The Depressant Effect of Fatty Acids on the Isolated Rabbit Heart* (34078)

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In previous investigations the intravenous infusion of long-chain saturated fatty acids to dogs produced massive thrombosis and death (1). When thrombosis was prevented by the prior administration of heparin, the administration of these fatty acids still resulted in death from myocardial failure which developed 5–10 min after the infusion was begun (2). Hemodynamic studies in these intact animals suggested that fatty acids might impair cardiac contractility. Deterioration of electrical conduction as monitored electrocardiographically also occurred.

In contrast, the infusion of long-chain, "unsaturated" fatty acids did not produce signs of toxicity. In plasma-free systems, it has been previously shown that both saturated and unsaturated fatty acids are highly toxic to cellular function and integrity. *In vitro* fatty acids block cellular metabolic activity in liver mitochondria by the uncoupling of oxidative phosphorylation (3). When albumin was incubated with the fatty acids, the toxicity to the mitochondria did not occur.

These different effects of saturated and unsaturated fatty acids upon the myocardium in the intact animal, despite the prevention of thrombosis, led to the present investigation of the effects of fatty acids upon the heart in a plasma-free system. In this study fatty acids were perfused through an isolated rabbit heart preparation. This preparation allowed for the more precise evaluation of the myocardial effects of both saturated and unsaturated fatty acids and yielded understanding about the lack of toxicity of unsaturated fatty acids in the intact animal.

Materials and Methods. An oxygenated Locke-Ringer solution (pH of 8) was infused through the isolated rabbit heart by the use of a modified Langendorf preparation (4). The solution was directed into the aorta and the aortic valves were forced closed by its pressure which was maintained at constant pressure of 30 mm Hg. The venous effluent was collected from the coronary sinus in a graduated beaker and represented coronary flow. The solution was oxygenated for at least 30 min before perfusion and was maintained at 39° in a thermostatically controlled water bath. The force of contraction was measured from the apex of the left ventricle. The percentage concentrations of the solutes in the Locke-Ringer solution were as follows: NaCl, 0.9; KCl, 0.042; $CaCl_2 \cdot 2H_2O$, 0.024; $NaHCO_3$, 0.03; dextrose, 0.2.

Three criteria were used to evaluate the effects of fatty acids upon the rabbit heart: coronary flow, heart rate, and amplitude of contraction. The heart rate and amplitude of contraction were recorded on a kymograph. The coronary perfusion was measured at 1-min intervals.

The fatty acids[3] (stearic and oleic) were converted to their sodium salts by gently warming with sodium hydroxide as has been previously described (5). One-tenth per cent solutions of fatty acids were used for all experiments. These solutions were adjusted to a pH of 8 by back-titrating with 0.1% hy-

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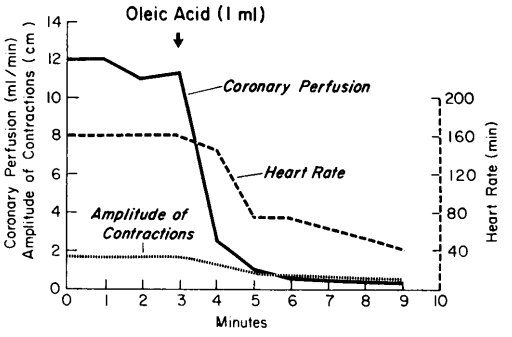

FIG. 1. The effects of 1 ml of 0.1% solution of oleic acid on the perfused rabbit heart. The amplitude of contraction was measured in centimeters and recorded on a kymograph. The coronary perfusion was measured as milliliters/minute. The heart rate was recorded in contractions/minute.

drochloric acid. A sodium chloride solution of a similar molarity and pH was used as a control perfusate. In addition, an albumin control solution, equal in molarity to the fatty acid solutions (3.5×10^{-4} molar), was prepared from 24.29 g of fraction V bovine albumin[4] added to 100 ml of distilled water.

