

## Exposure of Rats to Whole Body Gamma Rays Induces Early Alterations in Biliary Secretion

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**Aim:** To study the effects of whole body gamma irradiation on bile flow and bile composition in rats.

**Methods:** Three groups of male Sprague-Dawley rats were irradiated with a single 8 gray (Gy) fraction for 15-20 min and examined for bile secretion at different time intervals (18, 48, 72 h) after irradiation. For collection of biliary secretions, rats were anesthetized with intraperitoneal (i.p.) urethane (1.25 g/kg) and equipped with biliary cannulas inserted into the bile duct through the sphincter of Oddi. Bile was collected for 4 h following bile duct cannulation. Bile flow (bile-pancreatic juice) and biliary excretion of total proteins, cholesterol and total lipids were measured. Biliary activities of the hepatocellular alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the canalicular enzyme alkaline phosphatase (ALP) and biliary excretion of glucose were also assessed.

**Results:** After an 8-Gy fraction, no significant alterations occurred in bile flow. Biliary total protein concentration and outputs were significantly decreased following gamma irradiation, with 29.3% and 34.7% reductions in rats examined 72 h post-irradiation. Biliary total cholesterol and lipid concentrations and outputs were also significantly decreased, with the reduction being most marked during the 18 h-48 h post-irradiation measurement period. Biliary activity of ALT increased at 72 h post-irradiation, while that of AST was decreased at 48-72 h post-irradiation. Biliary ALP activity was significantly increased by the 2nd day post-irradiation. Biliary glucose secretion, which was very low in control rats, showed progressive increase over the study period and peaked at 48 h post-irradiation, coinciding with the increase in biliary ALP efflux.

**Conclusions:** Exposure of rats to whole body gamma rays induces early alterations in biliary secretion.

**Key Words:** Whole body gamma irradiation, bile secretion, rats

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## Tüm Vücut Gama Işınlamasına Maruz Kalan Ratlarda Biliyer Sekresyonlardaki Erken Değişiklikler

**Amaç:** Ratlarda tüm vücut gama ışınlanmasının safra akışı ve safra bileşimine olan etkisinin araştırılması.

**Yöntem ve Gereç:** 24 Sprague-Dawley rat her biri 6 hayvandan oluşan dört gruba ayrıldı. Kontrol grubu dışında kalan üç gruptaki ratlara 8 gray (Gy) dozunda, 15-20 dakika süreyle radyasyon verildi ve 18, 48, 72. saatlerde safra sekresyonu ölçüldü. Biliyer salgının toplanması için ratlar intraperitoneal (İP) uretan (1.25 g/kg) ile anestetize edildi ve oddi sfinkteri aracılığıyla biliyer kanülasyon yapıldı. Kanülasyondan sonra 4 h süresince safra toplandı. Safra akışı ve safradaki total proteinler, kolesterol total lipid düzeyleri ölçüldü. Safradaki Aspartat Aminotransferaz (AST), Alanin Aminotransferaz (ALT) düzeyleri ve kanaliküler enzim olan Alkalenfosfataz (ALP) ve safradaki glukoz düzeyleri ölçüldü.

**Bulgular:** 8-Gy fraksiyone uygulama sonrası safra akışında önemli bir değişiklik olmadı. Gama ışınlanmasını takiben 72 saatte incelenen ratlarda total protein konsantrasyonu % 29.3 ve atılımı % 34.7 oranında belirgin biçimde azaldı. Biliyer total kolesterol ve lipid düzeyleri de 18. - 48. saatte belirgin biçimde azaldı. Safra da AST düzeyleri 48 - 72 saat sonra azalırken, ALT aktivitesi 72. saatte arttı. Işınlamadan iki gün sonra safra ALP düzeyleri önemli ölçüde arttı. Biliyer glukoz düzeyleri kontrol ratlarda çok düşükken, çalışma periyodu boyunca belirgin ölçüde arttı. Artış 48 saatte en belirgini ve biliyer ALP artışına paraleldi.

