For the past fifty years investigators have attempted to show a relationship between high lipid diets and the production of atherosclerosis and thrombosis. Recently interest has shifted from a consideration of diets in the pathogenesis of atherosclerosis to a study of their association with alterations in the levels of coagulation factors as a possible cause of thrombus formation, a possibility suggested by Virchow many years ago. Thrombosis can be produced experimentally under certain conditions, enabling one to study the simultaneous alterations occurring in lipid fractions and in coagulation factors during thrombus formation. Thomas and Davidson have shown that it is possible to produce in rats a high incidence of thrombosis by dietary means. An investigation of the changes occurring in the hemostatic mechanism of those animals dying of thrombosis has revealed alterations in the coagulation factors. In addition to the latter alterations, changes in the thromboelastographic records of plasma obtained from such rats suggest that fibrinolytic activity is decreased. Other findings, such as changes in the stickiness of platelets, in animals fed a high fat diet have also been reported.

While most investigators have estimated changes in the level of coagulation factors using samples obtained when the animals were sacrificed, we have followed these changes sequentially in the living individual animals in an attempt to correlate the time of occurrence of changes in coagulation factors with the occurrence of thrombosis.

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Methods

Animals

White male rats of the Sprague-Dawley strain weighing either 100 or 200 g were divided into groups depending upon the diet administered. The animals were weighed weekly and autopsies were performed on all animals upon death or at the time of sacrificing. Routine histological staining procedures were used on formalin-fixed and paraffin-embedded tissues. In certain instances, fat stain (oil red O) and fibrin stain (phosphotungstic acid hematoxylin - PTAH) were used.

Diet

The diets suggested by Thomas et al. were used. A diet of 40% cow butter or cocoa butter was used as a means of producing infarcts while 40% peanut oil was incorporated into the basic diet converting it to an atherogenic one according to Thomas's classification. All diets were supplemented with 2% choline, 0.3% propylthiouracil, and 5% cholesterol. Diets were fed to the rats except for one experiment in which animals were force-fed daily 2 ml of the diet through a no. 10 polyethylene gastric tube inserted into the stomach. Purina chow was fed as a control diet.

Drawing of Blood Samples

Blood samples were drawn at weekly intervals from the tail vein of the rat following the application of heat and venous occlusion. A 2.0-ml siliconized syringe was filled to the 1.0-ml mark with veronal-buffed-oxalated-saline (VBOs) pH 7.35, and a clean venipuncture was performed using a 25-gauge needle. Blood was drawn to the 0.2-ml mark and the sample thus obtained was further diluted to the 2.0-ml mark, giving a final dilution of 1:10; after centrifugation at 2500 rev/min for 10 minutes, the plasma diluted to 1:20 was assayed for coagulation factors within two hours of venipuncture. Larger blood samples were obtained directly from the descending aorta of groups of six animals killed at weekly intervals. Clotting of the blood was prevented by the addition of one volume of 3.8% sodium citrate to nine volumes of blood.

Coagulation Studies

The following tests were performed on plasma.
Upper part: Changes in levels of coagulation factors of rats fed a 40% cow butter diet or Purina chow diet. Lower part: Relationship between weight and time and cause of death.

Results

A group of 40 rats weighing 100 g each was fed the diet containing 40% cow butter.
The changes in coagulation factor activity are shown in the upper portion of figure 1 and compared with values obtained from a control group of 20 animals fed a Purina chow diet. The values, expressed in per cent of normal on a semi-log scale, show that levels of Factors II, VII, and X rose over a period of six weeks, reached a peak, then began to fall. No elevation was observed in the levels of activity of Factors I or V (not shown). Activity of these factors also showed a decrease beginning several weeks before death of the animals. The initial low values for the coagulation factors may have been due to the stress of handling and shipping, since the first group of animals was placed on the diet immediately upon their receipt from the supply house. Mogenson\textsuperscript{10} has re-
ported such an effect of stress on the coagulation mechanism.

