

## Incorporation of Lipids into Herpes Simplex Virus Particles

By Y. ASHER, M. HELLER AND Y. BECKER

*Departments of Virology and Biochemistry, Hebrew University-  
Hadassah Medical School, Jerusalem, Israel*

(Accepted 1 July 1968)

### SUMMARY

The synthesis of lipids in BSC 1 cells and their association with herpes simplex virus was studied. Radioactive choline was found to be incorporated mainly into lecithin. Infection of cells with herpes simplex virus did not affect the synthesis of cellular lipoproteins. Infected cells, prelabelled with radioactive choline, yielded virus particles which contained radioactively labelled cellular lipids. Treatment of herpes virus particles with sodium deoxycholate caused the dissociation of the particles and the release of capsomeres and soluble proteins.

### INTRODUCTION

The chemical analysis of purified herpes simplex virus (Russell, Watson & Wildy, 1963) demonstrated the presence of DNA (6.5%), protein (70%), carbohydrate (1.6%) and phospholipids (22%) in the virus particles. The presence of lipids in the virus particles was correlated with the finding that virus particles are coated with distinct envelopes (Wildy & Watson, 1962; Epstein, 1962; Watson, Russell & Wildy, 1963). The cellular nature of the virus-associated envelopes was deduced from: (a) the presence of adenosine triphosphatase both in the virus envelopes and in the cellular membranes (Epstein & Holt, 1963), and (b) the ability of antibodies against uninfected cells to agglutinate enveloped particles (Watson & Wildy, 1963). Electron microscope studies by Siegert & Falke (1966) demonstrated that during the passage of herpes virus particles from the nucleus to the cytoplasm of the infected cells, the particles obtained their envelopes from the inner leaf of the nuclear membrane. The importance of lipids for virus infectivity was shown by the sensitivity of the infectious virus particles to treatment with ether (Holden, 1932), phosphatases (Amos, 1953) and sodium deoxycholate (Hochberg & Becker, 1968). The present study will describe: the effect of herpes virus infection on the incorporation of radioactive choline by BSC 1 cells; the association of choline labelled lipids with the herpes virus particles; the source and nature of the lipids associated with the virus particles, and the effect of sodium deoxycholate on the virus particles, capsids and capsomeres.

### METHODS

*Virus, cells and medium.* The HF strain of herpes simplex virus, which had been plaque purified, was propagated in the BSC 1 line of monkey kidney cells. Stock virus was prepared as previously described (Levitt & Becker, 1967). BSC 1 cells were grown in milk dilution or Roux bottles using Eagle's medium (1959). In order to permit

incorporation of radioactive choline, Eagle's medium without choline was used. Infection of cells, fractionation of cells and sucrose gradient analysis were carried out as previously described (Levitt & Becker, 1967; Olshevsky, Levitt & Becker, 1967; Becker, Olshevsky & Levitt, 1967; Hochberg & Becker, 1968). In order to prevent the synthesis of virus coat proteins, infected cells were incubated in Eagle's medium from which arginine was omitted (Becker *et al.* 1967). The nuclei from these cells, as well as from uninfected controls, were analysed in sucrose gradients. The regions in the gradients containing the virus capsids and capsomeres and the equivalent regions in sucrose gradients of the controls were recentrifuged in sucrose gradients before further analysis. The virus particles, capsids and capsomeres were treated with sodium deoxycholate (Nutritional Biochemical Co., U.S.A.) final concentration 1% (w/v) (Becker & Chen, 1966; Hochberg & Becker, 1968).

*Labelling of cells.* Cultures were grown in Eagle's medium containing 0.008 mM-leucine (1/100th the regular concentration) to enable the incorporation of radioactive leucine (Olshevsky *et al.* 1967).

When the cells were labelled with [<sup>3</sup>H]choline, the choline was omitted from Eagle's medium, which normally contains choline at a concentration of 1.0 mg./l. In most instances the cells were labelled with both tritiated and <sup>14</sup>C labelled compounds. Infected and control cultures were withdrawn at different time intervals, the cells were harvested and samples were treated with trichloroacetic acid, collected on Millipore filters and the radioactivity determined in a Packard liquid scintillation counter. [<sup>3</sup>H]Choline, [<sup>14</sup>C]thymidine, [<sup>14</sup>C]leucine and [<sup>3</sup>H]leucine (specific activities 2000, 54, 305 and 236 mc/m-mole, respectively) were obtained from The Radiochemical Centre, Amersham, England.

