

# Toxic Effects of Glucagon-Induced Acute Lipid Mobilization in Geese

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**ABSTRACT** The toxic effects associated with rapid lipid mobilization and a high plasma free fatty acid (FFA) concentration produced by glucagon were evaluated. Glucagon (0.5 mg/kg of body wt) was injected intravenously into nonfasting geese. The geese developed rapid respirations and high plasma FFA levels within 15 min after the glucagon injection; three of eleven died. Control geese, injected with saline, did not exhibit toxic signs. Peak FFA concentrations developed 15 min after glucagon and high levels persisted for over 90 min. Geese injected with glucagon frequently developed electrocardiographic abnormalities that included supraventricular tachycardia, premature ventricular contractions, and signs of myocardial ischemia. Light and electron microscopy revealed acute myocardial degeneration and fatty infiltration of the liver. The increase in plasma FFA concentrations and toxic effects were not prevented by pretreatment with nicotinic acid or propranolol.

## INTRODUCTION

Acute mobilization of lipid which results in high plasma free fatty acid (FFA) concentrations has been associated with toxic effects in dogs<sup>1</sup> and rabbits (2). Long chain fatty acids are known to produce cytotoxic effects in tissue cultures (3)

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and cause acute myocardial failure when injected intravenously in the unbound state into dogs (4). Infusion of norepinephrine has been associated with the development of fatty liver (5) and acute myocardial necrosis in dogs (6, 7). These effects in dogs can be prevented by propranolol that inhibits the usual increase in FFA produced by catecholamines.<sup>1</sup>

Birds mobilize lipid to a lesser extent in response to catecholamines or pituitary hormones (8, 9) than do mammals, but develop a prompt increase in FFA after injections of glucagon (10). Lipolysis in pigeon adipose tissue in vitro was stimulated by epinephrine and glucagon (11).

In this experiment, the biochemical and morphological effects of acute lipid mobilization produced by glucagon were studied in the goose (*Anser domesticus*).

## METHODS

Healthy, nonfasting geese of both sexes, weighing 2.5–7 kg were used. In group I, 11 geese were used. Under local 1% lidocaine anesthesia, an incision was made in the neck and the jugular vein was exposed and used for intravenous injections and blood sampling. After an initial blood sample was taken, glucagon<sup>2</sup> 0.5 mg/kg was given intravenously. Additional blood samples were taken 15, 30, 60, and 90 min for plasma FFA, blood glucose, and serum triglyceride determinations. Serum glutamic oxalacetic transaminase and lactic dehydrogenase activities were measured in four control and seven glucagon-treated geese. Electrocardiograms were taken on representative geese in all groups at intervals during the experiments. At the end of 90 min, all geese in group I were killed with pentobarbital. Samples of tissue were

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<sup>2</sup> Glucagon, USP, Eli Lilly & Co., Indianapolis, Ind.

TABLE I  
Mortality and Toxicity after Glucagon

Group	Injection	Interval between injection and sacrifice	No. in group	Toxic signs	Deaths	Heart lesions	Hepatic lesions
I	Glucagon	90 min	11	11	3	0	5, fatty changes
II	Saline control	90 min	5	0	0	0	1, slight fatty change
III	Glucagon	24 hr	10	10	2	6	8, fatty changes 1, hepatic necrosis
IV	Saline control	24 hr	5	0	0	0	1, slight fatty change
V	Nicotinic acid plus glucagon	90 min	6	6	1	1	3, fatty changes
VI	Propranolol plus glucagon	90 min	5	5	2	1	4, fatty changes

Toxic signs included rapid, labored respirations, tachycardia, vomiting, and prostration.

taken and prepared for light and electron microscopy. In group II, five control geese were injected with 0.9% saline instead of glucagon and had the same procedures as those in group I. Similar studies were performed in 10 geese (group III) given glucagon in the usual dose and allowed to survive 24 hr after the injection. Incisions were not made in these birds. Five control geese were injected with saline and allowed to survive for 24 hr (group IV).

In an attempt to inhibit the effects of glucagon, six geese were treated with nicotinic acid (group V) before they were given glucagon 30 min later. Two geese received nicotinic acid, 25 mg/kg by intraperitoneal injection; two others received the same dose intravenously, and two were given 50 mg/kg intravenously. Five geese were treated with propranolol, (group VI) 2 mg/kg, intravenously, 15 min before they were given glucagon. Geese in groups V and VI were killed 90 min after they received glucagon.

Tissue for electron microscopy was fixed in 1% phosphate-buffered osmium tetroxide, dehydrated in alcohol, and embedded in epon-Araldite. Sections were cut on a Reichert Om U2 ultramicrotome, stained with lead citrate and uranyl acetate, and examined with a Philips EM 300 electron microscope. Tissue for light microscopy was prepared by standard techniques. Sections were stained with Oil Red O to demonstrate the presence of lipid.

