To the Graduate Council:

I am submitting herewith a thesis written by Charlotte E. Behm entitled "The Effect of Stenotic Flow Conditions on Platelet Population." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Engineering Science.

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THE EFFECT OF STENOTIC FLOW CONDITIONS ON PLATELET POPULATION

A Thesis

Presented for the

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Degree

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ABSTRACT

In recent years both engineers and physicians have shown considerable interest in studying the various flow characterisitcs of blood in the vicinity of a vascular stenosis. Information from such studies will facilitate the understanding of the etiology, pathogenesis, diagnosis, and treatment of occlusive vascular disease.

The present investigation sought to determine if there was an acute effect on platelet population due to the flow conditions in the vicinity of a stenosis. Abdominal aortas of rats were occluded by approximately 53% by tying suture silk around the vessels. Blood samples were taken before opening the rats and one hour after the stenoses were induced. The results of this group of experiments were compared to the results of two control groups. To determine the effect of the incision alone, one group of rats had vessels exposed but no threads were placed around the aortas. In the second control group, threads were placed between the vena cava and the aorta, but the threads were not tied. This second control group was used to determine differences in platelet counts due to the manipulation of vessels during insertion of the thread.

The rats whose vessels were constricted had a decrease in platelet counts when compared to original values. This trend was opposite for the control groups, both of which had an increase in platelet count after surgery. The control group which had the thread placed between the vessels showed a smaller increase in platelet counts than the control group in which the vessels were only exposed.

The difference is believed to be due to platelet adhesion to damaged endothelium. The increase in platelet count in both control groups indicated that thrombocytosis developed as a result of surgery.

The peak Reynolds number for the flow was estimated to be 40.8, and it is speculated that a time-varying separated region of flow developed downstream from the stenosis. It is believed that the flow conditions present in the stenotic region contributed to the decrease in platelet counts. The platelets either may have adhered to the vessel wall or aggregated in the blood stream, possibly in the vicinity of the stenosis. Further research is needed to relate specific flow conditions in the vicinity of a stenosis to platelet activity.

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CHAPTER I

INTRODUCTION

A common condition is found in the arterial systems of mammals in which the vessels are narrowed at a particular site or sites. The narrowed areas, called stenoses, are sometimes "collar-like" in that they are approximately axisymmetric. These constrictions may be caused by several factors which include intravascular plaques and the impingement of ligaments or spurs on the vessel walls (22).

The condition of a stenosed vessel can lead to various complications. Clinical researchers have reported variations in blood of cardiac patients who have occlusive vascular diseases. Conditions such as increased viscosity, increased coagulation of the blood, and increased occurrence of thrombus formation have been reported (17, 68). A thrombus is defined as the formation of a blood clot inside a vessel; it may form whenever the flow of blood in the arteries or veins is impeded (43). Thrombi have been observed to deposit preferentially at certain sites in the circulation such as downstream from bifurcations, sharp bends, and constrictions such as stenoses (28).

Over a century ago, Virchow (63), as cited in an article by Dintenfass (16), suggested that three factors were responsible for thrombus formation: the alteration of blood content, the alteration of blood flow, and the alteration of the vessel wall. It has been reported or speculated by researchers that these three factors can be altered by the presence of a stenosis. First, the blood flow is

markedly changed by the nature of the converging and diverging sections of the stenosis. Secondly, vessel injury may result from the flow conditions in the area of the stenosis or downstream from the stenosis where a weakening and bulging of the artery commonly can be seen (post-stenotic dilatation). Finally, changes in blood content, as shown in the case of viscosity cited above, have been clinically linked to occlusive vascular disease.

The role of blood platelets in the formation of thrombotic conditions has been studied extensively, both in patients and in experimental procedures. Platelets appear in developing thrombi before any other elements (9). The platelet aggregation which is observed can be caused by several factors. There is also an increase in platelet concentration that can occur when the platelets adhere to a vessel wall due to injury to the wall. The understanding of the phenomena associated with platelet aggregation and adhesion is essential to understanding the mechanism of these changes that occur in the blood.

It has long been suspected that fluid mechanical factors play a large role in blood platelet function. Velocity gradients, flow separations, vortices, turbulence, and other flow conditions have been shown to affect platelet behavior (18,28,29,45). Blood flowing through a stenosis has the potential to experience all of these flow conditions; therefore, the effects on the platelets could be quite significant. The particular study described herein was designed as a "pilot study" to determine if a stenosis created in an artery could acutely affect the number of platelets in the blood stream.

In order to investigate if there was an effect on platelets due to stenotic flow conditions, stenoses were artificially created in rats. This condition was induced by tying a piece of suture silk around the abdominal aorta, producing a partial occlusion. Platelets were counted from blood samples taken before surgery and one hour after occlusion of the vessel. These counts were compared to counts of other rats that underwent similar surgery with no stenosis. In one control group, the rats were opened and the tissues around the aorta were pulled aside exposing the vessel. Rats in the other control group had their aortas exposed, and also had a piece of thread inserted between the aorta and vena cava, but the thread was not tied. The control groups were used in order to determine the effects of the incision and the movement of the vessel on the increase or decrease in platelet counts from the initial value. Attempts were also made to determine whether old or new platelets were preferentially affected by the flow conditions, and arteries were prepared for histological studies.

CHAPTER II

LITERATURE REVIEW

A literature search was conducted in areas related to vascular stenoses and platelets. Although no information was found relating platelet activity to stenoses, much information is available pertaining to certain cardiovascular diseases and their effects on platelets, as well as information pertaining to general platelet physiology. Some of this information is briefly summarized below. The flow conditions in the area of a stenosis (as determined primarily by model studies) and the effects on platelets subjected to some of these same flow conditions are also discussed. Finally, the phenomena of thrombocytopenia and thrombocytosis are reviewed, particularly as they relate to surgical procedures.

A. CLINICAL REPORTS OF OCCLUSIVE AND THROMBOTIC DISEASE

Clinical studies have shown that there is a significant relationship between occlusive diseases of the blood vessels and platelet function (47,60). Blood coagulation in patients with ischaemic heart disease, a deficiency of the blood due to constriction or obstruction of a blood vessel, has been studied and compared to normal subjects. The diseased patients demonstrated, among other conditions, significant differences from normal subjects in clot formation, platelet stickiness, and coagulation (40). Increases in blood viscosity have been studied extensively by Dintenfass (17,18). He stated that the viscosity of blood in certain cases of thrombosis and coronary

occlusion is up to tenfold greater than in normal blood. Baroldi commented on the genesis of thrombi in a recent article, saying that "the occlusive thrombus is never found in a normal coronary artery; on the contrary, it is always located in a vessel with an old, severe stenosis." (4).

The role of platelet aggrebility in the genesis of thrombosis is documented in the literature (70). Several clinical studies that deal with platelets and thrombosis, which today is a major cause of morbidity and mortality, have been reported (16,39). In order to evaluate the role that platelets play in the pathogenesis of recurrent deep vein thrombosis, twenty-seven patients with the condition were studied by Wu, Barnes, and Hoak (68,69). Both spontaneous platelet aggregation and circulatory platelet aggregates were enhanced in almost 50% of the patients. The abnormal platelet function persisted from weeks to months. In another study by Dintenfass (15), clots formed from blood thrombotic patients were found to be significantly stronger than clots formed from the blood of healthy persons. Gallus and coworkers (25) found that reduced platelet survival was seen in patients having recurrent venous thromboembolism despite anticoagulant treatment.

