Prolonged Application of Interferon and "Aging" of Human Diploid Fibroblasts (35541)

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Interest in the possible clinical use of interferon has raised the question of what effect prolonged or repeated application of exogenous interferon may have on cells. It has been shown in short-term experiments that purified interferon has no adverse effect on the synthesis of DNA, RNA, or protein in cultured cells, nor does it cause reduction in normal growth and division (1–3). This report concerns the effect of a constant level of exogenous interferon on the establishment, growth, and lifespan in culture of human diploid fibroblasts.

Materials and Methods. Cell cultures. Human foreskin cell cultures were prepared by trypsinization of neonatal foreskins. Dispersed cells were seeded in Falcon plastic flasks, in Eagles' basal medium (BME) with 20% fetal calf serum. When monolayers grew to confluency, serum was reduced to 10% and cultures were split 2 to 1 every 3 or 4 days for their entire lifetime in culture, anywhere from 40 to 60 passages. Cells were considered to be in their "decline phase" when they no longer could produce a confluent monolayer in 3 or 4 days after routine splits, and their useful lifespan was considered at an end when such transfers produced only a sparse growth of cells.

Production and assay of interferon. Two lots of human interferon (designated HIF-2 and HIF-3) were used in these studies. Both were produced by exposing established human foreskin cell cultures to Newcastle disease virus (NDV) (received from Dr. B. R. Forsyth, University of Vermont) at a concentration of 4×10^{-5} hemagglutination units/cell for 1 hr at 35°. Monolayers were then washed and BME with 5% fetal calf serum was used as a medium replacement. Culture fluids were harvested at 24 hr and were adjusted to pH 2 for 4 to 5 days, then neutralized and centrifuged at 100,000*g* before use. The double-stranded synthetic polynucleotide polyinosinic:polycytidylic acid (poly I:C) (Miles Laboratory, Elkhart, Indiana) was used to stimulate interferon production in certain experiments. Poly I:C at 10 or 40 μg/ml in medium containing 100 μg/ml of DEAE-dextran (mol wt 2×10^6, Pharmacia Fine Chemicals, Inc.) (4) was adsorbed on the cells for 1 hr at 35°. Monolayers were then washed, and the 5% fetal calf serum–BME medium was harvested after 24 hr (5). Interferon was assayed by the plaque reduction method using vesicular stomatitis virus (VSV), as previously reported (6). One PID_{50} unit was that dilution which reduced the plaques by one-half, and the reciprocal of that dilution was the titer of a given preparation.

Results. To test the effect of the presence of interferon on the establishment of primary cells in culture, three neonatal foreskins were trypsinized in the usual manner and the dispersed cells from each were seeded equally into 2 flasks–one containing growth medium and the other growth medium plus 6 units/ml of interferon. Cells in both interferon-containing and control cultures attached and grew normally. Medium was changed at 3-day intervals, and fresh human interferon was added to the appropriate cultures. When confluent monolayers were obtained, routine 2 to 1 splits were made. No significant differences were noted in growth or cell numbers in interferon-containing and control flasks.

Table I shows the titers obtained when serial dilutions of standard HIF-3 interferon were applied, in the plaque assay, to two cell lines and to their simultaneously carried in-

TABLE I. Sensitivity of Control and "Continuous-Interferon" Cell Lines to Standard Interferon Preparation HIF-3.

Cell line	Passage no.	Av titer of interferon
HF-8	15	210
HF-8(IF)	15	260
HF-6	8	620
HF-6(IF)	8	680

terferon-exposed lines. The cultures grown in the presence of a constant amount of interferon are seen to be equal in sensitivity to the control cultures. The concentrations of interferon in which the cultures were regularly maintained resulted in 100% plaque reduction in the case of HF-6(IF), and 80 to 96% reduction in the case of HF-8(IF), when tested by plaque assay. Two-to fourfold differences in sensitivity to the same interferon preparations were regularly and reproducibly observed among the individual HF cell lines.

