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Interferon stimulates cholesterol and phosphatidylcholine synthesis but inhibits cholesterol ester synthesis in HeLa-S3 cells

(human β-interferon/lipid metabolism/membrane structure/endocytosis/low density lipoprotein)

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ABSTRACT **Treatment of human HeLa-S3 cells (an epidermoid carcinoma line) with human β-interferon (640 units/ml) selectively alters lipid metabolism by increasing cholesterol synthesis per mg of cell protein as measured by 1-hr pulse-labeling of cells with [^{3}H]acetate. Cholesterol synthesis in interferon-treated cells is increased ≈60% at 24 hr after the beginning of treatment and ≈450% at 48 hr. Continuous labeling of interferon-treated cells with [^{14}C]acetate shows increased accumulation of label in cholesterol when normalized per mg of cell protein, as well as an increase in the specific activity of cholesterol in the treated cells. In contrast, interferon treatment decreases the accumulation of [^{14}C]acetate into cholesterol esters. The [^{14}C]acetate labeling of sphingomyelin, phosphatidylethanolamine, and triglycerides shows no change compared to untreated controls. The labeling of phosphatidylcholine was moderately increased in treated cells. The interferon-induced changes in lipid metabolism are a part of a coordinated response of cells to interferon treatment, characterized by reduced cell proliferation and cell motility and an increase in cell size and mass. The increased cholesterol synthesis is consistent with a model in which β-interferon treatment of HeLa cells inhibits the endocytosis of cholesterol-containing low density lipoprotein, which results in an increase in cholesterol synthesis.**

Interferons are inducible secretory cellular proteins that interact with discrete plasma membrane receptors and alter cellular gene expression (reviewed in refs. 1 and 2). Interferons inhibit viral replication by a number of distinct mechanisms and modulate cell structure and functions. Treatment of human skin fibroblasts and HeLa-S3 tumor cells with human β-interferon elicits a coordinated cellular response, including alterations in the plasma membrane–cytoskeleton complex. In HeLa-S3 cells, interferon treatment results in *increased* submembranous meshwork of microfilaments (3), rigidity of the plasma membrane lipid bilayer (4), and cell size (5), and *decreased* proliferation rate of cells (6), mobility of cell surface receptors for concanavalin A (7), and receptor-mediated endocytosis of concanavalin A (5). The development of marked alterations in the phenotype of interferon-treated HeLa-S3 cells requires 1–2 days although some effects can be detected within 12 hr after the beginning of treatment.

The plasma membrane lipid bilayer displays increased rigidity after treatment of HeLa-S3 cells with β-interferon for 30 min, but the rigidity reverts to the baseline value within 3–5 hr (4). However, 24 hr after the addition of interferon the plasma membrane lipid bilayer of the cells again is more rigid and remains so for at least 2 days (4). Chandrabose *et al.* (8) demonstrated that treatment of mouse S-180 sarcoma cells with mouse interferon results in decreased unsaturated fatty acid content of all major phospholipids in the cells. An increase in the ratio of saturated to unsaturated fatty acids in the plasma membrane of interferon-treated cells would be consistent with increased rigidity of the plasma membrane lipid bilayer.

In this communication, the time-dependent effect of β-interferon on lipid metabolism in HeLa-S3 cells is reported. Treatment of HeLa-S3 cells with β-interferon (640 units/ml) for 24 hr or longer results in increased accumulation of radioactive acetate in cholesterol but in decreased incorporation of acetate into cholesterol esters when expressed on a per mg of protein basis. The effects of β-interferon on lipid metabolism are selective in that there are no significant differences between control and treated cells in cumulative acetate incorporation per mg of cell protein into sphingomyelin, phosphatidylethanolamine, and triglycerides. After 24- or 48-hr treatment of cells with interferon, the cholesterol content is increased on a per cell but not on a per mg of protein basis. Expressing the results on a per cell basis is biased by the fact that the interferon-treated cells grow larger and increase in mass relative to control cells as the proliferation rate of the treated cells declines. Expressing results per ml of cell suspension is nearly equivalent to expressing the results per mg of protein as the total amount of cell protein in interferon-treated cultures relative to control cultures remains essentially unchanged over a 60-hr period of incubation. The data are expressed in this report per ml of cell suspension, per mg of cell protein, or per cell to emphasize different aspects of the results.