B. PLATELET AGGREGATION AND CLOTTING

In order to understand the mechanisms involved when blood is subjected to flow conditions as might occur in a stenotic region, a brief description of platelet physiology is given below. Platelets are aggregated by various agents that seem to act through three

stages. First, the platelets change shape from flat disks to spherical forms with various protrusions. This reaction seems to require only ADP. This step is rapidly followed by the adhesion of platelets to each other in loose aggregates. Lastly, the platelets release granules which contain ADP, thereby increasing its concentration in the plasma and creating the growth of platelet aggregates by a chain reaction. The aggregated platelets become much more closely packed during this release mechanism (8).

The activating agents diffusing into the bloodstream have a Schmidt number for diffusion, which is the kinematic viscosity divided by the diffusion coefficient (31), of approximately 10,000, so even with small Reynolds number flow as in small vessels there will be a distinct concentration layer of activator surrounding the mound of platelets. The shape and size of this layer depends on the velocity field. If the platelets are close enough to the platelet aggregate surface, they will stick to it. Under these conditions of surface activation, aggregate growth is determined by the rate at which the particles collide (51). Experiments have supported the collision hypothesis. Observation of the microcirculation, where the thrombotic process could be observed directly, has shown that the initial recognizable phenomenon has been the adhesive reaction between platelet and platelet, and platelet and endothelium (66).

It appears that there may be a difference in the aggregation of young and old platelets. Ginsburg (27) reported that in his experiments old platelets aggregated significantly less than young platelets. Also, young platelets disaggregated more readily than old ones.

Platelet aggregation can also be initiated by an initial adhesion of platelets to a surface (51). The platelets adherent to a surface differ from those in plasma. They are not removed under flow conditions that will remove platelet thrombi in the plasma. It appears that platelet adhesion to a vessel surface as compared to aggregation in the plasma is an irreversible process (6). When the vessel wall is injured, a chain of events is triggered which has as its final outcome the development of a thrombus (65). The response of the vessel wall to injury involves four mechanisms: adhesion of platelets to subendothelial connective tissue; release of platelet constituents; platelet aggregation; and blood coagulation (1).

The aggregation of platelets, clotting time of blood, and morphology of blood clots can be altered significantly by the flow of blood during the period of clotting. There are vast differences in the thrombi, which form in flowing blood, and clots, which form in static blood. The intravascular thrombi formed in static segments of the vascular tree have much the same structure of clots (47).

C. FLOW CONDITIONS IN STENOTIC REGIONS

Flow conditions in stenotic regions have been studied primarily by using models but also by some *in vivo* studies. One important incentive for these studies has been to learn more about possible fluid mechanic effects on the pathological conditions associated with such stenoses, and several reviews of this nature have previously been presented (see for example 23,24,33,54). The model studies usually involve flow through an axisymmetric constriction in a rigid tube with

varying degrees of severity of the constriction used. The fluid is considered to be homogeneous, incompressible, and Newtonian. Although blood is non-Newtonian (21), it is commonly treated as a Newtonian fluid except at very low rates of shear (23). Most model studies reported have been concerned with steady flow, but, even though the flow in animals is pulsatile, steady-flow studies can provide pertinent information (44). Besides steady flow models, there have also been model studies of oscillatory flow (73) and pulsatile flow (36), and *in vivo* studies using dogs (71).

Pressure drop. Narrowing in vessels such as stenoses are the sites of drops in pressure as the narrowing impedes flow (54). Pressure changes in the vicinity of a stenosis can be used to diagnose and evaluate the stenosis. Also one finds in the literature speculations which related changes in pressure along a stenosis to (a) an ingrowth of tissue near the throat (the minimum diameter) of the stenosis, (b) post-stenotic dilatation, and (c) flow to tissues distal to the stenosis and work of the heart.

For steady flow, the pressure reaches its minimum value slightly downstream from the throat of the constriction, then increases gradually until recovery (23,35,72). When the flow reaches the stenosis, the streamlines converge and the negative pressure gradient combines with viscous effects of the fluid to retard the flow (22). Lee and Fung (38) have developed equations for the pressure drop in bell-shaped constrictions for steady-state conditions. Forrester and Kozman (35) found similar values to theoretical

predictions by experimental measurements showing pressure drops along the stenosis. Theoretical studies on pulsatile flow showed pressure drops whose magnitudes varied considerably throughout the pulse cycle, but which oscillated about corresponding values predicted for steady flow having the same average volume rate of flow (36). Pressure drops across stenoses *in vivo* were studied in dogs by Young, Cholvin, and Roth (71) by inserting plugs into the femoral arteries to produce stenoses of varying degrees. They found that the pressure drop was related to the geometry of the stenosis and varied nonlinearly with instantaneous velocity.

Separated flow and stasis. It has been theoretically predicted (22) and experimentally shown (23) that if the adverse pressure gradient becomes large enough in a stenotic region of steady flow, the slowly-moving particles near the wall will reverse their flow directions and a separation region will develop. The fluid inside this region circulates as small eddies but does not move downstream with the main body of fluid. The extent of the separated region depends upon the Reynolds number and the geometry of the stenosis. For steady or pseudo-steady flow, it is felt that the relatively slow flow and stasis associated with a laminar separated region will lead to thrombus development which in turn may precipitate plaque development and/or emboli formation (23,24,35).

Localized zones of separated flow have also been predicted theoretically (36) and experimentally observed in oscillatory flow with time-dependent separation and reattachment points (73).

Turbulence. There has been considerable interest in speculating under what conditions turbulence may occur in the vascular system, and its potential effects on blood components and vessel walls. Young and Tsai (72) studied the development of turbulence in models of steady flow. They found that as the Reynolds number of the flow was increased beyond a critical point, the flow became increasingly unstable and at higher Reynolds numbers a transition to turbulence occurred. The critical Reynolds number was highly dependent upon the geometry of the stenosis. Other researchers have reported on the dependence of geometry and turbulence. The degree of turbulence will be different for different orifice diameters for the same upstream Reynolds number. Kim and Corcoran (33) found in steady flow studies that flow was laminar disturbed when the orifice was large but became fully turbulent as the diameter became small. *In vivo* studies with dogs showed that when the stenosis was greater than about 70%, turbulence was induced by the stenosis (71). In model studies of oscillatory flow (73), for Reynolds numbers slightly above the critical values the flow became turbulent and then returned to laminar prior to the next cyclic increase in the Reynolds number. Also for oscillatory flow, for a mildly constricted model, the critical Reynolds number was considerably larger than for steady flow. For severely constricted models, the critical Reynolds numbers were slightly less for oscillatory flow than for steady flow (73).

Velocity gradients and shear. Relatively large velocity gradients occur in the converging section of a severe stenosis,

resulting in possible deterioration of the endothelium of vessel walls due to shearing stresses (35). For steady flow, the maximum shear stress occurs in the converging portion and moves upstream from the throat with increasing Reynolds number (23). If separation occurs, the direction of the wall shearing stress is reversed in the separated region, which may have some effect on the vessel wall. There is an increase in the average velocity of flow at the throat of a stenosis. In model studies of a severe constriction there was a jetting of flow as it came through the stenosis (73). Thus large velocity gradients may occur near regions of high axial flow.