Interferon applied in growth medium to newly trypsinized cells did not confer as much protection at a given concentration as did the same concentration applied to cells in culture for 24 to 48 hr. Table II shows that 12 units/ml of human interferon applied for 20 hr to HF-8(IF) cells which were planted 24 hr previously reduced plaque counts by 91%. In another experiment, 12 units/ml on HF-8 cells which had been transferred 48 hr before gave 96% protection. However, when the same concentration of interferon was put directly into culture with newly trypsinized HF-8(IF) cells, even when trypsin was carefully drained, an assay 24 hr later showed the cells to be only 53% protected. Similar results were noted with HF-6(IF) cells, where 12 units/ml of human interferon conferred 100% protection from the challenge virus if applied to established monolayers, but only 50% if applied to newly trypsinized cells. Re-assay of medium from these cultures on 48 hr monolayers showed losses in titer of up to 87%. The possibility that residual trypsin might be acting upon the interferon was ruled out by addition of excess soybean trypsin inhibitor. In addition, when fresh human interferon was incubated with trypsin which had been in contact with foreskin cells for 30 min and the cells subsequently removed and trypsin inactivated with soybean inhibitor, no loss in titer was seen—the presence of the cells was necessary for the loss to occur. It, therefore, appears that freshly trypsinized cells bind or otherwise inactivate more interferon than established monolayers.

The protection of the cells remained fairly constant over a period of days once it was established, as shown in the case of HF-8(IF). These cultures were set up in 12 units/ml of interferon and replicates assayed each day for 4 days. The percentage reduction of plaques which was 53 after 24 hr incubation, was 70% at 45 hr and essentially the same for the next 2 days following.

Continuous-interferon and control cultures were exposed to NDV and to the double-stranded synthetic polynucleotide poly I:C to determine whether their ability to produce interferon was altered. Table III shows the results of these tests. HF-8 and HF-8(IF)

TABLE II. Differences in Protection Conferred by Standard Interferon HIF-3 upon Older Monolayers and Newly Trypsinized Cells.

	Cell line	Passage no.	Interferon conc (units/ml)	Duration of application of interferon (hr)	Percentage reduction of plaques
I.	HF-8(IF) (24-hr plant)	15	12	20	91
	HF-8 (48-hr plant)	19	12	20	96
	HF-8(IF) (newly tryp.)	19	12	24	53
				45	70
				71	69
				95	70
II.	HF-6(IF) (48-hr plant)	15	12	20	100
	HF-6(IF) (newly tryp.)	15	12	72	50

TABLE III. Production of Interferon by Control and "Continuous-Interferon" Cultures.

Cell line	Passage no.	IF conc in culture (units/ml)	Stimulating agent	Titer of IF produced
HF-8	16	—	NDV	1280
HF-8(IF)	16	5	NDV	1050
HF-8	27	—	NDV	1000
HF-8(IF)	27	12	NDV	300
HF-6	28	—	NDV	1000
HF-6(IF)	28	12	NDV	510
HF-8	27	—	poly I:C (10 μg/ml)	80
HF-8(IF)	27	12	poly I:C (10 μg/ml)	94
HF-8	27	—	poly I:C (40 μg/ml)	140
HF-8(IF)	27	12	poly I:C (40 μg/ml)	320

lines carried in 5 units/ml of interferon gave similar yields when challenged with NDV. However, when the HF-8(IF) line was carried in 12 units/ml somewhat less interferon was obtained from the continuous-interferon culture. HF-6(IF), carried in 12 units/ml, had a similar response. All cell cultures used to test for interferon yields were adjusted for slight differences in cell number by counting of replicate cultures, and equivalent cell-to-volume ratios were maintained during the time of interferon production.

When cultures were stimulated with poly I:C, little difference in interferon yields between the continuous-interferon and control cultures was seen.

The constant presence of interferon in the culture medium of the "IF" cell lines did not alter their lifespan in culture. The first pair of cell lines to be carried up to their decline phase was HF-8 and HF-8(IF), carried in 5 units/ml of human interferon. At passage 40, HF-8(IF) was noted to have fewer cells per standard bottle than did HF-8. After the next 2 serial passages, both lines were growing noticeably more slowly. By 44 passages, both lines were producing sparse growth and their culture was abandoned. A second pair of HF-8 and HF-8(IF) cultures were later carried to 48 passages with 12 units/ml of interferon in the HF-8(IF) culture. HF-6 and HF-6(IF) were carried from passage 3 to passage 35 in 12 units/ml of interferon. Routine splits were made up to passage 35 and good growth was obtained. About this time, these lines both began to decline and by 41 passages were no longer able to sustain twice-weekly transfers.

Conclusions and Summary. The presence of constant low levels of interferon in the growth medium does not affect the ability of primary human foreskin cells to establish themselves in tissue culture. In addition, continuous exposure of human foreskin fibroblasts to up to 12 units/ml of human interferon does not alter the limited lifespan of these cells *in vitro* (7). Cells continuously exposed to these levels of interferon are still able to respond normally and in a linear fashion to dilutions of interferon by becoming resistant to virus challenge.

