After centrifugation (500 g, 10 min) the aqueous phase was again removed. The resulting lipids were examined by thin-layer chromatography using procedure II with rat-brain lipid and a sample of sulphatides isolated from rat brain as controls. In order to locate the choline-containing lipids in these experiments the plates were sprayed with the Dragentorf reagent before charring.

In experiments analysing lipid extracts of cells pulse-labelled with [14C]acetate the plates were submitted to autoradiography by standard techniques. In some of these experiments, and similar ones involving 32P-labelled cells, the various labelled lipid classes were scraped from the thin-layer chromatography plate into toluene scintillating fluid and counted in a Packard Scintillation Counter.

**Radioactivity.** In investigations involving [32P]orthophosphate labelling, aqueous solutions were assayed by utilizing the Čerenkov radiation (Clausen, 1968) emitted in small glass vials enclosed in the standard scintillator vials. This method gave a value about 30%
of that obtained by placing a replicate sample on a square of filter paper, drying and placing in a toluene-based scintillating fluid. Samples (14C-labelled) were placed on filter-paper squares and counted as above.

Specific activities of the labelled phospholipids were determined with reference to the phosphorus content of a sample of the chloroform + methanol extract. Specific activities of the labelled neutral lipids were approximated by referring to the protein content of the original disrupted cell suspension. Protein was assayed by the procedure of Lowry et al. (1951) using bovine serum albumin as standard, and phosphorus by the procedure of Ames (1966) using a solution of potassium dihydrogen phosphate as standard.

RESULTS

Lipid analysis of control and infected cells.

No significant differences could be detected by thin-layer techniques in the unlabelled lipids of control and uninfected cells. Typical thin-layer chromatography patterns of these lipids and of controls after charring are shown in Fig. 1. The lipid extractions in these and the following experiments were carried out on cells which had been infected, or mock-infected as in controls, in Leibovitz medium in the presence of the small amount (0.02 %) of rabbit or calf serum derived from the inoculum. Preliminary investigations were carried out using [14C]acetate labelling of cells to ascertain if a different pattern of lipid synthesis could be induced in cells by these small amounts of serum or by changes in pH of the medium. No differences were detected using thin-layer chromatography autoradiographic techniques when these two parameters were altered within the ranges expected (i.e. pH 6.7 to 7.3). In fact, there were no noticeable changes in the pH of the medium on the infected cells using the well-buffered Leibovitz medium.

Effect of adenovirus infection on phospholipid metabolism

Although no changes in the lipid composition of HEK cells were detected following infection with adenovirus, it was possible that the rate of synthesis of certain lipid components had been altered. This possibility was studied by employing ‘pulse’ labelling techniques.

HEK cells were infected in suspension at a multiplicity of 200 p.f.u./cell and plated out as monolayers. At various times after infection replicate cultures were pulse-labelled with [32P]orthophosphate for periods of 1 hr (100 μC of label added to 5 x 10⁶ cells in 5 ml. of medium in Petri dishes). Under these conditions the infected cells would produce virus DNA at about 8 hr, the capsid antigens would be apparent at 10 to 12 hr, and the infectious virus would mature at 14 to 20 hr following infection (Mäntyjärvi & Russell, 1969; Russell et al. 1967a). Cells were scraped off the glass after incubation for 1 hr with label, washed with buffer, disrupted by sonic treatment and lyophilized. The cellular lipid was then extracted with the chloroform + methanol solvent. The specific activities of the extracted phospholipids were determined (Table 1) and indicated that a significant increase in phospholipid metabolism occurred relatively early in infection. Samples of the control and infected cell lipids were submitted to thin-layer chromatography using procedure II along with a standard rat-brain lipid. Portions of the thin-layer chromatography plates corresponding to the various lipid classes were scraped off and the radioactivities determined. When the radioactivities of the various phospholipid classes in infected and control cells were compared, the only significant difference was a slightly increased incorporation into phosphatidyl ethanolamine in infected cells at 24 hr.
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Effect of infection on incorporation of [14C]acetate into cellular lipids

Since the above experiments would only detect changes in the metabolism of phospholipids the metabolism of a larger spectrum of the cellular lipids was examined by pulse labelling with [14C]acetate.