D. EFFECTS OF FLOW CONDITIONS ON PLATELETS

There is much written in the literature concerning the effects on platelets of individual conditions of flow. Many of these flow conditions may be present in a stenotic region as has just been discussed. One can find in the literature both experimental data and speculations which suggest relationships between platelet activity and different flow conditions. A partial review of some of this information is presented below.

Stasis. "The key to coronary thrombosis," wrote Roberts in an editorial (53), "just as the key to thrombosis occurring anywhere in the body, is slow blood flow or relative stasis and sufficient time for the thrombus to form." Gardner (26) also observed that regions of stagnation or stasis are frequently sites of *in vivo* thrombus formation. Dintenfass (18) reported that at complete stasis,

viscosity as recorded by a cone-in-cone viscometer, increased to a very high value. Rieger and Schmid-Schonbein (52) found by using a rheoaggregometer that platelets remained dispersed in stasis and creeping flow and became aggregated only in the presence of a minimum amount of shear.

Flow separation. Morton and coworkers (45) have linked the occurrence of platelet thrombi to flow separation in that the formation of freely-floating aggregates were observed in separated flow regions. Hydrodynamic models suggested that sufficient activiation had occurred within a separated region so that platelets would stick on virtually every collision. This aggregation was described in terms of the collisions caused by a process that resulted from the fluid motion within the separated flow. There was a random stirring caused by red blood cell rotation and also by velocity gradients which caused platelets to collide (45).

Axial flow. The axial flow through a blood vessel has an effect on platelet activity. There is normally inward migration of red blood cells and platelets from the vessel wall due to the presence of an inwardly-directed force. This force is due either to the interaction of the flow field around the deformed particles with the tube wall, or, at higher flow rates, it is due to the inertia of the fluid (29). From model studies using different sized particles, Goldsmith (28) stated that the rate of inward drift of platelets is expected to be one-third of that of red cells. The large number of red cells continually interact, becoming deformed. This leads to an appreciable

dispersion of suspended particles such as platelets and the aggregation of red blood cells (28). The red blood cells would then occupy the most central position and the platelets, which otherwise would migrate inward, would be forced to flow close to the vessel wall. Not only are they in close proximity to the wall, which might lead to adhesion, but the platelets are moved out of the area of zero rate of shear at the center of the axial stream into a field of higher rates of shear near the wall (16).

Velocity gradient and shear stress. Dintenfass (14) has suggested that the velocity gradient represents a basic coagulation factor. The onset of clotting, which is measured by a rise in viscosity, depends upon the velocity gradient. Blood is non-Newtonian which means that blood viscosity is a function of the rate of shear (56).

The effects of flowing blood on blood viscosity have been studied using a cone-in-cone viscometer (15,19,55). When running various tests at 36 to 37°C, the viscosity of the blood was found to decrease as the rate of shear or velocity gradient increased. If coagulation proceeded at high enough rates of shear, no significant increase in viscosity could be observed and the blood viscosity at that point became directly related to hematocrit (15). Dintenfass (18) reported a reduced clotting time associated with an increase in the velocity gradient, and stated, "It was surprising to find that clotting was consistently more rapid when higher rates of shear were used. The latent period before the onset of clotting at low rates of shear may be as much as twice as long as the respective latent

period at high rates of shear." In other experiments, Goldsmith and coworkers (29) found that the thrombin-induced release of serotonin was appreciably greater in oscillatory flow (having a time average wall velocity gradient above 2000 sec^{-1}) than in the corresponding controls incubated at rest. In a different communication, Goldsmith (28) speculated that high and variable shear stresses could affect the function of platelets to release substances such as ADP and serotonin, and subsequently to aggregate.

There exist morphological differences between the clots of blood formed at high and low rates of shear (56). A thrombus obtained at low rates is intermediate between clots formed in a test tube and a thrombus formed at a high rate of shear. Low rate thrombi contain less platelet aggregates and are smaller in size. This kind of thrombus is common in cases of coronary occlusion (56). Klose and coworkers (34) reported that the more aggregable platelets, assumed to be the younger ones, form aggregates at low shear rates, whereas the less aggregable platelets, assumed to be the older ones, form aggregates only at higher shear rates.

Velocity increase. The mean velocity of blood flow has been found to have an effect on the rate of growth of platelet thrombi. To study this effect, Bengent and Born (8) examined the growth rate of thrombi produced in venules of similar size. They found that the growth rate increased with increasing mean blood flow velocities up to a sharp maximum. At higher blood flow velocities, shear increased to such an extent that it prevented more platelets from adhering.

They determined the velocity by following particles and assuming that the velocity profile in venules was not parabolic but flat almost to the walls, so that wherever the particle was, that was the mean velocity.

Vortex. Studies on the flow behavior of human blood in vessel obstructions producing vortices were carried out by Goldsmith (28). The model studies showed that the particles were subjected to first high and then much lower shear stresses as they entered the vortices. The author made speculations of such a situation *in vivo:*

> . . . a platelet passing through a region of very high shear stress, even though acting on it only for a millisecond, might become activated. If, once activated then within the vortex where it spends a relatively long time, the particle might stick to the wall or obstacle and thus promote thrombus formation. Furthermore, at the low shear rates prevailing, the collisions between platelets traveling along different streamlines would be more likely to produce non-separating aggregates, . . . Nevertheless, the fact that aggregates stayed within the vortex and indeed were observed to collect there, may be of significance in vivo if, for instance, at the site of a stenosis or an aneurysm, platelet aggregates were trapped near the vessel wall just distal to the expansion of the lumen.

Turbulence. The effect of turbulence on thrombus formation was studied by Smith, Stein, and coworkers (59,61). Two shunts were placed from the femoral artery to the femoral vein in each of six dogs. One shunt contained a turbulence-producing device, the other did not. Otherwise, the two shunts were identical. In each dog larger amounts of thrombi, by weight, accumulated in the turbulent system than in the laminar system. Deposits from the turbulent

system were 5.3 times heavier than from the laminar when weighed in the dried state and 3.4 times heavier when weighed in the wet state. The thrombi from the turbulent system were located downstream from the turbulent generator. Thrombi from the laminar shunt were obtained downstream from the dummy generator. Visual inspection showed no gross deposits upstream.

E. THROMBOCYTOPENIA AND THROMBOCYTOSIS

Certainly there are factors other than flow conditions which affect platelet activity and the number of platelets in the circulating blood. In the present investigation platelets were counted before and after surgery, and it is thus pertinent to briefly discuss the conditions of thrombocytopenia and thrombocytosis.

Thrombocytopenia. Thrombocytopenia is a condition in which there is a subnormal number of platelets in the circulating blood. Despite the number and diversity of disorders that may be associated etiologically, thrombocytopenia, according to Wintrobe (67), results from only three processes: (1) deficient platelet production; (2) accelerated platelet destruction; and (3) abnormal distribution or pooling of platelets within the body. These three processes are briefly discussed below.