No change in the ability to produce interferon in response to poly I:C was noted in continuous-interferon cell lines. Production of interferon in response to NDV challenge was unaltered by cells exposed to a constant 5 units/ml of interferon, but exposure to 12 units/ml caused moderately reduced yields.

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Hypocholesterolemic Effect of Rifampin in the Monkey (*M. fasicularis*) (35542)

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Rifampin, (Rifadin) a new antimicrobial agent introduced for the treatment of tuberculosis and other infections, is a semisynthetic antibiotic of orange-red color with molecular weight of 822.97. It has a macrolide-like ring structure and is the 3-(4-methyl piperazinyl iminomethyl) derivative of Rifamycin SV (1, 2).

In the course of chronic toxicity studies conducted in this laboratory it was noted that the oral administration of rifampin to the *M. fasicularis* monkey resulted in marked reductions of the serum cholesterol levels. These results are presented below.

Materials and Methods. A total of 48 cynomolgus (*Macaca fasicularis*) monkeys, males 3.21 ± 0.65 kg and females 2.62 ± 0.5 kg, were used. The monkeys were caged individually in an air-conditioned environment for approximately 90 days during which time they were stabilized to diet,[1] feed schedule, body weight, handling and dosing procedures; and a minimum of 3 blood samples were collected to establish base line hematologic and clinical chemistry values. The monkeys were grouped and divided equally by sex for administration of single daily oral doses of 40, 80, and 110–120 mg/kg of rifampin which was suspended daily in 10% aqueous gum acacia at a concentration of 80 mg/ml. Acacia vehicle and isoniazid (25 mg/kg/day) for tuberculosis prophylaxis were administered to controls. In addition, control and treated monkeys were given Tang[2] (5.0 ml) to increase the palatability of oral dosage forms.

[1] Purina Monkey Chow, Ralston Purina, Checkerboard Square, St. Louis, Missouri.

[2] Tang, General Foods Corporation, White Plains, New York.

Prior to drug administration, fasting blood samples were withdrawn from the femoral vein or artery at periodic intervals to determine the mean total serum cholesterol levels by the methods of Levine and Zak (3) with the Technicon AutoAnalyzer (4). Treatment groups 2 and 3 were initially administered dosage levels of 40 and 80 mg/kg, respectively, whereas group 4 animals were started at 110 mg/kg and raised to 120 mg/kg after approximately 60 consecutive days of treatment. Gastroenteric disturbances, characterized by vomiting, moderate anorexia, and intermittently loose and firm feces, were observed in several animals on the high dosage level during the initial 2 weeks of treatment. Drug administration was withdrawn on 5 males from the high dosage level for a 5-day period because of these adverse effects. Aside from these initial effects, rifampin was generally well tolerated at all dosage levels during the 180-day period.

Discussion. The results obtained from periodic determination of serum cholesterol levels in cynomolgus monkeys prior to, and during, the period of rifampin treatment are presented in Table I. The data indicate that marked reductions occurred in the serum cholesterol levels after oral administration of rifampin. An analysis of variance showed that percentage changes in cholesterol levels in all treatment groups were significantly different at the 95% level from the control group at all measurement intervals after treatment. Furthermore, there was no significant difference between groups that would suggest a dose–response relationship. Decreases in cholesterol levels of control monkeys were attributed to cyclic or hormonal influences. This influence was also reflected in treatment

TABLE I. Average Percentage Changes in Cholesterol Levels in Cynomolgus Monkeys After Oral Administration of Rifampin.

	Sampling period									
(weeks):	2		4		8		12		26	
Treatment	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
None (174 ± 35)[a]	—11.9	—16.0 (—13.9)[b]	—17.8	—26.1 (—21.7)	—18.5	—23.2 (—20.8)	—14.3	—26.7 (—20.5)	—4.9	—9.3 (—7.1)
40 mg/kg (154 ± 26)	—31.9	—45.2 (—38.5)	—40.1	—53.5 (—46.8)	—40.7	—50.7 (—45.7)	—43.6	—57.1 (—50.4)	—20.3	—41.2 (—30.8)
80 mg/kg (160 ± 40)	—44.5	—46.7 (—45.6)	—36.4	—47.9 (—42.7)	—46.7	—54.9 (—50.8)	—43.2	—49.3 (—46.3)	—28.5	—35.7 (—32.1)
110–120 mg/kg (162 ± 36)	—32.8	—53.8 (—43.4)	—51.7	—65.0 (—58.3)	—49.6	—56.9 (—53.2)	—50.7	—68.5 (—59.6)	—43.8	—45.5 (—44.7)

[a] Mean cholesterol levels (av of 3 pretreatment levels mg/100 ml).
[b] Group average change (%) in cholesterol levels.

group cholesterol levels as shown by the apparent increase in levels at the 26-week sampling period.