**Sonuç:** Ratların tüm vücut gama ışınlanması biliyer salgılarda erken değişikliklere yol açar.

**Anahtar Sözcükler:** Tüm vücut ışınlama, safra salınımı, rat

Received: September 12, 2005  
Accepted: October 10, 2006

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## Introduction

The effects of irradiation on the liver and other organs have been the subject of extensive research (1-3). Very few studies, however, have addressed the effect of irradiation on bile secretion and bile constituents (4,5). Bile secretion is an important function of the liver whereby the liver detoxifies drugs and xenobiotics. Bile also constitutes a major excretory route for cholesterol (6). Furthermore, an alteration in bile secretion has been proposed as an important cause of radiation enteritis, a complication that occurs frequently during abdominal radiotherapy (7). We therefore examined, in rats, whether whole body gamma irradiation of 8 Gray (Gy) led to alterations in bile secretion and constituents within the first three days after exposure to irradiation. Measurements were made in rats at 18, 48 and 72 h after their exposure to radiation.

## Materials and Methods

Male Sprague-Dawley rats weighing 130-140 g were used. Animals were housed under standardized conditions for light and temperature. Rats were randomly divided into four groups (n = 6/group) and three groups were irradiated with a single 8 Gy fraction for 15-20 min and examined for bile secretion at different times (18, 48, 72 h) after irradiation. The fourth group served as normal controls. Irradiation was performed through the use of <sup>137</sup>Cs gamma rays from the gamma cell-40 belonging to the National Centre for Radiation Research and Technology (NCRRT). The dose rate was 1 Gy/1.5 min at the time of the experiment. Rats were allowed access to laboratory chow and water until about 1 h before the study. To avoid variations due to circadian rhythms, all experiments were started at the same time of day at 9 a.m. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85-23, revised 1985).

### Surgical procedure

For measurement of bile secretion, rats were anesthetized with urethane (1.25 g/kg) given intraperitoneally. The bile duct was surgically exposed by a midline incision and cannulated with a polyethylene cannula inserted into the common bile duct at the sphincter of Oddi. The abdominal wall was covered with saline moist

gauze, and the rectal temperature was maintained at 37°C by a heating lamp to prevent hypothermic changes in bile flow. After an equilibration period of 30 min to allow bile flow to stabilize, bile was collected from each rat in 30-min fractions into pre-weighed vials for 4 h. Bile samples were immediately stored at -20 °C until the time of the assay. Rats were administered subcutaneous saline (1 ml/h) to correct for fluid loss during the experiment. At the end of the experiments, the rats were killed by exsanguination. Livers were quickly removed, weighed, and immersed in 10% formaldehyde solution for histological assessments. Mean bile flow was estimated gravimetrically, assuming a bile density of 1.0, and bile flow was expressed as  $\mu\text{L}/30 \text{ min/g liver}$ . The protein concentrations were determined in all groups studied in all bile samples over the 4 h study period. Bile collected from each rat was then pooled to provide an amount sufficient for further biochemical analysis.

### Analyses

Total protein in bile-pancreatic juice was measured spectrophotometrically using the assay method of Bradford (8). Bile was assayed for total cholesterol by the colorimetric method of Richmond et al. (9), which is based on enzymatic hydrolysis of cholesterol esters, oxidation of cholesterol by cholesterol oxidase, and colorimetric measurement of liberated hydrogen peroxide with 4-aminoantipyrine, phenol, and peroxidase. Colorimetric determination of total lipids with sulfophospho-vanillic mixture was used for biliary lipid measurement (10). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in bile were measured according to Reitman-Frankel colorimetric transaminase procedure (11), whereas colorimetric determination of alkaline phosphatase (ALP) activity was done according to the method of Belfield and Goldberg (12). Glucose concentrations in bile were measured enzymatically (10).

### Statistics

Results are expressed as means  $\pm$  S.E. for all data. Data were analyzed by one-way ANOVA, and post-hoc Duncan's multiple range test was used to compare different group means. P value less than 0.05 was considered significant. The number of rats used in experiments is presented in the text in parentheses. Output rates of total proteins, cholesterol and total lipids were determined by multiplying bile flow by corresponding concentrations.