Since platelet count remains quite constant under normal conditions, the production of platelets appears to be well regulated. Thus, one or more humoral controlling factors, such as a "thrombopoietin", have been postulated for many years, and it has even been

suggested that the control of thrombopoiesis may involve a negative feedback mechanism which contains a time delay resulting in an oscillation of platelet production (67). Wintrobe (67) stated that a deficiency in platelet production could result from a number of processes, but clinically those that depopulate the stem cell or megakaryocyte compartments are the most common.

Platelets injured either by intracorpuscular processes or (more frequently) extracorpuscular processes usually are removed from the circulation by the spleen, liver, and recticuloendothelial system (67). Thrombocytopenia may also result from increased platelet utilization in intravascular thrombi or on damaged endothelial surfaces.

With regard to abnormal platelet distribution or pooling, Wintrobe (67) stated that in the various disorders associated with splenomegaly platelet production is normal or even increased, but the majority of the platelets are sequestered in the enlarged extravascular splenic pool. He listed various disorders of the spleen and hypothermic anesthesia as possible causes for this abnormal platelet distribution or pooling.

Thrombocytosis. Thrombocytosis refers to the presence of an abnormally large number of platelets in the circulating blood. Transitory thrombocytosis may result from the mobilization of extravascular platelet pools (3) following the administration of epiniphrine or following vigorous exercise (67). However, McDonald (41) has reported results which indicate that factors other than a platelet

pool may be responsible for thrombocytosis. He injected mice with platelet-specific antisera that reduced the peripheral platelet count to essentially zero within four hours. Three days later the platelet count began to rise sharply, reached normal levels by the fourth day, and peaked above normal levels on the sixth day. It then decreased and fell slightly below the normal level before returning to normal near the twelfth day. By using radioactive tracers, he found that the most active thrombopoiesis was on the third day after antiserum treatment and the least activity was on days 6-2/3 to 7-2/3 (41). Because of the time delay in the increase in platelets, it appears that the recovery was not due to release from a splenic pool. McDonald (41) speculated that platelet stimulation and supression were due to a feedback mechanism by which platelets could affect the rate of thrombopoiesis. He also stated that his observations supported the concept of a humoral agent (thrombopoietin) as a platelet control.

Other forms of thrombocytosis may result from an accelerated platelet production or from the commonly observed release phenomenon. Moderate thrombocytosis often develops one or two days after major trauma or surgical operations, and may persist for one or two weeks (67). A rapid rise in platelet count immediately following splenectomy has been observed by several investigators (13,37,67). This finding has caused some researchers to speculate that the removal of the spleen as a "grave yard" for old and damaged platelets results in acute thrombocytosis. Davis and Ross (13) have noted that an elevated platelet count might be associated with either bleeding or thrombotic manifestations. However, in a study of fifteen patients undergoing

splenectomy, they found that despite relatively abrupt elevations in platelet counts shortly after surgery, none of the patients showed evidence of thrombotic phenomena (13).

Breslow and coworkers (11) showed that thrombocytosis could occur after a variety of major surgical procedures on sixteen patients. They found that there was an increase in the circulating megakaryocytes after surgery, peaking at three days at an average of a six-fold rise above preoperative levels, and returning to the original levels by seven days after surgery. The average platelet count did not become elevated until six to seven days after surgery.

Pepper and Lindsay (48) studied eleven patients undergoing extensive surgery, five of which had no blood transfusions. Ten of these patients showed a sharp decrease in platelet levels during the operative procedure or immediately following it. The platelet counts remained below preoperative levels for period of two to six days followed by a rise of the platelet levels three to ten days post-operatively. Platelet levels returned to their preoperative values between the tenth and fifteenth day postoperatively. Pepper and Lindsay (48) noted that their findings were different than those of Mustard (46) who reported thrombocytosis during and following extensive operations. Krizsa and coworkers (37) reported postoperative thrombocytosis in splenectomized mice and in their control group after "sham" operation (an incision over the spleen). The amount of platelet increase was considerably larger and persisted longer in the splenectomized group. However, nearly identical amounts of ^{75}Se-methionine given at various times after surgery appeared in the

circulating platelets of both groups of mice, and they concluded that increased platelet production did not play a decisive role in the development of post-splenectomy thrombocytosis.

CHAPTER III

EXPERIMENTAL PROCEDURE

A. COLLECTION OF SAMPLES

In order to determine if platelets aggregated or adhered to the vessel wall due to stenotic flow conditions, blood from rats was analyzed by taking two samples. One sample was taken before opening the animal and the second one hour after the stenosis was induced. Each sample was used for counting the number of platelets.

Male Sprague-Dawley rats were used in the study. The rats were obtained from the Comparative Animal Research Laboratory in Oak Ridge, Tennessee, and weighed between 409 and 474 grams on the morning of surgery. The rats were given food (Purina Rat Chow) and water *ad libitum* until prepared for surgery. They were anesthetized by an injection with a 25 gauge, 3 cc syringe of Sodium Pentobarbital (Nembutal, Abbott Laboratory, veterinary grade, 50 mg/cc) into the abdomen. The dose was approximately 0.55 cc for a 450 gm rat. Sodium Pentobarbital was found to be a commonly-used drug for research purposes. Many of the researchers that have been previously referenced used this drug to anesthetize rats for various purposes.

When the rats were anesthetized (when there was no reaction to twisting of the tail), they were prepared for surgery. Their left legs and abdomens were shaved with shears. Their legs were further shaved with a scalpel and soap and water. The leg was coated with petroleum jelly to facilitate "beading" of the blood sample when it

reached the surface of the skin. The initial blood sample was taken from the saphenous vein of the left leg. The vein was dilated by pressing the vein proximal to where the sample was to be taken. When the vein was clearly visible, it was pricked through the skin with a 25 gauge needle. The blood was collected into a blood diluting pipet (±1% Try-lyne Dade from Scientific Products or ±1% Fisherbrand #13-645-22 from Fisher Scientific Co.) filled to the 1.0 mark. It was then diluted with 1% ammonium oxalate solution to the 101 mark. The ammonium oxalate solution was used to lyse the red blood cells. The solution appeared cloudy when first mixed but turned clear when the red blood cells had lysed. Brecher and Cronkite (10) reported that 1% ammonium oxalate was used in their experiments in preference to other dilutents because the red cells are hemolyzed thus making the platelets more readily seen and identified. The platelets can be distinguished from other elements of the blood such as bacteria, yeasts, molds, and dust particles as the platelets are highly refractive. Also, adhesion to glass is minimal in the ammonium oxalate solution (10). The blood diluting pipet was then labeled and sealed with a Yankee Pipet Closure.

