Effect of Sendai Virus Infection on Lipid Metabolism in Chick Embryo Fibroblasts

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Received for publication 1 December 1971

Lipid metabolism in the "abortive" system, Sendai virus-infected primary chick embryo fibroblasts, was examined by using ³²P-orthophosphate, ¹⁴C-glucose, and ¹⁴C-glycerol as precursors. Incorporation of radioactivity from ³²P-orthophosphate and ¹⁴C-glucose into lipid was increased in infected cells. Synthesis of all individual phospholipids was about equally stimulated. There was also evidence for increased lipid synthesis in more productively infected monkey kidney cells. Incorporation of ¹⁴C-glycerol when at a high level in the medium was also increased. However, when this precursor was supplied in minute quantities of high specific activity, incorporation was inhibited. Even though incorporation of radioactivity from ¹⁴C-glucose was stimulated during long labeling periods, the uptake of this precursor during short pulses was inhibited in infected cells. The phenomenon of increased labeling of triglyceride in infected chick cells under certain conditions is discussed, in conjunction with the other effects, in terms of related changes in other virus-infected systems.

Sendai virus, a paramyxovirus, contains structural lipids. The lipids are part of the envelope of the virion acquired as the particle buds through the host cell plasma membrane. Although it is probable that at least some of the viral lipid is synthesized and incorporated into the cell membrane prior to virus infection (16, 32), it is of interest to determine whether virus replication and the consequent production of viral structural lipids affect lipid metabolism of host cells. A study of lipid metabolism in Sendai virus-infected cells may also give some information about the composition of animal cell membranes and about such virus-induced membrane alterations as are manifested by cell fusion (13) and by the appearance of new surface antigens (11).

During the course of this work, we learned that Shibuta and co-workers (26, 27) have shown that Sendai virus infection induces increased incorporation of ³²P into phospholipids of chorioallantoic membranes and cultured chick embryo (CE), monkey kidney (MK), and bovine kidney cells.

The principal system used in this work, Sendai virus-infected chick fibroblasts, has been described as abortive (7) because little infectious virus is released, although large amounts of viral nucleocapsid accumulate in the cytoplasm (3).

Sendai virus-infected MK cells were shown to produce about 10 times more virus than CE cells. These two systems were compared in similar experiments in an attempt to relate changes in lipid metabolism to the level of infectious virus production.

MATERIALS AND METHODS

Virus. Sendai virus was kindly supplied by H.G. Periera. Virus stocks were produced in the chorioallantoic fluid of 10-day-old embryonated eggs. Infectivity was assayed by inoculation of embryonated eggs and measurement of hemagglutinin production.

Cell cultures. Chick fibroblast tissue cultures were prepared from 9- to 12-day-old embryos as described by Rubin (25). Primary cells were seeded onto 90-mm plastic tissue culture dishes at a concentration of 10⁷ cells per dish in medium 199 containing 10% tryptose phosphate broth and 2% calf serum.

A continuous line of rhesus monkey MK cells (LLC-MK2) was grown in Eagle minimal essential medium with 10% calf serum. They were infected after 48 hr of growth.

Infection of nearly confluent cell monolayers at a multiplicity of infection (MOI) of 10 to 100 was carried out by either of two methods. In the first method, virus-containing chorioallantoic fluid (0.25 to 0.50 ml per plate) was added to half the plates (Sendai-infected). Where indicated, a similar volume of allantoic fluid, devoid of virus, was added to the remaining plates (sham-infected). Otherwise, no additions were made (controls). Radioactive precursors were added to all plates at the time of infec-

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tion. In the second method of infection, allantoic fluid, containing Sendai virus or virus-free, was added to cultures and later the medium was removed, cell monolayers were washed with warm phosphate-buffered saline (PBS), and warm fresh medium containing radioactive precursors was added.

Harvesting and fractionation of cells. After removal of medium, cell monolayers were washed once with PBS. Cells were usually dislodged by incubation for 10 min at room temperature in 2 mM ethylenediamine-tetraacetic acid in PBS and scraping with a rubber policeman, and then were transferred to centrifuge tubes and washed once more with PBS.

