# EFFECTS OF INHALED HF ON CHOLESTEROL METABOLISM IN GUINEA PIGS

by

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SUMMARY: Exposure to 5 mg HF/m$^3$ causes a significant increase in the plasma cholesterol levels in the guinea pig. Modifications of the cholesterol metabolism are due to the specific action of fluoride. Effects of HF on cholesterolemia are reversible however, and during a second exposure to HF, plasma cholesterol increases as in the first exposure. Cholesterol biosynthesis was studied. Acetate incorporation in intestinal tissue and lung was higher in intoxicated animals than in controls but mevalonate incorporation was comparable in the two groups. The enzyme catalyzing mevalonate synthesis, β-methyl-β-hydroxyglutaryl CoA reductase, could be activated by HF.

KEY WORDS: Cholesterol; Guinea pigs; HF; Intestinal tissue; Lung.

## Introduction

Hydrogen fluoride inhalation for 84 hours (10 mg HF/m$^3$) induces a significant increase in plasma cholesterol levels (1) in guinea pigs. The present study was undertaken to investigate which of two mechanisms might be involved in the development of hypercholesterolemia: a specific effects of hydrogen fluoride and/or an irritating action. The effects of controlled levels of hydrogen fluoride on plasma cholesterol were noted at various sampling times, as well as the reversibility of these effects.

## Method

Animals and experimental design: The male and female albino guinea pigs which were used weighed 350 g at the beginning of exposure. They were fed with commercially available pellets (purchased from Usine d'Alimentation Rationnelle "UAR", France) containing 20 ppm fluoride and with a fresh supply of carrots and vitamins. To study the effects of graded levels of hydrogen fluoride on plasma cholesterol, the animals were divided into five groups: A control group of guinea pigs was housed in a cylindrical plexiglass cage previously described (1) without gaseous HF. Four groups were exposed to 1.5, 3, 5 and 10 mg HF/m$^3$ respectively in the plexiglass cage for 84 hours. (Gaseous HF was produced by sending an aqueous solution of HF, by peristaltic pump, into a vaporization oven at 150°C. The desired level of HF in the atmosphere (1.5, 3, 5, and 10 mg HF/m$^3$) was obtained by varying the concentration and volume of the solution and by modifying the amount of purified air used to dilute the HF vapor. The level of fluoride was checked every 3 hrs for 84 hrs with an automatic captor by trapping the HF in a known volume

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of air on a dry caustic soda-impregnated filter and assaying with a specific electrode (2). During the study the average room temperature was 20°C and the humidity 72%.

For comparison of the effect of hydrogen fluoride and of hydrogen chloride, a group of animals was exposed to 5 mg $HCl/m^3$ for 84 hrs. The effect of hydrogen fluoride on animals presenting hypercholesterolemia was investigated on a group of male albino guinea pigs receiving the same diet wih 1% (w/w) additional cholesterol for seven days. All animals had free access to water and food during exposure and were fasted overnight before the blood samples were taken. On the other hand, the variations of plasma cholesterol concentration were studied for females as well as for males during prolonged exposure in the fluoride atmosphere (HF: 5 $mg/m^3$).

Plasma cholesterol of three groups of guinea pigs of both sexes (randomly selected and healthy) was evaluated on D : 0 (control). The animals were then exposed to a constant fluoride atmosphere at 5 mg $HF/m^3$. Each day a random selection of 5 animals was made and the value of plasma cholesterol was determined. Care was taken to insure that each animal was investigated every third day. For females, exposure was interrupted on the 15th day and re-exposure began on the 34th day and lasted until the 37th. The males were no longer exposed after day 14.

Technique: a) Cholesterol and fluoride determinations — Total cholesterol was estimated by the technique of Röschlau et al. (3). Plasma fluoride was determined by the method of Hall et al. (4). b) Statistical analysis: The dose factor is considered qualitatively (each day the animals receive a supplementary dose of HF). The homogeneity of the averages is tested (analysis of variances with repetition). If the averages differ significantly, the monotony of the response (amount of cholesterol) according to the dose of HF inhaled is studied. The response is supposedly linear. The linearity of the curve and its slope are tested.

Cholesterol metabolism: Materials — For incubation, tissues were immediately placed in cold saline. The ileum was opened longitudinally, flushed with cold saline, rinsed again and cut into 0.5 cm portions. Liver and lung were sliced. Ileum or liver or lung (200 mg) was placed in a flask with 2 ml of Krebs-Ringer buffer pH 7.5. Five experiments were prepared for each sample. After a 10 minute preincubation period at 37°C, in a metabolic shaker, the precursor was added: 0.25 µCi/ml of $|1\text{-}^{14}C|$-acetate or 0.25 µCi/ml of $|1\text{-}^{14}C|$-mevalonate following which the flasks were gassed 95% $O_2$ — 5% $CO_2$, capped and incubated for 90 minutes according to Turley et al. procedure (5) modified by Sablé and Sicart (6).