## Results

### Bile flow

Bile flow in control rats averaged  $34.43 \pm 2.22$  mL/30 min/g liver during the 4 h period of the study. Bile flow was reduced following exposure to 8 Gy gamma irradiation relative to control values, the reduction being maximal in rats examined at 48 h post-irradiation. Bile flow was reduced from the above control value to  $31.42 \pm 1.80$ ,  $28.91 \pm 3.20$  and  $31.84 \pm 1.70$   $\mu$ L/30 min/g liver. This corresponded to an 8.7%, 16% and 8.2% reduction in bile flow compared to the control group, respectively (Table 1).

### Biliary proteins

Exposure to 8 Gy significantly decreased biliary protein concentrations from control values of  $3.0 \pm 0.06$  to  $2.49 \pm 0.09$ ,  $2.43 \pm 0.08$  and  $2.12 \pm 0.09$  mg/ml at 18 h, 48 h and 72 h post-irradiation periods, respectively. Mean protein excretion rate was similarly significantly reduced by 24.3%, 26% and 34.7% at 18 h, 48 h and 72 h post-irradiation periods from  $103.23 \pm 6.74$  to  $78.19 \pm 4.82$ ,  $69.58 \pm 7.2$  and  $67.37 \pm 7.1$   $\mu$ g/30 min/g liver, respectively (Table 1).

### Biliary lipids

Exposure to 8 Gy markedly reduced biliary lipid concentration and biliary lipid output concentrations with respect to the control values. The concentration of total lipids in bile was reduced by 26.3%, 24% and 16.7% at 18 h, 48 h and 72 h post-irradiation periods from control values of  $2213.3 \pm 115.7$  to  $1631.67 \pm 68.8$ ,  $1684 \pm 123.3$  and  $1844 \pm 80.1$   $\mu$ g/ml,

respectively. Mean lipid output was reduced by 32.7%, 36.12% and 23% at 18 h, 48 h and 72 h post-irradiation periods from control values of  $76.21 \pm 3.98$  to  $51.28 \pm 2.8$ ,  $48.68 \pm 3.6$  and  $58.71 \pm 2.6$   $\mu$ g/30 min/g liver, respectively (Table 1).

### Biliary cholesterol

Total cholesterol concentration and output were also significantly decreased following irradiation. Cholesterol concentration in bile was reduced by 23.1%, 26% and 22.4% at 18 h, 48 h and 72 h post-irradiation periods from control values of  $119.8 \pm 4.81$  to  $92.1 \pm 4.18$ ,  $87.74 \pm 4.68$  and  $93.0 \pm 3.12$   $\mu$ g/ml, respectively. Mean cholesterol output was reduced by 29.6%, 38.4% and 28.2% at 18 h, 48 h and 72 h post-irradiation periods from control values of  $4.12 \pm 0.17$  to  $2.9 \pm 0.13$ ,  $2.54 \pm 0.14$  and  $2.96 \pm 0.1$   $\mu$ g/30 min/g liver, respectively (Table 1).

### Biliary glucose

Biliary glucose concentration was very low in control rats (19.4  $\mu$ g/ml). Biliary glucose concentration rose significantly by 9.3-fold as early as 18 h post-irradiation, reaching about 27-fold at 48 h post-irradiation, and 18.3-fold on the 3<sup>rd</sup> day post-irradiation compared to non-irradiated rats (Table 2).

### Biliary enzymes

Significant elevation in biliary activity of the hepatocellular enzyme ALT was seen at 72 h post-irradiation compared with non-irradiated control rats or those examined at 18 or 48 h post-irradiation. On the contrary, biliary AST activity was significantly suppressed

Table 1. Effect of single dose gamma irradiation on bile flow and biliary secretion of total proteins, total lipids and cholesterol in the rat.

