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MASSIVE THROMBOSIS PRODUCED BY FATTY ACID INFUSION *

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Previous investigations have shown that fatty acids affect the coagulation of blood (2–4). In particular, certain fatty acids greatly accelerated the formation of thrombi from rat or human blood in the Chandler apparatus [1] (6, 7). Long-chain, saturated fatty acids, such as stearic and palmitic, shortened the time of thrombus formation, whereas the unsaturated fatty acids of similar chain length (oleic, linoleic, linolenic, and arachidonic) did not influence the thrombus formation time. Short-chain, saturated fatty acids of chain length less than C12 were likewise without effect. The mechanism whereby thrombus formation was accelerated by long-chain, saturated fatty acids probably resulted from the activation of the Hageman or contact factor of plasma (7).

These observations relating fatty acids to thrombus formation *in vitro* have prompted additional investigations designed to test the effects of different fatty acids given intravenously to anesthetized dogs. Many previous workers have infused fatty acids (or their sodium salts) into animals. As early as 1889, Munk had noted that dogs developed hypotension and asystole after the intravenous injection of oleate or palmitate (8). Others have documented that fatty acids or their sodium salts, when given to animals intravenously, produced hemolysis (9, 10), capillary damage (11), and death (10–13). In none of these reports has thrombosis been alluded to as a pathological consequence of fatty acid infusion, nor has any differentiation been made between effects from different fatty acids.

In the present study, extensive thrombosis and death occurred after the intravenous infusion of long-chain, saturated fatty acids into dogs. These effects did not occur when unsaturated fatty acids or short-chain, saturated fatty acids were given. These more innocuous fatty acids did, however, induce some hypercoagulability of the blood as indicated by thrombus formation in a jugular vein segment isolated after the completion of the infusion.

MATERIALS AND METHODS

The sodium salts of different fatty acids were prepared in a 0.1% concentration (6). Control solutions consisted of a 0.9% NaCl solution and a hypotonic NaCl solution of the same molarity (0.097 M) as that used in the preparation of sodium stearate. A 0.1% suspension of bentonite and a 2% suspension of soybean phosphatide were prepared in 0.9% NaCl. The pH of all solutions was adjusted to 8.0. The preparations of long-chain fatty acids, both saturated and unsaturated, and of bentonite and soybean phosphatide appeared cloudy at room temperature. The physical state of the fatty-acid preparations associated with turbidity was important in the production of thrombosis and other effects. As has been previously discussed, the acceleration of thrombus formation *in vitro* was thought to be caused by the ionic micelle, a particle consisting of a negatively charged aggregation of fatty acid ions (6). Loss of activity resulted when the fatty acid concentration was reduced sufficiently for the solution to appear clear, or when the preparation was filtered through a bacterial filter that removed the particles responsible for turbidity and greatly lowered the fatty acid concentration (to about 3.5% of the original concentration).

Plasma FFA were determined by the method of Dole and Meinertz (14). The whole blood clotting time in silicone-coated test tubes was measured in 16 dogs (15). Determinations of the thrombus formation time of whole blood (6), one-stage prothrombin (16), two-stage prothrombin (17), accelerators (factors V and VII) (18), and fibrinogen were performed in 9 dogs before and 3

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[1] The thrombi produced in this system form in a moving stream of blood and are similar in structure to thrombi occurring in human thromboembolic disease (5).

to 8 minutes after the start of the infusion of a long-chain, saturated fatty acid. Blood for these determinations was obtained from a vein not previously used and in a limb different from the infusion site. A silicone-coated needle was used, and the blood was allowed to flow freely through plastic tubing into collecting test tubes. Plasma fibrinogen was measured by the determination of the total nitrogen in the clot formed after the addition of thrombin. The micro-Kjeldahl technique was used. Plasma levels of free hemoglobin were measured before and after fatty acid infusions in some experiments (19). The pH of arterial blood was determined with a Radiometer pH-meter, and it did not change after the fatty acid infusions.

Healthy mongrel dogs were anesthetized by intravenous sodium pentobarbital. An external jugular vein was exposed and a 3.4-cm segment isolated without disturbance of blood flow. The 0.1% fatty acid salt preparation, in an amount equal to 10 ml per kg of body weight, was infused into a foreleg vein over a 5-minute period through a blood transfusion filter set. This amount was selected on the basis of calculations that the plasma FFA levels of most dogs would approximately double if the infused substance remained in the plasma compartment for the duration of the infusion. Such an increase in FFA occurs physiologically after a variety of stimuli such as starvation, stress, or the injection of adrenaline (20). In a few dogs, the time of infusion was lengthened, or the quantity of fatty acid infused was reduced. Bentonite, 0.1%, and soybean phosphatide, 2%, were given in amounts equal to 10 ml per kg of weight over a 5-minute period.