MATERIALS AND METHODS

Cell Cultures. Human HeLa-S3 cells were grown in suspension culture at cell concentrations between 1×10^5 and 1.3×10^6 cells per ml in Eagle's minimal essential medium modified for spinner culture and supplemented with 4% fetal calf serum. For experiments, HeLa-S3 cell cultures that had attained a density of $1–1.3 \times 10^6$ cells per ml were harvested by pelleting at $800 \times g$, and the cells were resuspended at 2×10^5 cells per ml in fresh medium supplemented with 4% fetal calf serum and containing human β-interferon at 640 units/ml. Control cultures received no interferon. Cultures were maintained in suspension in 75-cm^2 Falcon tissue culture flasks on a reciprocating shaker set at 100 excursions per min.

For experiments on the cumulative incorporation of sodium [^{14}C]acetate (New England Nuclear; 57.5 mCi/mmol; 1 Ci = 37 GBq) into cell lipids, the radioactive precursor was added to tissue culture medium (0.125 μCi/ml) at the time of dilution of the cultures to a density of 2×10^5 cells per ml and addition of interferon. Twenty-four and 48 hr later 15-ml aliquots of the cell suspension were removed, pelleted at $800 \times g$, and washed three times with phosphate-buffered saline (P_i/NaCl; NaCl, 8 g/KCl, 0.2 g/NaH_2PO_4, 0.2 g/$CaCl_2$, 0.1 g/$MgCl_2 \cdot 6\ H_2O$, 0.1 g/pH 7.4, in 1 liter of solution). An aliquot of the cell suspension was analyzed on a Coulter

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Counter model ZM (Coulter Electronics) and the remainder was frozen at −20°C for lipid analysis.

For pulse-labeling of cell samples, sodium [^{3}H]acetate (New England Nuclear, 3.0 Ci/mmol) was added to the cell culture medium (1.5 μCi/ml) and the cultures were incubated for 1 hr at 37°C.

Lipid Analysis. Aliquots of HeLa-S3 cells, labeled with radioactive sodium acetate, were resuspended in 0.5 ml of P_i/NaCl in glass test tubes with Teflon-lined caps and sonicated for 10–15 sec with a Branson probe sonicator set at 50 W. After sonication, an aliquot was removed to determine protein concentration (9). The Folch *et al.* extraction procedure (10) was used and the lipids of the sonicated cells were isolated from the lower phase. The extracted lipids were dried under a gentle stream of nitrogen then resuspended and agitated (Vortex) in 1 ml of chloroform/methanol, 2:1 (vol/vol). Aliquots (50–100 μl) of the solubilized lipids were then analyzed by thin layer chromatography (TLC) on Silica Gel 60 TLC plates (Merck) and developed using hexane/ethyl ether/acetic acid, 90:10:5 (vol/vol). The lipid bands were visualized with iodine vapor. Under these conditions, neutral lipids can be readily separated with no detectable contamination from cholesterol. For determination of the radioactivity associated with regions on the chromatograph corresponding to the comigrating lipid standards, the spots were circled with a pencil, scraped with a scalpel, resuspended in 0.2 ml of distilled water, mixed with 4 ml of Liquiscint (National Diagnostics, Somerville, NJ) and assayed in a liquid scintillation spectrometer. Acetate incorporation into the lipids of interferon-treated HeLa-S3 cells is expressed relative to controls.

Interferon. Partially purified preparations of human β-interferon (1–3 × 10^7 units/mg protein) were provided by Julius S. Horoszewicz of Roswell Park Memorial Institute. Interferon activity was assayed on monolayers of human fibroblasts by a semimicro titration procedure based on inhibition of the cytopathic effect of vesicular stomatitis virus (11), using the National Institutes of Health human β-interferon standard (catalog no. G-23-902-527) for calibration. Interferon concentrations are expressed in terms of international reference units/ml.

