DETERMINATION OF PLATELET SURVIVAL USING AN ASPIRIN LABELING TECHNIQUE

by

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INTRODUCTION

A. STATEMENT OF THE PROBLEM

Early studies involving platelets showed a relationship between a decrease in circulating platelet number and excessive bleeding. Thereafter, thrombocytopenia was recognized as a hallmark of many bleeding disorders (7-9, 67). The causes of thrombocytopenic states were soon attributed to either decreased platelet production as seen in aplastic anemia or to increased platelet destruction as occurs in disorders such as idiopathic thrombocytopenic purpura (ITP)(15).

A subsequent phase of platelet research focused on patients with normal platelet numbers who still presented with excessive bleeding. The underlying process in this group of disorders involved abnormalities of platelet function (64). The pathologic mechanisms in thrombasthenia, storage pool disease, and the acquired defect induced by aspirin were elucidated.

Currently, much interest revolves around the concept of "hyper-functional" platelets (32, 43). Evidence is accumulating that increased platelet reactivity is implicated in the pathogenesis of arterial and venous thrombo-embolic disease (32). A common denominator in most of these patients seems to be a decreased platelet survival when measured in vivo (43).

Methods to treat platelet-induced thrombotic or embolic disease are now becoming available. Therapeutic agents such as dipyridamole,

alone or with aspirin, and sulfinpyrazone are effective in normalizing decreased platelet survival (30, 34). Attempts are being made to evaluate the effectiveness of these drugs in preventing thromboses and emboli in patients who have received prosthetic devices such as artificial heart valves, arterio-venous shunts or artificial blood vessels. Worldwide, controlled clinical trials are currently being performed to study the effects of these drugs in cerebrovascular disorders such as transient ischemic attacks, in coronary artery disease, in peripheral vascular disease and in acute renal disease (4, 5, 32).

A practical, clinically applicable method to identify individual patients as well as groups of patients with altered platelet survival is clearly needed. At the present time Chromium 51 (^{51}Cr) is used to label platelets in order to measure platelet survival in the circulation. Since this method is difficult, time-consuming and expensive, ^{51}Cr platelet survival is used primarily in research studies rather than as a routine clinical procedure. Recently, a much simpler and potentially clinically useful test for estimating platelet survival has been devised which utilized acetylsalicylic acid (ASA) as a platelet label (58). The present study was designed to evaluate this method in both normal subjects and in patients with artificial cardiac valves.

B. BACKGROUND

1. Platelets - Physiology and Function

Platelets are an important circulating cellular element of the blood. They are nonnucleated cells, with an average diameter of 2.0 microns (65, 66). Under normal conditions platelets circulate for approximately 10 days as smooth elliptical discs that are non-adherent

to each other or to normal vascular endothelium. The normal adult platelet count ranges from 150,000 to 350,000/cu. mm.

Platelets are produced by large (30 to 40 micron) multinucleated cells (megakaryocytes) in the bone marrow (28). Bits of megakaryocyte cytoplasm break off and are released through the sinusoids of the bone marrow into the peripheral circulation (22). A postulated hormone, thrombopoietin, is thought to control platelet production by stimulating various stages of megakaryocyte production and maturation and platelet release (18, 27-29).

A storage pool of platelets does not exist in the bone marrow (20). If the peripheral platelet count remains at a relatively constant level over a period of days, it can be assumed that the rate of production and rate of destruction are equal (27-29). Approximately one-third of the total circulating platelet numbers are stored in the spleen while the remaining two-thirds are contained within the vascular system (20, 46).

Platelets are necessary for effective hemostasis (64). Aggregates of platelets gather at a site of injury to form a primary platelet plug. The formation of a plug involves a series of platelet reactions: 1.) adhesion of platelets to collagen or other substances at the site of injury, 2.) the release of endogenous adenosine diphosphate (ADP) from the granules of the adherent platelets, and 3.) aggregation of surrounding platelets in response to ADP to form an enlarged platelet mass (32).

