Contribution of Atheromatous Gruel to Thrombus Formation.* (28934)

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Intravascular thrombosis is a frequent immediate cause of death in atherosclerotic individuals, certain lipids accelerate thrombus formation(1), and the necrotic core of an atherosclerotic plaque was observed (as early as 1768) to be rich in lipid(2). It is therefore plausible to postulate that thrombotic accidents may follow the luminal perforation of an atherosclerotic abscess. This possibility was studied by exposing lipids obtained from both experimental thromboatherosclerotic and from human coronary plaques to flowing blood in an apparatus designed for demonstrating thrombus formation(3).

Materials and methods. Yellow waxy material (plaque gruel) was obtained and pooled from the central cores of 30 aortic thromboatherosclerotic plaques of 10 cholesterol-fed rabbits which were killed immediately prior to collection of gruel. The thromboatherosclerotic plaques had been induced 8 weeks previously by intra-aortic insertion of zinc-coated magnesium aluminum alloy wire coils (4,5). For control purposes, the fibrous tissue of thrombosclerotic plaques which contained no gruel were obtained from stock-fed rabbits in which the thrombogenic wire had been inserted 8 weeks previously. For additional control purposes, a similar number of normal aortic samples consisting of media and endothelium were obtained from the stock-fed rabbits.

Each kind of pooled specimens was separately ground in glass Potter-Elvehjem type homogenizers in the presence of distilled water, until finely divided suspensions resulted. Samples from each suspension were dried, weighed, and the main bulk of the suspension adjusted by appropriate dilution to the desired concentration. Aliquots of the adjusted suspensions were then compared for thrombogenic activity as described below.

In addition to these simple suspensions of the original tissues, lipid extracts and lipid-free residues of each of the original tissues were prepared in the following manner. Aliquots of the adjusted suspensions were extracted with 25 volumes of chloroform-methanol mixture in a ratio of 4:1, the extract filtered through paper, evaporated to dryness below 50°C and reextracted with petroleum-ether. The petroleum-ether solutions were evaporated in separate homogenizer cylinders, the solvent-free lipid mixtures homogenized and suspended in water sufficient to make the volume equal to that of the adjusted suspension originally taken. The defatted residues insoluble in chloroform-methanol were individually transferred to homogenizers, ground and resuspended in similar fashion.

A second pooled collection of yellow waxy material was obtained from plaques present in 3 human coronary arteries. This material was not homogenized, extracted or treated in any way; portions of the original substance were studied directly.

Thrombus formation time was determined using a moving column of blood flowing in a circular tube which was rotated on 3½″ diameter turntable set at an angle of 30° to the horizontal. The apparatus was that of Chandler(3) slightly modified in that tubing having a smaller diameter was used. Rabbits were bled by heart puncture, using siliconized needles and syringes; 9 parts of blood were drawn into 1 part of 3.8% sodium citrate previously placed in the syringe barrel. The citrated blood was stored in siliconized tubes from 15 to 120 minutes at 0°C after which time 0.5 ml in a siliconized pipette was mixed with 0.1 ml of 0.25 M $CaCl_2$. A test suspension 5, 20, 25, 30, or 40 μl in volume, was added to the $CaCl_2$ just prior to the addition of blood. Three-tenths μl of recalcified blood containing the test suspension was drawn into an 11½″ length of "Tygon" brand, formulation R-3603, plastic tubing of 1/16″ internal diameter; the ends of the tubing were

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approximated with the aid of a collar, and the circle so formed was rotated at 10 rpm at room temperature. For control purposes, similar volumes of water were used instead of test suspension.

Portions of the gruel pooled from the human coronary artery plaques were placed in the tubing at the point of approximation of its ends (rather than being mixed as a suspension with recalcified blood).

Two intervals of time were measured per test; the starting point for each was the moment of recalcification. The end-point for the first interval was the moment when the leading end of the column of blood slipped back from its initial position by 20° in the direction of rotation of the tubing (this was called displacement time), and coincided with the appearance of specks of solid at the leading edge of the blood columns. The end-point for the second interval was the moment when the column of blood moved around as a unit in a complete circle, (this was called orbit time), and coincided with the appearance of a solid plug at the leading edge. Equal weight was accorded to the two intervals in evaluating thrombogenic activity, and the average used for comparative purposes.