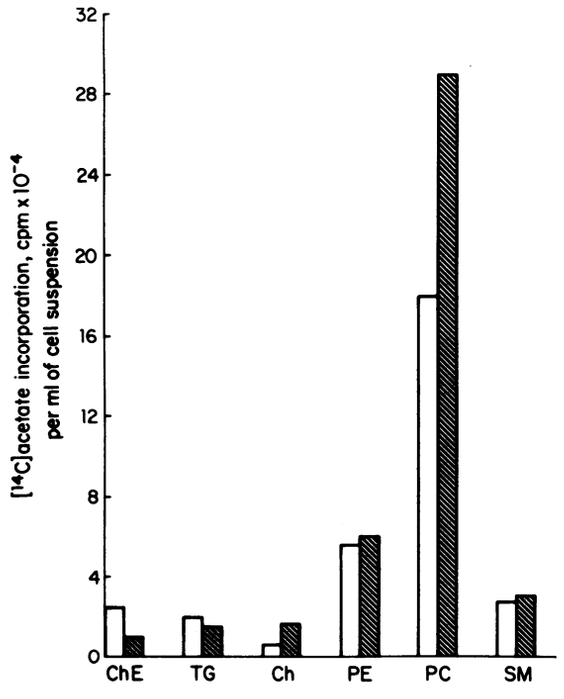

FIG. 1. Effects of interferon on accumulation of [^{14}C]acetate in cellular lipids. HeLa-S3 cells were pelleted and diluted to a density of 2 × 10^5 cells per ml in medium containing β-interferon (640 units/ml) and [^{14}C]acetate (0.125 μCi/ml). Control cultures received no interferon. After incubation for 48 hr, total cellular lipids were extracted and aliquots containing approximately equivalent amounts of radioactivity separated by thin layer chromatography. The radioactivity associated with cholesterol (Ch), triglycerides (TG), cholesterol esters (ChE), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM) was determined in lipid extracts from control (open bars) and interferon-treated HeLa (hatched bars) cells. The recorded counts represent mean values per ml of cell suspension of duplicate samples from three separate experiments. A change of >25% in acetate incorporation into cellular lipids of interferon-treated HeLa-S3 cell is significant.

RESULTS

Effects of Interferon on Acetate Incorporation into Cell Lipids. Treatment of HeLa-S3 cells with β-interferon results in alterations in [^{14}C]acetate incorporation into lipids, with notable changes in incorporation into cholesterol, cholesterol esters, and phosphatidylcholine. Fig. 1 shows that interferon treatment (640 units/ml, 48 hr) caused an ≈3-fold increase in incorporation of radioactive acetate into cholesterol but an ≈50% decrease in incorporation into cholesterol esters measured per ml of culture. Incorporation of [^{14}C]acetate into phosphatidylcholine was increased 50% by interferon treatment. Radioactive acetate nonspecifically labels the cell lipids.

Calculated on a per cell basis the incorporation of radioactive acetate into cholesterol was increased 6-fold in the interferon-treated cultures, whereas that into cholesterol esters showed little if any change. Qualitatively similar effects on acetate incorporation into cholesterol and cholesterol esters were observed already 24 hr after the beginning of interferon treatment, but the effects were not as pronounced as at 48 hr.

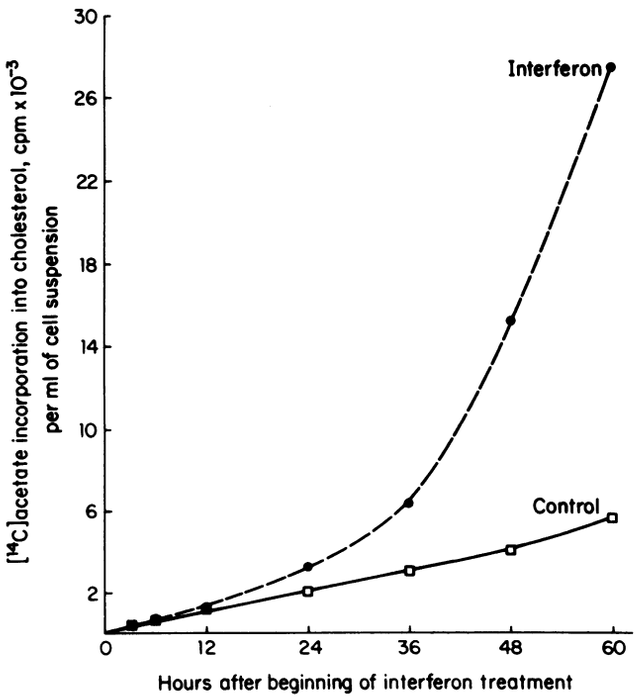

FIG. 2. Effect of interferon on cumulative incorporation of [^{14}C]acetate into cholesterol in HeLa-S3 cells. At various times after the addition of interferon and [^{14}C]acetate, incorporation of the labeled precursor into cholesterol per ml of cell suspension was determined in lipid extracts from control (□) and interferon-treated (●) HeLa cells. The results from three separate experiments, each done in duplicate, were averaged.