Plasma FFA was determined by the method of Dole (12). Serum triglycerides were measured by the method of Van Handel and Zilvermit (13). Blood glucose was measured by the Hoffman method (14) on the Auto-Analyzer. Serum glutamic oxalacetic transaminase (SGOT) activity was determined by the Karmen method (15) and lactic dehydrogenase (LDH) activity was as-

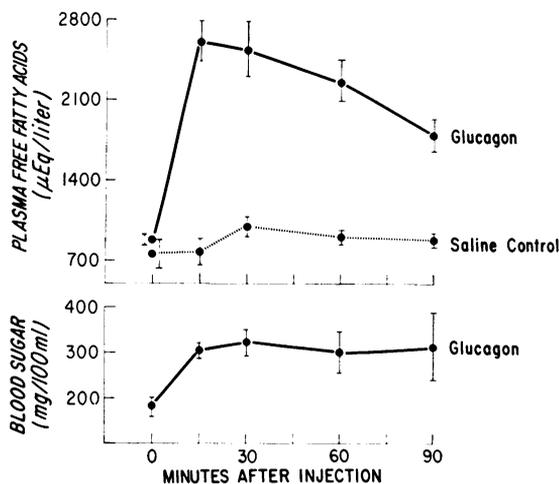


FIGURE 1 Effect of glucagon upon FFA and blood glucose concentrations. Glucagon, 0.5 mg/kg, intravenously, caused a prompt increase in FFA and blood glucose concentrations.

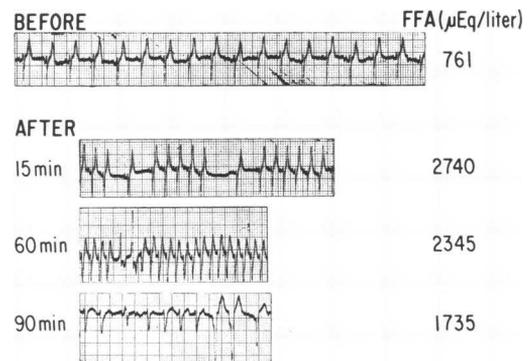


FIGURE 2. Electrocardiogram of a goose treated with glucagon. This tracing was taken before and after the administration of glucagon, 0.5 mg/kg. Within 15 min the FFA increased from 761 to 2740  $\mu$ Eq/liter and the electrocardiogram showed an increase in cardiac rate and periods of sinus arrest. Plasma FFA remained high. At 60 min, supraventricular tachycardia was present. By 90 min the rate had slowed, but the bizarre complexes suggested a ventricular rhythm or aberrant conduction with supraventricular arrhythmia. The goose died shortly after the last tracing was taken.

TABLE II  
Effects of Nicotinic Acid and Propranolol on FFA Response to Glucagon

Group	Drug	No. of geese	Plasma FFA ( $\mu$ Eq/liter)					
			Before drug	After drug	Min after glucagon (0.5 mg/kg)			
					15	30	60	90
I	None	11	873 $\pm$ 42	—	2611 $\pm$ 174	2540 $\pm$ 237	2272 $\pm$ 174	1789 $\pm$ 127
V	Nicotinic acid	6	650 $\pm$ 31	682 $\pm$ 21 (30 min)	2400 $\pm$ 220	2466 $\pm$ 125	2241 $\pm$ 206	1976 $\pm$ 156
VI	Propranolol	5	765 $\pm$ 57	1036 $\pm$ 104* (15 min)	3195 $\pm$ 159	2900 $\pm$ 192	3137 $\pm$ 349‡	—

Plasma FFA values represent mean values  $\pm$  SEM.

\* Represents a significant difference from the mean value of group V ( $P < 0.05$ ).

‡ Represents a significant difference from the mean value of the group given glucagon alone ( $P < 0.05$ ).

sayed by the method of Wroblewski and LaDue (16).

Statistical analysis of the values for plasma FFA concentrations, serum triglyceride determinations, and serum SGOT and LDH activities were performed by the *t* test.

### RESULTS

Within 10 min after the geese were injected with glucagon, toxic signs developed. Characteristic toxic signs included tachycardia, rapid respira-

tions, vomiting, defecation, and prostration. Some geese exhibited progressive deterioration and died within 90 min. The mortality figures are shown in Table I. In contrast, control birds did not exhibit toxic signs and none died.