HEK cells were infected in suspension and plated out in monolayers and replicate cell cultures were labelled with [14C]acetate for periods of 6 hr in two experiments and 1 hr in a third experiment. After incubation with the label the cells were scraped from the glass, washed, disrupted, lyophilized and extracted with lipid solvents. In all of these experiments an increase in the incorporation of [14C]acetate into the total extracted lipids was demonstrated in the earlier stages of infection. At later times the specific activities of the lipids extracted from infected cells showed a marked decrease (Fig. 2). When these lipid extracts...
were separated by thin-layer chromatography using procedure I, the acetate label was found to have been incorporated into a wide variety of lipids (Fig. 3). Measurement of radioactivity by both microdensitometer tracing of the autoradiogram and scintillation counting of thin-layer fractions (Table 2) suggested an increased incorporation of the label into the triglyceride fraction occurring 12 to 18 hr after infection. This was confirmed by using thin-layer chromatography procedure III which separated the neutral lipids more effectively. In this case, it was also evident that the increased incorporation of label into the triglyceride fraction was accompanied by a corresponding decrease in the incorporation into cholesterol (Fig. 4). By pulse labelling cells at 72 hr after infection (when the infected cells showed massive cytopathic effect and the uninfected cells were in a tightly packed monolayer and showing signs of 'ageing') it was still possible to obtain some incorporation of [14C]acetate into cellular lipids from the infected cells. Most of this appeared in the triglyceride fraction. In contrast, the control cells showed a pattern of incorporation similar to that seen at earlier times.

### Table 1. Specific activities of ³²P-labelled cell phospholipids

<table>
<thead>
<tr>
<th>Time of adding label (hr after infection)</th>
<th>Specific activity [³²P] (counts/min./mg lipid P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected</td>
</tr>
<tr>
<td>1 to 2</td>
<td>1415</td>
</tr>
<tr>
<td>3 to 4</td>
<td>1776</td>
</tr>
<tr>
<td>5 to 6</td>
<td>1960</td>
</tr>
<tr>
<td>7 to 8</td>
<td>3000</td>
</tr>
<tr>
<td>23 to 24</td>
<td>4240</td>
</tr>
</tbody>
</table>

![Graph](image_url)

Fig. 2. Specific activities of lipids (counts/min./μg. protein) extracted from cells 'pulse' labelled with [¹⁴C]acetate. In Expt. a cells were pulse-labelled for 6 hr periods, and in Expt. b for 1 hr periods. ■, Infected; □, control.
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It was interesting that a significant increase in $[^{14}C]$acetate incorporation into lipids could be obtained even after infection with u.v.-irradiated virus. In this experiment the cells were infected with either irradiated virus (which had lost over 5 log. units of infectivity) or unirradiated virus. The cells were labelled with $[^{14}C]$acetate from 6 to 8 hr after infection and harvested for lipid extraction in the usual way. Table 3 (Expt. A) shows that the specific activity of the extracted lipid from cells infected with irradiated virus is comparable to that from the normal infected cells, and both are approximately twice that of uninfected cells. Further thin-layer chromatography using procedure III on these lipid extracts indicated that in this experiment also there was a significant incorporation of the labelled acetate into the triglyceride fraction of the lipids from both infected cell samples (Table 3). This result suggested that the increased lipid metabolism may have been induced not by the infective virus but by a component of the virus capsid. Accordingly, a further experiment (B) was carried out to study the effect of purified virus antigens on the cellular lipid metabolism.
Table 3 indicates that neither the hexon nor the fibre antigen had any effect, but that both the penton and penton base component induced small but significant increases in uptake of label. An additional experiment (C) using the penton base component alone confirmed that this virus component could stimulate $[^{14}C]$acetate incorporation into cellular lipids.