The rat was the placed on a rat operating table (Fisher Scientific Co.) and its four legs were fastened to the table. The abdomen was opened with a scalpel creating a straight incision approximately 6 cm in length. The skin and outer muscle layer were held apart by clamps that were held by elastic extending from the bottom of the operating table. The intestines were placed to one side but remained inside of the body cavity and the aorta was exposed by gently

pulling away the tissues around it with gauze. When the aorta was cleared of tissues, a blunt probe was placed under the aorta from the right side and then between the vena cava and aorta into the tissue connecting them. The probe was moved around slightly to insure that it was not directed toward the wall of the vena cava (if the probe were on the wall of the vena cava, when it was pulled back toward the right there would be blood visible through the vena cava as it flowed across the area that had been previously blocked by the probe). The probe was then gently pushed through the tissue toward the left. A cotton swab was placed on the other side of the tissue and the tissue was split by rubbing the swab against the probe. The probe was pushed through approximately one centimeter past the vessels. An 18 gauge needle, which had been dulled with a file and was threaded with a piece of size one silk suture (J. A. Deknatel and Son, Inc.) and a piece of size 4-0 suture (Ethicon), was placed on the end of the probe. Both probe and needle were moved back to the right between the vessels. The string was held on the right side and the needle was pulled back to the left until it cleared the vessel area. The needle was then pulled out so as to leave only the thread. The smaller thread (size 4-0) was looped around the aorta and marked with fingernail polish to get an approximate measurement of the circumference of the vessel. This thread was then removed. The aorta diameter was measured with a caliper and the caliper was measured by a micrometer. After taking these measurements, the remaining thread was tied once around the aorta and left for about thirty seconds. If there was no excess bulging upstream to the tie, which would indicate that it was tied too

tightly and therefore blocking too much blood flow, the thread was tied again making a square knot. The excess thread was cut close to the knot. Saline was squirted into the wound with a syringe to avoid drying of the tissues and a large piece of gauze (4 x 4 inches) that was coated with petroleum jelly was placed over the wound. The rat was removed from the operating table and placed on its back until one hour after the tying of the vessel, when another blood sample was taken.

There were also experiments performed that did not involve surgery. These experiments were used as controls to determine if there were any effects due only to the injection of Nembutal. Two rats were first bled from the left leg as described above. After an hour, another sample was taken from the right leg.

There were also two methods of surgery used for control subjects. One method was the same as the procedure described above except that it was terminated at the point that the artery was exposed. This procedure was performed to find the effects on platelet count and size due to cutting the rat open and moving the tissues. The second control method involved the same procedure as outlined above except that it was terminated at the point where the threads were in place between the vena cava and the aorta. This procedure was performed in order to determine if there was any additional effect due to separating the aorta from the vena cava and stretching the aorta as it was moved during the insertion of the threads. The control rats were bled again one hour after the artery was exposed or the threads were in place, whichever situation applied.

The second bleeding procedure (at the end of the one-hour period) was the same for all three experiments. The rat was placed on the operating table again. The vena cava was rolled slightly to the right. A piece of Parafilm, which was folded over itself three times to make it stiff, was placed under the vena cava and the vein was punctured with a 27 gauge needle. The blood was quickly drawn into the blood diluting pipet from the Parafilm. A cotton swab coated with petroleum jelly was placed over the puncture to cause stasis of the blood so that the puncture would close by the clotting of blood. This healing usually took approximately one to two minutes but the swab was left on the vessel long enough to prepare the blood sample.

A further study was conducted simultaneously with the studies just discussed but has not yet been completed. The purpose of this additional investigation was to determine if there were platelets adhering to the vessel wall in the vicinity of the stenosis. After the second sample of blood had been taken from the rats in the two control groups, their aortas were measured and then tied as in the stenosis induced group, using the same procedure that has been previously described. After the stenoses were induced, the rats were closed with silk sutures, either with the same 4-0 silk mentioned above and threadable reusable semicircular needles or with sutures with needles permanently attached to the silk (Ethicon, size 000). The muscle layer was sewn with continuous stitches. The outer layer (skin) was sewn with individually-tied stitches. The rats were placed in separate clean cages, covered with a cloth, and taken to a warm room to recover. After various periods of time (ranging from one to

sixty days) the segment of the abdominal aorta containing the stenosis was removed from the rat and prepared for histological studies. Following a procedure described by Karnovsky and Roots (32), the segments were fixed overnight in a solution of 10% formalin containing 1% calcium chloride. They were then stored in a sucrose-1% gum arabic solution. Acetylthiocholine iodide is needed (and as yet not available) before the staining can proceed. The Histology Department at the University of Tennessee Memorial Research Center will assist in this portion of the study.

B. ANALYSIS OF THE BLOOD SAMPLES

The blood platelets were counted in a hemacytometer (American Optical, Spencer, Bright-Line, Improved Neubauer, 0.1 mm deep) by placing a No. 1½ coverslip over the chamber and filling it with blood-ammonium oxalate solution from the blood diluting pipet. The samples were mixed by inverting the pipet several times; then some of the solution was drawn out by running it over a paper towel before filling the chamber. The samples were taken in duplicate with mixing in between the filling of each chamber. The chambers were placed under a petri dish with a piece of wet cotton and sealed with tap water around the edges of the inverted petri dish to avoid drying-out of the sample. The samples were left for twenty minutes to allow for the settling of the platelets.

The platelets were counted by use of a phase microscope (American Optical, Spencer) using a dark phase contrast 45x objective. Each side of the chamber has a grid of twenty-five squares in rows and

columns of five. The total number of platelets in the four corners and center squares was counted. Since each square has an area of 0.04 mm^2 and a volume of 0.004 mm^3, and the blood was diluted with 100 parts ammonium oxalate, this count was multiplied by 5,000 to give the number of platelets per cubic milimeter of blood. The two values obtained from the two chambers of the hemacytometer were averaged to obtain the count for that particular sample.

The platelet counts were analyzed statistically by using a t test for independent samples (20) to determine if there were significant differences in the original platelet counts for all three groups. The t test for paired observations (20) was used in each group to compare the platelet counts from the original sample with the platelet counts after surgery. This test was used because the two samples were not independent, as the sample after surgery was dependent upon the sample taken before surgery.

CHAPTER IV

DISCUSSION OF RESULTS

A. DATA LISTING

The platelet counts obtained for the rats undergoing surgery are shown in Table 1 and graphed in Figure 1. The categories of data listed in Table 1 are: (a) Sample 1, platelet count from the leg before opening the rat; (b) Sample 2, platelet count from the vena cava one hour after surgery; (c) the difference in the two platelet counts, the second one minus the first; and (d) the percentage difference, the difference divided by the first sample (from the leg) and that value multiplied by 100. The average value of percent difference listed includes the value of the standard error. Each value listed in the Table is the average value of the duplicate counts that were taken of each blood sample.