Glucagon produced a threefold increase in FFA within 15 min (Fig. 1). Mean FFA values increased from 873 to 2611  $\mu$ Eq/liter and remained elevated for the rest of the experiment. A simul-

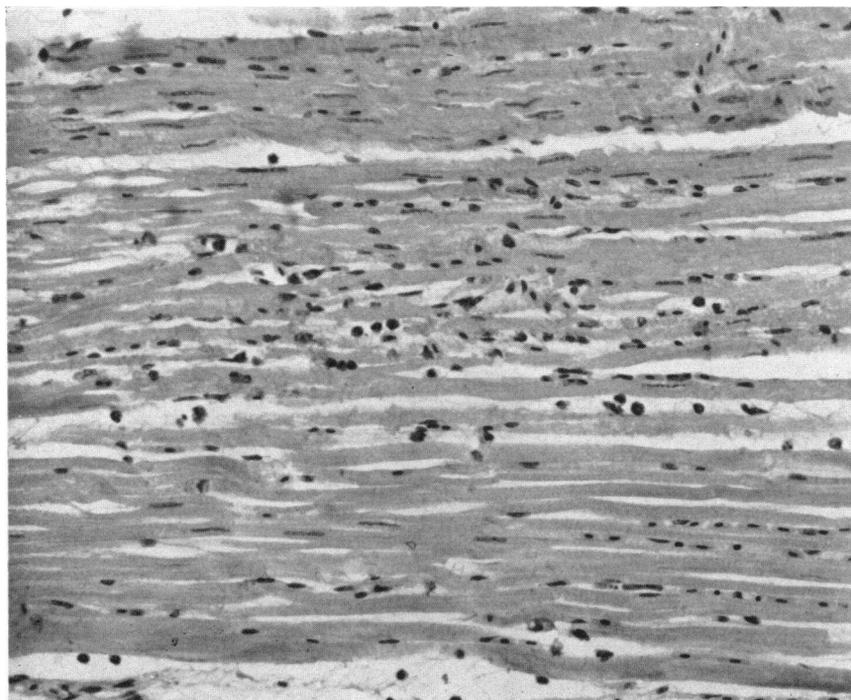


FIGURE 3 Photomicrograph of a myocardial lesion from a goose injected with glucagon and allowed to survive 24 hrs. Changes of early myocardial necrosis with leukocytic infiltration are present. H and E,  $\times 250$ .