The various fatty acid solutions, albumin, sodium chloride, or the mixed solutions were added to the perfusion media from a syringe attached to an adjoining Y tube located just above the cannulated aorta. The solutions were added to the entering perfusion solution over a 2-min period. These solutions immediately mixed with the perfusing solution as it entered the coronary arteries. The injected fatty acids were added in equal concentration and at the same rate. Since part of the experiment required a mixture of fatty acid and albumin (in equal volumes), it was necessary to mix the fatty acid solutions that were added alone with an equal volume of the Locke-Ringer solution before they were added to the perfusing solution to assure a constant concentration and rate throughout the experiment.

Results. When 1.0 ml of either oleic acid or stearic acid was perfused through the heart, a precipitous drop in the coronary flow occurred. The heart rate slowed progressively. The amplitude of contraction decreased until complete cessation of heart activity occurred (Figs. 1 and 2). No heart ever recovered from these effects. The effects produced by all perfusions of fatty acid and control solutions are shown in Table I.

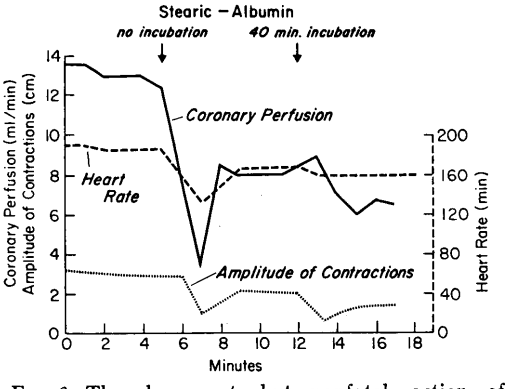

FIG. 3. The depressant, but nonfatal, action of stearic acid–albumin mixture (not incubated) and after 40 min of incubation.

When albumin was added to the stearic acid solution and then perfused, serious toxicity no longer occurred. With the immediate perfusion of the stearic acid–albumin mixture (not incubated), there was a fall in the coronary perfusion to as low as one-half to two-thirds of the original flow with minimal recovery (Fig. 3). No toxic effects occurred when the stearic acid–albumin solution was incubated for 2 hr at a temperature of 80° and then perfused (Fig. 4). Asystole occurred when the concentration (molarity) of the albumin was reduced to one-half in the stearic acid–albumin mixture with only 5 min of incubation. However, when this same

[4] Armour Co., Chicago, Illinois.

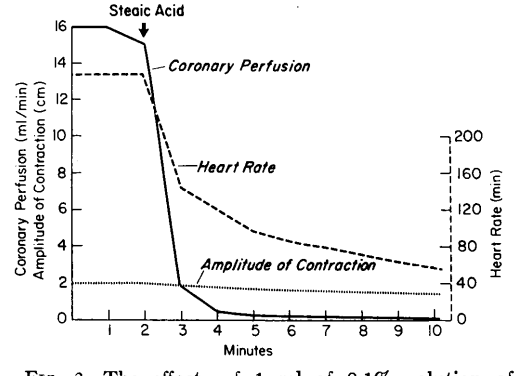

FIG. 2. The effects of 1 ml of 0.1% solution of stearic acid on the isolated rabbit heart.

TABLE I. Results of Fatty Acid Perfusion of the Isolated Rabbit Heart.

Perfusing solution	No. of hearts perfused	Toxicity		
		Eventual asystole	Partial depression[a]	None
Stearic acid 0.1%	7	7		
Stearic acid and albumin (1:1)				
No incubation	4		4	
40-min incubation	1		1	
2-hr incubation	1			1
4½-hr incubation	1			1
Stearic acid and albumin (2:1)				
5-min incubation	4	4		
4½-hr incubation	1			1
Oleic acid 0.1%	13	13		
Oleic acid and albumin (1:1)				
No incubation	4			4
10-min incubation	4			4
4½-hr incubation	1			1
Oleic acid and albumin (2:1)	2			2
Oleic acid and albumin (4:1)	2			2
Oleic acid and albumin (8:1)	1			1
NaCl control	7			7
Albumin control	4			4
Perfusion stopped	2	2		

[a] Varying degrees of: (1) reduction in coronary flow; (2) diminution of amplitude of contraction; (3) reduction of heart rate.

mixture had been incubated for 4½ hr, cardiac activity continued.