After completion of the infusion, the isolated segment of the jugular vein was clamped off, as described by Wessler (21). Fifteen minutes after the initial clamping, the vein segment was tied off, opened up longitudinally, and examined for the presence of a thrombus. If the animal did not survive the infusion, the vein segment was clamped at time of death and inspected similarly 15 minutes later for the presence of a thrombus. With this particular technique, the two factors important in thrombus formation are stasis and hypercoagulability of the blood confined in the isolated vein segment.

In the event of death, the chambers of the heart and the great vessels were opened and examined for the presence of thrombi immediately after breathing stopped. In most instances, the examination was made when cardiac contractions were still occurring. Specimens of appropriate vessels, lung, heart, liver, spleen, kidney, and brain were taken for subsequent histologic examination.

RESULTS

The long-chain, saturated fatty acids almost invaribly caused the death of the animal within 3 to 5 minutes after the beginning of the infusion. Electrocardiographic monitoring revealed an initial increase in the heart rate, followed by profound depression of the ST segment, and later by an irregular cardiac rhythm and periods of asystole. A loud systolic murmur could be auscultated at about the same time that the electrocardiographic changes appeared.

At post-mortem examination, these dogs had large thrombi [2] in the inferior and superior vena cavae and in the right heart extending into the pulmonary artery. The chambers of the heart were opened immediately after breathing stopped and while the cardiac muscle was still irregularly contracting.[3] Occasionally the left atrium and left ventricle contained thrombi. Thrombi in the aorta and peripheral arteries occurred at times. Microscopic sections of the viscera usually were not abnormal. In some instances, however, microscopic thrombi did occur and demonstrated layering of fibrin and cellular elements, thus suggesting that they formed in moving blood.

In contrast, the infusion of either long-chain, unsaturated fatty acids or the short-chain, saturated fatty acids did not kill any animal, nor were there overt signs of toxicity. Table I summarizes the results from the infusion of various fatty acids. Nineteen dogs were given long-chain, saturated fatty acids; 16 died, and 15 of these had large thrombi in the heart and great vessels. When fatty acids of shorter chain length were used, the toxic effects of their infusion disappeared. The critical change occurred from C14:0 to C12:0. Thus, 13 dogs infused with short-chain, saturated fatty acids sustained no demonstrable ill effects. Infusions of five, different, unsaturated fatty acids were given to 16 dogs. These acids included oleic and erucic, both with one double bond, and the polyunsaturated acids linoleic, linolenic, and arachidonic. None of these fatty acids was lethal.

Ten dogs were infused with a sodium chloride solution of the same molarity and pH as the solutions of fatty acid salts. This control solution

[2] The term "thrombus" is used to indicate intravascular clotting during the life of an animal. Such thrombi often will not have the classic structure of clots formed from moving blood. The structural pattern of layering of blood elements will not develop if the clotting is rapid, or if, like a propagated thrombus, the thrombus develops in a static blood column.

[3] In this connection, it should be noted that dogs killed with sodium pentobarbital and immediately autopsied did not have thrombi; the blood in the cardiac chambers was still fluid.

TABLE I

Results of fatty acid infusions

Substance infused	No. of dogs	No. of deaths	Massive thrombosis
Saturated fatty acids			
Long-chain			
C22:0	2	2	2
C18:0	9	8	7
C16:0	6	4	4
C14:0	2	2	2
Total	19	16	15
Short-chain			
C12:0	3	0	0
C10:0	4	0	0
C6 :0	6	0	0
Total	13	0	0
Unsaturated fatty acids			
C18:1	3	0	0
C18:2	4	0	0
C18:3	3	0	0
C20:4	3	0	0
C22:1	3	0	0
Total	16	0	0
Bentonite	2	2	2
Soybean phosphatide	2	0	0
Control – NaCl	10	0	0

produced no observable systemic toxic effects. The infusion of the opalescent soybean phosphatide suspension was likewise without toxicity. A 0.1% suspension of bentonite, a substance activating the Hageman factor and thereby initiating blood clotting, produced death and massive thrombosis in the 2 dogs to which it was given.