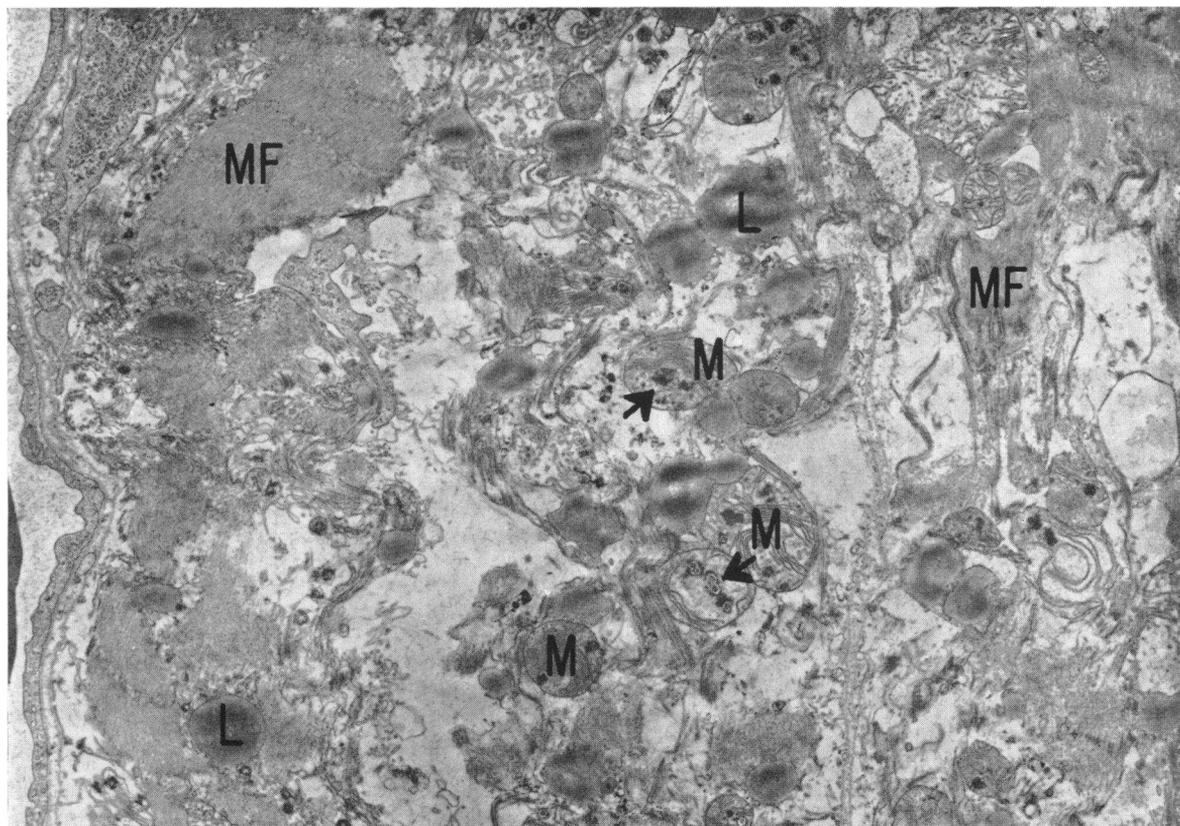


FIGURE 4 Electron micrograph of the myocardial lesion shown in Fig. 3. Note the distorted and irregular mitochondria (*M*). Arrows point to intramitochondrial osmiophilic inclusion particles. Myofibrils (*MF*) showed areas of destruction. Lipid Particles (*L*) were frequently seen.  $\times 7330$ .

taneous increase in blood glucose also occurred. Nicotinic acid did not prevent the rise in FFA induced by glucagon (Table II). Geese treated with propranolol before receiving glucagon developed even higher FFA levels than those given glucagon alone. Neither nicotinic acid nor propranolol inhibited the adipokinetic influence or the toxic effects that resulted from the injection of glucagon.

Geese, given glucagon, developed electrocardiographic changes frequently. An example is shown in Fig. 2. Sinus tachycardia and premature ventricular contractions were common. In others, sinus arrest, supraventricular tachycardia, ventricular tachycardia, and signs of myocardial ischemia were observed. In group III, 6 of 10 geese that developed electrocardiographic abnormalities had myocardial lesions at necropsy. Control geese did not develop electrocardiographic changes.

Serum glutamic oxalacetic transaminase (SGOT) and lactic dehydrogenase (LDH) ac-

tivities were determined in a small number of birds. Both control geese (group II) and glucagon-treated geese (group I) developed progressive increases in SGOT and LDH activities, but the glucagon-treated geese had higher values. Glucagon-treated geese had an increase in SGOT activity from a base line value of  $49 \pm 10$  units to  $206 \pm 73$  at 90 min ( $P > 0.05$ ) whereas control geese SGOT values increased from  $25 \pm 6$  to  $68 \pm 14$  units at 90 min, ( $P > 0.05$ ). Greater changes were seen in LDH activity. The values in glucagon-treated geese increased from a base line of  $406 \pm 115$  to  $1311 \pm 261$  units at 90 min, ( $P < 0.01$ ). Control geese increased from  $310 \pm 30$  to  $750 \pm 50$  units, ( $P > 0.05$ ). Part of the increase in enzyme activities in both groups may have resulted from tissue damage due to the operative incisions and hematoma formation after collection of the blood samples.