Lowering of serum cholesterol levels has been reported after oral administration of polyene macrolide antifungal antibiotics (5) and the nonantibiotic *N*-methyl neomycin (6). It was suggested that these might exert their effects on cholesterol lowering by preventing absorption–resorption of cholesterol (5) or by complexing with bile acids (6).

In contrast to the polyene antifungal antibiotics, rifampin is readily absorbed from the gastrointestinal tract. In addition, rifampin was shown to have an enterohepatic circulation and choleretic activity (7). It is of interest to speculate whether the choleretic activity of rifampin or complexing with bile acids might be bases for the hypocholesterolemic effect observed in the cynomolgus monkey after oral administration of rifampin.

Summary. The oral administration of rifampin to cynomolgus monkeys at dosage levels of 40, 80, and 110–120 mg/kg for 180 consecutive days resulted in marked lowering of serum cholesterol levels. Choleretic activity or complexing with bile acids by rifampin may be a mechanism(s) for the observed hypocholesterolemic effect.

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Effects of Thyroxine and Cold-Acclimation on Hepatic Fatty Acid Metabolism in the Hamster[1] (35543)

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A previous report from this laboratory (1) showed that during the process of cold-acclimation, a relatively higher degree of fatty acid unsaturation occurred in hamster whole liver. In the rat, however, the fatty acid desaturating mechanism does not appear to be operating as cold-acclimation fails to increase the proportion of unsaturated hepatic fatty acids (1, 2).

The mitochondria represent the site of oxidative metabolism in the liver cell and are known to undergo structural and functional changes during cold-acclimation (3, 4). Indeed, cold-acclimation results in a decrease in total fatty acid unsaturation in rat liver mitochondria (2) and induces changes in the proportion of individual fatty acids of both rat (2) and hamster (5) liver mitochondria. Furthermore, the thyroid gland is known to participate in an animal's response to a cold environment (3), effect a number of parameters of fatty acid metabolism (6–9), and produce structural and functional changes in liver mitochondria (10).

The present experiments were performed, therefore, to evaluate in the hamster the role of the thyroid gland and cold-acclimation on fatty acid unsaturation as well as changes in individual fatty acids in both whole liver and liver mitochondria.

Methods and Materials. Adult male hamsters were caged singly and cold-acclimated at 5 ± 2° for 6–7 weeks. Warm-acclimated hamsters were kept at room temperature (23 ± 2°) for 4–5 weeks. Both the warm- and cold-acclimated animals were divided into three groups: controls, prophylthiouracil (PTU)-treated animals and thyroxine-treated animals. PTU and thyroxine were administered for 10 days prior to sacrifice by ip injection. L-Thyroxine was administered as the sodium salt at a dose of 2 mg/100 g of body weight while the dosage of PTU was 5 mg/100 g of body weight (11).

Animals were maintained on synthetic diets and were given distilled water *ad libitum*. The diet had the following composition(%): promine R (25), corn starch (58.8), corn oil (65), Briggs salt mixture (4), alphacel (4), vitamin mixture (2), and methionine (0.2). The fatty acid analysis of the diet expressed in percentage composition was as follows: palmitic (12.40); stearic (2.65); oleic (26.00); linoleic (57.00); and linolenic (1.95).

Animals were killed by decapitation, the livers were removed and two samples were taken: one for whole liver total fatty acid extraction and one for mitochondrial preparation. The methods used for mitochondrial isolation and fatty acid extraction have previously been described (2).

A Barber-Colman gas chromatograph was used to separate and quantitate the fatty acids. The operating conditions of this instrument have been outlined previously (2). Peak area of the fatty acids was obtained with the model 205 Disc Chart Integrator. The fatty acids from 14 to 22 carbons in chain length are included in this study. The fatty acid designations used give the number of carbon atoms and double bonds present: thus myristic is 14:0, palmitic 16:0, palmitoleic 16:1, stearic 18:0, oleic 18:1, linoleic

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TABLE I. Hamster Whole Liver Percentage Unsaturated Fatty Acid Composition; A Comparison Between Treatments.[a]

Comparison percentage unsaturation	DBM	Significance (.05 level)	CI
Warm C vs warm PTU (65.52 ± 0.56) (63.70 ± 0.45)[b]	1.82	NS	(—0.29, 3.93)
Warm C vs warm T_4 (65.02 ± 0.56) (64.05 ± 0.45)	1.47	NS	(—0.64, 3.58)
Warm C vs cold C (65.52 ± 0.56) (65.84 ± 0.36)	1.32	NS	(—0.79, 3.43)
Cold C vs cold PTU (66.84 ± 0.36) (65.48 ± 0.48)	1.36	NS	(—0.75, 3.47)
Cold C vs cold T_4 (66.84 ± 0.36) (66.81 ± 0.71)	0.03 D = 2.11	NS	(—2.08, 2.14)

[a] Abbrev.: DBM = difference between means; CI = 95% confidence interval; C = control; PTU = propylthiouracil; T_4 = thyroxine; NS = not significant; D = mean square times Q value.