A reduction in the amount of stearic acid infused, from 0.1 to 0.01%, prevented the usual thrombosis and death. When the time of the usual 0.1% infusion of stearic acid was increased from 5 minutes to 30 or 40 minutes, no toxic effects appeared. When the concentration was given in a 10-minute infusion, however, massive thrombosis and death resulted.

Thrombus formation in the isolated jugular vein segment occurred in most dogs receiving long-chain fatty acids, whether saturated or unsaturated. In Table II, the jugular segment thrombi are classified as absent, small, or as large if over 1.5 cm long. Long-chain, saturated fatty acids caused jugular thrombosis in 9 of 11 dogs. Short-chain fatty acids produced thrombi less often, in 5 of 13 instances. The infusion of unsaturated fatty acids, in contrast to their lack of effect in causing systemic thrombosis, caused jugular thrombi in 14 of 16 animals. The injection of the hypotonic control NaCl solution resulted in jugular thrombi in only 2 of 10 dogs. A 0.9% NaCl solution produced no jugular thrombi in 5 dogs.

TABLE II

Thrombi in isolated jugular vein segments after fatty acid infusions

Substance infused	No. of dogs	Jugular vein thrombi		
		Absent	Small	Large*
Saturated fatty acids				
Long-chain	11	2	2	7
Short-chain	13	8	2	3
Unsaturated fatty acids	16	2	5	9
Soybean phosphatide	2	2	0	0
Control – hypotonic NaCl	10	8	1	1
Control – 0.9% NaCl	5	5	0	0

*Over 1.5 cm long.

At this point, it should be indicated that previous reports over the past 30 years have stressed especially the hemolysis resulting from the infusion of fatty acids into animals (9). Because hemolyzed erythrocytes have been incriminated in the causation of hypercoagulability of the blood and thrombosis, it was considered important to determine whether there was a relationship between the extent of hemolysis and the production of thrombosis. Accordingly, the plasma hemoglobin was measured before and after the various infusions (Table III). Since these solutions were hypotonic, all resulted in some hemolysis. The amount of hemolysis was highly variable and could not be correlated with intravascular clotting. In fact, infusion of the long-chain, saturated fatty acids usually was associated with the least hemolysis. Thus hemolysis was not a major factor in thrombosis or death.

TABLE III

*Hemolysis of dog blood before and after infusion of fatty acids**

Before	After		
	Saturated fatty acids		Unsaturated fatty acids
	Long-chain	Short-chain	
32.3±17.5 (SD)	84±52.2	148.2±53.4	104.6±75.9

* Mean values ±SD in milligrams per 100 ml free hemoglobin.

Blood coagulation tests were carried out *in vitro* before and after the infusion of long-chain, saturated fatty acids (either C18:0 or C22:0). An attempt was made to obtain blood before the death of the animal and while blood was still fluid and circulating. The results of blood clotting tests are given in Table IV. The two-stage prothrombin, fibrinogen, and thrombus formation times were slightly and variably reduced after fatty acid infusion. The one-stage prothrombin time and accelerators were not changed. The whole blood clotting time in silicone-coated tubes was decreased, from an average of 32 minutes to 17 minutes. These changes were variable, probably due to rapid death and poor mixing of the fatty acid with the blood generally. To indicate the extremes, in 2 dogs the silicone clotting time decreased from 20 to 2 minutes; in another dog, it actually increased from 33 to 44 minutes. This suggests the possibility of an inhibitor.

Many investigators have shown that the plasma FFA have a rapid turnover (20). This may occur because of their prompt metabolism by muscle and liver, or possibly from rapid diffusibility. In 25 of our experiments, the plasma FFA concentrations were measured before the fatty acid infusion and exactly at the time of completion of the infusion. They did not change significantly, despite the administration of an amount designed to raise the plasma concentration by 100%. This lack of change was observed with all three groups of fatty acids infused. The plasma FFA concentration was 517 ± 218 μM before the infusions of long-chain, saturated fatty acids and 490 ± 170 μM immediately afterward ($p > 0.5$). With the short-chain, saturated fatty acid infusions, the values were 604 ± 192 μM before and 584 ± 166 μM afterward ($p > 0.5$). With the long-chain, unsaturated fatty acids, the values were 446 ± 130 μM before and 483 ± 141 μM afterward ($p > 0.2$).