Mean serum triglyceride values increased in the

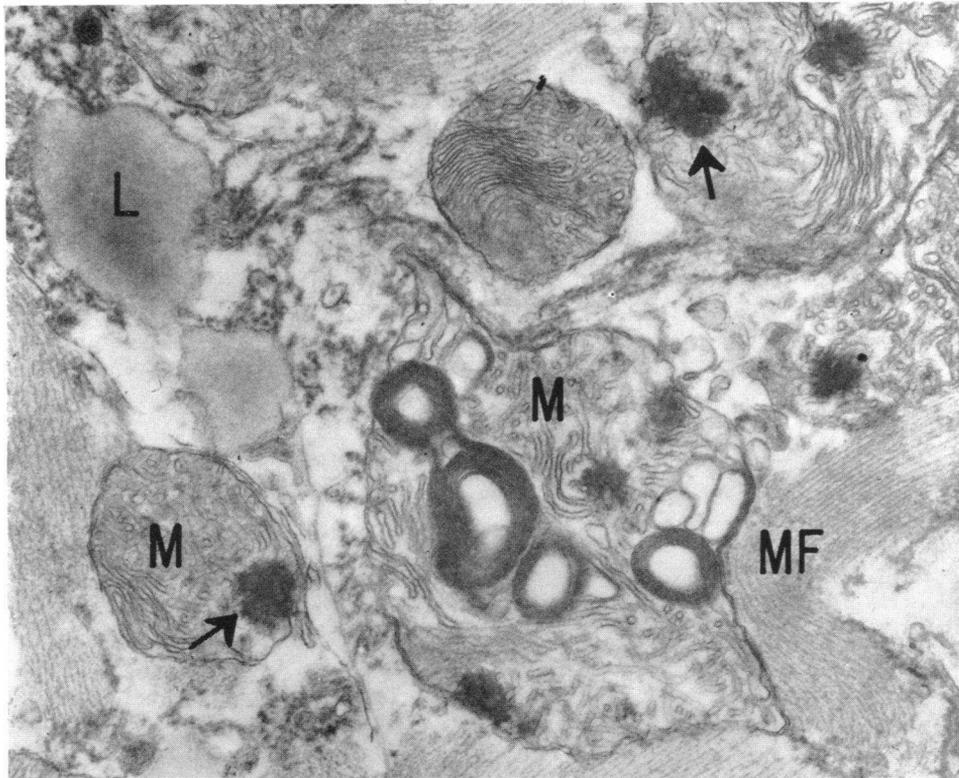


FIGURE 5 Electron micrograph of the lesion shown in Fig. 4 which demonstrates intramitochondrial changes at greater magnification. Note the myelin figures in the distorted mitochondrion (*M*) in the center. Arrows point to inclusions. Myofibril (*MF*).  $\times 36,000$ .

glucagon-treated geese (group I) from  $115 \pm 31$  to  $182 \pm 52$  mg/100 ml at 90 min whereas the control values (group II) decreased from  $176 \pm 100$  to  $119 \pm 68$ . None of the changes in triglyceride concentrations were statistically significant.

Although 6 of the 22 geese given glucagon died within 90 min after the injection, myocardial lesions were found in only two. Twelve in this group had fatty livers. In contrast, 6 of 10 geese allowed to survive for 24 hr after receiving glucagon had myocardial lesions at necropsy. In the latter group, eight had fatty infiltration of the liver and one had acute hepatic necrosis.

The typical myocardial lesion on gross examination consisted of a focal hemorrhagic area that was usually found in the ventricular wall. On histological examination areas of focal hemorrhage were seen. In one of the myocardial lesions from a goose in group III (Fig. 3), leukocytic infiltration and focal areas of early myocardial necrosis were conspicuous features. Small fat droplets were present in the lesion when the sections were stained with

Oil Red O. Examination of the cardiac lesions with electron microscopy revealed destructive changes in the myofibrils, distortion of mitochondrial structure, and osmiophilic intramitochondrial inclusions in addition to the changes observed with light microscopy (Figs. 4 and 5). An electron micrograph demonstrating myocardium from a normal goose is shown in Fig. 6.

Hepatic changes seen in the glucagon-treated geese consisted chiefly of fatty infiltration that was presented grossly as a yellowish-tan liver. Histologically, lipid vacuoles were seen in the hematoxylin and eosin stained specimens of liver (Fig. 7 B). These areas took up a stain for lipid, Oil Red O, which is shown in Fig. 7 C. Electron microscopy confirmed the changes seen with light microscopy (Fig. 7 A). In one goose in group III, hepatic necrosis was found in addition to the fatty infiltration. Electron micrographs and photomicrographs of normal goose liver are shown in Fig. 8 A, 8 B, and 8 C.

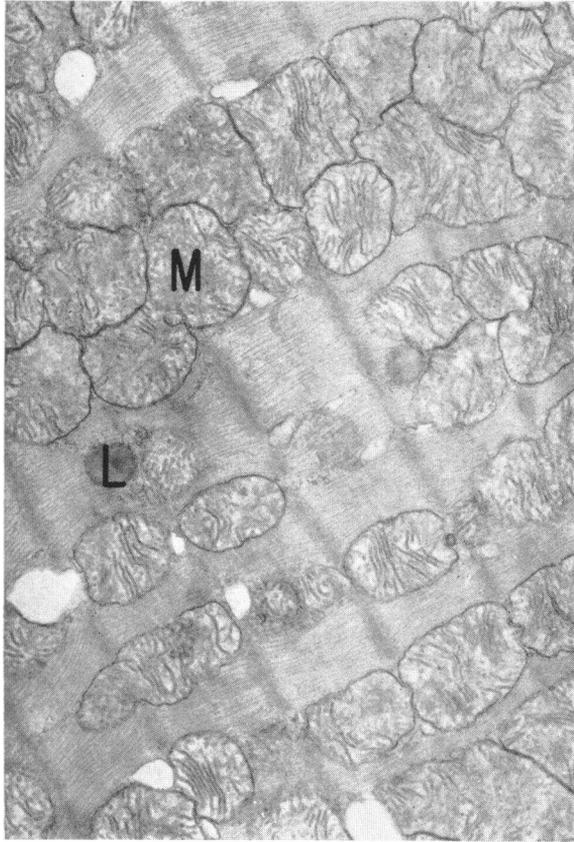


FIGURE 6 Electron micrograph of normal goose myocardium.  $\times 13,300$ .