Lipids were obtained by extracting cells overnight with 5 to 10 ml of chloroform-methanol (2:1; all ratios are expressed in volumes) per mg of dry cells and by repeating the extractions with similar volumes of chloroform-methanol (2:1) and chloroform-methanol (1:2) at 40°C for 1 hr. Lipid extracts were dried and washed by the method of Folch, Lees, and Sloane Stanley (10).

Often delipidated cells additionally were extracted twice with 5 ml of 30% ethanol per mg of dry cells to remove small-molecular-weight material. Subsequent extraction of the insoluble residue with 5% trichloroacetic acid did not solubilize any additional material. Delipidated, ethanol-extracted ^{14}C -glucose-labeled residues were hydrolyzed in sealed tubes with 2 N HCl at 100°C for 3 hr. The hydrolysates were then extracted with light petroleum and diethyl ethers. The aqueous residue was neutralized with Amberlite IR-45 (OH^-) and passed through a small column of Dowex 50 (H^+) to remove amino sugars and amino acids. The neutral eluate was chromatographed in ethyl acetate-acetic acid-formic acid-water (18:3:1:4) with several monosaccharides as markers. Protein content of the delipidated residue was determined by the method of Lowry et al. (18).

Lipid analysis. Methods previously employed for analyses of bacterial lipids (5) were used to examine lipids of control and infected cells. Neutral lipids were resolved on Silica Gel G plates (20 by 20 cm) in light petroleum (bp 40 to 60°C)-diethyl ether-acetic acid (85:15:2). Chromatography of neutral lipids from ^{14}C -glycerol-labeled cells showed radioactive diglyceride and triglyceride only. When ^{14}C -glucose was used, the fatty acids of these lipids were labeled also.

Phospholipids were chromatographed on plates (40 cm long) of Silica Gel H in chloroform-methanol-water (65:35:4). This system resulted in resolution as good as was achieved with two-dimensional systems (4). Labeled ^{14}C -lipids were also deacylated and chromatographed on paper in isopropanol-ammonia (2:1) for resolution of the glycerylphosphoryl derivatives or in ethyl acetate-acetic acid-formic acid-water (18:3:1:4) for 18 hr, which readily resolved the glycerylphosphoryl derivatives (R_F 0 to 0.2) and the glycerol (R_F 0.6) arising from glycerides.

Materials. CE were obtained from Sykes (Ireland) Ltd. All components of growth media were obtained from Burroughs Wellcome, London. Tissue culture dishes were from Nunc, Denmark. Tripalmitin, diolein, monoolein, sphingomyelin, and free fatty acids were obtained from Sigma Ltd., London. Phosphatidylcholine was prepared from egg yolks

(21), phosphatidylinositol from yeast (30), and phosphatidylethanolamine from *Mycobacterium phlei* (5). Radioactive chemicals were from the Radiochemical Centre, Amersham.

RESULTS

Gross effects of virus infection on host cells.

Under the conditions of Sendai virus infection used here, CE cells released 5 to 10 infectious progeny particles per cell during the first 24 hr after infection. Infected MK cells produced 10 times as many infectious particles. Virus-specific ribonucleic acid (RNA) was first detectable in CE cells at about 2 to 3 hr postinfection, and infectious virus first appeared in the medium after 6 hr (2). The onset of the typical refractile appearance occurred between 6 and 12 hr and was pronounced after 18 to 24 hr of infection. A small amount of cell fusion could also be seen by 24 hr postinfection. Sham-infected cells did not display any of these features; they were indistinguishable from control cells. Infection with Sendai virus for periods far exceeding the onset of obvious morphological changes did not affect growth of the cells as determined by protein content, cell count, or dry weight (Table 1). From this, it appears that Sendai, in common with many other paramyxoviruses, is not rapidly cytopathogenic. No effect on total lipid content was observed over a 24-hr infection period (Table 1).