Results. Displacement and orbit time varied from 2½ to 150 minutes, depending upon the test suspension and particular blood sample used. Control blood samples exhibited shorter thrombus formation times as they aged; fresh samples showed times as long as 150 minutes; after storage at 0° for 2 hours, such a sample might show a time of only 48 minutes. For this reason the thrombus formation time of each blood specimen was repeatedly determined each day using volumes of distilled water equal to each of the various volumes of test suspension. In this way comparison was facilitated between test suspensions run with different citrated blood specimens. The thrombus formation time of each test suspension was expressed as a percentage of that of an equal volume of water, the latter being taken as 100. Representative data are given in Table I. Thrombus formation time was usually shortest with the suspensions of ground arterial tissue, while plaque gruel and plaque fibrous tissue showed similar thrombus formation times.

TABLE I. Comparative Thrombus Formation Time with Homogenized and Untreated Native Solids.

Type of suspension	Amt used-solids Vol: μl	Dry wt: μg	Thrombus formation time*
A. Homogenized Native Solids			
Plague gruel (from thromboatherosclerotic plaque)	5	.100	70
	30	.300	45
	40	.400	34
Plaque tissue (from thrombosclerotic plaque)	5	.100	70
	30	.300	42
	40	.400	41
Arterial tissue	5	.100	39
	20	.200	20
	40	.400	16
B. Homogenized Lipid Extracts			
Lipid from plaque gruel	5	.500	100
" " " tissue	25	.125	100
" " arterial "	5	.250	100
C. Homogenized Fat-Free Residues			
Fat-free gruel residue	5	.750	90
" " tissue "	5	.500	50
D. Untreated Native Solid			
Solid gruel (human)	—	—	85
	—	—	96
	—	—	65
	—	—	85

* Expressed as percentage of the time taken for thrombus formation compared with that taken when equal volume of water was used.

In 16 separate direct comparisons between atherosclerotic plaque gruel and simple sclerotic plaque tissue at various concentrations, the thrombus formation time was shortest using gruel in 7 trials; the two materials showed equal times in 8 trials, while simple plaque tissue produced the shortest thrombus time only once. Lipid extracted by solvents from atherosclerotic plaque gruel, simple sclerotic plaque tissue, or normal arterial tissue did not appear thrombogenic. Thrombus formation, moreover, was not significantly hastened by the fat-free residues from gruel but was shortened by similar types of residues from normal arterial tissue. Samples of gruel pooled from a number of atherosclerotic plaques in a human heart failed to shorten thrombus time significantly when used as a solid placed directly in the tubing at the joint.

Discussion. The relatively rapid formation of a cholesterol and lipid rich gruel in the thromboatherosclerotic plaque(5) furnishes a

ready and easily available source of this material for various *in vitro* studies.

Although the present experiments suggested that gruel from an experimental atherosclerotic plaque did hasten the formation of a thrombus in the Chandler apparatus(3), its thrombogenic propensity was no greater than that of fibrous tissue obtained from simple thrombosclerotic plaques, and very much less than that of normal aortic tissue. Moreover, the lipids extracted from experimental plaque gruel did not possess significant thrombogenic activity. Finally, native gruel obtained from human coronary plaques had no discernible thrombogenic properties.

These results suggest to us that the lipid component of the gruel of an atherosclerotic plaque even in the human subject probably does not play an important part in the occurrence of luminal thrombosis. However, this does not mean that the luminal rupture of an atherosclerotic process may not initiate thrombosis. Substances other than lipid may be present which activate the process of platelet aggregation or some other phase of the thrombosis phenomenon. Then too, luminal blood rushing into the cavity walls of a ruptured plaque may expose such blood to still living but badly damaged and possibly potently thromboplastic tissue.

Summary. Gruel obtained from experimental thromboatherosclerotic plaques and from human coronary artery plaques was tested in *in vitro* studies for its possible thromboplastic potency. Gruel from experimental thromboatherosclerotic plaques was moderately thrombogenic, but that obtained from the human plaque appeared to be non-thrombogenic. The lipid extract of the experimental gruel failed to exhibit any thrombogenic propensity.

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Glucose Metabolism in the Rat During Starvation and Refeeding Following Starvation.* (28935)

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Reports of impaired glucose tolerance in fasting and starved animals have led to a popular concept of "starvation diabetes"(1), although impaired glucose tolerance does not necessarily reflect diabetes nor does starvation necessarily decrease glucose tolerance(2). There are certainly profound changes in the metabolism of glucose in starvation, and a marked "supernormal lipogenesis" in refeeding following starvation(3) which is apparently related primarily to abnormal glucose metabolism. Refeeding following starvation often results in fatty livers which may be sufficiently severe to result in degenerative changes(4-6). These, and perhaps other metabolic abnormalities which accompany refeeding following starvation, often cause physiological "stresses" which may be manifested either acutely or chronically. In the cardiovascular system particularly, tissue damage in refeeding might be expected to be manifested chronically in terms of hypertensive and/or atherosclerotic cardiovascular disease(7).

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Methods and materials. Experiment 1.