	Bile flow		Bile proteins		Bile cholesterol		Bile lipids	
	$\mu$ L/30min/g liver	mg/ml	$\mu$ g/30min/g liver	$\mu$ g/ml	$\mu$ g/30min/g liver	$\mu$ g/ml	$\mu$ g/30min/g liver	
Control	$34.43 \pm 2.22$	$3.00 \pm 0.062$	$103.23 \pm 6.74$	$119.8 \pm 4.81$	$4.12 \pm 0.17$	$2213.1 \pm 115.7$	$76.21 \pm 3.98$	
18 h post-irradiation	$31.42 \pm 1.80^{NS}$	$2.50 \pm 0.087^*$	$78.19 \pm 4.82^*$	$92.10 \pm 4.18^*$	$2.90 \pm 0.13^*$	$1631.7 \pm 86.80^*$	$51.28 \pm 2.81^*$	
48 h post-irradiation	$28.91 \pm 2.20^{NS}$	$2.43 \pm 0.078^*$	$69.58 \pm 7.20^*$	$87.74 \pm 4.68^*$	$2.54 \pm 0.14^*$	$1684.0 \pm 123.3^*$	$48.68 \pm 3.60^*$	
72 h post-irradiation	$31.84 \pm 2.10^{NS}$	$2.12 \pm 0.086^*$	$67.37 \pm 7.10^*$	$93.00 \pm 3.12^*$	$2.96 \pm 0.10^*$	$1844.0 \pm 80.1^*$	$58.71 \pm 2.60^*$	

Results are means  $\pm$  S.E. Data were analyzed by one-way ANOVA and means of different groups were compared by Duncan's multiple range test. Two-tailed probabilities of less than 0.05 were considered significant. Statistical comparisons between the saline-treated control and irradiated groups are indicated by asterisks.

Table 2. Effect of single dose gamma irradiation on biliary secretion of AST, ALT, ALP and glucose in the rat.

	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Glucose (µg/ml)
Control	49.80 ± 2.66	30.00 ± 3.78	29.16 ± 1.58	19.40 ± 12.10
18 h post-irradiation	48.40 ± 5.78	37.00 ± 1.41	28.13 ± 3.31	180.17 ± 46.00*
48 h post-irradiation	34.53 ± 2.86*	32.50 ± 2.10	41.05 ± 4.80*	551.30 ± 64.30*
72 h post-irradiation	31.80 ± 0.58*	67.25 ± 6.52*	23.95 ± 3.20	355.60 ± 61.00*

Results are means ± S.E. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan's multiple range test. Two-tailed probabilities of less than 0.05 were considered significant. Statistical comparisons between the saline-treated control and irradiated-groups are indicated by asterisks.

by the 2<sup>nd</sup> and 3<sup>rd</sup> days post-irradiation compared with non-irradiated control rats or those examined at 18 h post-irradiation (Table 2). The biliary activity of the canalicular enzyme ALP was significantly increased in bile examined 48 h post-irradiation compared to other groups (Table 2). This coincided with the maximal increase in biliary glucose leakage.

**Histology**

The liver of control rats revealed the characteristic hepatic architecture (Figure 1A). Sections examined as early as 18 h after exposure to whole body gamma irradiation revealed variable degrees of cellular lesions, oedema, cellular infiltration, cloudy swelling, necrosis, damaged bile ducts and epithelial cells. The nuclei suffered from pleomorphism and the chromatin clumped adjacent to the inner membrane of the nuclear envelope (Figure 1B). Changes increased in severity over the next 48-72 h post-irradiation (Figure 1C). Sections examined 72 h after exposure showed marked cellular infiltration and increased fibrous tissue (Figure 1D).

**Discussion**

Very little information is available concerning gamma irradiation in rats, particularly with regard to bile flow and bile composition. Data obtained from 8 Gy whole body gamma irradiated rats are presented in this study. Results indicated that a non-significant reduction in bile flow is registered in rats at 18 h, 24 h, and 72 h post-irradiation. Scanff et al. (5) showed that bile flow in mixed neutron and gamma irradiated pigs decreased significantly on the 1st day then returned to control values for the next 3 days post-irradiation.

It was of greatest interest to study early alterations in bile composition after radiation exposure. Scanff et al. (13) studied the dose-related changes in bile acids to identify potential bio-indicators of radiation-induced gastrointestinal injuries. They found that the proportion of dihydroxylated bile acids was increased at 8 Gy in the pool of total bile acids, which might explain radiation-induced diarrhea.