Table 2. Distribution of $[^{14}C]$ label in cellular lipids using acetate precursor

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>0 to 6 hr after infection</th>
<th>6 to 12 hr after infection</th>
<th>12 to 18 hr after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected</td>
<td>Control</td>
<td>Infected</td>
</tr>
<tr>
<td>Lecithin/sphingomyelin</td>
<td>62.2</td>
<td>61.3</td>
<td>63.4</td>
</tr>
<tr>
<td>Sulphatides</td>
<td>2.2</td>
<td>3.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>9.9</td>
<td>8.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Cerebrosides</td>
<td>2.1</td>
<td>2.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Monoglycercides</td>
<td>4.7</td>
<td>6.0</td>
<td>5.9</td>
</tr>
<tr>
<td>Cholesterol/diglycerides/free fatty acids</td>
<td>12.5</td>
<td>14.1</td>
<td>12.1</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>4.3</td>
<td>3.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Cholesterol esters</td>
<td>1.0</td>
<td>0.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The effect of virus infection on glycolipid synthesis was examined by infecting cells in the presence of $[^{14}C]$acetate, harvesting cells 3 days later, extracting lipid in the usual manner and then hydrolysing with methanolic sodium hydroxide, as described in Methods. Thin-layer chromatography and resultant autoradiography (Fig. 5) showed stimulation of incorporation of $[^{14}C]$acetate on infection, but there was no significant difference in the pattern of incorporation of the label.

Purification of virus and removal of contaminating lipid

The possibility that adenovirus contained small but significant amounts of hitherto undetected lipid components enclosed within the capsid was examined by radioactive-labelling techniques. In assessing the specificity of such small quantities, the efficacy of the purification procedure in removing non-specific components of a similar chemical nature is of crucial importance. Accordingly, the standard purification procedure for adenovirus was critically examined for its efficacy in removing $^{32}$P-labelled phospholipid components. In the first experiment (1), cells $(20 \times 10^6)$ were infected with virus and 6 hr later $^{32}$P-orthophosphate $(5 \mu C/mL)$ was added. As a control, uninfected cells were labelled under the same conditions. After 3 days, the cells were shaken or scraped from the glass and washed well, and suspensions of labelled cells were each added to fluorocarbon extracts of infected, unlabelled cells $(100 \times 10^6 \text{ cells})$. After further homogenization in fluorocarbon the extracts were submitted to the purification procedures outlined in Methods and in Fig. 6. The distribution of the label with respect to the virus was readily ascertained using the Čerenkov procedure. The virus fraction at each step in the purification procedure was detected by its opalescence. The proportion of the label which was attributable to phospholipid was determined by lyophilizing the relevant fraction and extracting with chloroform + methanol. Further analysis was carried out by thin-layer chromatography where necessary. Fig. 6 illustrates the purification of unlabelled virus in the presence of $^{32}$P-labelled uninfected cellular material. After extraction with fluorocarbon a large amount of phospholipid was carried through to the later purification steps, and even after three centrifugations in caesium
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chloride there was a small but significant amount of label associated with purified virus. This purified virus fraction and the parallel fraction derived from labelled infected cells were desalted by filtration through Sephadex G-25. The two purified virus suspensions were then disrupted by heating at 56° for 1 min. (Russell et al. 1967b), lyophilized and extracted with chloroform + methanol. Some label was extracted from both preparations; in this experiment (no. 1 in Table 4) there was considerably more lipid [32P] in virus particles purified from labelled infected cells than in those purified from labelled uninfected cells. Examination by thin-layer chromatography of the lipids at the earlier stages of purification showed that no major differences could be detected between the classes of lipids derived from the infected and control cells, and that these lipids were mainly in the lecithin/sphingomyelin group. The specific activities of the labelled lipids were determined at an early stage of purification when there was sufficient lipid to determine the phosphorus content. On the assumption

Fig. 4. Autoradiogram of thin-layer chromatography of lipids from cells pulse-labelled with [14C]acetate (procedure III). Cells were pulse-labelled 18 to 24 hr after infection. Microdensitometer tracings of the autoradiograms are also shown.
that similar specific activities would be appropriate at the last stage of virus purification, an estimate could then be made of the number of phospholipid molecules associated with each virus particle. Table 4 indicates that a significantly higher number of phospholipid molecules appeared to be associated with the virus when the label was derived from the infected cell lipids. However, in view of the obvious difficulty of removing contaminating cellular phospholipids, it was impossible to state with any certainty from this result that the phospholipids were an integral component of the virus particle. Accordingly, attempts
were made to remove extraneous phospholipid by a more rigorous purification involving further steps of treatment with trypsin, nucleases and sodium deoxycholate (Expt. 2). In these later experiments parallel control preparations included infected cells labelled with $^{32}$P before infection and also cells infected with virus in the absence of arginine when no complete virus is manufactured (Russell & Becker, 1968). These additional treatments significantly reduced the amount of lipid in association with the virus (Table 4) and suggested that at least the major part of the phospholipid found in association with the purified virus in the first experiment was non-specifically attached to the virus particle and not an integral component.