It is important to emphasize that the interferon-induced changes in cumulative acetate incorporation into lipids per ml of cell suspension were selective for cholesterol, cholesterol esters, and phosphatidylcholine. No significant differences were observed between control and interferon-treated cultures in the uptake of acetate into sphingomyelin, phosphatidylethanolamine, and triglycerides per ml of cell suspension.

Time Course of Interferon Effects on Cumulative [^{14}C]Acetate Incorporation into Cholesterol and Cholesterol Esters. Cumulative incorporation of [^{14}C]acetate into cholesterol and cholesterol esters was investigated as a function of time after the beginning of interferon treatment. After 12-hr treatment with interferon, the level of incorporation of [^{14}C]acetate into cholesterol, expressed per ml of cell suspension, was similar in control and interferon-treated HeLa cells (Fig. 2). However, by 24 hr after the beginning of treatment the cumulative incorporation of acetate into cholesterol was increased in interferon-treated HeLa-S3 cells by ≈50% as compared to controls. At 60 hr after the beginning of treatment, the level of [^{14}C]acetate accumulation into cholesterol in interferon-treated cells was ≈5 times that in control cells.

As shown in Fig. 3, at 24 hr after the beginning of interferon treatment, [^{14}C]acetate incorporation into cholesterol esters appeared to be slightly inhibited in interferon-treated cells relative to controls. From 36 to 60 hr the inhibition of acetate accumulation into cholesterol esters in interferon-treated cells became more marked and, by 60 hr, the inhibition was ≈50%. Furthermore, the parallel [^{14}C]acetate incorporation into cholesterol (Fig. 2) and cholesterol esters (Fig. 3) in both control and interferon-treated HeLa-S3 cells indicates that the acetate pool is not being depleted.

To define interferon effects further, we measured the protein content and cell number, as well as the accumulation of [^{14}C]acetate into cholesterol and cholesterol esters in control and interferon-treated cultures as a function of time. In Fig. 4, the values obtained for each parameter per ml of interferon-treated cell suspension are expressed as percentages of those for controls. The protein content remains essentially constant while the cell number in interferon-treated relative to control cultures decreases markedly with time. This is consistent with the previously reported results that interferon treatment of HeLa-S3 cells causes a progressive increase in cell volume (7, 12). Thus, as the interferon-treated cells increase in size relative to control cells, the protein content per interferon-treated cell also increases. The relative increase in acetate incorporation into cholesterol of interferon-treated cells considerably exceeds the increase in relative protein content. It is evident that the time course of inhibition of cumulative acetate incorporation into cholesterol esters in the interferon-treated cultures closely parallels the inhibition of cell proliferation.

The Effects of Interferon on Cholesterol and Cholesterol Ester Synthesis in HeLa-S3 Cells. To assess whether the effects of interferon treatment on the incorporation of acetate into cholesterol and cholesterol esters were related to effects on the rates of synthesis in HeLa cells, cultures were incubated with [^{3}H]acetate for 1 hr at 37°C at various times after the addition of interferon (640 units/ml). The choice of [^{3}H]acetate in the short-term labeling experiments is premised on the relatively low specific activity of [^{14}C]acetate. Fig. 5 shows that pulse-labeling of cholesterol with [^{3}H]acetate was not increased per ml of cell suspension by 10 hr after the beginning of interferon treatment but that it was increased by ≈60% at 24 hr and by ≈450% at 48 hr. Fig. 6 shows that

