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Influence of Unsaturation on Fibrinolytic Activity of Salts of Fatty Acids. (32416)

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(Introduced by G. G. Wright)
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Sodium salts of long chain saturated fatty acids have been reported to accelerate blood clotting and artificial thrombus formation *in vitro*(1,2) and to cause massive thrombosis and death in dogs(3,4). Unsaturated fatty acids are relatively inactive. We have found recently that sodium salts of long chain fatty acids can induce lysis of bovine fibrin films, and in this case it appeared that the unsaturated acids were more active than the saturated. No lysis occurred on heated plates. These preliminary findings are confirmed and extended in the present report.

Materials and methods. Fibrin films were prepared by dissolving Armour bovine fibrinogen (2.5 mg/ml) and Parke, Davis bovine thrombin, topical (50 NIH units/ml) in sodium borate buffer pH 7.7(5). Fibrinogen solutions were sterilized by filtration and 10 ml volumes were added to 8.5 cm Petri dishes. With the plates on a level surface, 0.5 ml of thrombin solution was added dropwise to the fibrinogen while the mixture was gently swirled to assure thorough distribution. Highly purified fatty acids were purchased from Applied Science Laboratories, State College, Pa. They were converted to their sodium or potassium salts by gentle warming with sodium (test no. 1) or potassium (tests 2-5) hydroxide and were adjusted to pH 8.1 with HCl. Two-fold dilutions of the fatty acids in sodium borate buffer were placed on fibrin films in 0.02 ml amounts. The lowest concentration which caused liquefaction sufficient for complete perforation of the fibrin film after incubation at 37°C for at least 4 hours was taken as the end point. (Identical results were obtained with oleate when the system was adjusted to pH 7.4).

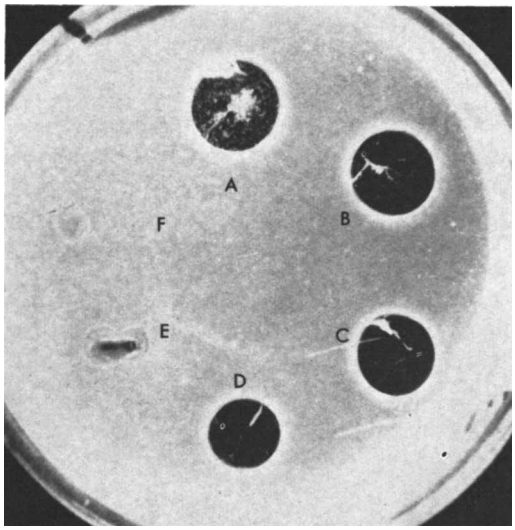

FIG. 1. Lysis of unheated bovine fibrin by sodium myristate. (A) 20 mM, (B) 10 mM, (C) 5mM, (D) 2.5 mM, (E) 1.25 mM, (F) 0.63 mM.

Results and discussion. Fig. 1 shows the activity of 2-fold dilutions of sodium myristate. At concentrations of 20, 10, 5, 2.5 and 1.25 mM, 0.02 ml amounts placed on the fibrin film caused complete perforation of the film. Liquefaction caused by 0.63 mM concentration was insufficient to perforate the film.

Saturated fatty acids having 16, 18, 20, 22 and 24 carbons were compared with acids with one unsaturated bond (Table I). Palmitic and palmitoleic acids appear to be

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TABLE I. Influence of Unsaturation on Fibrinolytic Activity.

Fatty acid		Active concentration, mM — Test No. 1	2	3	4	5
Palmitate	C16:0		5	.3	.3	
Palmitoleate	C16:1		.5	.3	.3	
Stearate	C18:0		.6	1	1	
Oleate	C18:1	.2	.06	.2	.08	
Elaidate	C18:1		.06	.2	.2	
Arachidate	C20:0		1	5	1	
Eicosenoate	C20:1					.08
Behenate	C22:0		>10	>10	>10	
Erucate	C22:1		.03	.02	.04	
Lignocerate	C24:0					>10
Nervonate	C24:1					.08

equally active. Presence of one unsaturated bond in C18 and C20 acids increases activity roughly 10-fold. Presence of one unsaturated bond in C22 and C24 acids increases activity well over 100-fold.

The data in Table II suggest that although one unsaturated bond increases activity, further unsaturation may decrease it.

In Table III it can be seen that saturated fatty acids of chain lengths 12 to 20 are fibrinolytic.