Effects of Sendai virus infection on the incorporation of radioactive precursors into lipids of host cells. The first procedure for Sendai virus infection of cells was initially used routinely. Infecting virus inoculum as well as radioactive precursors were left in cell growth medium throughout the experiment. Under these conditions, a marked increase in phospholipid metabolism was observed at all infection periods (Fig. 1). The ratio of the amount of ^{32}P incorporated by infected cells to that incorporated by control cells was approximately constant from 3 to 24 hr postinfection; a mean value of 1.55 ± 0.14 ($\pm$ standard error from mean) was found for this ratio over five time points. The early lag in labeling of the phospholipid fraction has also been observed by Pfefferkorn and Hunter (23) in CE cells. They also demonstrated the metabolic stability of newly synthesized phospholipid in virus-infected and uninfected CE fibroblasts, suggesting that the increased metabolism of lipid phosphorus in Sendai virus-infected cells is due to stimulated synthesis of phospholipid.

By using the same conditions and ^{14}C -glucose as lipid precursor, a less pronounced stimulation of lipid synthesis was observed at all infection periods between 3 and 12 hr (Fig. 2). The effect in these and other experiments was always well established after 12 hr of infection. However,

TABLE 1. *Effects of Sendai virus infection on growth and composition of primary chick fibroblasts*

Cells	Micrograms of protein per plate ^a	Micrograms of lipid per mg of protein	Micrograms of lipid P per mg of protein ^b
Control (48 to 96 hr after seeded)	908 ± 84.40 (37)	557 ± 40.56 (4)	5.10 ± 1.05 (2)
Infected (1 to 48 hr post-infection)	1.04 ± 0.06 (29)	0.99 ± 0.18 (3)	1.06 ± 0.14 (2)

^a Usually determined by the weight of delipidated cells. Protein content of unfractionated cells, as determined by the method of Lowry et al. (18), with bovine serum albumin as the standard, corresponded closely to the weight of delipidated cells.

^b Estimated as described previously (5).

^c Results from infected cells are expressed as ratios of the value to the corresponding control value. Little effect on total cell count was noticed after infection for about one cell generation (22 hr). For example, in one experiment control cultures contained an average of 6.6×10^6 cells per plate, and infected cultures contained an average of 5.7×10^6 cells per plate.

FIG. 1. Effect of Sendai virus infection on $^{32}\text{PO}_4$ incorporation into phospholipids of chick embryo fibroblasts. Each point represents the mean of two experiments. $^{32}\text{PO}_4$ (131 μCi per plate in the first experiment and 14.8 μCi per plate in the second experiment) was added to all plates after 48 hr of growth. Half the plates were infected at the same time. Incorporation into control (○) and infected (□) cells is expressed as a percentage of that incorporated into control cells after 24 hr of labeling.

when ^{14}C -glycerol was supplied in the medium, incorporation of ^{14}C into lipid at all infection periods was inhibited (Fig. 3). A similar effect on MK cells was evident (Table 2) indicating that

FIG. 2. Effect of Sendai virus infection on ^{14}C -glucose incorporation into lipids of chick embryo fibroblasts. Incorporation of ^{14}C -glucose, 335 $\mu\text{Ci}/\mu\text{mole}$ (2.5 μCi per plate), into control (○) and infected (□) cells is expressed as a percentage of that incorporated into control cells after 12 hr of labeling. Each point represents the mean of two experiments.

the phenomenon was not confined to the less productive system.

The only differences between the experiments shown in Fig. 1–3 were the types and amounts of radioactive precursors in the growth media. Because it was unlikely that these minute chemical differences would alter the metabolism of the cell, two other explanations for the effect on ^{14}C -glycerol incorporation were suggested. First, virus infection could limit intracellular availability of some precursors, and this would be

particularly noticeable in the ^{14}C -glycerol experiments because the only glycerol in the medium was that supplied in the radioactive form ($3.4\text{ }\mu\text{g}$ of glycerol as compared with 8.4 mg of unlabeled glucose and 1.7 mg of phosphate ion per plate).

FIG. 3. Effect of Sendai virus infection on ^{14}C -glycerol incorporation into lipids of chick embryo fibroblasts. Each point represents the mean of two experiments. Glycerol- $U\text{-}^{14}\text{C}$, $13.3\text{ }\mu\text{Ci}/\mu\text{mole}$ ($0.22\text{ }\mu\text{Ci}$ per plate in the first experiment and $0.50\text{ }\mu\text{Ci}$ in the second experiment, each containing 1.7 to $3.5\text{ }\mu\text{g}$ of glycerol), was added to all the plates. Half the plates were infected at the same time. Incorporation into control ($\circ$) and infected ($\square$) cells is expressed as a percentage of that incorporated into control cells after 12 hr of labeling.