Similar experiments were carried out using $[^{14}C]$acetate labelling techniques. Radioactivity extracted by the lipid solvents could also be detected in the purified virus fraction. Analysis

### Table 3. Effect of infective virus, irradiated virus and virus antigens on $[^{14}C]$acetate incorporation into lipids

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells infected with virus</td>
<td>100</td>
<td>221</td>
<td>125</td>
<td>42</td>
<td>20</td>
</tr>
<tr>
<td>Cells infected with irradiated virus</td>
<td>96</td>
<td></td>
<td></td>
<td>53</td>
<td>14</td>
</tr>
<tr>
<td>Cells + hexon antigen $^\dagger$</td>
<td>141</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells + fibre antigen $^\ddagger$</td>
<td>131</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells + penton antigen $^|$</td>
<td>168</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells + penton base antigen $^|$</td>
<td>162</td>
<td>123</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected cells</td>
<td>54</td>
<td>135</td>
<td>87</td>
<td>57</td>
<td>10</td>
</tr>
</tbody>
</table>

* Label present 6 to 8 hr after infection.
† Expt. A.
$^\dagger$ $10^7$ cells + 1.4 mg. hexon antigen (1 x $10^{15}$ virus particle equivalents).
$^\ddagger$ $10^7$ cells + 50 µg. fibre antigen (3 x $10^{15}$ virus particle equivalents).
$^\|$ $10^7$ cells + 340 µg. penton antigen (5 x $10^{15}$ virus particle equivalents).
$^\|$ $10^7$ cells + 300 µg. penton base antigen (5 x $10^{15}$ virus particle equivalents).

### Table 4. Lipid phosphorus present in purified adenovirus

<table>
<thead>
<tr>
<th>Source of radioactivity</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected cells</td>
<td>Infected cells</td>
</tr>
<tr>
<td>Total radioactivity $^{32}$P counts/min.</td>
<td>$1.5 \times 10^8$</td>
<td>$1.4 \times 10^8$</td>
</tr>
<tr>
<td>Radioactivity extracted by lipid solvent $^{32}$P counts/min.</td>
<td>36</td>
<td>740</td>
</tr>
<tr>
<td>Specific activity of phospholipid (counts/min./µM P)</td>
<td>$2.8 \times 10^8$</td>
<td>$3.9 \times 10^8$</td>
</tr>
<tr>
<td>No. of atoms of lipid P*</td>
<td>$7.8 \times 10^{12}$</td>
<td>$1.1 \times 10^{14}$</td>
</tr>
<tr>
<td>Total protein (µg.)</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>No. of particles†</td>
<td>$3.0 \times 10^{11}$</td>
<td>$2.9 \times 10^{11}$</td>
</tr>
<tr>
<td>No. of phospholipid molecules/particle</td>
<td>16</td>
<td>380</td>
</tr>
</tbody>
</table>

* Calculated from Avogadro's number.
† Calculated from the measured total protein in purified virus and an assumed virus particle molecular weight of $10^9$. 

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by thin-layer chromatography using long-term autoradiography indicated that the radioactivity could be attributed to the lecithin/sphingomyelin group, confirming that this type of lipid, and not the neutral lipid group, was associated with the purified virus.

![Diagram](image)

**Fig. 6.** Distribution of radioactivity derived from $^{32}$P-labelled uninfected cells and measured by Čerenkov counting during the purification of adenovirus. Extracts of labelled, uninfected cells were added to extracts of unlabelled, infected cells and the virus purified as in Methods. The virus fractions were located by their opalescence (shaded black) and the entire fraction taken forward to the next purification step. Various fractions were examined for their phospholipid content (P.L.) by ascertaining the percentage of radioactivity which was soluble in chloroform + methanol (2:1).

**DISCUSSION**

It appears likely that membranes containing lipid play a role in many stages of adenovirus infection from entry of the virus into the cell to the final assembly of the infectious particles (Lonberg-Holm & Philipson, 1969; Mäntyjärvi & Russell, 1969). This is in spite of the fact that previous investigations (Green & Piña, 1963) have demonstrated the absence of a significant amount of lipid in the adenovirus particle itself. The studies described here were made to clarify these problems.