## DISCUSSION

The mobilization of lipids from adipose tissue in the intact organism is complex and reflects the interaction of hormonal, nervous, and nutritional factors. The homeostatic process is potentially vulnerable to a great many pathological processes operating on the controlling mechanisms. Changes in the rate and degree of lipid mobilization provoked by these processes might have extensive effects upon body metabolism or upon the metabolism of certain organs. Carlson, Boberg, and Högstedt have written an excellent review of this subject (17).

The greater proportion of FFA mobilized from adipose tissue is taken up in liver and muscle. After injection of labeled albumin-bound palmitic acid-1- $^{14}\text{C}$ , about one-third of the recovered activity was found in the liver and one-third in muscle (18, 19). In man, a significant arteriovenous difference of FFA has been found across

the myocardium, indicating uptake of FFA (20). The mechanisms involved in the uptake of FFA in various organs have not been clearly delineated. It appears that the uptake was proportional to the amount of FFA perfusing an organ per unit time (plasma concentration times flow) (17).

Since FFA appears to be taken up by various organs in amounts proportional to the concentration in plasma, increased lipid mobilization will cause increased uptake of FFA in various target organs. If this results in a concentration of FFA greater than the oxidative needs of the cells, either storage or increased oxidation of the fatty acids may have an important influence on metabolism, since fatty acids uncouple oxidative phosphorylation *in vitro* (21).

Agents stimulating lipid mobilization increase the lipid content of the liver. Thus, administration of epinephrine (22) or norepinephrine (5, 23) caused a fatty liver. Evidence suggests that the development of a fatty liver after norepinephrine was caused by the increased influx of FFA to plasma and not by norepinephrine directly. Feigelson, Pfaff, Karmen, and Steinberg (5) found that intraportal infusion of norepinephrine did not cause a major increase in plasma FFA or in liver triglyceride concentration. Carlson and Liljedahl (24) observed no increase in hepatic lipids when the lipid mobilizing effects of norepinephrine were inhibited by nicotinic acid in dogs. Feigelson et al. (5) also showed that the composition of the triglyceride fatty acids of the fatty liver that developed after norepinephrine was similar to that of adipose tissue triglycerides.

An increased lipid content of the heart was found after the administration of epinephrine or norepinephrine (23, 25). Both catecholamines have produced acute myocardial necrosis in animals (6, 7). These lesions did not occur in dogs when the usual rise in plasma FFA, in response to norepinephrine, was inhibited by pretreatment of the dogs with the adrenergic blocking agent, propranolol.<sup>1</sup>

In the present study, glucagon caused a marked rise in FFA. Similar results have been observed in birds by others (10, 26). This increase in FFA was not inhibited by pretreatment with nicotinic acid or propranolol. In association with the high concentration of FFA, geese given glucagon developed toxic signs, myocardial lesions, and fatty