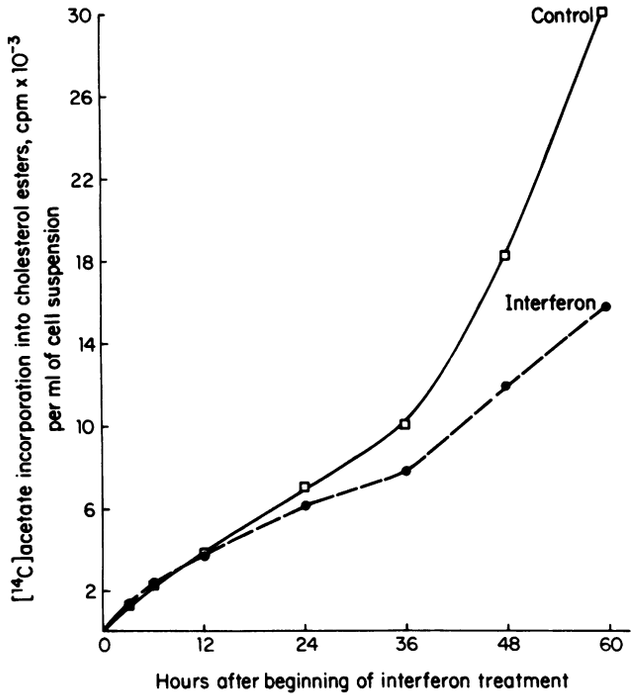

FIG. 3. Effect of interferon on cumulative incorporation of [^{14}C]acetate into cholesterol esters in HeLa-S3 cells. At various times after the addition of interferon and [^{14}C]acetate, the incorporation of the labeled precursor into cholesterol esters was determined per ml of cell suspension in lipid extracts from control (□) and interferon-treated (●) HeLa cells. The results from three separate experiments, each done in duplicate, were averaged.

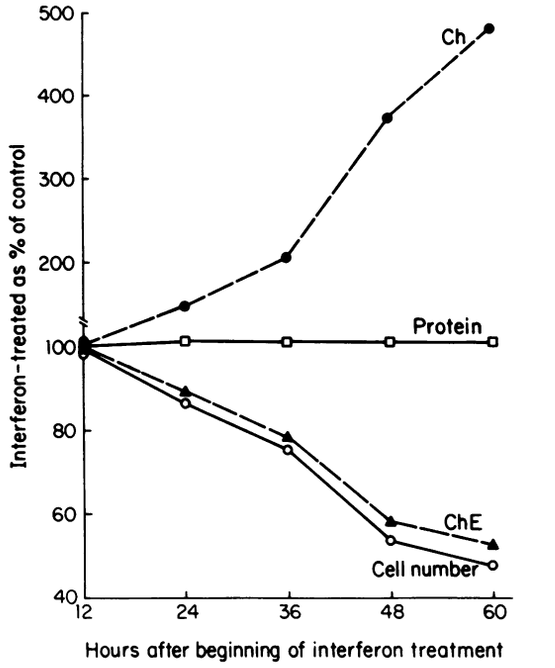

FIG. 4. Effects of interferon on cell number, cellular protein content, and cumulative [^{14}C]acetate incorporation into cholesterol and cholesterol esters in HeLa-S3 cells. At various times after the addition of interferon and [^{14}C]acetate, 5-ml samples of the control and interferon-treated cell suspensions were pelleted and washed in phosphate-buffered saline. An aliquot of each cell suspension was used to determine cell number; the rest of the cells was sonicated, and aliquots were removed to determine protein content. The total cellular lipids were then extracted from the remaining cells and the labeled acetate incorporated into cholesterol and cholesterol esters was measured. Protein concentration (□), cell number (○), and [^{14}C]acetate incorporation into cholesterol (●) and cholesterol esters (▲) were calculated per ml of cell suspension; the values for interferon-treated HeLa cells are expressed as percent of control values. The results represent means of three separate experiments each performed in duplicate.