In addition to their primary role in the arrest of bleeding, platelets also participate in the formation of fibrin (64-66). Platelet factor 3 is contained within platelet membranes and is a substrate for several reactions leading to the conversion of prothrombin to thrombin.

After thrombin catalyzes the conversion of fibrinogen to fibrin, a permanent arrest of bleeding usually occurs.

2. Past and Present Platelet Research

Early research focused on quantitative defects of platelets in diseases such as ITP. A major accomplishment was the differentiation of the mechanisms causing thrombocytopenia. It was recognized that thrombocytopenia could result from increased peripheral destruction as in that due to ITP, decreased platelet production as occurs in aplastic anemia or increased splenic sequestration as is associated with the hypersplenic syndromes. In each of these situations a decreased peripheral platelet count may lead to an increased incidence of bruising, hemorrhage or death (7-9).

Qualitative platelet disorders were the next area researchers began to study (64). Platelet function disorders that were previously poorly understood were correlated with defects in the various stages of normal platelet function. For example, platelets from patients with von Willebrand's disease failed to demonstrate normal adherence to a column of glass beads; patients with storage pool disease had a decreased amount of ADP in the dense granules of their platelets, and platelets from patients with thrombasthenia failed to respond when stimulated with exogenous ADP (49, 64). Most of these qualitative defects in platelet function are associated with normal peripheral platelet counts.

In the last few years interest has turned away from the hemorrhagic states and toward detection and evaluation of platelet "hyperfunction" (increased platelet reactivity) which is very often associated with accelerated platelet destruction. The tests which are currently used

to identify platelet hyperreactivity are platelet aggregation, detection of circulating platelet aggregates, platelet adhesiveness and most importantly, platelet survival.

3. Tests to Detect Platelet Hyperfunction

Carvalho measured platelet reactivity in the aggregometer in patients with Type IIa hypercholesterolemia (13). The platelets from these patients required 10-1000 fold less aggregating agent to induce a maximum aggregation response than platelets from normal subjects.

Hoak and Wu developed a method to detect circulating platelet aggregates in the peripheral blood (71). Freshly drawn blood was added to tubes containing (ethylenediaminetetra-acetate) EDTA alone and EDTA plus formalin. After gentle centrifugation a platelet count was performed on each of the supernatants. A comparison of the two counts will show whether platelet aggregates were present in the blood sample, since platelet aggregates in the tube with both the EDTA and formalin will be fixed and centrifuged away resulting in a lower platelet count. A second method for detecting platelet aggregates measures the pressure required to force fresh blood through a screen with a pore size of approximately 20 microns. Platelet aggregates will plug the screen and result in a higher filtration pressure. More recently, industry has developed a sophisticated instrument that will size and count circulating platelet aggregates (52).

Platelet adhesiveness measures the ability of platelets to adhere to a column of glass beads (49). Unfortunately, this test is difficult to perform and often gives spurious results.

The first attempt to evaluate platelet survival involved direct transfusions of whole blood from donors with elevated peripheral

platelet counts to recipients who were severely thrombocytopenic (33). Survival, estimated to be 4-8 days, was determined by performing serial platelet counts. Because of the obvious drawbacks of this method, improved techniques were developed.

Several radioisotope methods were evaluated but each presented its own inherent difficulty (1, 67). To date, the best and most frequently used radioisotope label is ^{51}Cr. While the method is accurate, it is both time-consuming and expensive. In brief, a unit (450 ml) of blood is removed from the patient, the platelets are separated by differential centrifugation, and the ^{51}Cr label is added and allowed to incubate for 15 minutes at room temperature (1). After incubation, the platelet suspension is centrifuged and the residual radioactivity is determined in the supernatant. The labeled platelets are resuspended in a solution containing platelet poor plasma and saline. Ascorbic acid is added to prevent the label from binding to the red cells, and the labeled platelets are reinfused into the patient. Subsequently, blood samples are drawn at 30 minutes, 2 hours, 4 hours and then daily until the radioactivity disappears. Platelet survival by this method is 9-11 days with a T_{50} (time in days until 50% of the radioactivity has disappeared) of 3.3 to 5.5 days (59).