From the data presented in our study, it is evident that 8 Gy gamma irradiation induced changes in biliary excretion of total proteins, cholesterol, and total lipids, in biliary activities of hepatocellular ALT, AST, ALP and in glucose content. In the present work, a marked decrease has been observed in the level of biliary total proteins in irradiated rats. This drop coincides with the decrease in serum total proteins reported by other workers in irradiated rats, which may be due to radiation damage to the liver (14).

Manciluae et al. (15) reported that after whole body gamma irradiation there was significant inhibition in liver DNA, RNA and protein synthesis. Reddy and Sasira (16) showed an increase in protease activity and a decrease in protein content during the post-irradiation period due to lysosomal damage. Roushdy et al. (17) suggested that the decrease in protein in irradiated rats might be the result of changes in the permeability of the liver.

The present data also showed that 8 Gy whole body gamma irradiation caused a decline in biliary total cholesterol and total lipid concentration which was marked at 18 h and 48 h post-irradiation. Scanff et al. (4) studied the effect of 6 Gy whole body gamma irradiation on bile composition in pigs. They concluded that cholesterol and phospholipid concentrations were not

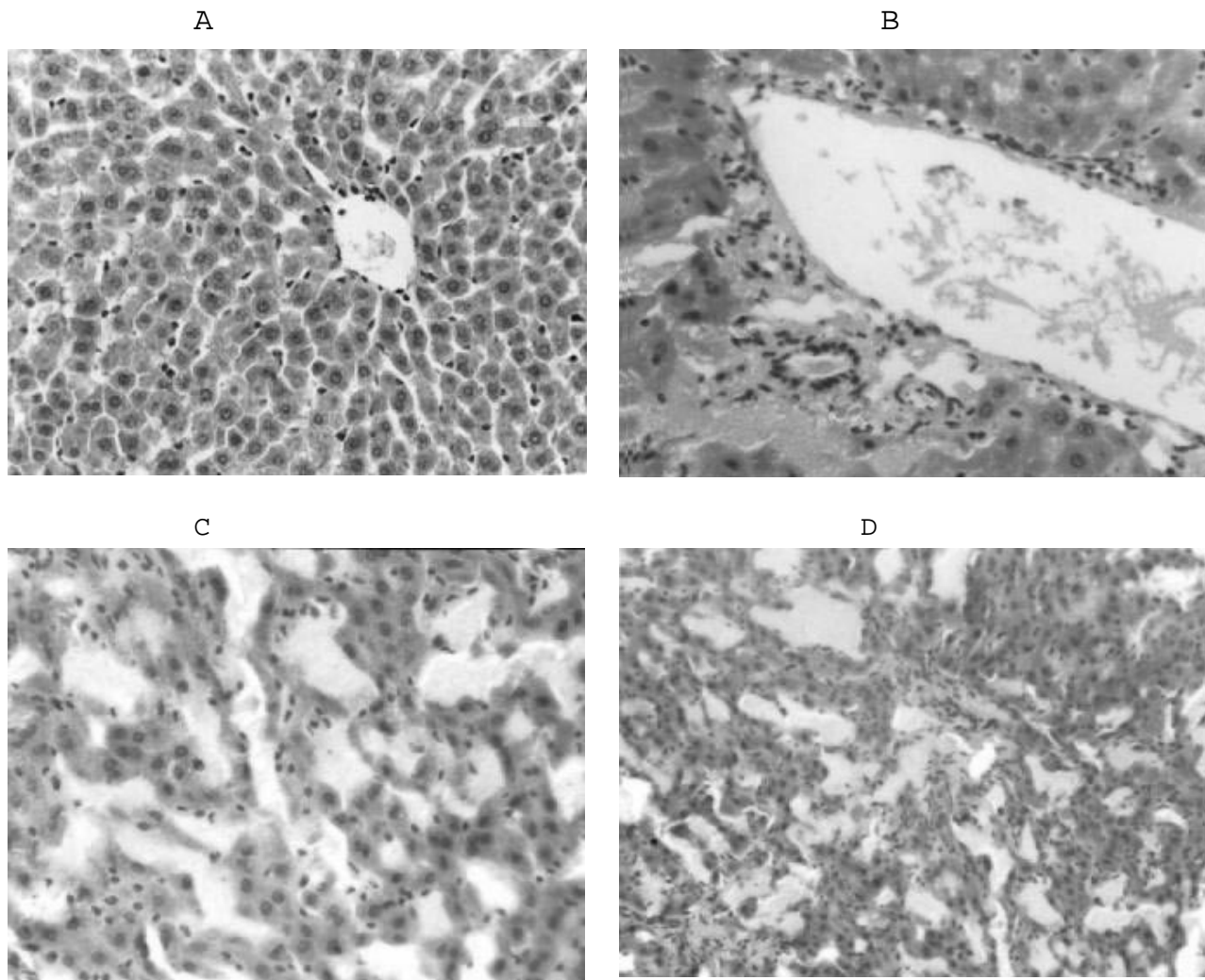


Figure 1. Hematoxylin and eosin stained section from (A) control rat liver; (B) rat liver 18 h after exposure to 8 Gy whole body gamma irradiation showing expanded portal vessels, necrosis and damaged bile ducts; (C) rat liver 48 h after exposure to 8 Gy whole body gamma irradiation showing marked increased in fibrous tissues and edematous spaces - the hepatocytes show cloudy swelling and nuclear pleomorphism; and (D) rat liver 72 h after exposure to 8 Gy whole body gamma irradiation showing chronic cellular infiltration. The hepatocytes nuclei show margination of chromatin, angular nuclei, non-prominent nucleolus and hypernuclei.

altered. It is likely that the different results are due to species differences. In their study in the hamster, Feurgard et al. (3) found that 8 Gy gamma irradiation decreased plasma levels of cholesterol, triglycerides and phospholipid concentrations 2 days post-irradiation, but this reduction was followed by an increase in all these values on the 6th day post-irradiation. Changes in the activities of hepatic HMGCoA reductase, the rate-limiting enzyme for cholesterol synthesis, and in hepatic cholesterol 7 $\alpha$ -hydroxylase, the rate-limiting enzyme