There were four conditions that led to elimination of data which were not included in Table 1. Gruner and Endresen (30) reported that the normal platelet count for rats is approximately one million per cubic milimeter; the acceptable range for the first sample taken from the leg vein was from 800,000 to 1,300,000 platelets/mm^3. Samples with values not in that range were discarded. Another reason for elimination of some data was due to the process of using the Parafilm for the second bleeding. It was difficult to always get an adequate sample of blood on the Parafilm. If the first sample taken from the vein was not successful (for example, if a clot formed in it) then it

TABLE 1

EFFECT OF VARIOUS SURGICAL TECHNIQUES ON PLATELET COUNTS OF RATS

Group Tested	Sample 1 (Plt/mm^3)	Sample 2 (Plt/mm^3)	Difference (Plt/mm^3)	Percent Difference
Exposed Artery No Thread				
Rat AA	912,500	1,040,000	127,500	14.0
Rat BB	802,500	952,500	150,000	18.7
Rat CC	915,000	1,090,000	175,000	19.1
Rat DD	825,000	1,017,500	192,500	23.3
Average Value	863,750	1,025,000	161,250	18.8 ±1.90
Thread Between Vessels Not Tied				
Rat EE	875,000	925,000	50,000	5.7
Rat FF	1,060,000	1,152,500	92,500	8.7
Rat GG	1,010,000	1,140,000	130,000	12.9
Rat HH	1,025,000	1,265,000	240,000	23.4
Average Value	992,500	1,120,625	128,125	12.7 ±3.87
Thread Tied Stenosis Induced				
Rat II	940,000	692,500	-247,500	-26.3
Rat JJ	842,500	687,500	-155,000	-18.4
Rat KK	933,000	820,000	-113,000	-12.1
Rat LL	932,500	877,500	-55,000	-5.9
Rat MM	875,000	917,500	42,500	4.9
Average Value	904,600	799,000	-105,600	-11.6 ±5.33

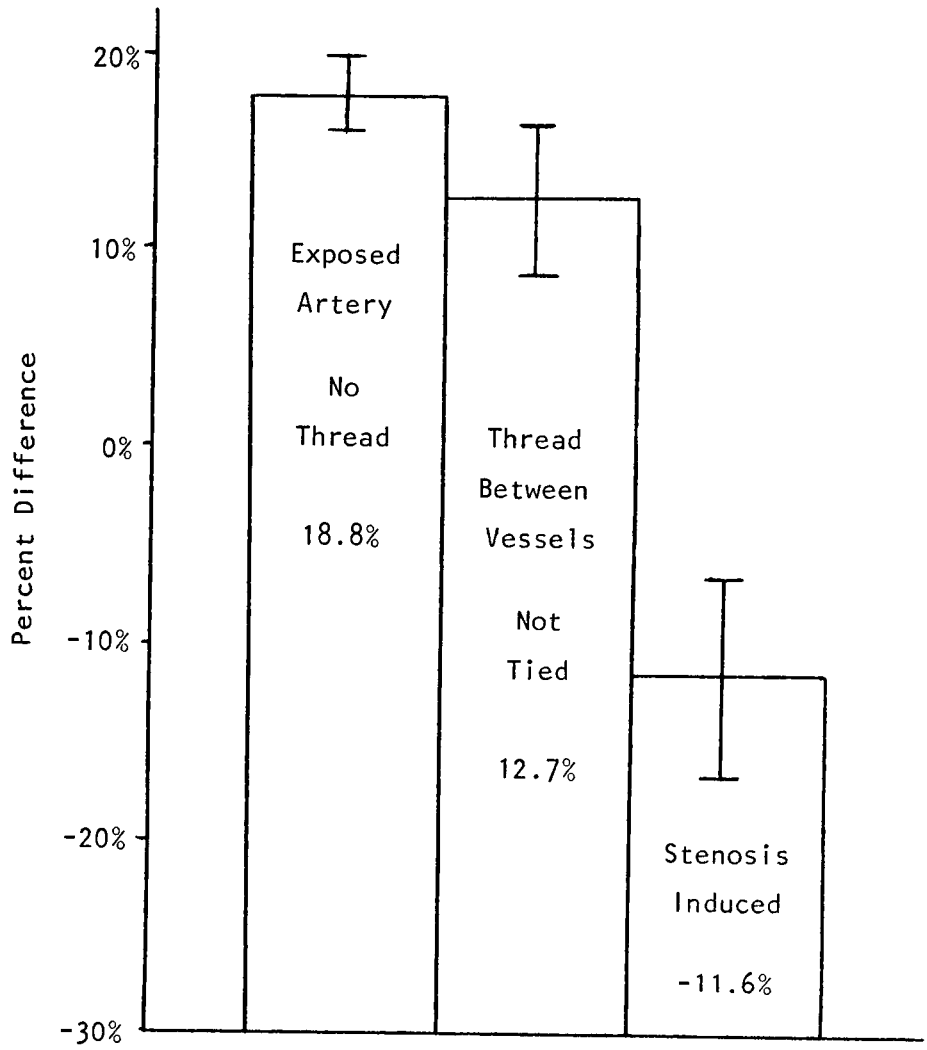


Figure 1. Changes in Platelet Counts of Thirteen Rats
Bars represent standard errors.

was difficult to obtain a suitable sample from a second puncture. Data were also eliminated when they did not fit into the range of values that was statistically significant as determined by the t test (64). The final reason for elimination of data was due to poor operating procedure which was evident when there was bleeding from the vena cava when the probe was inserted between the vessels.

Twenty-five rats were used for the study, with thirteen producing data that were used for the final results. Four rats had unacceptable initial counts; two rats had low platelet counts of 780,000/mm^3 and 777,500/mm^3 and two rats had high counts of 1,480,000/mm^3 and 1,532,000/mm^3. An unsuccessful second bleeding, the one from the vena cava, eliminated four more rats. The second count for these four was very low compared to the first count in all three situations: the artery exposed with no thread (two eliminated), thread between the vessels but not tied (one eliminated), and the vessel tied (one eliminated). In three of these samples, clots were visible in the pipet. Even though it was suspected that the platelet counts would be low, the samples were nevertheless counted and ranged from 241,500 to 530,000/mm^3. The first samples were all in the normal range. One value from the thread between, not tied group was eliminated because the vena cava was accidentally punctured as the thread was inserted between it and the aorta. Bleeding was stopped but the second sample obtained one hour later had a platelet count of 497,500/mm^3 which was, as expected, much lower than other values in this control group (see Table 1). The initial count was normal.

The other three data points were eliminated by statistical analysis. A t test (64) was performed on all data and a 95% significance level was used. Of the three values that were eliminated by statistics, there was one value in the artery exposed, no thread category and two values in the stenosis-induced category.

The twenty-five rats that underwent surgery fell into these categories: eight tested in the artery exposed, no thread category; seven in the thread between the vessels but not tied category; and ten whose vessels were tied to produce stenoses. When the samples were eliminated as discussed above there remained four samples in each of the control groups and five samples in the stenosis-induced group.

B. PLATELET COUNTS

The data show two important results: (1) thrombocytosis was observed one hour after surgery and (2) platelet counts decreased due to constriction of the vessels. These results will be discussed following a discussion of the significance of the data.

The data were analyzed using the t test (20). The platelet counts of rats following the exposure of the artery with no thread were higher ($p < 0.001$) than the original counts. The platelet counts of rats following the thread placed between the vessels but not tied surgery also were higher ($p < 0.05$) than the original counts. The platelet counts of rats following the induced stenosis were lower ($p < 0.05$) than the original platelet counts. There was no noticeable change in platelet counts due to the Nembutal only, based on observation

of two rats. The values of the original counts, analyzed by the t test (20) were not different between the three groups ($p > 0.05$).

The percent increase in counts in the exposed artery with no thread group was greater than the increase in the thread between the vessels but not tied group. The stenosis induced group showed a decrease in percent difference as compared to the thread between the vessels but not tied group and a larger decrease in percent difference as compared to the exposed artery with no thread group.

