THE IMPORTANCE OF LIVER IN NORMAL AND SILICOTIC LUNG-LIPID HOMEOSTASIS: 3. TRIACYLGLYCEROLS

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• *Early lung silicosis in rats activates the liver for triacylglycerol (TG) biosynthesis. The newly formed liver TG seems mobilized to the silicotic lung, as indicated by increased lung TG content and specific activity. These TG changes are associated with decreased TG content and increased TG specific activity in the liver. Serum TGs are also decreased at the study times of 6, 24, 72, and 144 h post intrapulmonary silica treatment.*

INTRODUCTION

It has previously been shown that the silicotic lung accumulates lipids.[1-3] Recently we demonstrated that early silicotic lung mobilizes phospholipids and cholesterol from the liver. The silicotic lung also causes increased cholesterolgenesis and phospholipidgenesis in liver. The *de novo* formed liver cholesterol and liver phospholipids are then transported to the lung via the blood. The lung takes up the lipids as indicated by increased lung-lipid content.[4,5]

The purpose of this study is to determine if acutely injured lung by quartz intratracheal injection induces similar changes in the liver triacylglycerols (TG). If this occurs, it will suggest a generalized liver-lipid metabolism induced by the acute inflammatory lesion in the silicotic lung.

METHODS

Animals

Sprague-Dawley male rats, 200-225 g body weight, were anesthetized by ether. A midline incision 1.0 cm long was made on the anterior neck surface directly over the submaxillary gland. By blunt dissection, the trachea was exposed, and 0.75 ml of either saline or silica DQ 12[1] (100 mg silica/ml saline) was instilled intratracheally through a 20 G needle. After a 3-15 sec pause in respiration, all animals recovered. Wound closure was made with prolene suture (Ethicon, Inc.).

A control group of 10 rats was neither surgically manipulated nor exposed to ether or saline.

Chemical Methods

Lipid extraction from tissue and serum. To 1-1.5 g of lung or liver, 10 ml of chloroform-methanol (2:1) Folch reagent was added.[6] The tissues were homogenized for 30 sec using a polytron homogenizer. The homogenizer was rinsed 2 times for 15-25 sec with 10 ml of the chloroform-methanol mixture. The washings were added to the original homogenate in a 35 ml glass-stoppered centrifuge tube, thoroughly mixed, and allowed to stand for 16-20 h. The supernate obtained by centrifugation for 15 min at 1000 rpm was dried by adding 1-3 g of anhydrous sodium sulfate and allowing it to stand for more than 1 h.

One ml of serum was added to 20 ml of Folch reagent,[6] mixed thoroughly, and treated in the same manner as the tissue homogenate.

All lipid analyses were done on aliquots of these extracts.

Triacylglycerol (TG) determination. The triacylglycerols were determined according to a method described by Dole and Meinertz[7] and modified by Goldfinch and Prudhoe.[8] The extraction procedure uses nonane; isopropanol transesterification and hydrolysis of the acylglycerols with 0.1 M NaOH in isopropanol; splitting of glycerol into formaldehyde by periodate; and the development of color by reacting the formaldehyde formed with ammonium acetate-buffered acetylacetone.

Thin layer chromatographic (TLC) separation of lipid classes.[9]

5-ml aliquot of the Folch tissue and the serum extracts were dried at 60-70° under a N_2 stream. Cholesterol, cholesterol palmitate, triolein, diheptodecanoyl-lecithin, and stearic acid were added as carrier to the extract to visualize the various components of the silica G plates. The solvent system contained petroleum ether, ether, and glacial acetic acid (82:16:12). The lipid spots on the TLC plates were stained by a 0.2% solution of 2,7-dichlorofluorescein-ethanol spray reagent.