*Isolation and identification of [<sup>3</sup>H]choline labelled lipids.* The fractions from sucrose gradients containing virus particles, capsids and capsomeres were extracted with chloroform and methanol (1:1) and were left overnight at room temperature. A solution of 0.1 M-KCl was added (Bligh & Dyer, 1959), the samples were centrifuged and the upper methanolic-aqueous phase was removed by aspiration and was discarded. The lower chloroform phase was evaporated to dryness under reduced pressure. Further purification of lipids was achieved by dissolving the residue in chloroform + methanol (2:1) and adding 0.1 M-KCl in a proportion of chloroform + methanol + KCl 8:4:3 (Folch, Lees & Sloane-Stanley, 1957). After centrifugation, the upper phase was removed and methanol was added in order to precipitate the protein present in the interphase. Samples of the clear solution were placed on thin layer chromatoplates (silica gel HR, obtained from Merck Co., U.S.A., 0.25 mm. thick), along with 50 to 100 µg. of ovolecithin as carrier. The plates were developed in a chloroform + methanol + 28% NH<sub>3</sub> (70:30:2) solvent system by ascending chromatography to a distance of 14 cm. The spots were visualized with iodine vapour, the different areas were marked and scraped directly into counting vials. Before the addition of 12 ml. scintillation liquid to each vial, 1 ml. of a solution of 10% triton (X-100) prepared in absolute ethanol was added to the powder to facilitate phospholipid extraction. After thorough mixing the radioactivity was determined in a Packard scintillation counter.

## RESULTS

*The effect of virus infection on choline incorporation by BSC1 cells*

In order to determine the effect of herpes virus infection on lipid synthesis in BSC1 cells, the latter were labelled with [ $^3\text{H}$ ]choline throughout the infectious cycle, beginning 3 hr after infection. Infected cells were also labelled with [ $^{14}\text{C}$ ]thymidine in order to compare lipid synthesis with the course of viral DNA synthesis (Olshevsky *et al.* 1967). At different time intervals the cells were fractionated and the amount of radioactivity was determined in samples taken from the total cell homogenates, as well as from the nuclear and cytoplasmic fractions.

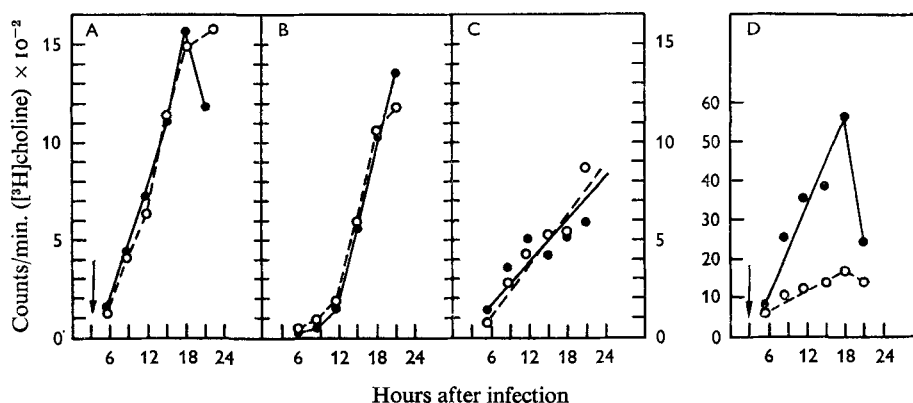


Fig. 1. The effect of herpes virus infection on choline and on thymidine incorporation into BSC1 cells. [ $^3\text{H}$ ]Choline incorporation into total cell homogenate (A), nuclei (B) and cytoplasm (C). [ $^{14}\text{C}$ ]Thymidine incorporation into total cell homogenate (D). ●—●, Infected; ○----○, uninfected. Arrow indicates time at which [ $^3\text{H}$ ]choline or [ $^{14}\text{C}$ ]thymidine were added.

Table 1. *Effect of choline on herpes virus production*

Choline in the medium		Virus yield (p.f.u./ml.)		
mg./ml.	Relative concentration	Expt 1	Expt 2	Expt 3
0	0	$1.4 \times 10^7$	$3.2 \times 10^8$	$4 \times 10^8$
0.001	1	$1.6 \times 10^7$	$2.7 \times 10^8$	$6 \times 10^8$
0.010	$\times 10$	$0.6 \times 10^7$	$2.6 \times 10^8$	$6 \times 10^8$
0.100	$\times 100$	$6.0 \times 10^7$	$2.2 \times 10^8$	$6 \times 10^8$
1.000	$\times 1000$	$1.2 \times 10^8$	$2.4 \times 10^8$	$8 \times 10^8$

Infection of the cells with herpes simplex virus was found to have no effect on choline incorporation (Fig. 1 A). Analysis of the cell fractions demonstrated that radioactive choline was incorporated into the nuclei only 8 hr after the addition of the isotope (Fig. 1 B), and that incorporation of radioactive choline into the cytoplasm of the cells started immediately after the addition of the isotope (Fig. 1 C) indicating that choline is incorporated into lipids in the cell cytoplasm. Omission of arginine from the medium, which was found to decrease protein synthesis and to inhibit the formation of virus particles (Becker *et al.* 1967), did not affect choline incorporation in the

infected and uninfected control cells. The results were identical to those presented in Fig. 1 (A, B, C).