In the present study, the ability of certain long chain fatty acids to cause fibrinolysis has been studied only on bovine fibrin films. Any conclusions concerning the importance of these findings will of course require further study in other assay systems. In his investigations of nonenzymatic activation of human plasminogen by various organic compounds, von Kaulla(6) found certain short chain saturated fatty acids to be active; tests with unsaturated and long chain fatty acids were not reported. Although, in our work, the inactivity of long chain fatty acids on heated fibrin films suggests that the lysis of unheated films may be caused by activation

TABLE II. Influence of Polyunsaturation.

Fatty acid		Active concentration, mM — Test No. 1	2	3	4	5
Oleate	C18:1	.2	.06	.2	.08	
Elaidate	C18:1		.06	.2	.2	
Linoleate	C18:2	.2	.5	.3	10	
Linolenate	C18:3		.1	.6	.6	
Eicosenoate	C20:1					.08
Arachidonate	C20:4			1	1	

TABLE III. Influence of Chain Length on Activity of Saturated Fatty Acid Salts.

Fatty acid		Active concentration, mM — Test No. 1	2	3	4
Laurate	C12	5	10	3	10
Myristate	C14	1	.6	3	1
Pentadecanoate	C15		1	6	3
Palmitate	C16		5	.3	.3
Stearate	C18		.6	1	1
Nonadecanoate	C19		6	3	1
Arachidate	C20		1	5	1
Behenate	C22		>10	>10	>10

of plasminogen, we have not ruled out a direct nonenzymatic dissolution of fibrin as in the case of surfactants(7).

Active interest in fibrinolysis caused by fatty acids seems justified because fatty acids are reported to cause significant effects on blood clotting systems *in vivo*. For example, a lethal intravenous infusion of fatty acids in dogs is associated with massive thrombosis if stearic acid is injected(3), and with pulmonary edema and hemorrhage in the case of oleic acid(8). In man, ingestion of linolenic acid can reduce elevated thrombotic tendency (9).

A role of plasminogen activator in preventing formation of thrombi was suggested to Gans(10) by the finding that increased plasminogen activator occurs as early as does hypercoagulability following intravenous administration of endotoxin to dogs. Man and animals have great capacity for effecting rapid changes physiologically in plasma concentrations of fatty acids, fatty acid compounds and fatty acid binding materials as

well as of both fibrinolytic and thrombotic inhibitors. Various changes also occur in pathologic states. It seems reasonable to suspect a possible role of fatty acids in regulation of fibrin formation and digestion.

Summary. We have found that sodium or potassium salts of some long chain fatty acids induce fibrinolytic activity on unheated bovine fibrin films. The influence of chain length and unsaturation on ability to induce fibrinolysis has been studied. Unsaturated fatty acids seem to be more active than the saturated compounds. The presence of one unsaturated bond appears to make little difference in activity of C16 fatty acids. In C18 and C20 acids, it increases activity roughly 10-fold. In C22 and C24 acids, it increases activity more than 100-fold. A possible role of fatty acids in regulation of fibrin formation and digestion is suggested.

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Effect of Mineral Oil Ingestion on Growth and Liver Lipid Composition in the Rat.* (32417)

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Paraffin oils and hydrocarbons have been found to be absorbed and metabolized when supplied with the diet(1,2). The ingestion of such oils has been associated with a decrease in the absorption of carotene, fat soluble vitamins, and other nutrients(3-8). Mineral oil enhanced the development of essential fatty acid deficiency(9) and caused growth retardation, alopecia, priapism and even death in rats when fed at the 10% level(10). However, Oser *et al*(11) found that rats fed petroleum exhibited none of these problems. The gross and microscopic appearance of the tissues and organs remained normal.

In addition to small quantities of mineral oil used for medical purposes, food additives, and food packaging materials in most countries, mineral oils, such as paraffin oil and transformer and spindle oils are used as adulterants in cooking oils, particularly in India. In certain cases, the edible oil may be adulterated with as much as 90% of mineral oil. In the present study, the influence of ingested mineral oils such as paraffin oil, U.S.P.; transformer and spindle oils on the growth rate and lipid class composition of the liver lipid in rats was investigated. Since edible oils adulterated with mineral oil would also be heated during the process of cooking and frying, the effect of mildly thermally oxidized mineral oils admixed with the fresh edible oil was investigated.

Materials and methods. Eight groups each consisting of 4 male weanling rats (Holtzman Strain) were fed *ad libitum* for 4 weeks a purified diet containing 10% by weight

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