Although this method is accurate it is time-consuming (about 4-6 hours are required to prepare the unit of labeled platelets, and reinfuse the patient), requires sterile conditions and exposes the patient to a source of radioactivity. Because of these drawbacks and its expense, this method is not in general clinical use.

Platelet aggregometry has also been used to estimate platelet survival (51). Aspirin is known to inhibit platelet aggregation induced

by collagen or epinephrine in vitro. By administering aspirin to subjects and serially measuring the aggregation response until full reactivity returns, normal survival was found to be 4-7 days. However this method failed to correlate with the ^{51}Cr method in patients with ITP.

Within the last year a new method for estimating platelet survival using aspirin to label the platelets was proposed by Stuart, Oski and Murphy (58). Until the last few years the mechanism by which acetylsalicylic acid interacts with platelets was not understood. Now however, it has been shown that aspirin inhibits the synthesis of prostaglandins from arachidonic acid found in the platelet membranes (53). An enzyme, cyclo-oxygenase, is responsible for incorporating moelcular oxygen into the arachidonic acid to give endoperoxide intermediates which are then converted to prostaglandin metabolites. Aspirin specifically inhibits cyclo-oxygenase activity and this inhibition persists for the life span of the platelet (38, 54, 58, 63). If prostaglandin synthesis in the platelet membrane is artificially stimulated, various metabolites of prostaglandin synthesis, such as malonaldehyde may be measured spectrophotometrically (44, 45, 54).

The method proposed by Stuart involves first measuring a baseline value of malonaldehyde and then administering aspirin to the patient (59). The aspirin inhibits lipid peroxidation of all platelets circulating at that time. Malonaldehyde production increases as platelet renewal occurs, until the baseline value is approximated. The time required for recovery from the aspirin-induced platelet defect gives an estimate of platelet survival. The results obtained by Stuart show excellent correlation with ^{51}Cr studies.

4. Relationship of Platelets to Thromboembolic Disease

Clinical disorders associated with decreased platelet survival can be roughly categorized into four groups. Each group is associated with a mechanism which results in accelerated destruction of the circulating platelets. The mechanism can involve: (1) an immunological process, (2) intravascular destruction, (3) destruction due to abnormalities of the blood vessel wall, or (4) intrinsic abnormalities in the platelet.

a. Immunological Process

Platelets may become coated with an antiplatelet antibody which results in accelerated platelet removal by the reticuloendothelial system (7-9). Often, bone marrow production of platelets may increase sufficiently so that overt thrombocytopenia does not occur. If however, the bone marrow cannot compensate, a falling platelet count may be observed. This disorder may occur spontaneously, may be drug induced or may be a secondary process associated with diseases such as lupus erythematosis, or chronic lymphocytic leukemia.

b. Intravascular Destruction

Intravascular destruction may lead to decreased platelet survival but is not always associated with thrombocytopenia. Patients with disseminated intravascular coagulation (DIC) or prosthetic heart valves often demonstrate a compensated state.

Decreased platelet survival has frequently been reported in patients who have received prosthetic heart valves (6, 30, 56, 62). However thrombocytopenia has not been associated with decreased platelet survival in these patients. Usually the peripheral platelet counts are in the

normal range (200,000-400,000/mm^3) (30, 60). Hence the bone marrow compensates for decreased platelet survival by increasing platelet production to maintain a normal peripheral platelet count.

The mechanism leading to shortened platelet life span has not been fully elucidated. The prosthetic valves may damage the platelets so that they are removed prematurely from the peripheral circulation by the reticuloendothelial system (62). Platelets may also be consumed at an increased rate due to formation of thrombi (30). Thromboembolism occurs at a higher frequency than normal in patients who have received artificial cardiac valves. This complication may result from stimulation of platelets by the artificial surface. Platelets may adhere to the surfaces near the site of the valve, release endogenous ADP, aggregate, and eventually embolize to the central nervous system or other site (61).

c. Vascular Destruction

Vascular destruction of platelets can be seen in many conditions and again a compensated state may exist so that thrombocytopenia does not occur. Cerebrovascular transient ischemic attacks (TIA) may arise from the formation of platelet thromboemboli upon carotid atherosclerotic plaques (19, 48). At the present time two cooperative studies are underway to evaluate the effect of antiplatet drugs in this group of patients (21, 41). The design of the study is to evaluate the effects of treatment on the incidence of transient ischemia, stroke, and survival (32).