Examination of the individual values of percent difference in platelet counts in each group shows that they are different from one group to another. There were only two values that were similar for the two control groups, 23.3 for Rat DD and 23.4 for Rat HH. All other values for the exposed artery with no thread group were higher than the three other values for the thread between the vessels but not tied group. The stenosis-induced group showed a decrease in count with nearly every rat in that group. The one rat that had an increase, Rat MM, showed an increase that was below the mean of either control group and even lower than any of the individual values for either control group.

Thrombocytosis. The results from the first control group of rats having the vessels exposed but no thread inserted around the aorta showed thrombocytosis one hour after surgery. The mechanisms involved in this increase in platelet count are unknown, as was discussed in Chapter II, but it is interesting to speculate on some possible causes for the increase. The platelet count increase due to

surgery was probably not due to an accelerated platelet production because the production has been reported to take three to four days (11,41). The increase was shown to be unrelated to the Nembutal because samples taken from rats that were anesthetized but not opened showed no increase in count. Also, based on the results of other investigators (11,41,48), it seems unlikely that the increase in platelet count was due to the release of platelets from a "platelet pool". After excluding the three hypotheses above, it is speculated that the increase in platelet count may have been caused by some kind of a release reaction possibly triggered by the release of epinephrine or other drugs as a result of the surgical procedure.

Platelet decrease due to stenosis. The data reported from this study showed an effect on platelets due to the stretching and manipulation of a vessel and especially to the presence of a stenosis. It is speculated that many of the platelets were adhering to the walls around the stenosis because there was evidence of possible adhesion to the vessel wall due to vessel damage even before the thread was tied (seen by comparing the second control group to the first control group). A stenotic condition could have caused further vessel damage and therefore could have caused increased platelet adhesion.

The effect of mechanical trauma on vessels has been studied previously. Ashford and Freiman (2) performed an experiment to determine if there was increased platelet adhesion due to the injury of the vessel. These investigators pinched the femoral veins of Sprague-Dawley rats with forceps. The vein was pinched with a force sufficient to cause edema but not bleeding. The vessels were fixed

for study ten minutes later. These vessels were compared to others that were exposed by separating them from surrounding tissue. Even after such a short period of time, there was evidence of endothelial cell damage and platelet adhesion in the pinched group. Even though the mechanical force was much greater than in the experiments being reported here, it is significant to note that there was a change due to outside mechanical force as compared to controls that are very similar to one control group studied in the current study. Therefore, one may expect damage due to separating and stretching the vessel slightly when thread was inserted between the vessels but was not tied.

Other investigators have also shown a relationship between endothelial damage and platelet adhesion. Stemerman (62) selectively removed endothelium by the insertion of a balloon catheter into a vessel. He reported that there was platelet adhesion to the damaged wall within ten minutes. Baumgartner and coworkers (5,7) also reported platelet adhesion to endothelium of a vessel damaged by a balloon catheter.

Other speculations may be made as to why platelets were removed from the circulating blood in the stenosis induced group of the present investigation. Some platelets may have been damaged and removed from the circulation. Others may have formed aggregates, potentially producing thrombotic conditions due to the flow conditions present. By knowing only the platelet counts, it is impossible to know the reason or reasons for the decreases. If the staining procedure previously described proves successful, there will be information

available towards determining the amount and location of adhesion that resulted from the stenosis. Some further discussion of the results, particularly as they may be influenced by flow conditions, is contained in the next chapter.

C. OTHER STUDIES

Initial studies. The study reported herein was preceded by an investigation in which platelets were counted from fifty-four rats before and after surgery using similar procedures to those described in Chapter III. The reason that the results of this initial study are not reported is that the platelet counts before surgery were lower than normal and there was a large amount of scatter in the data. It is believed that the primary reason for the poor data was that platelets were adhering to glass, since nonsiliconized, heparinized glass vials were used for collecting the samples. A glass Pasteur pipet was also used to transfer the blood to a small piece of Parafilm from which it could be drawn in to the blood diluting pipet. However, platelets are known to adhere to glass (50), and heparin apparently did not prevent this adhesion.

Despite the low counts in the initial study, the trend of the results was similar to that seen in the reported study. There was an increase in platelet count in the exposed artery with no thread group and a slightly smaller increase in the thread between the vessel but not tied group. The value for the percent difference in the thread tied, stenosis induced group was much lower than in either control group.

Platelet sizing. An attempt was made to determine if either young or old platelets were preferentially removed from the circulation. McDonald, Odell, and Gosslee (42) showed that platelet age was related to size. Attempts in the present study to size the platelets were unsuccessful due to the inability to acquire an adequate amount of platelet-rich plasma that was needed for the sizing instrument (Electro Zone/Celloscope (Particle Data Inc.)). Blood samples for the platelet sizing were taken soon after the samples for counting were taken. Many trials were made, using techniques of (a) collecting the blood in tubes and letting it settle, (b) centrifuging blood that was collected from the rat into heparinized vials, (c) centrifuging the blood which had been collected in heparinized vials and exposed to ethylenediaminetraacetic acid (EDTA) and heparin in a double-dose tube before transferring it into the plastic tubes used in the centrifuge, and (d) collecting the blood directly into a double-dose tube and then centrifuging the blood.

The fourth method, used for approximately half of the rats reported in the present study, was the most successful but was not consistently successful. McDonald, Odell, and Gosslee (42) reported that rat blood could be successfully centrifuged but their work was performed with approximately ten mililiters of blood. It appears that the sizing studies could be completed if the blood samples were larger. If only one sample were taken from the rat, possibly from the aorta downstream from the stenosis, this sample could be compared to samples taken from the same location in rats that did not have a constricted aorta. Further studies need to be made in order to determine the relationship between platelet age and stenotic conditions.

Platelet adhesion study. This investigation, as discussed in Chapter III, has not been completed and the histological study cannot proceed until acetylthiocholine iodide is obtained. However, if successfully completed, this study will indicate if platelets adhered to the vessel walls in the vicinity of the stenoses. It will also indicate the locations with respect to the stenoses at which adherence occurred.

CHAPTER V

SPECULATION ON FLOW CONDITIONS AND PHYSICAL PARAMETERS

It is desirable to know the specific stenotic flow conditions which may have caused the decrease in platelet count. The flow conditions, however, are not known and can only be speculated. An attempt will be made to use various results either from this study or studies reported in the literature to estimate the flow conditions in the vicinity of the stenosis.

In order to obtain an estimate of the degree of the stenosis, measurements were taken of the vessel as described in the experimental procedure. To obtain the normal size of the aorta, a thread was placed around the vessel and marked at the place where the two sides crossed. The vessel diameter was also measured with calipers. Later, when the rat was sacrificed, the string around the stenosis was separated from the vessel. Then a needle was inserted through the ring of thread and the thread was cut from around the needle with a scalpel. The length of the thread was measured with a micrometer to obtain the outside circumference of the vessel at the throat of the stenosis.

The above procedures gave an estimation of the outside dimensions. To estimate the inside radius, the thickness of the vessel wall (determined to be approximately 0.076 mm in initial studies) was subtracted from the outside radius. Also subtracted was the radius of the cross-section of the thread, measured to be 0.064 mm for the thread used to measure the normal vessel size and 0.241 mm for the thread used

to tie the vessel. The average value found for the normal inside area was calculated to be 1.9 mm^2 with a range of 1.3 mm^2 to 2.9 mm^2. These values were obtained by averaging the values obtained from the caliper and thread methods. The values for the percent decrease in area due to stenosis ranged from 45% to 61% with a mean of 53%. There was no apparent association between the degree of constriction and the platelet counts. This method of measurement was obviously approximate.