In addition to the above coagulation tests performed on the tail vein samples, the one-stage prothrombin time (Quick) showed a slight shortening (from 14 seconds to 12 seconds) at six weeks but a significant prolongation to 18 seconds by the end of three weeks. The partial thromboplastin time initially was 36 seconds, at six weeks 45 seconds, and at eight weeks 57 seconds (normal range 30 to 40 seconds). Thromboses were present in seven of twenty-one (33%) animals dying spontaneously in this group. The morphologic appearance of the thrombi and infarcts was similar to that reported by Thomas's group.\(^6\)

The lower portion of the graph shows the time and cause of death, together with the weights of the animals. The shaded portion indicates the weekly mean weights, with one standard deviation, of animals fed the diet. The weights of those dying are in most instances significantly lower than the mean values.

Since the animals did not eat sufficient amounts of the diet containing cow butter and lost weight, rats were force-fed in order to assure an adequate caloric intake. Two groups of 20 animals each were force-fed either a diet containing peanut oil or one containing cocoa butter. Analysis of plasma samples obtained from the tail veins at weekly intervals is shown in figure 2. The shaded area represents the normal range of values. By the end of the first week the levels of activity of all coagulation factors decreased.

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**FIGURE 3**

Changes in coagulation factor activity of rats fed a diet containing 40% cocoa butter
○—○, 40% peanut oil ×—×, and Purina chow ●—●.

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significantly regardless of the kind of fat in the diet. By the end of the second week, the fall in coagulation factors was more marked and many of the animals in each group died. Bleeding from the genitourinary or upper-respiratory tracts was observed in most animals before death. Autopsy findings revealed that one animal died of aspiration of the diet. All animals had hemorrhages in the lungs, seven in the kidneys with two exhibiting adrenal hemorrhage. Histological examination of the livers of these animals revealed a moderate degree of fatty changes around the central veins.

Figure 3 shows the changes that occurred in coagulation factors in two groups of rats consisting of twenty animals each and weighing 200 g fed ad libitum a diet containing either 40% peanut oil or 40% cocoa butter. A control group of twenty animals was fed Purina chow. All animals were acclimatized to the surroundings of the laboratory for a period of one week prior to the feeding of the experimental diet.

Increases in levels of activity of Factors II, VII, and X followed a pattern similar to that observed in animals fed the diet containing cow butter. After ten to eleven weeks on the diet, levels of activity of all coagulation factors of the animals fed the cocoa butter diet began to fall. Animals fed the cocoa butter diet died approximately two weeks after coagulation factors started to fall. The incidence of thrombosis in the group fed cocoa butter was less than in the group fed cow butter with one of twenty showing such evidence at autopsy. Coagulation studies of animals dying of thrombosis in both the cocoa butter and cow butter groups revealed that there was a decrease of factors at the last time determinations were made prior to death. Similar coagulation factor changes, however, were observed in those animals where thrombosis was not found.

Figure 4 shows the relationship between the weight and the time and cause of death. In general, the animals that died weighed less than the mean weights of the group as determined at the time the animal died. Animals on the peanut oil diet did not lose as much weight as those fed cocoa butter. Hemorrhage was observed in five animals and two animals developed jaundice prior to death. No pathological cause for death other than marked inanition was found in nine animals.

In addition to the coagulation studies, we have followed the levels of the hematocrit of control animals and those fed the cocoa butter or peanut oil diets. At the tenth week, the hematocrit (mean and sd) in nine rats fed peanut oil was 44 ± 2%, for nine animals fed cocoa butter 48 ± 5%, and for fourteen in the control group 51 ± 5%. Only the values for the animals fed the peanut oil diet were significantly lower than those for the normal rats (P = 0.02 to 0.05).

Discussion
Changes in the levels of coagulation factor activity of rats fed high fat diets have been followed at weekly intervals. One or two weeks before death in animals where thrombosis was found, there was a fall in the levels of coagulation factors previously elevated above the range of normal. This decrease may be a result of either overutilization of plasma factors (fibrination—defibrination) as suggested by some investigators12 or, alternatively, liver cell damage with subsequent fall in coagulation factors. The appearance of jaundice in a number of animals would serve to substantiate this latter point. Liver cell damage may have been more marked when the animals were force-fed the diets and only gradual when fed the cow butter or cocoa butter diets ad libitum.