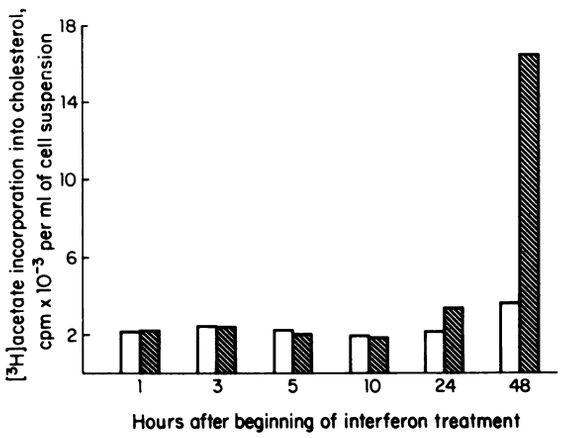

FIG. 5. Effect of interferon on cholesterol synthesis in HeLa-S3 cells. At various times after the beginning of treatment, the incorporation of [^{3}H]acetate into cholesterol per ml of cell suspension during a 1-hr incubation was determined in lipid extracts of control (open bars) and interferon-treated HeLa-S3 (hatched bars) cells. The results represent means of data from two separate experiments each performed in duplicate.

pulse-labeling of cholesterol esters was decreased by ≈30% per ml of cell suspension at 24 hr after the beginning of interferon treatment, but that at 48 hr it was ≈40% greater than that in control cells. Since cholesterol synthesis is enhanced at 48 hr after the beginning of interferon treatment, an increase in acetate incorporation into cholesterol esters through esterification of newly synthesized cholesterol is plausible.

The Effect of Interferon on Cellular Cholesterol Content. Since interferon treatment of HeLa cells results in markedly increased synthesis of cholesterol, the cholesterol content of interferon-treated HeLa-S3 cells was determined by gas/liquid chromatography. As shown in Table 1, interferon treatment of HeLa-S3 cells for 24 or 48 hr increased the total cholesterol content per cell by ≈10% and ≈30%, respectively, as compared to control values. When results are expressed per mg of cell protein, no significant differences in cholesterol content are evident between control and interferon-treated HeLa-S3 cells.

The specific activity of cellular cholesterol (i.e., cumulative incorporation of [^{14}C]acetate into cholesterol per μg cholesterol) was enhanced in HeLa-S3 cells after interferon treatment. At 24 hr after the beginning of treatment, the specific activity of cholesterol in interferon-treated cells (1700 cpm/μg) was ≈1.3 times that in controls (1300 cpm/μg). At

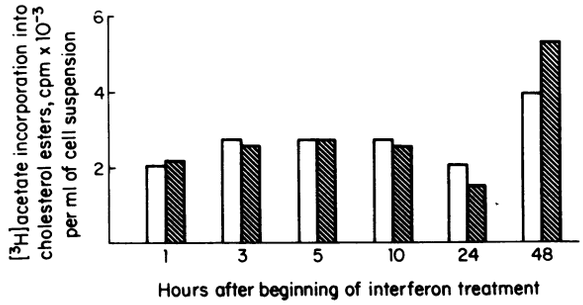

FIG. 6. Effect of interferon on cholesterol ester synthesis in HeLa-S3 cells. At various times after the beginning of treatment, the incorporation of [^{3}H]acetate into cholesterol esters per ml of cell suspension during a 1-hr incubation was determined in lipid extracts of control (open bars) and interferon-treated HeLa-S3 (hatched bars) cells. The results represent means of data from two separate experiments each performed in duplicate.

Table 1. Effect of β-interferon on cholesterol content of HeLa-S3 cells

Treatment	Cholesterol content	
	μg per 10^7 cells	μg/mg of protein
Control: 24 hr	16.6 ± 0.5	9.2 ± 0.5
Interferon: 24 hr	18.7 ± 0.5	9.4 ± 0.4
Control: 48 hr	16.2 ± 0.6	9.6 ± 0.6
Interferon: 48 hr	21.1 ± 0.9	8.6 ± 0.8

Aliquots of ≈1 × 10^7 cells were pelleted and washed, and total cellular lipids were extracted and separated by thin layer chromatography. The cholesterol bands were scraped and transferred to sintered glass funnels, washed three times with 5 ml of diethyl ether, and dried under a gentle stream of nitrogen. The cholesterol content of the samples was determined by gas/liquid chromatography using coprastanol as a standard (13). Results represent mean ± SD for duplicate determinations in two separate experiments.