The flow rate through the vessel was an important parameter. This time-varying rate could be measured by an electromagnetic flowmeter if there were a probe available that was small enough to fit the abdominal aorta of a rat. There was not one available for this study that was small enough. The flow rate in rats has been measured by Pfeffer and Frohlich (49) by using an electromagnetic flowmeter. The flow was measured in the ascending aorta, which is larger than the abdominal aorta. The researchers reported pressures and flow rates of rats anesthetized with Urethan, pentobarbital, and ether. It was interesting to note the differences due to the anesthesia. Pentobarbital caused a rise in heart beat rate compared to the other two groups (352 beats/min), a slightly lower cardiac output, a higher diastolic blood pressure, and a lower maximum flow acceleration.

The average value for the peak flow for the pentobarbital group was 3.8 ml/sec. This value was used to estimate the flow through the abdominal aorta by comparing the distribution of flow in the rat to the flow in the human. Burton (12) reported that of the blood volume pumped by the heart of a person at rest, a total of 5,800 ml/min, 750 goes to the brain, 250 to the heart, 1,100 to the kidneys, 1,200 to the

muscles, 500 to the skin, 1,400 to the abdomen, and 600 to other. At the point where the stenosis was tied, the blood to the brain, heart, and kidney had already been distributed. It was estimated that all the blood to the abdomen, one-fourth of the muscle, one-sixth to the skin, and one-tenth of that to other was left to be distributed after leaving the stenosed area. This leaves 1843/5800 or 0.32 of the amount coming from the ascending aorta. By using the amount cited above, that would mean that the rough estimate of peak flow rate would be 0.32 x 3.8 ml/sec or 1.2 ml/sec. It is assumed that the volume rate of flow through the aorta has not been reduced because of the stenosis, since it is generally felt that mild and moderate stenoses (the stenosis in the present investigation would be classified as "moderate") have little effect on the flow rate (54,71).

The blood pressure of rats has also been reported. Pfeffer and Frohlich (49) reported that in the ascending aorta of rats anesthetized with pentobarbital, the systolic pressure was 103 mm Hg, the diastolic pressure was 63 mm Hg, and the mean pressure was 79 mm Hg. Smith (58) reported that the mean femoral arterial pressure of rats also anesthetized with pentobarbital was 140 mm Hg.

Young, Cholvin, and Roth (71) studied pressure drops across stenoses induced by the insertion of plugs into femoral arteries of dogs, and gave values for the pressure drop according to percent reduction of area due to the stenosis. The smallest percentage stenosis studied in dogs was 53% (the mean value for the present study), which exhibited a mean pressure drop of 3.5 mm Hg. The pressure drop increased with the percent stenosis up to a value of 92%, which had a

drop of 30 mm Hg. There was likely some pressure drop due to the stenosis induced in the present study, as the reduction in area was estimated to be 53%. However, the pressure drop would be extremely difficult to measure due to the small size of the vessel, and in the present study, it was felt that experimental error would be larger than the pressure drops recorded. If a larger animal were used, a stenosis could be induced by inserting a bypass containing a known stenosis shape. It would also be easier to measure the pressure drop if pressure taps were included in the bypass.

The viscosity of rat blood has also been reported in the literature. Shorrock and Hillman (57) reported that the absolute viscosity of rat blood at body temperature was 6.4 centipoise and the relative viscosity was 9.76. The values were from normal rats and not necessarily representative of what may be present in the area around a stenosis due to the shear stresses present there. Zingg, Morgan, and Anderson (74) reported that viscosity of whole rat blood changed with shear rates. At a rate of 230 sec^{-1}, the viscosity was 4.3 centipoise, which was much lower than the viscosity reported by Shorrock and Hillman. The values of other rates reported are at 115 sec^{-1} (4.9), 46 sec^{-1} (6.5), and 23 sec^{-1} (8.1). Only at the rate of 46 sec^{-1} were the values close to the reported normal values. Therefore, what is reported to be the value of viscosity for normal rats may be different from that of rat blood in a stenotic region. Perhaps blood samples could be taken from that region. Again, a larger animal would be necessary because the area of the stenosis is very small in a rat, and there is only a small volume of blood that could be experiencing

this change in viscosity at any one period of time. In articles cited in Chapter II, it was reported that the circulating blood of patients with occlusive diseases had increased viscosities. Perhaps a similar study could be done on rats or other animals with artificially-induced stenotic conditions.

The value of the Reynolds number for the flow of blood in the abdominal aorta of rats was estimated using some of the values given above. The value for the Reynolds number was calculated as described by Smith and coworkers (59) in that measured or known blood flow and inside diameter was used and also an assumed normal value for the density of blood was used. The specific gravity was assumed to be 1.06; therefore the absolute density would be 1.06×10^{-3} gm/mm^3. The density of the blood does not normally vary over 10% from that value (59) and since this calculation was only an approximation, that value for density was used. The value reported above for absolute viscosity of 6.4×10^{-2} poise was used. The inside diameter was calculated to be 1.55 mm. The peak velocity was used, and was found by dividing the peak volume rate by the area which gave a value of 159 mm/sec. The calculation of the peak Reynolds number follows:

$$Re = \frac{\rho VD}{\mu} = \frac{(1.06 \times 10^{-3}\ gm/mm^3)(159\ mm/sec)(1.55\ mm)}{6.4 \times 10^{-3}\ gm/mm \times sec} = 40.8$$

If the Reynolds number were calculated using an average velocity during the ejection part of the cardiac cycle it is estimated to be approximately 60% of the above calculated value, based on flow curves presented by Pfeffer and Frohlich (49). If the Reynolds number is

calculated using the average velocity over the total cycle of one heart beat, then it is approximately 35% of the value calculated above, based upon the flow curves presented in Pfeffer and Frohlich (49). This latter result is consistent with that calculated from data presented by Young, Cholvin, and Roth (71).

Flow in a constricted tube at a low Reynolds number of twenty-five for steady flow has been reported by Kozman and Forrester (35). They reported that there was separated flow in flow with a Reynolds number greater than twelve. Therefore, there is a possibility that there was separated flow in the rats in the present study. This separated flow could have also caused stasis and therefore platelet aggregation as described previously. Kozman and Forrester (35) also found large velocity gradients present in flow of Reynolds number twenty-five in the area of a severe stenosis. These large velocity gradients were associated with shearing stresses that could cause deterioration of vessel walls. If the vessels in the present study experienced endothelial damage, there was time for platelet adhesion due to the injury before the second blood sample was taken.

Several of the flow conditions that have been found to be present in stenotic regions could have been present in the *in vivo* rat studies that have been described. Any one of the flow conditions could have partially caused the decrease in platelet count that was shown in the results. It is difficult to know which flow condition or conditions was present. Further investigations are needed, including those mentioned previously. Larger animals should be used in these studies so that the geometry of the stenosis can be regulated

more accurately, and the acquisition of samples and the measurement of flow parameters can be facilitated.

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