The omission of choline from the culture medium did not interfere with the replication of the herpes simplex virus as indicated by the increased rate of DNA synthesis in the infected cells (Fig. 1 D) which occurs in the cell nuclei (Olshevsky *et al.* 1967), and by the virus yield obtained from cells incubated in medium lacking choline which was almost identical to the virus yield obtained from cells incubated in Eagle's medium containing the regular choline concentration (0.001 mg./ml.) (Table 1).

#### Association of prelabelled lipids with herpes virus particles

In repeated experiments it was found that labelling of cells with radioactive choline during infection did not result in the labelling of virus particles. BSC1 cells were therefore labelled with [<sup>3</sup>H]choline for 4 or 5 days before infection with the virus. To

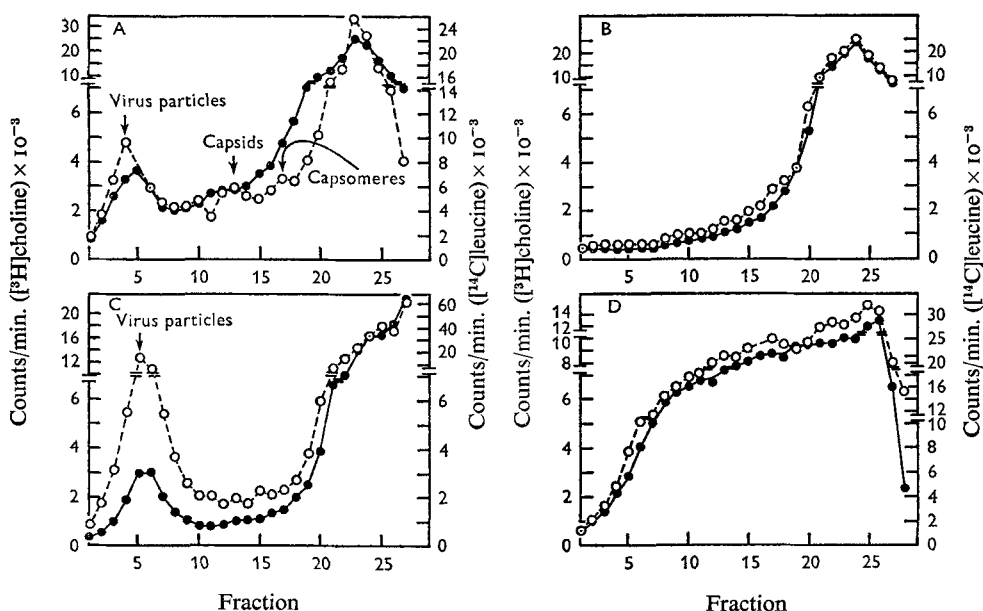


Fig. 2. Distribution of prelabelled lipids in sucrose gradients of nuclei and cytoplasm of infected and uninfected cells. Sucrose gradient analysis of nuclei from infected (A) and uninfected (B) cells and sucrose gradient analysis of cytoplasmic fractions from infected (C) and uninfected cells (D). ●—●, [<sup>3</sup>H]choline; ○---○, [<sup>14</sup>C]leucine. The bottom of the gradient is to the left.

prevent the incorporation of radioactive choline into lipids during the virus growth cycle, fresh medium containing an excess of unlabelled choline was added to the cell cultures immediately after virus infection. It was found that when the amount of choline in the medium was increased ten- or 100-fold, the virus yield was unaffected (Table 1). However, in two experiments 1000-fold concentration of choline caused an increase in the virus yield (ten- and twofold, respectively) and in one experiment it did not affect the virus titre. In addition to labelling with choline the cells were labelled during infection with [<sup>14</sup>C]leucine to label the viral proteins (Becker *et al.* 1967; Olshevsky *et al.* 1967). Uninfected controls were similarly labelled.

Sucrose gradient analyses of the nuclei from infected cells (Fig. 2A) demonstrated that radioactive choline was present in a large amount at the top of the sucrose gradient and was also distributed throughout the gradient. A definite peak of both radioactive lipids and proteins was found in the region of the sucrose gradient which contains the virus particles (Becker *et al.* 1967). This result was different with uninfected nuclei (Fig. 2B), in which all the labelled protein and lipids were retained at the top of the sucrose gradient. From such an analysis it could not be excluded that cellular structures, such as lipid-containing membranes, present in the infected nuclei, sediment throughout the sucrose gradient contaminating the viral structures. To determine if the choline labelled lipids non-specifically associated with the virus particles, a homogenate from uninfected nuclei labelled with choline was mixed with a homogenate from infected unlabelled nuclei. The mixture was centrifuged in a sucrose gradient and the radioactivity in each fraction was determined. It was found that the virus band did not contain choline labelled components. This result indicated that the lipids present in the virus band were probably attached to the virus particles.

Sucrose gradient analyses of the cytoplasmic fractions demonstrated a clearer difference between infected and control cells. A distinct virus band, labelled both in the proteins and the lipids, was isolated from the cytoplasmic fraction of infected cells (Fig. 2C), while most of the lipids were retained at the top of the gradient. A completely different situation was found in the cytoplasm of uninfected cells (Fig. 2D). In these cells the lipids and proteins were associated with large, heterogenous structures which sedimented throughout the sucrose gradient.