TABLE 2. Effects of Sendai virus infection on incorporation of ^{14}C from ^{14}C -glycerol into lipids of monkey kidney cells^a

Cells	Period of labeling or labeling and infection (hr)	Incorporation of radioactivity into lipid (counts/min/mg of protein)
Control	12	4,100
	18	5,600
Infected	12	1,635
	18	2,710

^a Glycerol- $U\text{-}^{14}\text{C}$ ($0.50\text{ }\mu\text{Ci}$ containing $3.5\text{ }\mu\text{g}$ of glycerol) was added to all culture bottles at the time of addition of Sendai virus to half of them. Six bottles were combined for each time point.

Second, it was also considered possible that the allantoic fluid in which virus was suspended could contain trace amounts of glycerol, thus lowering the specific activity of that added to the infected cells' medium with a consequent apparent reduction in glycerol incorporation into lipid and other components. To test this second possibility, sham infection and the second procedure for virus infection were used (Table 3). A number of points emerge from these experiments. There was increased synthesis of lipid at extended periods of infection (12 hr and upwards) as shown by increased incorporation of ^{32}P and ^{14}C from ^{14}C -glucose. The extent of the effect varied considerably (a feature of all of these experiments) as also did the incorporation of radioactivity into controls; a linear relationship between time and isotope incorporation was not always maintained. In a few cases, the addition of Sendai virus-free allantoic fluid had a small stimulatory effect on lipid synthesis. However, the results in general indicate that control and sham-infected cells do not differ considerably. Sham-infected cells, at least at the earlier stages of infection, displayed some of the reduced ^{14}C -glycerol incorporation characteristic of infected cells. This inhibitory effect of sham infection due to lowering of specific activity of added radioactive precursors by trace amounts of glycerol in allantoic fluid could partially explain the effect attributed to infection in Fig. 3, because, in the presence of much higher levels of added glycerol, the more usual stimulatory effect on lipid synthesis of infection was obvious (Table 3). Synthesis of lipid of MK cells was stimulated after 6 hr of Sendai virus infection (Table 3). There was some evidence that this was preceded at short infection periods by inhibited incorporation or uptake, a point which is examined below.

Pattern of labeling of glycerides in infected cells. Lipids from normal CE cells labeled with ^{32}P were chromatographed on thin-layer plates, located with iodine, and counted. Phosphatidylcholine and phosphatidylinositol each accounted for over 40% of radioactivity after a 3-hr labeling period with no appreciable labeling of other phospholipids. With longer labeling (12 hr), phosphatidylcholine accounted for 77% , phosphatidylinositol for 13% , phosphatidylethanolamine for 10% , and sphingomyelin for about 0.3% of radioactivity. These findings are in general agreement with more complete analyses of the rates of labeling of phospholipids of cultured chick fibroblasts (P. L. Marion, Ph.D. thesis, University of California, Berkeley, 1970).

A comparison of thin-layer chromatograms of intact lipids and paper chromatograms of

TABLE 3. Comparison of sham infection and usual control with two methods of Sendai virus infection on incorporation of radioactive precursors into lipids of chick embryo and monkey kidney cells^a

Cells	Period of labeling (hr)	Type of cells	Infection procedure, period of exposure to virus	Incorporation of radioactivity into lipid (counts/min per mg of protein)			
				³² P _O ₄	¹⁴ C-glucose	¹⁴ C-glycerol	
						3.4 µg/plate	625 µg/plate
Chick embryo	6	Control	I, 6 hr	33,300	3,295	29,700	475
		Sham-infected		32,800	3,300	25,850	500
		Sendai-infected		37,300	2,910	20,400	520
	12	Control	I, 12 hr	81,000		36,800	880
		Sham-infected		86,500		38,100	823
		Sendai-infected		104,100		31,300	1,630
	24	Control	I, 24 hr		16,040		
		Sham-infected			18,630		
		Sendai-infected			22,150		
	24	Control	II, 1 hr		16,380		
		Sham-infected			16,900		
		Sendai-infected			19,200		
Monkey kidney	0.5	Sham-infected	II, 0.5 hr		587		
		Sendai-infected			418		
	1.5	Sham-infected	II, 0.5 hr		978		
		Sendai-infected			1,063		
	6.5	Sham-infected	II, 0.5 hr		3,180		
		Sendai-infected			5,060		