[b] Mean ± SEM; 16 animals/treatment.

18:2, linolenic 18:3, arachidonic 20:4, and docosahexanoic 22:6. Three fatty acids, designated as X_1, X_2, and X_3, were not positively identified as standards could not be obtained. However, from previously reported fatty acid compositions of rat liver (12), the authors feel that X_1 may represent 5,8, 11-eicosatrienoic; X_2, 8,11,14-eicosatrienoic;

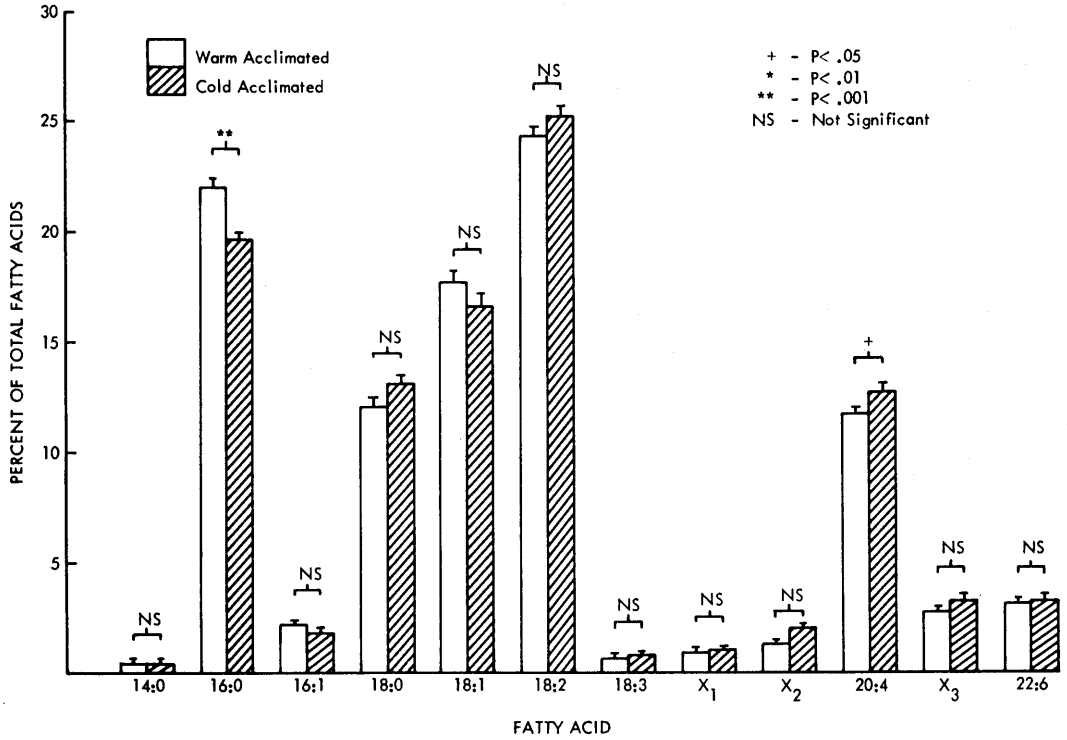

FIG. 1. Hamster: warm-acclimated controls compared to cold-acclimated controls; percentage fatty acid composition of whole liver.

and X_3, docosapentanoic acids.

The Studentized Range Test (13) was used for testing comparisons among treatment means for total fatty acid unsaturation of whole liver and liver mitochondria. The difference between means of individual fatty acids was statistically analyzed in accordance with the Student's *t* test.

Results and Discussion. Table I shows that neither cold-acclimation nor PTU or thyroxine treatments produce any significant changes in the degree of total unsaturation of whole liver fatty acids. The data suggest, therefore, that during cold-acclimation the fatty acid desaturating mechanism is not utilized to any significant extent which is consistent with the data that have been reported for the cold-acclimated rat (2).