The ability (Fig. 2) to recover herpes virus particles from the nuclei and cytoplasmic fractions of the infected BSC 1 cells (both labelled with radioactive lipids) raises the question of the nature of the virus particles. Preliminary studies had demonstrated that virus particles obtained from the nuclei do not have an envelope, while the virus particles which were obtained from the cell cytoplasm contain a distinct envelope. It is likely, therefore, that the association of lipids with herpes virus particles begins in the nuclei, but the complete envelope is obtained only after passage through the nuclear membrane into the cell cytoplasm.

#### *Analysis of lipids in the cells and in the virus bands*

To determine the nature of the choline-labelled lipids appearing in the virus particles, virus specific bands were taken from the sucrose gradients of infected nuclei. In such gradients, besides the band of virus particles, two additional bands were seen: the first above the band of virus particles consists of incomplete capsids and the second seems to contain viral capsomeres. The different viral components, labelled with radioactive leucine, were isolated from the sucrose gradient and rebanded (Fig. 3). It can be seen that the different components were obtained in almost homogenous bands.

The different virus specific bands were removed, extracted as described in Methods and analysed by thin-layer chromatography (Table 2). Most of the radioactive choline which was utilized by the BSC 1 cells was incorporated into lecithin (the amount ranged from 58 to 95%) and the rest (from 26 to 3%) was incorporated into sphingomyelin and lysolecithin. The same results were obtained from all the virus specific bands as well as from the nuclei and cytoplasm of uninfected cells.

*The significance of the presence of lipids in the bands of virus particles*

In view of the distribution of radioactive lipids in the sucrose gradients (Fig. 2 A, C) it was important to determine whether the lipids are part of the virus particles or constitute a cellular contaminant. Cells, prelabelled with radioactive choline, were infected with herpes simplex virus. Some of the infected cultures were incubated in the presence of arginine in the culture medium and some in its absence. Cells infected in the absence

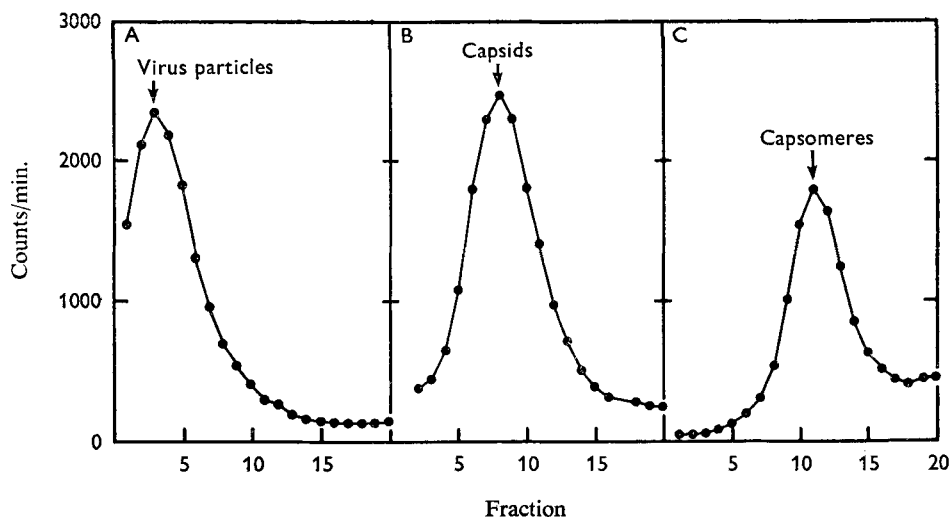


Fig. 3. Rebanding of virus components. Bands of virus particles (A), capsids (B) and capsomeres (C), labelled with [ $^3\text{H}$ ]leucine were obtained from sucrose gradients and rebanded in sucrose gradients.

of arginine are regarded as better controls than uninfected cells since replication of viral DNA and the inhibition of the cellular macromolecular processes take place in the absence of arginine (Becker *et al.* 1967). The bands of virus particles, capsids and capsomeres were isolated from infected nuclei in sucrose gradients. Regions similar to the former were removed from the sucrose gradients of the nuclei which were obtained from cells incubated in the absence of arginine.

Recentrifugation of the virus particles (Fig. 4A), which had been obtained from the cytoplasm of infected cells, demonstrated the presence of radioactive lipids in the virus band. Rebanding of a similar portion of the sucrose gradient of cytoplasm from cells which had been incubated in the absence of arginine (Fig. 4A), demonstrated the absence of virus particles and the presence of a low concentration of radioactive lipids. Similar results were obtained with virus particles obtained from the nuclear fraction of infected cells (Fig. 4B). This result indicated that the lipids in the virus bands were associated with the virus particles and only part of them was contamination. A different situation was found with the two bands which contained capsids (Fig. 4C) and capsomeres (Fig. 4D). These bands were closer to the top of the sucrose gradient and contained lipids which sedimented into the gradient as indicated by the increase in the amount of radioactive lipids present in the sucrose gradients of infected cells which were incubated in the absence of arginine.