The protective property of albumin was even more pronounced after albumin was combined with oleic acid. With the oleic acid–albumin mixture, coronary perfusion decreased only slightly and promptly returned to the range of the original level (Fig. 5). The heart rate or amplitude of contraction did not change. The protective effect of al-

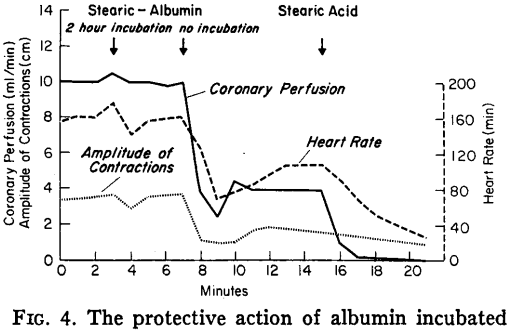

FIG. 4. The protective action of albumin incubated 2 hr with stearic acid. The subsequent addition of stearic acid–albumin (not incubated) had a depressant action, followed by the irreversible toxic effect of stearic acid added alone.

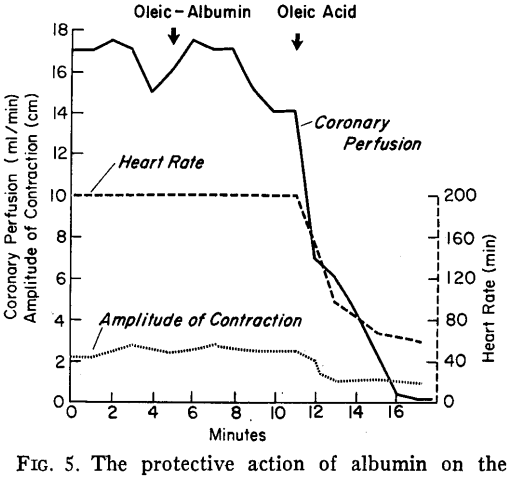

FIG. 5. The protective action of albumin on the usual cardiac depression induced by oleic acid. The subsequent addition of oleic acid alone produced irreversible toxic effects.

bumin with oleic acid resulted even without any previous incubation period. The concentration of albumin could be reduced to as much as one-eighth of the molarity of the oleic acid and cardiac asystole did not occur (Table I).

Control solutions (sodium chloride and albumin) were infused in the same manner as the tested solutions. In no instance did cessation of heart activity occur. Only transient effects occurred. The albumin solution produced a slight increase in the coronary flow with return to the original level occurring in 3–4 min. There was often a reduction in the amplitude of contraction as well as the heart rate. These minor changes were followed by prompt recovery to the original levels (Fig. 6). The sodium chloride perfusions were accompanied by varying responses, especially in the decreased perfusion rate, which then returned to the baseline. Amplitude and rate fell only transiently.

The perfusion was shut off in two heart preparations to deprive them of oxygen and nutrients. The hearts progressively deteriorated to complete asystole much like the hearts that were perfused with the fatty acid solutions.

Discussion. When a protein-free solution of either saturated or unsaturated fatty acid was infused through the isolated rabbit heart, both coronary flow and cardiac contractions ceased. This similarity of toxicity of both saturated and unsaturated fatty acids was in contrast to the lack of toxicity from unsaturated fatty acids when infused into the intact animal (2). The addition of albumin to the unsaturated fatty acid, oleic acid, immediately blocked its toxicity to the isolated rabbit heart. This suggests that the binding of fatty acids to albumin which occurs when they are incubated together prevented their usual toxic effects on the myocardium. Although previous investigators have demonstrated this detoxifying capacity of albumin in cellular systems, they have not indicated any *in vivo* or *in vitro* differences nor any differences between different kinds of fatty acids, (3, 6, 7).

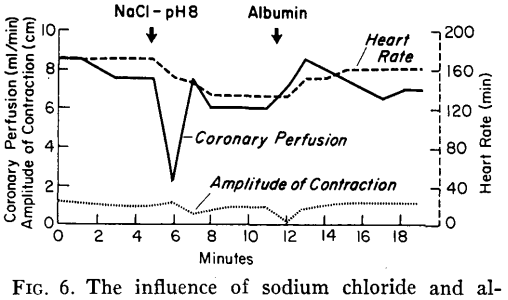

FIG. 6. The influence of sodium chloride and albumin solutions on the coronary flow, heart rate, and amplitude of cardiac contraction in the isolated, perfused rabbit heart.