Although total fatty acid unsaturation is not effected by cold-acclimation, changes in individual hepatic fatty acids occur as seen by a relative decrease in palmitate and an increase in arachidonate (Fig. 1). These findings may reflect changes in the triglyceride to phospholipid ratio for Therriault and Poe (14) showed a decrease in triglycerides with no change in total phospholipids of hepatic lipid from chronically cold-exposed rats.

PTU and thyroxine treatments also produce marked changes in individual fatty acids of liver from warm-acclimated hamsters (Fig. 2). The large increases in palmitate and oleate as a result of thyroxine most likely represent mobilization from adipose tissue as it is known that these two fatty acids comprise 70% of the total fatty acids of this tissue (1). Similar findings have been reported in the thyroxine-treated rat by Ellefson and Mason (7). The decrease in stearate may represent increased desaturation of this particular fatty acid forming oleate since this conversion is known to be stimulated by thyroxine in the rat (8). The decrease in the polyunsaturated fatty acids linoleate, X_2 and arachidonate, following thyroxine injection are striking and may represent increased utilization. However, this is contrary to what has been reported in the rat (7), but since we are dealing with percentage composition,

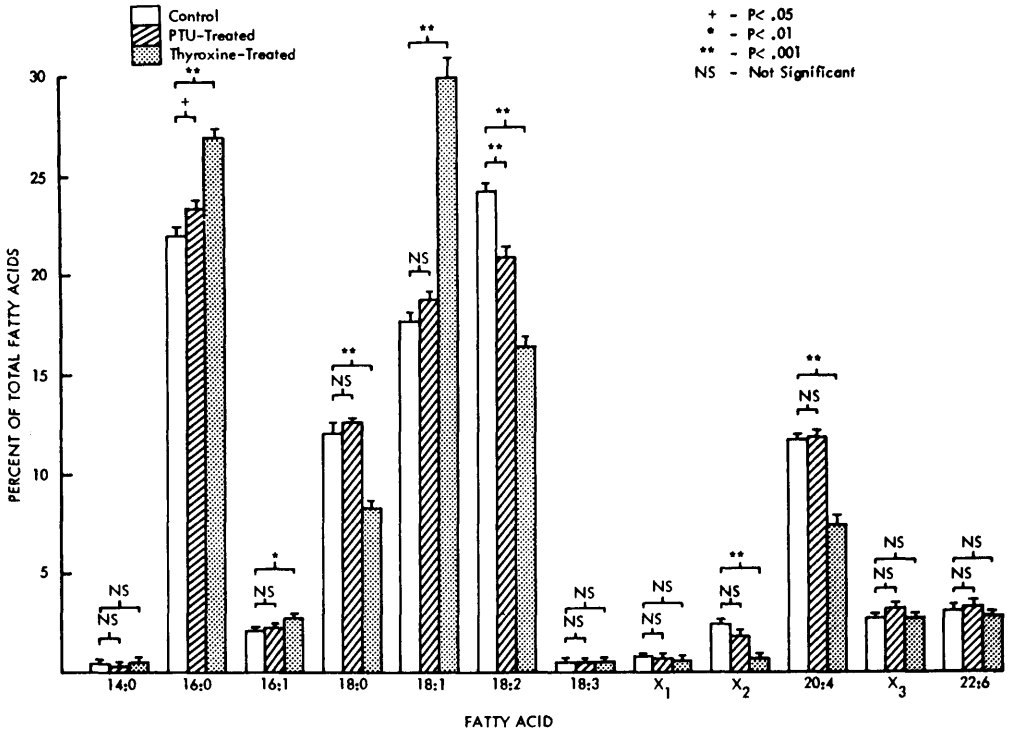

FIG. 2. Warm-acclimated hamster: percentage fatty acid composition of whole liver.

TABLE II. Hamster Liver Mitochondria Percentage Unsaturated Fatty Acid Composition; A Comparison Between Treatments.[a]

Comparison percentage unsaturation	DBM	Significance (.05 level)	CI
Warm C vs warm PTU (68.99 ± 0.53) (67.96 ± 0.41)[b]	1.03	NS	(−0.56, 2.62)
Warm C vs warm T_4 (68.99 ± 0.53) (62.53 ± 0.37)	6.46	Significant	(4.87, 8.05)
Warm C vs cold C (68.99 ± 0.53) (68.85 ± 0.23)	0.14	NS	(−1.45, 1.73)
Cold C vs cold PTU (68.85 ± 0.23) (69.50 ± 0.32)	0.64	NS	(−0.89, 2.24)
Cold C vs cold T_4 (68.85 ± 0.23) (65.26 ± 0.40)	3.59 D = 1.59	Significant	(2.00, 5.18)

[a] Abbrev.: DBM = difference between means; CI = 95% confidence interval; C = control; PTU = propylthiouracil; T_4 = thyroxine; NS = not significant; D = mean square times Q value.