Table 2.  
Radioactivity (counts/min.)

Lipid	Radioactivity (counts/min.)																		
	Fractions from nuclear gradients						Fractions from cytoplasmic gradients												
	Virus particles			Capsids			Capsomeres			Soluble			Virus particles			Soluble			
Expt. ....	1	2	3	1	2	3	1	2	1	2	1	2	1	2	1	2	1	2	
Neutral lipids	44	42	45	37	33	24	30	44	52	28	67	44	37	24					
Phosphatidyl ethanol-amine	37	32	34	29	29	36	33	35	34	29	53	67	35	28					
Lecithin	3,282 (89%)	1,119 (58%)	3,536 (93%)	1,647 (64%)	1,431 (75%)	4,234 (92%)	1,351 (70%)	5,882 (95%)	1,469 (81%)	3,091 (71%)	45,900 (95%)	2,446 (88%)	3,588 (66%)	6,464 (93%)					
Sphingomyelin	149 (4%)	506 (26%)	97 (3%)	112 (4%)	226 (11%)	166 (14%)	343 (17%)	167 (3%)	104 (6%)	689 (16%)	1,224 (3%)	135 (5%)	1,468 (26%)	228 (3%)					
Lysolecithin	71 (2%)	75 (4%)	55 (1.5%)	659 (26%)	64 (3%)	231 (5%)	69 (3%)	57 (1%)	53 (3%)	159 (3%)	366 (4%)	60 (2%)	97 (2%)	60 (0.9%)					
Phosphatidic acid, phosphatidylserine, phosphatidylinositol	33	36	26	34	37	29	36	53	42	91	99	37	51	122					
Total	3,695	1,946	3,793	2,566	1,915	4,725	1,956	6,238	1,804	4,339	48,191	2,789	5,472	6,926					

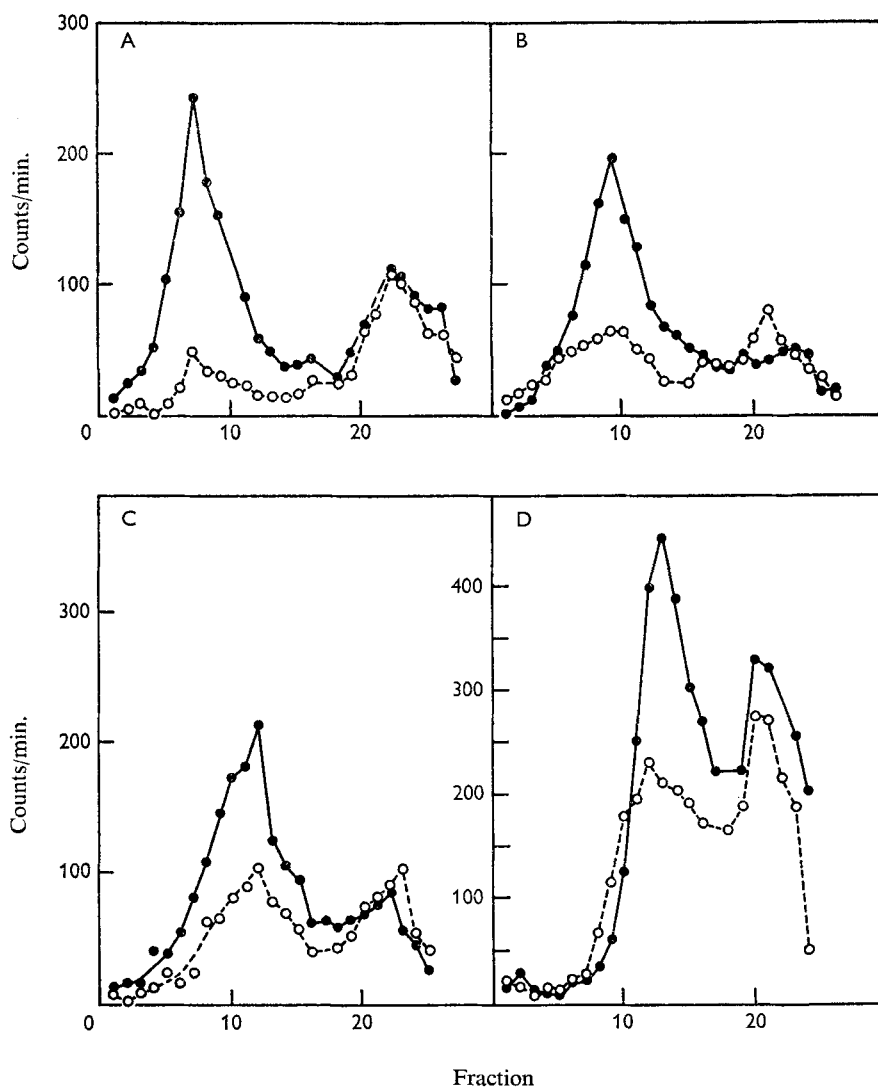


Fig. 4. Association of cellular lipids labelled with [ $^3\text{H}$ ]choline with virus particles, capsids and capsomeres isolated in sucrose gradients. Sucrose gradients (15 to 30%) on materials taken from previous gradients in the regions of: A, virus particles from the cytoplasmic fraction; B, virus particles from the nuclear fraction; C, capsids, and D, capsomeres. ●—●, [ $^3\text{H}$ ]-Choline-containing lipids associated with virus specific components synthesized in infected cells in the presence of arginine; ○—○, in absence of arginine.