The depressant action of stearic acid was prevented only by prolonged incubation with albumin whereas the toxicity from oleic acid was blocked by mixing with albumin immediately before perfusing the heart. The explanation of the different effects of long-chain saturated and unsaturated fatty acids (both in the intact dog and in the isolated rabbit heart) may relate to their rate of binding to albumin. Unsaturated fatty acids are bound rapidly both *in vivo* and *in vitro* while saturated fatty acids bind slowly (8, 9), perhaps because of poorer solubility.

Stearic acid and oleic acid also differ in their solubility as sodium salts. Long-chain saturated fatty acids exist as colloidal particles in aqueous suspensions known as micelles (10, 11). The size of the micelle in 0.1% concentration of stearic acid at pH of 8 was found to be as large as 5–8 μ (2). Because of the micelle formation, the possibility of mechanical clogging of the capillary bed in the isolated heart was entertained. However, oleic acid, which also produced irreversible toxicity to the myocardium, was more soluble as a sodium salt. It seems unlikely that it produced vascular bed occlusion. It has been previously found that phospholipids, like fatty acids, exist as micellar particles and, in spite of their colloidal state, they were not toxic to dogs when given intravenously (2). Stearic acid injected into the foreleg veins of dogs produced acute myocardial failure and death. Postmortem tissue sections utilizing special stains for fatty acids revealed no evidence of obstructive lesions in the heart vessels (2).

There is both experimental and chemical evidence that fatty acids play an important role in normal myocardial metabolism (12–19). Bing found that in the postabsorptive state three-fourths of the energy expen-

diture of the heart was sustained by fatty acids (17). In the fasting state as much as 42% of the fatty acids removed by heart muscle were free fatty acids while 58% were esterified fatty acids. Gold *et al.* estimated that 24% of the energy source for the myocardium is from free fatty acids (19). Myocardial uptake of free fatty acids increased as arterial free fatty acid level increased (18).

Whether the plasma free fatty acids ever are toxic to the myocardium in the intact animal remains conjectural, but some indirect evidence points to this possibility. Glucagon administration to geese produced very high circulating levels of free fatty acids (20). Some geese died from heart failure. Others had electrocardiographic abnormalities. At autopsy myocardial lesions were found. Rosenbaum *et al.* noted that high plasma concentrations of nonesterified fatty acids, produced by injecting sympathomimetic amines, have been associated with the production of cardiac lesions (21) and Hoak *et al.* also found myocardial lesions on electron microscopy after catecholamine infusion (22). Catecholamines act upon the hormone-sensitive lipase in adipose tissue and release nonesterified fatty acids. It was speculated that their toxicity was produced by an altered permeability of the myocardial cell membrane as a result of the hyperlipemic effect. Another possible cause for this toxicity to the myocardium is that upon the release of nonesterified fatty acids the available binding sites of serum albumin are exceeded. Should this occur, the fatty acid that is normally albumin-bound and acts as a metabolic substrate might become an intracellular blocker of oxidative phosphorylation as demonstrated by Helsinki (4).

Summary. The toxic effects of stearic and oleic acids on the rabbit myocardium were investigated in a plasma-free system using a modified Langendorf isolated heart preparation. A 0.1% stearic acid or 0.1% oleic acid solution was added to the solution which perfused the coronary vessels. The coronary flow, and the rate and amplitude of contractions progressively deteriorated until there was death of the heart. Equimolar albumin incubated with the fatty acid solutions prevented this toxic effect. The time of incubation was important to the blocking of toxicity for stearic acid but not for oleic acid. No incubation period was required to prevent the toxicity of the oleic acid when combined with albumin. This suggested a difference in the rate of albumin–fatty acid binding for different fatty acids. Unbound fatty acids, saturated or unsaturated, were extremely toxic to the heart.

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