Extraction and separation of lipids: At the end of the incubation period, the tissues were rapidly removed, placed in a flask containing a chloroform-methanol mixture (2:1 v/v) and homogenized. Total lipids were extracted by Folch's method, then fractionated into cholesterol and cholesterol esters by thin layer chromatography on silica gel G coated plates (0.25 mm thickness, Merck, Darmstadt, West Germany) in acetic acid-diethyl ether — light petroleum ether (1:20:80, v/v). After development of the chromatogram, the silica gel plate areas corresponding to the labeled lipids were scraped off and put into a vial containing 10 ml liquid scintillation mixture. The radioactivity was measured in a Packard Tricarb 3320 liquid scintillation spectrometer.

## RESULTS

The specific effect of hydrogen fluoride in the development of hypercholesterolemia is shown by plasma cholesterol determinations in two groups of guinea pigs. One was exposed to HF (5 mg/m$^3$) the other to HCl (5 mg/m$^3$), a gas the irritant action of which is similar to HF. Guinea pigs exposed to hydrogen fluoride presented a plasma fluoride concentration of 2,000 µg/liter whereas, in animals exposed to HCl, levels were 200 µg/liter. Cholesterol levels were significantly increased ($p < 0.001$) in the fluoride group compared with levels in the HCl-exposed group. Mean values were 1.58 mmole/liter in the fluoride group, 0.68 mmoles/liter in the chloride group. Therefore, increase of cholesterol is due to a specific effect of hydrogen fluoride.

To study the effects of different concentrations of hydrogen fluoride on cholesterol levels, the animals were divided into four groups and exposed to 1.5, 3, 5 and 10 mg HF/m$^3$ for 84 hours. Only when guinea pigs were exposed to 5 and 10 mg HF/m$^3$ was plasma fluoride concentration increased compared with controls (Table 1). Modifications in plasma cholesterol concentration were also observed (Table 2). Cholesterol levels were significantly increased in groups exposed to 5 and 10 mg HF/m$^3$, but no cholesterol increase was registered in guinea pigs exposed to 1.5 or 3 mg HF/m$^3$ for four days or more.

Table 1

Plasma Fluoride Levels of Guinea Pigs Exposed to Fluoride Graded Levels

| HF in Atmosphere (mg/m$^3$) | Plasma F (mg/l) |
|---|---|
| 1.5 | 1.2 ±0.19 |
| 3 | 1.4 ±0.21 |
| 5 | 2 ±0.23 |
| 10 | 2.5 ±0.25 |
| 0 (control) | 0.2 ±0.15 |

Table 2

Plasma Cholesterol Levels of Guinea Pigs Before and After Exposure to Fluoride Graded Levels (Total Cholesterol: mmol/l)

| Groups | n | Before Exposure m | Before Exposure S.D. | mg HF/m$^3$ | After Exposure m | After Exposure S.D. |
|---|---|---|---|---|---|---|
| 1 | 6 | 0.740 | 0.123 | 1.5 | 0.683 | 0.089 |
| 2 | 6 | 0.725 | 0.092 | 3 | 0.713 | 0.107 |
| 3 | 15 | 0.870 | 0.200 | 5 | 1.580 | 0.430 |
| 4 | 46 | 0.850 | 0.490 | 10 | 1.530 | 0.630 |

m = mean; S.D. = standard deviation; n = number of animals

Figure 1

Plasma Cholesterol Level (mmole/l) of Male Guinea Pigs during HF Exposure (5 mg/m³). 1: Start of Exposure; 2: End of Exposure.

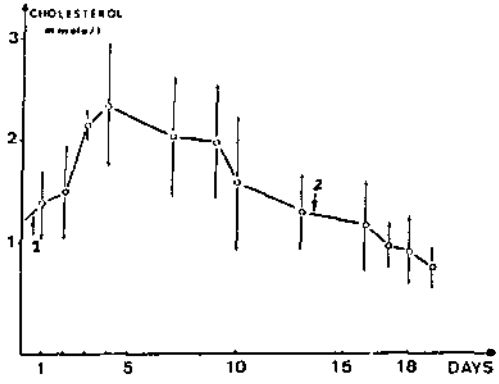
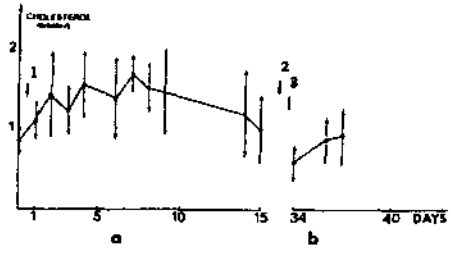



Figure 2

Plasma Cholesterol Level (mmole/l) of Female Guinea Pigs during First HF Exposure [a] and during a Second HF Exposure [b] after Two Weeks without Intoxication. 1: Start of First Exposure; 2: End of First Exposure; 3: Start of Second Exposure.