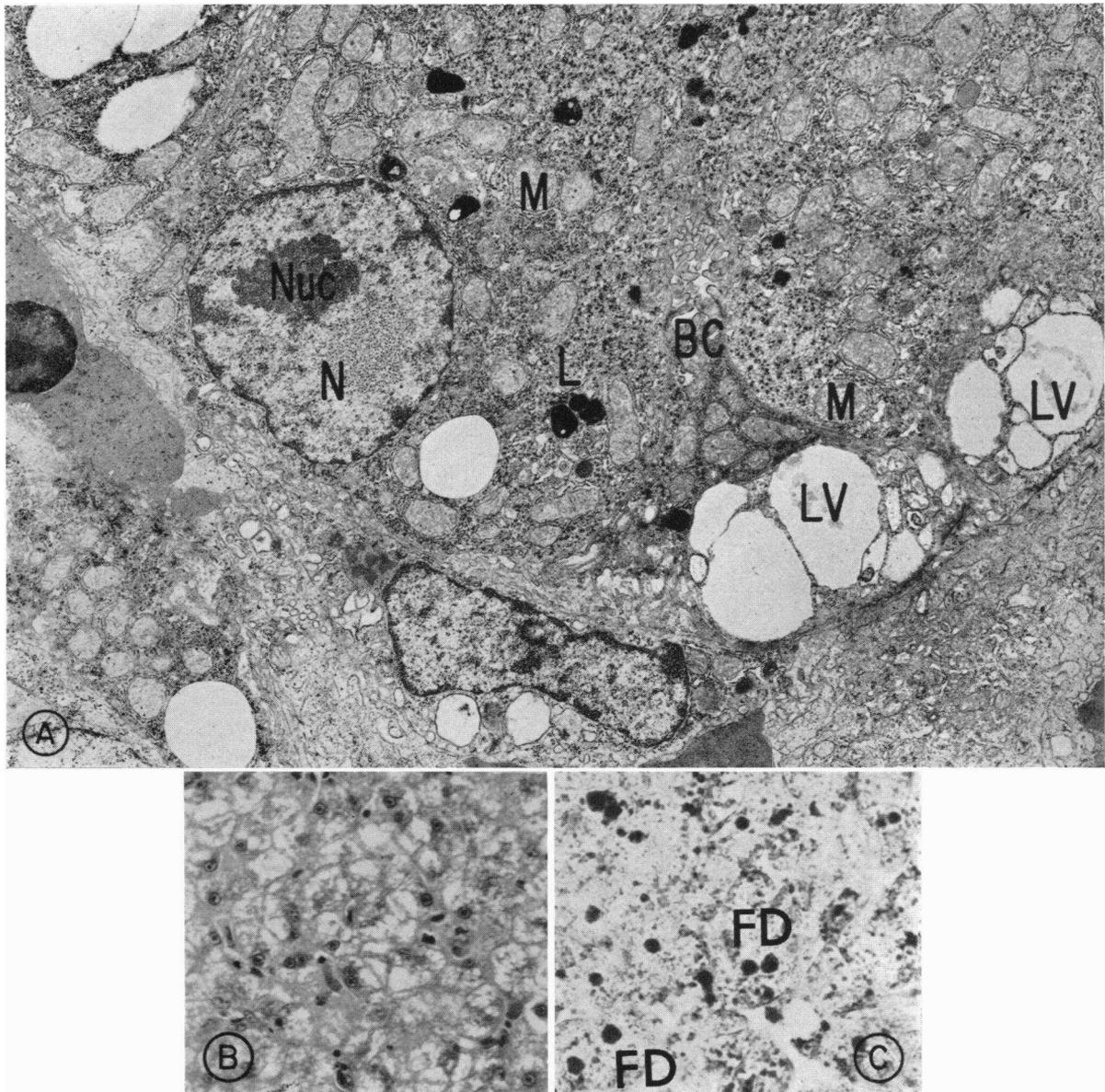


FIGURE 7 A Electron micrograph showing fatty changes in the liver of a goose given glucagon. *N*, nucleus; *Nuc*, nucleolus; *M*, mitochondrion; *BC*, bile canaliculus; *L*, lysosome; *LV*, lipid vacuoles.  $\times 7390$ .

FIGURE 7 B Photomicrograph showing fatty infiltration of the liver of a glucagon-treated goose. *H.* and *E.*,  $\times 375$ .

FIGURE 7 C Photomicrograph demonstrating intrahepatic lipid (FD-fat droplets) in the specimen shown in Fig. 7 B. Oil Red O stain,  $\times 375$ .

livers. The glucagon-treated geese also developed increased serum glutamic oxalacetic transaminase and lactic dehydrogenase activities. Serum triglyceride concentrations increased slightly in the glucagon group and decreased in the control geese.

The myocardial lesion produced by glucagon was similar or identical to that produced in dogs by norepinephrine<sup>1</sup> or in rabbits by injections of

ACTH (27). All of these agents were potent stimuli for lipid mobilization in the particular species used. Electron microscopy revealed extensive destruction of myofibrils and mitochondria. Distorted, but intact mitochondria were frequently found to have osmiophilic inclusions.

Fatty liver was also a common finding in the geese given glucagon. Hepatic necrosis was found