^a Infection procedures were as described in Material and Methods. ³²P-orthophosphate (233 µCi) or glucose-*U*-¹⁴C (2.5 µCi) was added to each plate. In one set of the glycerol experiments, 0.5 µCi of glycerol-*U*-¹⁴C was added to each plate, and in the second set this was supplemented with unlabeled glycerol.

deacylated lipids from ³²P-labeled control and infected cells showed equally stimulated synthesis of all classes of phospholipids after infection. However, when lipids obtained from infected cells exposed to small amounts of ¹⁴C-glycerol of high specific activity were examined by the same means, a consistent alteration of the pattern was evident (Fig. 4). The amount of label in the bulk of lipids, the phospholipids, was reduced, thereby accounting for the reduced radioactivity in total lipid, and there was increased label in glycerol from neutral glyceride over that in control cells. Under these conditions, there was equal suppression of synthesis of all classes of phospholipids, as shown by paper chromatography of the glycerylphosphoryl derivatives and thin-layer chromatography of intact lipids (Fig. 5). The additional radioactivity in the glycerol from infected-cell neutral lipid was shown by thin-layer chromatography to arise from triglyceride (Table 4), and the reproducibility of the effect was also established. Also, under certain conditions (as when infection periods were extended) incorporation of ¹⁴C from glucose into CE cells and their lipids was inhibited, but incorporation into triglyceride was considerably increased (Table 4). However, the effect was

absent from cells labeled with ¹⁴C-glucose under the usual conditions (Table 4) and also when high levels of glycerol were present in the medium. Increased incorporation of ¹⁴C from ¹⁴C-glycerol into triglyceride was absent in MK cells (Table 4) under the conditions which brought about decreased overall incorporation into lipid (Table 2). Considerably more of the radioactivity from ¹⁴C-glycerol entered the triglyceride of normal MK cells than CE cells. Thus, it appeared that even when overall incorporation was inhibited by Sendai virus infection, increased triglyceride synthesis took place in the CE cell system.

Early effects of Sendai virus infection on glucose uptake by CE cells. The first suggestion offered for the effects of Sendai virus infection on the incorporation of trace amounts of glycerol was that its uptake was inhibited. To determine if this was a more general effect of virus infection, the uptake of glucose, a normal component and major carbon source of the medium, was examined using the assay system of Hatanaka et al. (15). Normal and infected cells were exposed to pulses of ¹⁴C-glucose at various times during the first 6 hr of infection. The pulse was ended by removal of medium and thorough washing of the monolayer; then cells were suspended in 1 ml of

FIG. 4. Pattern of labeling of total glycerides from Sendai virus-infected cells with inhibited ^{14}C -glycerol incorporation. Experimental details are described in Fig. 3. Lipids were deacylated and the water-soluble material (about 80% of lipid radioactivity) was passed through a small column of Dowex 50(H^+) (Bio-Rad Laboratories) and chromatographed on Whatman no. 1 paper in ethyl acetate-acetic acid-formic acid-water (18:3:1:4), descending for 18 hr. Hatched area is material from 46-hr-infected cells and clear area is that from control cells. (Sham-infected cells were identical to control cells.)

water and homogenized briefly by vibration with a Vortex mixer. Samples were then counted for radioactivity (Table 5). A definite and significant decrease in the uptake of glucose was observed in all experiments at short pulse periods, even in the first 30 min after exposure to virus. The time of termination of this effect varied independently of length of infection period. In one experiment, inhibition of uptake terminated within 15 min after the addition of glucose, whereas in others there was evidence for the effect after 20 to 30 min of labeling. It is possible that infection by Sendai virus changes the surface of CE cells so that the rate of uptake of glucose is slower than that in normal cells, and only after a short but variable lag time is the impaired transport system able to provide an intracellular concentration of glucose sufficient to support the stimulated metabolism induced by virus infection. If, as in the case of experiments where minute quantities of glycerol are available in growth medium, extracellular concentrations of precursors are very low, the disrupted or numerically decreased transport structures may never be able to supply