Exposure time to HF (5 mg/m³) in relation to plasma cholesterol levels was studied in males and females (Figs. 1 and 2). For female guinea pigs, a test of homogeneity of the means (D : 0 and D : 9) shows a significant difference ($F_{46}^{8} = 4$) at 0.001. Analysis of the variance shows that between D : 0 and D : 9, the curve crossing through the set of points does not significantly deviate from linearity ($F_{46}^{1} = 1.15$ N.S.) and furthermore that the slope is very significant ($F_{46}^{1} = 24$ at 0.001). After withdrawing the animals from the fluoride atmosphere for a period of 10 days, and again exposing them plasma cholesterol increased but the new increase was smaller (Tables 3, 4, 5). For male guinea pigs, a test of homogeneity of the means (D : 0 and D : 4) shows a significant difference. Analysis of variance shows that between D : 0 and D : 4, the curve crossing through the set of points does not significantly deviate from linearity ($F_{22}^{3} = 0.7$ N.S.) and furthermore that the slope is very significant ($F_{22}^{1} = 22.7$ at 0.001). However in male guinea pigs, the increase

Table 3

Cholesterol Levels of Female Guinea Pigs Before HF Exposure (5 mg/m³) (Total Cholesterol: mmol/l)

| Groups | n | m | $s^2$ |
|---|---|---|---|
| A | 5 | 1.066 | 0.027 |
| B | 5 | 0.902 | 0.020 |
| C | 5 | 0.876 | 0.042 |
| A+B+C | 15 | 0.948 | 0.182 |

m = mean; S.D. = standard deviation; n = number of animals

Table 4

Cholesterol Levels of Female Guinea Pigs at Different Sample Times During HF Exposure (5 mg/m³) (Total cholesterol: mmol/l)

| Days | 0 | 1 | 2 | 3 | 4 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Groups | A+B+C | A | B | C | A | B | C | A | B |
| n | 15 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| m | 0.948 | 1.134 | 1.440 | 1.262 | 1.584 | 1.420 | 1.738 | 1.547 | 1.510 |
| S.D. | 0.182 | 0.243 | 0.545 | 0.317 | 0.424 | 0.519 | 0.229 | 0.313 | 0.529 |

m = mean; S.D. = standard deviation; n = number of animals

Table 5

Cholesterol Levels of Female Guinea Pigs at Different Sample Times During a Second HF Exposure (Total cholesterol: mmol/l)

| Days | 34 | 36 | 38 |
|---|---|---|---|
| n | 12 | 5 | 6 |
| m | 0.678 | 0.907 | 1.050 |
| S.D. | 0.228 | 0.280 | 0.372 |

m = mean; S.D. = standard deviation; n = number of animals

Table 6

Cholesterol Levels of Male Guinea Pigs at Different Sample Times During HF Exposure (5 mg/m³) (Total cholesterol: mmol/l)

| Days | 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Groups | A+B+C | A | B | C | A |
| n | 15 | 5 | 5 | 5 | 5 |
| m | 1.190 | 1.384 | 1.472 | 2.140 | 2.350 |
| S.D. | 0.15 | 0.601 | 0.491 | 0.190 | 0.641 |

m = mean; S.D. = standard deviation; n = number of animals

of plasma cholesterol is faster and the maximum value of cholesterol is reached more quickly than in females (Table 6).

These variations led to the study of the effect of HF on the rate of cholesterol biosynthesis by means of [1-$^{14}$C]-acetate and [1-$^{14}$C]-mevalonate incorporation in controls and intoxicated animals. In the HF group, the experiment was performed when serum cholesterol had reached its maximum value. Acetate incorporation in intestinal tissue and lung was higher in intoxicated animals that in controls (Table 7) but mevalonate incorporation was comparable in the two groups (Table 8). In contrast, when the same experiment was performed

Table 7

|1-$^{14}$C|-acetate incorporation in Cholesterol of Ileum, Lung and Liver of Controls and Intoxicated Animals

| | Controls | Exposed |
|---|---|---|
| Ileum: | | |
| m | 1907* | 8730 |
| S.D. | 1165 | 628 |
| n | 4 | 4 |
| difference statistically significant at P<0.001 | | |
| Lung: | | |
| m | 1910 | 7663 |
| S.D. | 1233 | 3874 |
| n | 4 | 4 |
| note dispersion of levels in exposed guinea pigs. | | |
| Liver: | | |
| m | 2250 | 2863 |
| S.D. | 860 | 705 |
| n | 4 | 4 |

m = mean; S.D. = standard deviation; n = number of animals

* dpm/gram tissue/hour.