Specific activity (SA) determination of TG. The TG spots on the TLC plates were scraped off into scintillation vials. To each vial, 2 ml of glacial acetic acid was added and 10 ml of POPOP-PPO toluene solution [3 g/l of PPO (2,5-diphenyloxazole) and 150 mg/1 of POPOP (1,4-bis-2-(methyl-5-phenyloxazolyl)-benzene)]. The vials were mixed and the amount of radioactivity in each vial determined on a Beckman 1-S 250 liquid scintillation counting system. The amount of quench for each sample was determined by using ^{14}C-toluene solution as the internal standard. The amount of the TG found in each Folch extract was related to the amount of radioactivity obtained from the TLC; thus the SA was calculated.

Statistical analysis. Because of the three differing treatment groups of rats and the various animal-sacrifice times, a Duncans New Multiple Range statistical analysis[10] was used to evaluate the data at all time periods. A 95% confidence level was chosen for the significance level. Hence data indicated in the figures as "significant" are significant at the 95% or higher confidence level.

RESULTS

We report changes in TG in the liver, lung, and serum at four different time intervals after intratracheal instillation of quartz into rats. Three aspects of TG are shown: (a) change of the density (concentration) in a certain organ, (b) change in total TG, and (c) rate of biosynthesis of TG.

Liver Triacylglycerol (Fig. 1)

The liver TG concentration decreased to minimal levels (60-70% of normal values) 6 h following the intrapulmonary administration of either saline or saline plus silica, then slowly increased toward normal TG levels (Fig. 1A). However, at 144 h following the silica treatment, the TG concentrations were still significantly depressed when compared to those of nontreated control rats. By 144 h, the saline-treated animals had a greater liver TG concentration than the silica-treated rats (Fig. 1A).

The total TG-liver content indicated a greater decrease of TG at 6, 72, and 144 h in the silica rats than in the saline rats. The liver TG content in the saline rats returned more rapidly to normal values whereas the saline-silica treated rats liver TG content plateaued at 60% below normal (Fig. 1B).

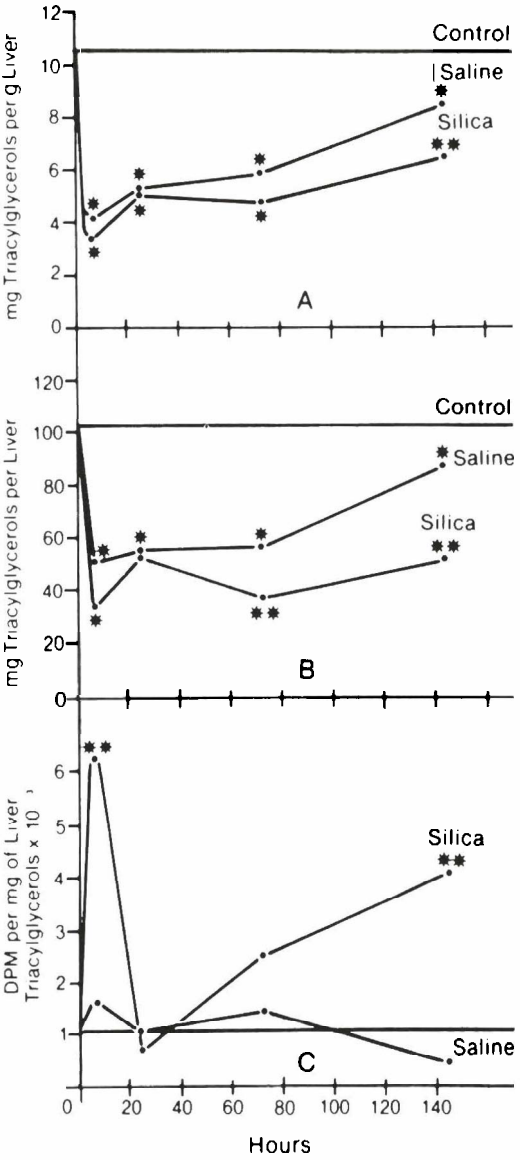

FIGURE 1. Triacylglycerol (TG) changes in the liver following intrapulmonary injection of saline or silica. Graph A—TG concentration (mg/g liver): $F = 28.44$. Graph B—TG content (mg/liver): $F = 18.24$. Graph C—SA of TG in liver: $F = 4.23$. *Significantly different from control rat group ($p \leq .05$). **Significantly different from both control and saline rat groups ($p \leq .05$). Each data point represents the mean TG value of 7-14 rat livers at various times. For graphs A, B, and C, number (n) of saline-group individuals and silica-group individuals, at the time intervals studied, were as follows:

Interval (hours)	Saline (n) A	B	C	Silica (n) A	B	C
6	7	7	7	7	7	7
24	7	7	6	8	8	8
72	7	14	14	7	13	14
144	7	13	13	7	13	13

Control group n = 9

The liver-TG SA was increased 6 times above the control value at 6 h, and 4 times above the control value at 144 h, post silica treatment. A significant decrease of TG-SA to control levels occurred at 24 h in the silica rats then rose at 72 and 144 h post silica treatment. The TG-SA in the saline-treated rats was not significantly altered from that of control values.

Lung Triacylglycerol (Fig. 2)

Six hours following intrapulmonary silica treatment in rats, a significant (80% of control value) decrease in lung TG concentration resulted. The lung-TG concentration then rose to 50% below normal levels at 24 h, followed by a decrease to a plateau level of approximately 70% below normal values at 72 and 144 h.

Intrapulmonary saline treatment also elicited an 80% decrease from control levels in lung TG levels. The lung TG level then rose to 39% below control levels at 24 and 72 h, and increased to 20% below normal by 144 h (Fig. 2).

The lung-TG content decreased to 72% of control values at 6 h, rose to control values at 24 and at 72 h, then significantly increased 1.6 times above control values by 144 h post silica treatment. The TG content of lung in rats given intrapulmonary saline also initially decreased to 72% of control at 6 h, rose from 72% to 29% below control lung TG content at 24 and 72 h respectively, then reached control lung TG content at 144 h post saline treatment (Fig. 2B).

Serum Triacylglycerol Results (Fig. 3)

At all time periods studied, a significant decrease from control levels of serum TG levels occurred in both the saline and silica treated rats. The serum TG levels in the silica rats were significantly less than those in the saline rats at 72 h. At the later time intervals the differences between the two groups of rats were the same at 76-44% below control levels (Fig. 3A).

At 6 h the silica rats' serum TG SA was significantly increased, being 1.7 and 1.3 times above both the saline and control rat values respectively. By 24 h, the serum TG SA decreased 37% below control values, then rose to control levels at later sampling periods; i.e., 72 and 144 h post silica treatment (Fig. 3B).

The serum TG SA of the saline rats was significantly decreased from control values at 24 h, then returned to normal levels at 72 h but fell slightly again at 144 h.

DISCUSSION

The dynamics of changes in TG, irrespective of whether they refer to total amount per organ, degree of concentration, or rate of synthesis (SA), result from two types of reactions. The first is nonspecific trauma—stress in early periods (6 h). The second is reaction to silica, or sometimes to saline, in later periods.

Six hours following silica or saline instillation into rat lungs, there occurs a rapid decrease in lung TG concentration accompanied by a significant increase in lung TG