FIG. 5. Pattern of labeling of intact glycerides from Sendai-infected cells with inhibited ^{14}C -glycerol incorporation. Experimental details are described in Fig. 3. Lipid was chromatographed on plates of Silica Gel H (40 cm long) in chloroform-methanol-water (65:35:4). Standard phospholipids were located with molybdenum blue reagent (8). Radioactive lipids from control and 42-hr-infected cells were located with iodine, and the chromatograms were divided into 1-cm bands and counted. Hatched area indicates material from infected cells.

an intracellular concentration of nutrient sufficient to support the potentially stimulated metabolism.

Cells labeled for very short periods, harvested, and disrupted by suspension in water were centrifuged at 1,000 rev/min for 10 min. Generally 85 to 90% of the radioactivity was in the

TABLE 4. *Distribution of incorporated radioactivity in neutral and phosphoglycerides of infected cells^a*

Cells	Radioactive precursor	Period of labeling (hr)	Per cent incorporation of total lipid radioactivity (counts/min per mg of protein)		
			Phosphoglyceride	Triglyceride	Diglyceride
Control CE cells	¹⁴ C-glycerol (3.5 μ g per plate)	1 to 12	92.68 $\pm$ 0.62 (7) ^b	1.48 $\pm$ 0.07 (7)	2.87 $\pm$ 0.73 (7)
		24 to 48	89.00 $\pm$ 0.91 (4) ^c	3.09 $\pm$ 1.37 (4)	5.75 $\pm$ 0.93 (4)
	¹⁴ C-glycerol (860 μ g per plate)	12	87.00 ^d	4.10	8.30
	¹⁴ C-glucose	12	83.00		11.10
Infected CE cells	¹⁴ C-glycerol (3.5 μ g per plate)	3 to 12	91.85 $\pm$ 1.45 (6)	3.47 $\pm$ 0.08 (6)	2.75 $\pm$ 1.18 (6)
		24 to 48	84.25 $\pm$ 3.57 (4)	9.23 $\pm$ 3.67 (4)	3.77 $\pm$ 0.54 (4)
	¹⁴ C-glycerol (860 μ g per plate)	12	87.00	3.30	8.30
	¹⁴ C-glucose	12	85.90		10.80
Control MK cells		48	72.80		20.30
Control MK cells	¹⁴ C-glycerol (3.5 μ g per plate)	12	42.50	51.00	0
		18	47.00	44.30	2
Infected MK cells	¹⁴ C-glycerol (3.5 μ g per plate)	12	47.00	50.00	0
		18	48.50	38.50	0

^a The types of experiments carried out are described in Fig. 2 and 3 and Table 2. Lipids from ¹⁴C-glycerol-labeled cells were chromatographed on plates of Silica Gel G in light petroleum-diethyl ether-acetic acid (85:15:2), and the three groups of glycerides were located with iodine, cut out, and counted for radioactivity. Lipids from ¹⁴C-glucose-labeled cells were deacylated and chromatographed on paper as described in Fig. 5.

^b Figure in parentheses refers to the number of chromatographic determinations at different time points from two experiments.

^c Number of determinations carried out at different time points in two experiments which were separate from those quoted for the shorter periods.

^d Results of one experiment.

supernatant fluid. In the case of cells pulsed for 5 min, 75 to 78% of this radioactivity was free glucose, about 11% corresponding to hexose mono- and diphosphates and about 2% to non-chromatographable material. Therefore, the phenomenon examined at short pulse periods is largely glucose transport. After 1 hr of labeling, only 47% of radioactivity was in the form of free glucose. At extended periods of labeling (6 hr and over) there was no appreciable free glucose in the cell.