Table 8

|1-$^{14}$C|-mevalonate incorporation of Ileum and Lung of Controls and Intoxicated Guinea Pigs

| | Controls | Exposed |
|---|---|---|
| Ileum: | | |
| m | 6649* | 4219 |
| S.D. | 1767 | 1329 |
| n | 4 | 4 |
| difference not statistically significant | | |
| Lung: | | |
| m | 10764 | 11276 |
| S.D. | 4381 | 4116 |
| n | 4 | 4 |
| difference not statistically significant | | |

m = mean; S.D. = standard deviation; n = number of animals

* dpm/gram tissue/hour.

during the serum cholesterol decrease in intestinal tissue of intoxicated animals, acetate incorporation was low (2203 ±546 dpm/g. ileum/h.). The rate of acetate incorporation in the liver was much lower than that in ileum and lung. The rates of sterol synthesis are similar in control and exposed groups (2250 ±860 compared to 2863 ±705 dpm/g. liver/h.).

## Discussion

Fluoride inhalation produces a more constant impregnation of fluoride than oral ingestion. The percentage of fluoride metabolized by the latter is only about 45%. Therefore, in this study, fluoride inhalation was chosen. Guinea pigs received 0.75, 1.5, 2 and 4 mg of gaseous HF/day plus about 1.5 mg of fluoride in the diet per day. Control guinea pigs received only 1.5 of dietary fluoride.

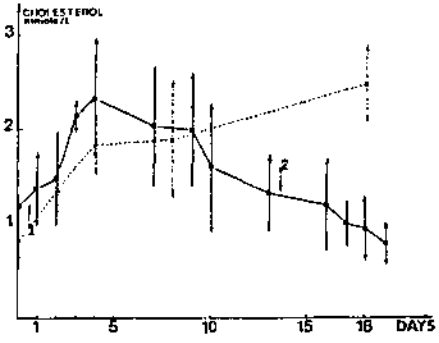
The specific action of HF on cholesterol metabolism is shown by comparing the effects of HF and HCl. Acetate and mevalonate incorporation suggests that β-OH, βmethylglutaryl CoA reductase (E.C.1.1.134), the enzyme catalyzing mevalonate synthesis, could be activated by HF. The enzyme might then be inhibited by the high quantity of cholesterol produced in the first phase of intoxication as previously demonstrated for the regulation of cholesterol biosynthesis in different mammalian species (7,8). The regulation of cholesterol biosynthesis in the guinea pig, however, seems to be different. Some authors have pointed out that guinea pig and rabbit cholesterolemia, in contrast to that in the rat, progressively increases with a cholesterol-rich diet slowly reaching a maximum value (6). Thus it seems that exogenous cholesterol does not produce a negative feed-back control in this species.

To assess this hypothesis, plasma cholesterol and |1-$^{14}$C|-acetate incorporation were studied in cholesterol-fed guinea pigs. In contrast to results observed

in intoxicated groups, plasma cholesterol and |1-$^{14}$C|-acetate incorporation slowly increased and no diminution of the parameters was observed when the diet was continued (Figure 3.) Thus endogenous cholesterol seems to be able to produce a negative retrocontrol on its own biosynthesis but exogenous sterol, contained in a cholesterol-enriched diet, should not cause such inhibition. Furthermore, effects of exogenous and endogenous cholesterol are cumulative. When effects of HF inhalation on the plasma cholesterol level and |1-14C|-acetate incorporation were studied in cholesterol-fed guinea pigs, acetate incorporation in ileum was higher in intoxicated animals than in controls, both groups receiving a cholesterol rich diet (10480 ±5147 compared to 5290 ±2823 dpm/g. ileum/h.). The effects of HF on cholesterolemia are reversible when exposure to HF is discontinued. During a second exposure, however, cholesterol biosynthesis increases again (Figure 2).

Figure 3

Comparative Effects of Endogenous (●——●) and Exegenous (*----*) Cholesterol on Guinea Pig Cholesterolemia during Exposure to HF and Cholesterol-Rich Diet Respectively.

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