In the group of animals force-fed the diets, it is postulated that one of the constituents of the diet may have proved toxic to the liver causing the marked decrease of coagulation factors. Naeye11 has reported that a patient treated with propylthiouracil developed a decrease in coagulation factors with bleeding with a subsequent return to normal values after vitamin K administration. In our experiment this drug was present in equal amounts in both the peanut oil and cocoa
butter diets. It is possible that its effect on the liver is similar to that of Dicumarol and that this drug may have evoked the marked fall in coagulation factors which in turn precipitated hemorrhage.

Although not elevated during the course of the feeding experiments, levels of activity of Factors I and V decreased gradually, beginning several weeks prior to death. This fall in activity occurred simultaneously with that observed for Factors II, VII, and X. For this reason we are inclined to consider over-utilization as a significant part of the causative mechanism responsible for a decrease of
coagulation factors before the animals die of thrombosis.

It is our belief that the increase in coagulation factors is an expression of a hypercoagulable state since a similar picture is seen in patients with proven thrombosis. This observation has been reported by a number of investigators who noted hypercoagulability in patients with thrombo-embolic diseases. Changes in the first phase of the coagulation process were found in such patients using the diluted thromboplastin generation test or thromboplastin activation test. Numerous investigators, however, have not noted any change in the coagulation mechanisms that would be indicative of an increased tendency on the part of the blood to clot. Lack of unanimity of opinion on this subject has been reviewed by Merskey. Using rats fed a high fat diet, Davidson et al. presented evidence for hypercoagulability, with an associated serum defect in the thromboplastin generation test and a prolonged silicone clotting time. Elevations of the levels of Factors I, II, V, VII, VIII, IX, and X were noted with an increase in the platelet count and decrease in fibrinolytic activity. Merskey, using rats fed a diet containing 40% beef fat, has reported decreased activity of Factors IX. The significant lengthening of the partial thromboplastin time in our studies is in agreement with this finding. Again, overutilization of coagulation factors or a decrease of those formed by the liver and affecting the stage of thromboplastin formation, may be the reason for this observation. Wohl has reported, in addition to the impaired thromboplastin generation, an elevation of fibrinogen levels and the development of anemia in animals fed the diet containing beef fat. Our observations made at weekly intervals showed a pattern of elevation of levels of coagulation factor activity followed by a decrease, and thrombosis in some. These changes may explain the discrepancies among investigators performing similar experiments on rats, some of whom found an elevation while others found a decrease of coagulation factors. This is understandable when one performs only one series of assays at the time the animal is sacrificed and when levels of activity of the factors are either elevated, normal, or decreased.

The relationship of platelets to the pathogenesis of thrombosis was not investigated in this study. Numerous workers have intimated that changes in the number and stickiness of platelets may play an important role in both clinical and experimental observations in the presence of lipemia. Alterations in the fibrinolytic process in patients and in animals have been shown by several workers to be associated with hyperlipemia. Decreased fibrinolytic activity as gauged by thromboelastographic studies has been found also in rats fed a high fat diet. It may be that other factors such as alterations in fibrinolytic activity or platelet change may be critical in precipitating thrombosis.

**Summary**

High fat diets were given *ad libitum* or *force-fed* to rats. The incidence of thrombosis in animals fed a cow butter diet was higher than in those fed a cocoa butter diet. No thrombosis was found in rats fed a diet containing peanut oil. Coagulation studies performed at weekly intervals on blood samples obtained from a tail vein of rats fed the diets *ad libitum* showed that there was a gradual but marked increase in the levels of Factors II, VII, and X of animals fed a diet containing cow or cocoa butter, but not one containing peanut oil. Several weeks prior to death, however, the level of all coagulation factors, including Factors I and V, fell. A significant prolongation of the values for the partial thromboplastin time was found in animals killed at the time when levels of plasma coagulation factors decreased. The pattern of changes in concentration of plasma coagulation factors was similar in the groups of animals dying with proven thrombosis and in those in which thrombosis was not found. All rats force-fed the above two diets died within two weeks, showing a marked fall in all coagulation factors and no thrombosis.
Weight loss was more pronounced in the groups of animals fed the diet containing cow butter and cocoa butter than in animals fed Purina chow. Autopsy findings were: intraventricular thrombi with myocardial infarction, lung hemorrhage, pneumonia, and rarely jaundice. No cause of death other than inanition was discernible in some of the animals. Marked fatty infiltration of the liver was a feature of all animals autopsied.

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Blood Coagulation Changes in Rats Fed High Fat Diets
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