The increased incorporation of glucose after 5 to 6 hr (Table 5) was only partially accounted for by the increased synthesis of phospholipid. There was also increased incorporation into the delipidated residue. A mean value of 1.80 ± 0.23 ($\pm$ standard error from mean) over seven time points was found for the ratio of the total amount of ¹⁴C (from ¹⁴C-glucose) incorporated by infected cells to that incorporated by control cells. This material was hydrolyzed and treated as described above. Only about 1% of the radioactivity was in the form of covalently bound fatty

acids; over 10% was neutral monosaccharides, principally glucose, galactose, mannose, and some unknowns. The remaining radioactivity apparently was amino acids and amino sugars. This excess radioactivity in virus-infected cells could be due to formation and accumulation of virus nucleocapsid (3) or other virus-specific intracellular components.

DISCUSSION

In this paper we present a large body of observations of changes in lipid metabolism in chick fibroblasts resulting from Sendai virus infection. Some of the observations are as yet unexplainable and may be specific to the system used, which is "abortive" in the sense that little infectious virus is produced although large amounts of virus precursors accumulate within infected cells (3). Considerably more work is required to determine if the observations are closely linked to this abortive infection or are valid for cells more actively producing virus.

TABLE 5. *Effects of Sendai infection on ^{14}C -glucose uptake by chick embryo fibroblasts^a*

Expt no.	Period of pulse (min)	Type and length of infection	Uptake of radioactivity (counts/min per mg of protein)		Per cent inhibition of uptake
			Control cells	Infected cells	
1	5	II, ^b 1 hr	56,100	32,400	42.4
	10		62,500	46,000	26.4
	15		81,600	81,600	0
2	10	II, 4 hr	65,200	50,500	22.5
	20		81,500	64,500	20.9
3	15	II, 6 hr	81,000	72,500	10.5
4	30	I, 30 min	4,250	3,340	21.5
	60	I, 60 min	5,310	6,040	
	300	I, 300 min	13,100	28,200	

^a ^{14}C -glucose (330 $\mu\text{Ci}/\mu\text{mole}$) (5 μCi) was added to each plate in experiments 2 and 4, and ^{14}C -glucose (3 $\mu\text{Ci}/\mu\text{mole}$) (19 μCi) was added in experiments 1 and 3. Cell sheets were washed three times with phosphate-buffered saline. Cells were removed with a rubber policeman, centrifuged and washed twice more in the tubes before counting.

^b I and II refer to the first and second Sendai infection procedures, respectively.

Shibuta et al. (26, 27) demonstrated increased phospholipid synthesis in cells both productively and abortively infected with Sendai virus after pulses of ^{32}P throughout the infection cycle. Figard and Levine (9) also found much greater incorporation of labeled precursors (glucose and acetic acid) into lipids of Rous sarcoma virus (RSV)-induced tumors on chorioallantoic membranes when compared with uninfected membranes. In this system stimulated synthesis could be related either to virus replication or to malignant cell transformation. On the other hand, Pfefferkorn and Hunter (23) and Waite and Pfefferkorn (31) showed that there was a decrease in phospholipid synthesis in primary CE cells after infection by a lipid-containing arbovirus, although virus was actively released by the cells.

The results of McIntosh, Payne, and Russell (20) on the lipids of hamster embryo kidney (HEK) cells after adenovirus infection show remarkable similarities between their system and ours. In view of the dissimilarities in the structure of the two viruses (different types of nucleic acid, different capsid symmetry, and the absence of a lipoprotein envelope in adenovirus) and their different modes of replication, it is difficult to assign a common role to the phenomenon of stimulated lipid synthesis. The demonstration here that incorporation into nonlipid components

is also stimulated after Sendai virus infection suggests that there is a generalized enhancement of metabolism in virus-infected cells. In the case of influenza virus infection (16) and in tumors induced in chorioallantoic membranes by RSV (9), it is probable that stimulated lipid synthesis is part of an overall enhanced metabolism. This applies particularly to the work with transformed cells (9) in which incorporation of both ^{14}C -glucose and ^{14}C -acetate into all lipids, with the possible exception of sphingomyelin, was increased. Moreover, the effects on lipids were accompanied by greatly stimulated glucose oxidation and amino acid incorporation.