#### *The effect of sodium deoxycholate on herpes simplex virus*

Initial studies (Hochberg & Becker, 1968) demonstrated that herpes simplex virus particles were dissolved by sodium deoxycholate, suggesting that the virus particles contain lipid components which are essential for their integrity. It was also found that virus DNA can be released from virus particles by sodium deoxycholate treatment (Becker, Dym & Sarov, to be published). To study the effect of sodium deoxycholate,

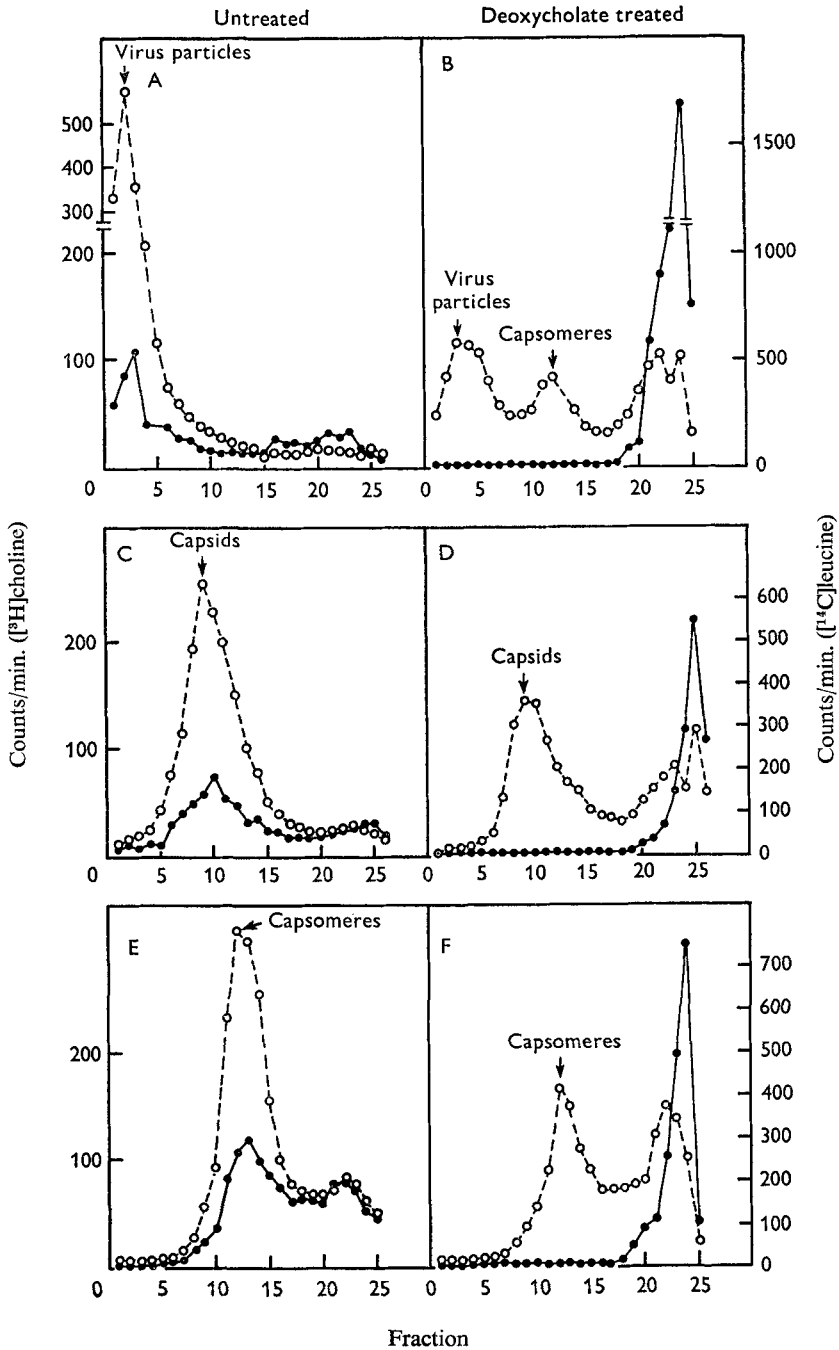


Fig. 5. Effect of sodium deoxycholate on virus particles, capsids and capsomeres. A, Virus particles; B, virus particles treated with 1% sodium deoxycholate; C, capsids; D, capsids treated with 1% sodium deoxycholate; E, capsomeres; F, capsomeres treated with 1% sodium deoxycholate. ●—●, [<sup>3</sup>H]Choline; ○---○, [<sup>14</sup>C]leucine.

virus particles, capsids and capsomeres labelled in the proteins and the lipids were treated with the detergent before rebanding in sucrose gradients. It was found (Fig. 5A, B) that the lipids associated with the virus particles were dissolved by sodium deoxycholate and banded at the top of the sucrose gradient. Also, some of the virus particles were disrupted by the detergents and gave rise to protein particles which banded in the region of capsomeres, and to proteins which banded at the top of the sucrose gradient. The rest of the virus particles remained in the virus band.