The report by Green (1959) that a significant increase in phospholipid metabolism occurs
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on infection has been confirmed. It appears unlikely that this stimulation reflects a diminution of pool size of available substrate, since the same phenomenon was observed with both acetate and phosphate precursors. The increased phospholipid synthesis reflected a general increase in the synthesis of all the major phospholipid components. On the other hand, by $[^{14}C]$acetate labelling we were able to demonstrate a significant difference in the pattern of synthesis of the neutral lipids. Under our conditions this stimulation could be detected relatively early in infection, at a time considerably before production of virus DNA and capsid proteins. By this time the particle has entered the nucleus and is at least partially uncoated (Lawrence & Ginsberg, 1967; Lonberg-Holm & Philipson, 1969; Morgan et al. 1969) and transcription of the early virus messenger RNA has begun (Lucas & Ginsberg, 1970; Thomas & Green, 1969). It is not known whether this lipid stimulation reflects the formation of membranes necessary for these early events or is a prelude to the later synthesis and assembly of particles. It is of interest that a similar increase in lipid synthesis has been described in cells infected with vaccinia (Gaus & Youngner, 1963) and poliovirus (Cornatzer et al. 1961; Penman, 1965).

The markedly increased uptake of $[^{14}C]$acetate into triglycerides in infection is of uncertain significance. The possibility that this was a non-specific event in dying cells was examined by testing 'aged', uninfected cells, but apparently simply 'old' HEK cells do not synthesize triglycerides at an increased rate. Changes in environmental conditions also did not produce a similar pattern of uptake of label (Rothblat, 1969). Because the triglyceride formation might represent merely the accumulation of fat droplets in infected cells, efforts were made in parallel experiments to stain neutral lipids in infected and control cells (J. A. Armstrong & W. C. Russell, unpublished observations), but no differences could be detected in several such attempts. It is of course possible that the differences observed might be the result of some pool size changes in the divergent pathways from acetate to cholesterol and to the triglycerides or fatty acids. It may be of significance that very considerable changes have been observed in the lipids associated with fowlpox virus infection (White et al. 1968) where squalene, an intermediate in the cholesterol biosynthetic pathway, appeared to accumulate in both the infected cells and in the purified virus particle. No squalene production could be detected, however, in the adenovirus-infected cells. It is also of interest that alterations in the cholesterol pathway have been associated with various neoplastic conditions (Gore & Popjak, 1962; Siperstein, Fagan and Morris, 1966).

The finding that irradiated virus is almost as efficient in stimulating lipid metabolism as unirradiated virus suggested that the event initiating the stimulation was related to the structural components of the virus and not to a mechanism under control of a virus gene. The subsequent experiments involving interaction of the cells with the purified capsomere components appeared to substantiate this view and, in particular, pointed to the crucial role of the penton base component. It should be noted, however, that in these experiments large quantities of these components were added - significantly more than would have been the case had purified virus seed alone been used. In one experiment the stimulation of lipid metabolism was considerably less than when using the complete particles, and it may be that size considerations play a part in the interaction (Ryser, 1967).

Previous investigations (Pereira, 1958; Valentine & Pereira, 1965) had shown that the penton component was responsible for the so-called 'early cytopathic effect'. This phenomenon is manifested by a rounding of the cells when in contact with this antigen and, when mixed in suspension, by the inability of cells to spread normally (Russell et al. 1967a). Presumably these effects can be related to changes in cellular membranes and are reflected in the alteration in the lipid metabolism noted.
Under conditions of extensive purification, we were unable to demonstrate any intrinsic lipid in the adenovirus particle, confirming the earlier report of Green & Pina (1963). Morgan et al. (1969) made thin section studies of the early stages of adenovirus type 7 infection and suggested that the core was surrounded by a 'membrane' which was apparent just as the infecting virus approached the nuclear membrane of the cell. It is conceivable that this 'membrane' could represent a protein or glycoprotein shell rather than a lipid-containing membrane. Our studies have also demonstrated the fact that small quantities of extrinsic lipid are adsorbed very readily to virus particles and survive many purification procedures.

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REFERENCES


Lipids and adenovirus infection


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