Because the Sendai virion acquires its membrane-like outer coat by budding through the plasma membrane of a host cell, it could be expected that this is provided for by increased synthesis of membrane components. Even in this system where few virus particles are released, virus infection may induce such stimulated synthesis. We present evidence that this stimulatory effect begins at an early stage in the replication cycle (before virus-specific RNA synthesis is detectable), suggesting that it may not be directed by the virus genome but may be brought about by the mere presence of either the virus or some component of it in the cell. McIntosh et al. (20) showed that increased lipid synthesis in adenovirus-infected HEK cells could be induced by a purified component of the virus capsid. However, the demonstration by Shibuta et al. (26) that ultraviolet-inactivated Sendai virus does not induce increased phospholipid synthesis argues against this possibility for our system, and, because it is now known that Sendai virions possess an RNA-transcriptase (29), it can be assumed that virus-specific RNA synthesis may begin immediately after uncoating.

In the case of picornavirus infection of mammalian cells (6, 22), the need for new membranous structures has also been used to explain increased phospholipid synthesis, although the virions contain no lipid (1, 22). Recent evidence, however, indicates that newly synthesized phosphatidylcholine is incorporated into cytoplasmic membranes other than those of the virus-specific RNA-synthesizing complex (24). Lipid-containing membranes may also play a role in several stages of adenovirus replication (17, 19, 20).

Much of the work on the effects of Sendai infection on ^{14}C -glycerol incorporation into lipids may not be relevant to an understanding of the biochemistry of virus replication because free glycerol is not normally supplied to the cells. However, the phenomenon was responsible for discovery of the effect of infection on glucose

uptake by CE cells. There are several features common to the transport of glucose and glycerol in erythrocytes from a number of sources. The transport of each substrate meets with many of the criteria for facilitated diffusion outlined by Stein (28). In the context of our present work, it is possible that virus attachment or replication blocks in some way the transport systems for glycerol and glucose. One can imagine that when there are low levels of these precursors in the medium, inactivation of a few transport molecules could considerably reduce precursor intake. When there are high levels of precursors in the medium, the reduced number of available transport sites is still sufficient to allow uptake to proceed at a slightly reduced pace, but this is not obvious due to stimulated lipid synthesis. Again, considerably more work is required to definitively establish these assumptions and the connection between glucose and glycerol transport and to show the role of altered transport properties in infection.

The inhibitory effect of Sendai infection on the uptake of glucose is the opposite of the effects of RSV and murine sarcoma virus (MSV) transformation (14, 15). Cells transformed by both sarcoma viruses exhibit a marked and specific increase in sugar uptake. It is interesting that phosphoric acid uptake was not stimulated in MSV-transformed mouse cells (15). The absence of Sendai virus-mediated inhibition of phosphate uptake in the present experiments would provide for the markedly enhanced synthesis of ^{32}P -labeled phospholipid in infected cells as compared to ^{14}C -glucose-labeled lipid.

The observed increase in triglyceride synthesis in infected CE cells under conditions of overall depressed precursor incorporation is a phenomenon we find difficult to explain. Some of the lipids of infected cells may be synthesized at an enhanced rate under all conditions. Triglycerides and phosphoglycerides arising from either glucose or glycerol are synthesized by acylation of α -glycerophosphate to yield phosphatidic acid, after which the pathways diverge. It is conceivable that in infected cells there is increased activity of the phosphatidic acid phosphatase or the diglyceride acylase. However, if this were so one would expect substantially more triglyceride synthesis when ^{14}C -glucose was a precursor. There may be two pools of triglyceride in the cell or two separate pathways for its biosynthesis. The effect is interesting since there was increased percentage of incorporation of ^{14}C -acetate into triglyceride under conditions of increased total lipid synthesis in both RSV-induced tumors (9) and adenovirus-infected HEK cells (20). There-

fore, the conditions under which increased synthesis occurs in Sendai infection are the opposite of those in other systems. One set of conditions necessary to demonstrate this effect involved extended infection with resulting decreased overall incorporation from ^{14}C -glucose. At the early stages of adenovirus infection of HEK cells, incorporation of ^{14}C -acetate into lipid was stimulated, but at later times the specific activities of the lipids extracted from infected cells showed a marked decrease.

ACKNOWLEDGMENTS

We thank Mairead Kelly for skilled technical help. The work was supported by the Irish Cancer Society, and P. B. was supported by the National Science Council, Ireland.

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