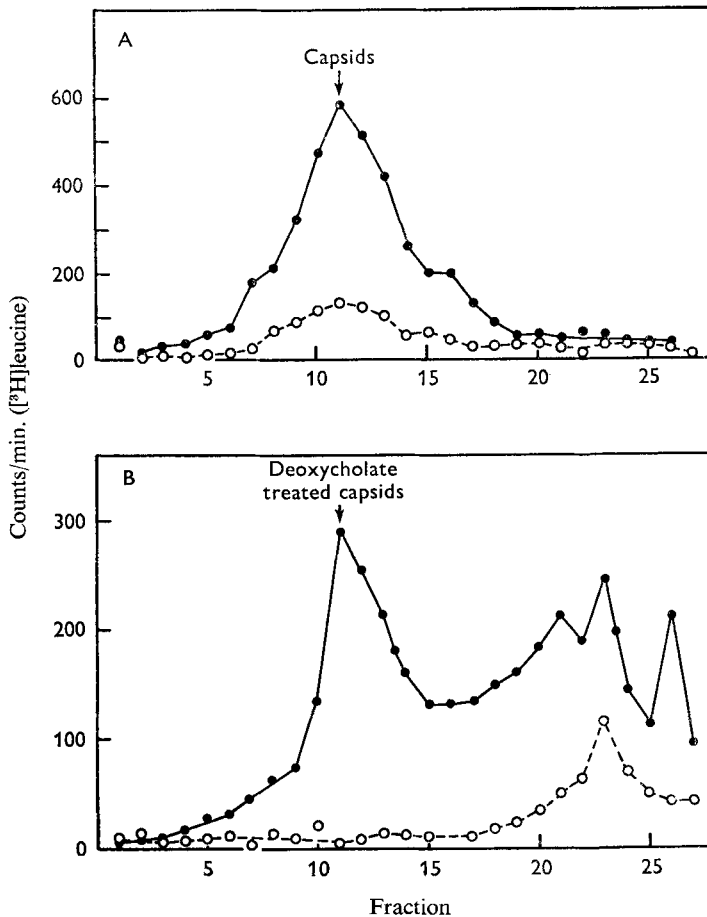


Fig. 6. Rebanding of the capsid bands obtained from sucrose gradients of infected and control nuclei. A, Rebanding of viral capsids and the same region from sucrose gradients of uninfected cells labelled with  $^3\text{H}$ ]leucine. B, Rebanding of the same viral capsids and equivalent uninfected cellular material after treatment with sodium deoxycholate. ●—●,  $^3\text{H}$ ]Leucine-labelled capsids from gradients of infected nuclei; ○----○, the same region but from sucrose gradients of nuclei from uninfected cells.

Sodium deoxycholate removed the lipids from the capsid (Fig. 5C, D) and capsomere (Fig. 5E, F) bands, removing also part of the radioactive proteins. However, the capsids were not degraded into capsomeres (Fig. 5C, D). The nature of the association between the lipids and the proteins is not yet known.

The effect of sodium deoxycholate on viral capsids and capsomeres was also used to distinguish between virus specific structures and proteins associated with them. Nuclei from infected and uninfected cells were labelled with radioactive leucine and analysed in sucrose gradients. The capsid band, and also the analogous region in the sucrose gradient of uninfected nuclei were removed, each was divided into two portions: one was centrifuged without treatment (Fig. 6A) and the second was treated with sodium deoxycholate before recentrifugation (Fig. 6B). It can be seen that the capsids sedimented in a distinct band (Fig. 6A, infected). Similarly, the analogous region from the sucrose gradient of uninfected nuclei demonstrated the presence of radioactive material (Fig. 6A, control). After treatment with sodium deoxycholate (Fig. 6B, infected) part of the radioactive proteins present in the viral capsids was dissolved and banded at the top of the sucrose gradient. However, all the radioactive material present in the control gradient (Fig. 6B, control) was dissolved by the detergent and retained at the top of the sucrose gradient. Similar results were obtained with the capsomere band.

#### DISCUSSION

The present communication deals with the effect of virus infection on lipid synthesis and the association of cellular lipids with herpes simplex virus particles. The BSC 1 cells were found to incorporate radioactive choline mainly into lecithin and to a small extent into sphingomyelin and lysolecithin. However, in contrast to poliovirus which stimulated the incorporation of choline by infected cells (Penman, 1965), herpes simplex virus did not affect the incorporation of choline by the host cells. The synthesis of lipids by BSC 1 cells was not affected, even in the absence of arginine (Becker *et al.* 1967).