TABLE IV

Blood clotting tests after the infusion of long-chain, saturated fatty acids

	Before	After	p
One-stage prothrombin time, seconds	12.2 ± 2.3	12.7 ± 2.8	>0.5
Two-stage prothrombin, units	206 ± 29	185 ± 39	>0.05
Test for accelerators, factors V and VII, %	107 ± 21	108 ± 21	>0.8
Fibrinogen, mg per 100 ml	348 ± 100	320 ± 100	>0.05
Thrombus formation time, minutes	4.7 ± 1.2	3.7 ± 1.6	>0.1
Clotting time of whole blood in silicone-coated tubes, minutes	32 ± 11	17 ± 11	<0.001

DISCUSSION

It is not clear why dogs tolerate the infusion of unsaturated fatty acids, whereas they die from saturated fatty acids of similar chain length. A difference in thrombotic effect was anticipated from previous work *in vitro* (5, 6). The toxic effect on the myocardium, likewise limited to the long-chain, saturated fatty acids, was distinct from the induced intravascular clotting. Significantly, one dog died from stearic acid infusion, but did not have any thrombosis upon immediate autopsy. We suppose that myocardial failure in this animal occurred before there was time for intravascular clotting. Furthermore, when clotting was prevented in animals by pretreatment with heparin, these fatty acids still produced electrocardiographic abnormalities and impaired cardiac contractility (22). Neither the thrombotic nor the myocardial effects were manifested if the duration of the infusion was prolonged, or the concentration of the infused fatty acid was considerably reduced.

Although fatty acids constitute a major energy source for the working cells of the body, it has long been appreciated that in plasma-free systems they are highly toxic to cellular function and integrity (23–25). This was found to be true of both saturated and unsaturated fatty acids. In a recent study, Helinski and Cooper demonstrated an enzymatic disturbance that might account for the toxicity of fatty acids (26). Fatty acids blocked metabolic activity in liver mitochondria by uncoupling oxidative phosphorylation. When infused into dogs under the conditions of our experiments, the long-chain, saturated fatty acid group, as represented by stearic acid, is highly toxic. The other group, of which oleic acid is a prototype, however, is almost completely harmless. A possible explanation for the great difference in action might be that unsaturated fatty acids are rapidly bound and so rendered nontoxic with respect to both thrombosis and the effect of the myocardium.

Under physiological conditions, most of the circulating FFA is complexed with the plasma albumin, and smaller quantities are complexed with lipoproteins and erythrocytes (27–29). In our study, unbound fatty acids were infused into the blood. In all probability, as a fatty acid and blood mixed intravascularly, there was a strong tendency for the fatty acid to be bound to the plasma albumin and other blood elements. Perhaps oleic acid, given intravenously, may be rapidly bound to the albumin of dog blood and therefore exerts little toxic effect. Fredrickson and Gordon indicated that the binding of sodium oleate with albumin was very rapid, occurring as soon as mixing of the two substances occurred (20). They made no mention of the rate of binding of stearate to albumin. Goodman has studied the number of serum albumin binding sites for stearate, oleate, and linoleate, as well as for other fatty acids (27). His data do not suggest that there are fewer binding sites of the serum albumin for saturated than for unsaturated fatty acids, and therefore do not provide any explanation for a probability of more rapid binding of unsaturated fatty acids. In fact, lipoproteins had more binding sites for stearate than for linoleate (28). Since all incubations of fatty acids with albumin were carried out for 48 hours, the rate of binding for different fatty acids was not reported.

If stearic acid is more slowly bound, it might exert its toxic effects before binding occurs. Studies are now in progress to test the validity of this hypothesis. It has already been demonstrated that the incubation of stearic acid and bovine albumin for some time prevented the usual shortening of thrombus formation time (6). Furthermore, the mixing of stearic acid with bovine albumin under special conditions of temperature has been shown to prevent the expected thrombosis after the intravenous infusion (22).

Although long-chain, unsaturated fatty acids did not produce generalized thrombosis or evidence of toxic damage to the heart, they did induce some hypercoagulability of the blood as demonstrated by the thrombi formed in the isolated jugular vein segments. Similarly, short-chain, saturated fatty acids caused jugular thrombi in 5 of the 13 attempts. The infusion of hypotonic saline also led to jugular thrombi in 2 of 10 dogs. This suggests that with both the unsaturated and the short-chain fatty acids, hemolysis may have contributed to a degree in the formation of jugular vein thrombi in some animals. Since the infusion of the long-chain, unsaturated fatty acids usually caused jugular vein thrombi (in 14 of 16 experiments), it appears that they exerted some effect upon coagulation beyond the contribution from hemolysis. Perhaps some activation of coagulation mechanisms occurred as these fatty acids entered the blood and before they became bound to the plasma proteins. This effect was not sufficient to cause intravascular clotting in moving blood.