for bile acid synthesis, were noted following radiation exposure (3). In intestinal mucosa of the rat exposed to 4 Gy of whole body gamma irradiation, cholesterol, DNA and protein synthesis in crypt cells were lowered 1 and 2 days after irradiation, over-expressed after 4 days and subsequently returned to normal level (18).

The assessment of hepatic and biliary ALT, AST, and ALP aims to evaluate the degree of hepatic functional disturbances. Cholestasis is associated with a marked increase in the release of canalicular membrane enzymes

into bile (19). The changes in the activity of transaminases due to radiation were found to be contradictory. Some authors recorded an elevated activity while others found a decrease. The present results showed that radiation affected biliary ALT, AST and ALP levels. These data are in harmony with those reported by Cheng et al. (20). Changes in activities of biliary enzymes may be due to drastic physiological effects caused by irradiation interaction with the cellular membrane, mitochondria or through the action of free radicals. Hence, the increase in ALT activities obtained in our results may be related to extensive breakdown of liver parenchyma with subsequent enzyme release, or to increase in permeability of the cell membrane that could enhance the movement of enzymes from their sites of production, while the decrease in some enzyme activities (AST) can be attributed to the inactivation of their biosynthesis (15).

Our results also showed that whole body gamma irradiation at 8 Gy evoked a sharp increase in biliary

glucose levels, reaching 27-fold at 48 h post-irradiation, the time when the increase in biliary ALP efflux was marked. Hyperglycemia has been observed after radiation exposure, which may be attributable to the diminished utilization of glucose by irradiated tissues (21) or to the indirect effect of radiation exposure which accelerated the process of gluconeogenesis (22). The increase in biliary glucose might also reflect impairment in glucose transport as a result of radiation injury to biliary epithelial cells. Increased biliary glucose has also been observed following biliary epithelial damage by other toxic substances (23,24).

In summary, exposure of the rat to 8 Gy whole body gamma irradiation induced early alterations in hepatic function. Results are distinct from those reported in other species (e.g. pigs) and imply that bile flow is not severely impaired early after exposure to gamma rays; however, marked reductions were observed in the secretion of proteins, lipids, and cholesterol, while biliary glucose secretion increased.

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