The distribution of the labelled lipids in the sucrose gradient of the cytoplasmic fractions of uninfected cells demonstrated that radioactively labelled lipids were associated with heterogenous structures, presumably the membranes of the reticulum (Becker, unpublished results). In cells infected with herpes simplex virus an altered sedimentation behaviour of such structures was found (Fig. 2C, D). This could be correlated with the release of ribosomes from the cytoplasmic membranes which takes place in infected BSC 1 cells (J. Levitt & Y. Becker, unpublished results).

Isolation of herpes simplex virus particles from nuclei of infected cells, prelabelled with radioactive choline demonstrated the association of cellular lipids with the virus particles. The presence of lipids in virus particles which were released from the nuclei to the cytoplasm may be correlated with the presence of a distinct envelope (Wildy & Watson, 1962). The association of lipids with those virus particles still present in the nuclei of the infected cells was suggested by the sensitivity of nuclear virus particles to treatment with sodium deoxycholate. It is of interest that 60 to 70% of the virus particles were dissolved by the detergent as indicated by the appearance of capsomeres which banded in the sucrose gradient. The virus particles which were not affected by the detergent might represent virus particles which still lack lipids. It is therefore suggested that during the assembly process of herpes simplex virus particles in the nuclei of the infected cells, cellular lipids are integrated into the coat of the virus particles to form the lipid envelope, rendering them sensitive to sodium deoxycholate treatment. The envelope is completed during the passage of the virus particles through the nuclear membrane to the cytoplasm.

The results presented in this communication confirm the analytical and morphological findings that an integral step in the formation of herpes virus particles is the interaction of the latter with the nuclear membrane (Siegert & Falke, 1966). Further work is needed to analyse the nature of the virus and cellular proteins present in the mature herpes virus particles.

One of the authors (Y. A.) is a M.Sc. student at the Institute of Microbiology, Hebrew University-Hadassah Medical School, Jerusalem. The skilled technical assistance of Miss Miriam Adler is greatly appreciated.

## REFERENCES

- AMOS, H. (1953). The inactivation of herpes simplex virus by phosphatase enzymes. *J. exp. Med.* **98**, 365.
- BECKER, Y. & CHEN, Y. (1966). The reaction of vaccinia virus with deoxycholate. *Israel J. med. Sci.* **2**, 417.
- BECKER, Y., OLSHEVSKY, U. & LEVITT, J. (1967). The role of arginine in the replication of herpes simplex virus. *J. gen. Virol.* **1**, 471.
- BLIGH, E. G. & DYER, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911.
- EAGLE, H. (1959). Amino acid metabolism in mammalian cultures. *Science, N.Y.* **130**, 432.
- EPSTEIN, M. A. (1962). Observations on the fine structure of mature herpes simplex virus and on the composition of its nucleoid. *J. exp. Med.* **115**, 1.
- EPSTEIN, M. A. & HOLT, S. J. (1963). Adenosine triphosphatase activity at the surface of mature extracellular herpes virus. *Nature, Lond.* **198**, 509.
- FOLCH, J., LEES, M. & SLOANE-STANLEY, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *J. biol. Chem.* **226**, 497.
- HOLDEN, M. (1932). Nature and properties of virus of herpes. *J. Infect. Dis.* **50**, 218.
- HOCHBERG, E. & BECKER, Y. (1968). Adsorption, penetration and uncoating of herpes simplex virus. *J. gen. Virol.* **20**, 231.
- LEVITT, J. & BECKER, Y. (1967). The effect of cytosine arabinoside on the replication of herpes simplex virus. *Virology* **31**, 129.
- OLSHEVSKY, U., LEVITT, J. & BECKER, Y. (1967). Studies on the synthesis of herpes simplex virus. *Virology* **33**, 323.
- PENMAN, S. (1965). Stimulation of the incorporation of choline in poliovirus-infected cells. *Virology* **25**, 148.
- RUSSELL, W. C., WATSON, D. H. & WILDY, P. (1963). Preliminary chemical studies on herpes virus. *Biochem. J.* **87**, 26.
- SIEGERT, R. & FALKE, D. (1966). Electron microscopical study on the envelopment of herpes virus hominis in cultured cells. *Arch. ges. Virusforsch.* **19**, 230.
- WATSON, D. H. & WILDY, P. (1963). Some serological properties of herpes simplex virus particles studied with the electron microscope. *Virology* **21**, 100.
- WATSON, D. H., RUSSELL, W. C. & WILDY, P. (1963). Electron microscopic particle counts on herpes virus using the phosphotungstate negative technique. *Virology* **19**, 250.
- WILDY, P. & WATSON, D. H. (1962). Electron microscopic studies on the architecture of animal viruses. *Cold Spring Harb. Symp. quant. Biol.* **27**, 25.

(Received 1 March 1968)