[b] Mean ± SEM; 16 animals/treatment.

these changes may be an inverse resultant of higher percentages of other fatty acids. Regardless of the mechanism(s) which may be involved, it is readily apparent that thyroxine can drastically alter the distribution and metabolism of individual hepatic fatty acids in the hamster.

With regard to liver mitochondria, cold-acclimation does not affect the degree of total fatty acid unsaturation (Table II). In the

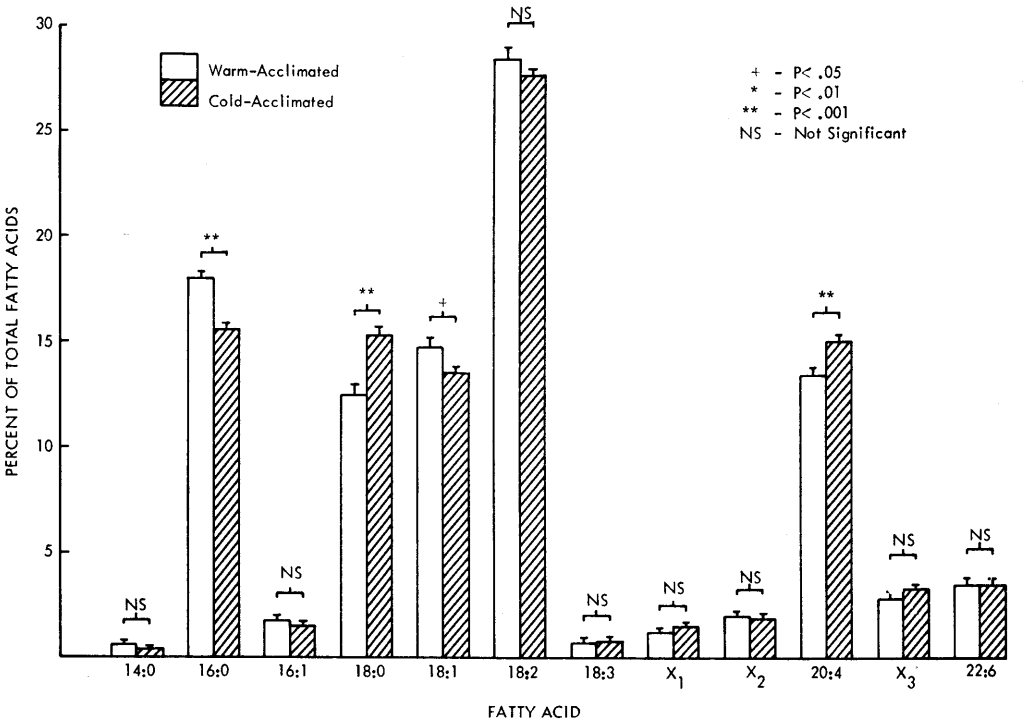

FIG. 3. Hamster: warm-acclimated controls compared to cold-acclimated controls; percentage fatty acid composition of liver mitochondria.

rat, however, the cold-acclimated state did induce a significant decrease in total unsaturation (2). This finding suggests that differences exist between these two species concerning mitochondrial function in the cold.

Cold-acclimation does produce a number of changes in individual mitochondrial fatty acids as shown by decreases in palmitate and oleate and increases in stearate and arachidonate (Fig. 3). These changes may represent structural alterations in the lipoprotein components of the mitochondrial membrane. Indeed, studies on essential fatty acid deficiency (15, 16) have shown that the fatty acid composition of mitochondria is altered which is associated with changes in both their structure and function. Furthermore, Green and Fleischer (17) have suggested that the stability of the phospholipid component of the oxidative phosphorylating apparatus of mitochondria depends, in part, on the nature of the fatty acids of the phospholipids. The data suggest, therefore, that changing the environmental temperature can modify the biochemical components of mitochondria. Such modifications may play a role in the increased heat production characteristic of the cold-acclimated state.

Table II and Fig. 4 show the effects of PTU and thyroxine on total mitochondrial unsaturation and individual fatty acids from warm-acclimated animals. Thyroxine treatment results in a decrease in total unsaturation and produces marked changes in individual fatty acids as evidenced by increases in palmitate, stearate, arachidonate and X_3 and by a decrease in linoleate and X_2. These findings appear unrelated to those demonstrated in whole liver. Apparently thyroxine can alter the fatty acid composition of liver mitochondria independent of its effect on whole liver.