Activation of the Hageman factor has been considered a possible mechanism in the causation of thrombotic disease (30, 31). This factor normally circulates in an inactive form. Any agent capable of activating it might well produce thrombosis, since the Hageman factor initiates the early phases of blood clotting. In this study, two substances believed to activate the Hageman factor did, in fact, cause thrombosis when given intravenously. These were the long-chain, saturated fatty acids and bentonite (7).

If Hageman factor activation was the mechanism of the thrombotic effect from the long-chain, saturated fatty acid, it would be suspected that the blood clotting tests reflecting active Hageman factor would be altered. Shortening of the silicone clotting time reflects well the extent of *in vivo* Hageman factor activation. In this study, there was shortening of the silicone clotting time of blood in almost all dogs. It seems likely that activation of the Hageman factor with subsequent *in vivo* plasma thromboplastin generation was the mechanism of the intravascular clotting induced by the fatty acid infusion. After plasma thromboplastin had formed, the massive intravascular clotting was very rapid and probably did not allow time for either uniform mixing or the progressive depletion of clotting factors in the circulating blood. Undoubtedly, the concurrent decline of cardiac function was also a factor in this matter. The intravenous infusion of particulate matter and thromboplastins ordinarily produces a deficiency in certain clotting factors (32), or even incoagulable blood, the defibrination syndrome. Fatty acid infusion, on the other hand, induced no, or only slight, reduction of clotting factors.

The recent demonstrations that diets high in

saturated fat may produce thrombosis and organ infarction may have some relationship with the thrombosis resulting from long-chain, saturated fatty acids in our study. Whereas Thomas and Hartroft found that butter fat and lard fed to rats caused coronary thrombosis, they noted no thrombosis after corn oil feeding (33). Corn oil was apparently not thrombotic because of its predominantly unsaturated fatty acid composition. These experiments in rats have been confirmed by Gresham and Howard (34, 35). Saturated fat (butter, beef fat, or synthetic stearates) caused thrombosis and infarction. Rats fed linoleate or oleate did not develop thrombi. Extending these findings to another species, Hartroft, Suzuki, and O'Neal found that the thrombosis-producing diet of butter fat induced both thrombosis and atherosclerosis in the dog (36). In addition, hypercoagulability of the blood was reported in rats fed such a diet (37).

The relationship of stress to episodes of thrombosis and to sudden death in man has long intrigued investigators. The early work of Cannon and Gray suggested that adrenaline secretion might be a mediating factor in causing hypercoagulability of the blood (38). Since both stress and adrenaline stimulate a prompt increase in the plasma FFA, Poole reasonably speculated that FFA might be involved in the initiating of thrombosis (39). Saturated fatty acids make up 37.8% of the FFA composition of plasma (40). De Long, Uhley, and Friedman found that stress in rats accelerated the blood clotting times (41). Rats under stress also had a rise in FFA (42). The stress of repeated breeding, unilateral nephrectomy, and administration of ACTH produced thrombosis in female rats (43). Obviously, these interrelationships require much further work before any conclusions are possible.

SUMMARY

1. Long-chain, saturated fatty acids produced extensive thrombosis and death when given intravenously to dogs. Thrombi were found in the veins and chambers of the heart and occasionally in the peripheral arteries. Thrombosis and death did not occur if the fatty acids were infused more slowly or in lower concentration.

2. This fatty-acid-induced thrombosis probably represented an example of intravascular clotting occurring from rapid activation of the Hageman factor. The clotting time of whole blood in silicone-coated tubes was shortened by fatty acid infusion. Thus a hypercoagulable state of the circulating blood was demonstrated before the occurrence of massive thrombosis.

3. Similar infusions of long-chain, unsaturated fatty acids and of saturated fatty acids of chain length C12 and below did not cause massive thrombosis or death. They did, however, induce some hypercoagulability of the blood as indicated by the formation of thrombi in the jugular vein segments isolated after the completion of the fatty acid infusions.

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