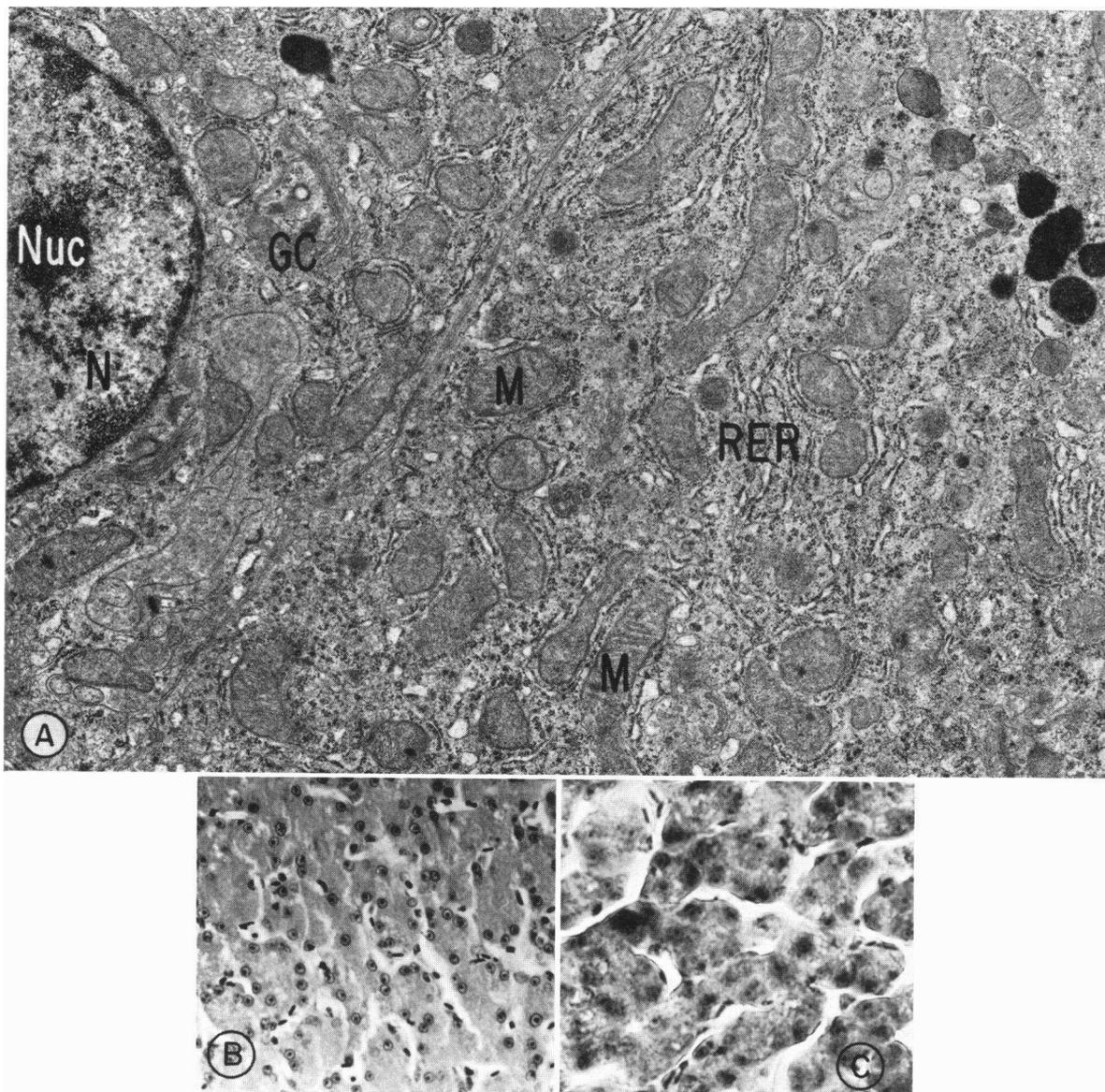


FIGURE 8 A Electron micrograph of normal goose liver. *N*, nucleus; *NUC*, nucleolus; *GC*, golgi complex; *M*, mitochondrion; *RER*, rough-surfaced endoplasmic reticulum.  $\times 13,800$ .

FIGURE 8 B Photomicrograph of normal goose liver. *H and E*,  $\times 240$ .

FIGURE 8 C Photomicrograph of normal goose liver stained with Oil Red O,  $\times 360$ .

in one bird 24 hr after glucagon. Extensive lipid was demonstrated by staining methods, and electron microscopy revealed extensive vacuolization of the hepatic cells.

The dose of glucagon used in this study was pharmacologic, and produced a potent lipid mobilizing effect. In most instances, when glucagon was given to normal human subjects, an immediate depression of plasma FFA occurred followed by a later rise (28, 29). The effect was

much less, however, than the large change we observed in geese. In an *in vitro* study, using human adipose tissue obtained at operation, glucagon produced little or no lipolytic effect (30). In different species, glucagon has been shown to stimulate FFA release *in vitro* (31, 32).

The relevance of these observations to clinical disease is speculative, but similar mechanisms may operate in the pathogenesis of myocardial and hepatic disorders. The hypothesis that very high

plasma concentrations of FFA or a decrease in the rate of oxidation of fatty acids by the cell may result in accumulation of sufficient fatty acids to prove detrimental to cellular structure and function is an interesting concept. Diphtheria toxin produced acute myocardial lesions similar to those induced by catecholamines (6). This toxin also inhibited the oxidation of long chain fatty acids by heart homogenate preparations (33).

Significant elevation of plasma FFA concentrations were found in patients with acute myocardial infarction and cerebral vascular occlusion (34). More recently, patients with serum FFA concentrations above 1200  $\mu$ Eq/liter after acute myocardial infarction had an increased prevalence of serious arrhythmias and a higher mortality (35).

It is unknown whether fatty acids play a causative role or whether they merely reflect other metabolic or hormonal changes in patients with acute myocardial infarction. Such a differentiation is of considerable importance, since means are available for lowering plasma FFA concentrations in man.

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