48 hr the specific activity of cholesterol in interferon-treated HeLa cells (5000 cpm/μg) reached a value ≈2.5 times that in controls (2000 cpm/μg). The increased specific activity of cholesterol probably reflects enhancement of endogenous cholesterol synthesis in HeLa-S3 cells at 24 and 48 hr after the addition of interferon.

DISCUSSION

The results presented show that β-interferon treatment of HeLa-S3 cells markedly enhances cholesterol synthesis within 24 hr after the beginning of interferon treatment, while it inhibits the synthesis of cholesterol esters on a per mg protein basis. The inhibition of acetate accumulation into cholesterol esters over the course of 60 hr of interferon treatment of HeLa-S3 cells correlates with the decreased proliferation rate of interferon-treated cultures, while cholesterol synthesis shows a marked increase during interferon treatment. In addition, acetate accumulation into phosphatidylcholine is moderately increased in interferon-treated cells. These effects of β-interferon on lipid metabolism are selective as accumulation of labeled acetate in sphingomyelin, phosphatidylethanolamine, and triglycerides is similar in control and interferon-treated cultures.

Interferon treatment of HeLa-S3 cells also results in a somewhat greater cholesterol content per cell as compared to controls; however, on a per mg protein basis, cholesterol is not increased in the treated cell. The bulk (80–95%) of cholesterol in cells is in the plasma membrane (14), with little cholesterol in mitochondria, endoplasmic reticulum, and other organelles (15, 16). Thus, the increased cholesterol content of interferon-treated HeLa-S3 cells probably represents an increased cholesterol content of plasma membranes, and may at least in part reflect the demonstrated increase in cell volume of interferon-treated cells (12). Although it is tempting to speculate that an observed increase in cholesterol content of interferon-treated HeLa-S3 cells is directly related to an increased rigidity of the membrane lipid bilayer (4), our evidence does not support this conclusion. The increased rigidity of the membrane lipid bilayer in interferon-treated cells could reflect an increased cholesterol to phospholipid ratio, an increased proportion of saturated relative to unsaturated fatty acyl chains (8) or changes in the interactions among the protein and lipid constituents of the plasma membrane.

The marked enhancement of cholesterol synthesis in HeLa-S3 cells develops between 24 and 48 hr after the beginning of interferon treatment. Previous studies on the time course of development of the effects of interferon treatment on HeLa-S3 cell proliferation (6, 12), concanavalin A receptor mobility (7), membrane rigidity (15), organization of submembranous microfilaments (12) and cell volume (7, 12)

have shown that 24–48 hr is required for the development of maximum interferon effects.

The receptor-mediated endocytosis of the cholesterol-containing low density lipoprotein (LDL) from the serum lipoprotein fraction tightly regulates cholesterol synthesis in cultured cells (17, 18). When rat granulosa cells are grown in medium deficient in lipoproteins, [^{14}C]acetate incorporation into cholesterol is markedly increased, whereas the incorporation of acetate into cholesterol esters is reduced (19, 20). Interferon treatment has been found to inhibit both receptor-mediated endocytosis and pinocytosis. Interferon inhibits the internalization of concanavalin A in HeLa-S3 cells (5), and of horseradish peroxidase and vesicular stomatitis virus in mouse L cells (21, 22). Interferon treatment of thioglycolate-elicited mouse macrophages results in inhibition of pinocytosis of horseradish peroxidase and fluorescent dextran (23).

Interferon treatment of HeLa cells results in a marked increase in [^{14}C]acetate incorporation into cholesterol and an inhibition of incorporation into cholesterol esters. We hypothesize that interferon treatment inhibits receptor-mediated endocytosis of LDL and therefore the uptake of exogenous cholesterol, which secondarily results in an increase in endogenous cholesterol synthesis.

However, an increased acetate incorporation into cholesterol is also consistent with an increased hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase activity in interferon-treated HeLa-S3 cells. Under physiological conditions, the enzyme HMG-CoA reductase catalyzes the rate-limiting reaction of cholesterol synthesis. The enzyme has been shown to be sensitive to hormonal regulation. For example, treatment of several cell lines with insulin results in an increased acetate incorporation into cholesterol and a parallel increase in HMG-CoA reductase activity (24).

Further experiments are needed to establish the mechanism by which interferon increases cholesterol synthesis in cells in the presence of serum in the medium.

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