From the present experiments it is not possible to assess the significance of the data in relation to the action of thyroxine on mitochondria. However, it is proposed that by bringing about changes in the fatty acid composition, thyroxine can sufficiently modify the structure of the mitochondrial membrane to cause altered enzymatic activity and permeability. The effects of thyroxine are dramatic and may represent an important site of

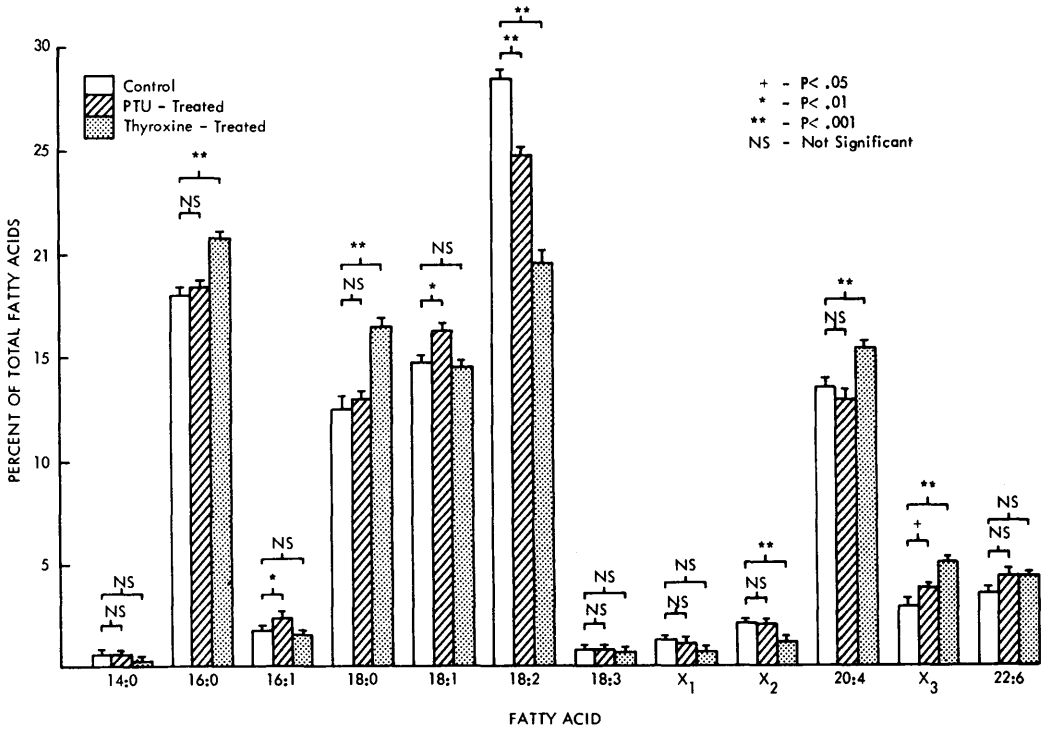

FIG. 4. Warm-acclimated hamster: percentage fatty acid composition of liver mitochondria.

hormone action for it is well documented that the thyroid hormones can affect both the structure and function of mitochondria (10, 18).

The present experiments fail to show analogous effects between cold-acclimation and thyroxine treated, warm-acclimated animals regarding the fatty acid composition of mitochondria. In the rat, however, Patton and Platner (2) have reported similarities between cold-induced changes in mitochondrial fatty acids and those occurring as a result of thyroxine treatment.

Summary. Relative concentrations of fatty acids from whole liver and liver mitochondria of cold-acclimated hamsters were analyzed by gas chromatography. Involvement of the thyroid gland was studied by the administration of PTU and thyroxine to both warm- and cold-acclimated animals. No change was observed in total unsaturation of either whole liver or mitochondrial fatty acids as a result of cold-acclimation but changes did occur in the composition of individual fatty acids. These were a decrease in palmitate and an increase in arachidonate in whole liver while mitochondria showed increases in stearate and arachidonate and decreases in palmitate and oleate. Injection of warm-acclimated animals with thyroxine also resulted in no change in whole liver unsaturation but did produce a decrease in the total unsaturation of mitochondria. Marked effects were also observed in individual fatty acids following thyroxine treatment as evidenced by increases in palmitate and stearate and a decrease in linoleate in mitochondria while in whole liver there were increases in palmitate and oleate and decreases in stearate, linoleate, and arachidonate. Analogous effects on fatty acid metabolism between thyroxine-treated, warm-acclimated hamsters and cold-acclimation were not observed.

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