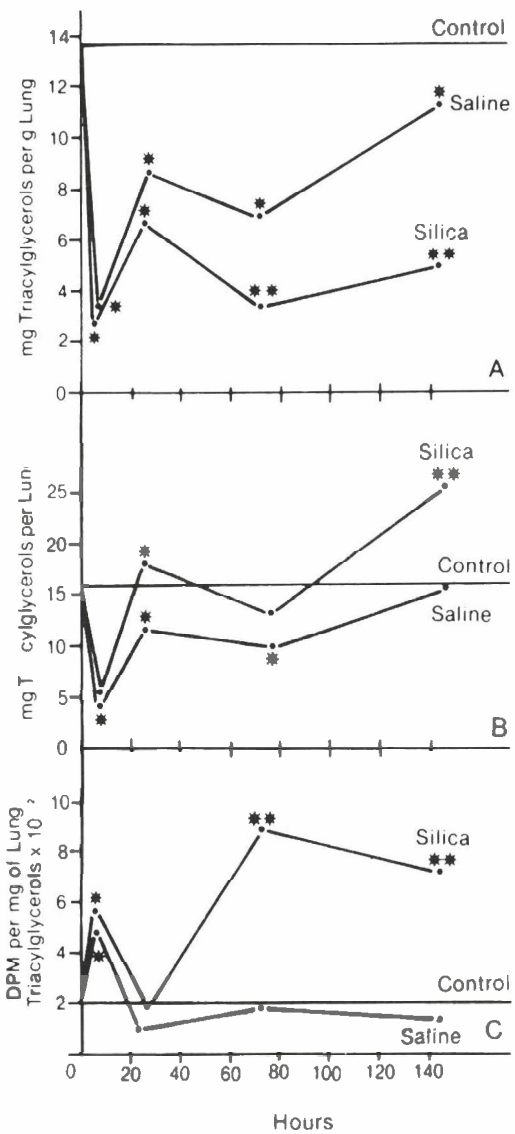

FIGURE 2. Triacylglycerol (TG) changes in rat lung following intrapulmonary injection of saline and silica. Graph A—TG concentration in the lung (mg/g wet lung weight): F = 26.66. Graph B—TG content of lung (mg/lung): F = 21.76. Graph C—SA of TG in liver (dpm/mg lung triacylglycerol): F = 15.53. *Significantly different from control rat ($p \leq .05$). **Significantly different from control and saline rat groups ($p \leq .05$). Each data point represents the mean TG value of 7-14 rat lungs at various times. Number (n) of saline-group individuals and silica-group individuals, at the time intervals studied, were as follows:

Interval (hours)	Saline (n) All graphs	Silica (n) All graphs
6	7	7
24	7	8
72	14	14
144	13	13

Control group n = 9

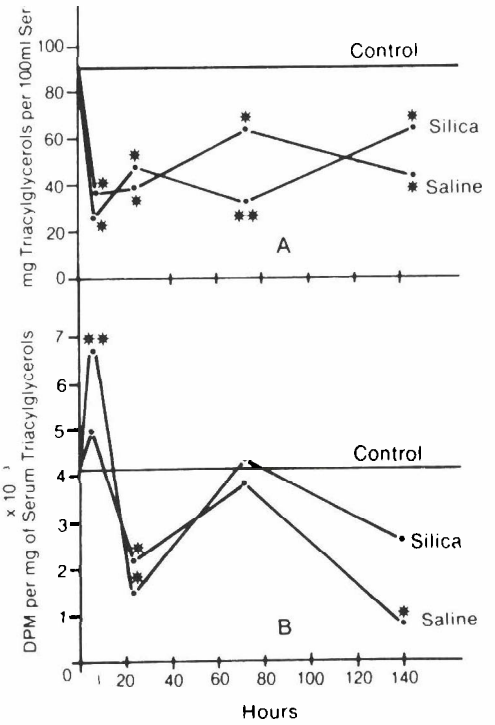

FIGURE 3. Triacylglycerol (TG) changes in rat serum following intrapulmonary injection of saline and silica. Graph A—Serum TG level (mg/100 ml serum): F = 6.16. Graph B—Serum TG SA (dpm/mg of serum TG): F = 2.96. *Significantly different from control rat group ($p \leq .05$). **Significantly different from both control and saline rat groups ($p \leq .05$). Each data point represents the mean TG value of 6-14 samples at various times. For graphs A and B, number (n) of saline-group individuals and number of silica-group individuals. at the time intervals studied, were as follows:

Interval (hours)	Saline (n) A	Saline (n) B	Silica (n) A	Silica (n) B
6	7	7	7	7
24	6	7	8	8
72	13	13	14	14
144	13	12	12	13

Control group n = 9

SA. This finding suggests that the lung has responded to these stressful situations by losing TG. This loss in turn may be attributed to a loss of surfactant, which contains TG,[11] due to the intrapulmonary "saline wash" as discussed in our previous study.[4,5] The lung has the ability for phospholipid formation.[12–14] This pathway also contains intermediate compounds for TG formation. Because lung surfactant is important for maintaining pulmonary function, it is probable that lung TG are utilized for formation of dipalmitoyl lecithin, the principal lipid in lung surfactant.[14]

We recently demonstrated a significant decrease of phospholipid concentration in the early saline- and silica-treated rat lung and further demonstrated an increase of lung and liver phospholipid biosynthesis.[4]

Since TG is an important source of energy for living systems, its utilization as an energy source for maintaining lung lipid homeostasis may be a third reason for the rapid loss of TG in this stressful situation. We suggest that all three of the above processes probably play a role in the decreased concentration of TG in lung tissue in intrapulmonary saline-treated rats.

A fourth probable reason for the decrease in lung TG levels in the silica-treated rats is the dramatic increase in lung weight.[4,5] However, only in silicotic rats is the lung TG decrease associated with a significant increase in liver TG SA, suggesting that the silicotic lung has triggered the liver for TG biosynthesis. We reported a similar activation of liver for phospholipidgenesis[4] and cholesterolgenesis.[5] Thus the early trauma, while inducing silicotic lung injury, apparently causes the liver to respond with a generalized lipidgenesis.

The early silica insult to rat lungs causes the liver to respond by losing TG. This TG loss is associated with a concurrent loss of cholesterol[5] and phospholipid.[4] We thus conclude that the early silicotic lung causes lipid loss from the liver.

Since lung TG content, lung cholesterol

content,[5] and lung phospholipid content[4] increase, we conclude that the silicotic lung accumulates lipids associated with its increasing size. However, the silicotic lung-lipid concentration on a mg/g basis is decreased for all times studied.[4,5]

The SA of silicotic lung TG, cholesterol,[5] and phospholipids[4] are all increased. Therefore we believe that silicotic pulmonary tissue utilizes lipids made in both lung and liver; however, the significant amounts originate from the liver, being transported to the lung by the blood.

The serum TG levels are depressed in silicotic-lung animals, suggesting that some tissue, such as the lung, is picking up TG from the serum. The SA of serum TG is increased only at 6 h, indicating an earlier uptake of the nonlabeled serum TG and an outpouring to the serum, probably from the liver, of *de novo* formed TG probably being taken up by "pulmonary tissue." This concept is supported by the observation that at 6 h the SA of liver TG is increased 6 times as compared to the SA of control rat liver TG.

The saline-treated lung does not activate the liver for TG synthesis. This finding is supported by our previous findings showing that the saline insult to the lung is not a sufficient stimulus to increase liver lipidgenesis; i.e., phospholipidgenesis[4] and cholesterolgenesis.[5] Saline instillation into lung does result, however, in mobilization of lipids, including TG, from the liver to the lung. This process is suggested by a significant decrease of the content of liver TG, phospholipid, and cholesterol.[4,5]

In summary, the TG changes in the liver, lung, and serum of acute silica-induced lung injury correlate with previous findings[4,5] to the effect that the silicotic lung has the ability to cause both a lipid mobilization from the liver to the lung and increased liver lipidgenesis.

The foregoing work was supported by grant HL19633 from the Public Health Service.

We express deep gratitude to Dr. Ronald L. Misiorowski for his interest and advice, and particularly for developing and providing us with computer programs to facilitate our statistical analysis. The crystalline quartz was kindly given to us by Prof. A. G. Heppleston, Institute of Occupational Medicine, Roxburg Place, Edinburgh, England.

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(Received December 14, 1978)