Other vascular endothelial abnormalities may lead to platelet destruction. Conditions such as thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, acute glomerulonephritis, pre-eclampsia and malignant hypertension may not only damage platelets, but also destroy

red blood cells at an accelerated rate (11, 16, 26, 31, 36, 37, 40, 47).

Some investigators feel that platelets participate in the formation or occlusion of atherosclerotic lesions (32). Possibly therapeutic drugs such as dipyridamole may interrupt the disease process or at least prevent the terminal occlusive event (3).

Altered platelet survival has been implicated in the thrombosis of arteriovenous shunts such as those used for chronic hemodialysis because of increased reactivity of the platelet with an artificial vascular surface (34, 35). Sulfinpyrazone has been shown to reduce the incidence of occlusion of dialysis shunts.

Traditionally, venous thrombosis has been thought to occur via activation of coagulation without primary involvement of platelets (32). For this reason the classical treatment of venous thrombosis has been anticoagulant drugs rather than "antiplatelet" drugs (50). Recently, some evidence has been put forth which implicates platelets in the initiation of venous thrombosis in a sub-set of patients with this disease (55, 64, 72). If so, some benefit might be derived from the use of drugs which inhibit platelet function. If platelets do have a role in the pathogenesis of venous thrombosis in some patients, a thorough evaluation of platelet reactivity and survival would be necessary (50).

Homocystinuria is another disease entity which has an associated vascular abnormality with thromboembolic complications. Harker studied platelet survival and fibrinogen consumption in four patients (31). They noted that platelet consumption was markedly increased and postulated that the formation of thrombi could be attributed to

interaction of the platelets with the abnormal endothelial surface induced by homocystinemia. In addition, they found that dipyridamole was effective in restoring shortened platelet survival to normal.

d. Intrinsic Platelet Abnormalities

Wiscott-Aldrich syndrome presents a number of symptoms, one of which is thrombocytopenia (64). Both megakaryocytes and platelets have altered structure and autologous platelets have decreased survival while homologous platelets have normal survival. May-Hegglin anomaly exhibits thrombocytopenia as one of its presenting symptoms but variable platelet survival for both autologous and homologous platelets has been reported (64).

Clearly, altered platelet survival is associated with a wide range of disorders. In some of these conditions, detection of decreased platelet life span is necessary to predict which patients or group of patients are at risk for thromboembolic disease. Potentially, a battery of tests including platelet survival may be used to look for platelet hyperfunction. The same battery of tests could be used to evaluate effectiveness of potential therapeutic agents in controlled clinical trials.

The University of Oregon Health Sciences Center is fortunate in having an active program of study and treatment for patients with valvular heart disease. Since prosthetic valvular dysfunction may result in accelerated platelet destruction but a normal peripheral platelet count, platelet survival was measured in a heterogenous group of patients with artificial cardiac valves. If this method can detect decreased platelet survival in patients with defective valve prosthesis the utility and

efficacy of the aspirin platelet survival technique will be confirmed. Moreover further studies in other groups of patients suspected of having decreased platelet survival will become feasible.

MATERIALS AND METHODS

A. REAGENTS

N-ethylmaleimide

N-ethylmaleimide (NEM), supplied by Eastman Kodak, Rochester, New York was prepared daily in a concentration of 100 mM by adding 1.25 g of NEM to 100 ml of 0.15 M phosphate buffered saline (PBS). A working solution of 1 mM concentration was prepared by diluting the stock solution 1/100 with PBS. The NEM powder was stored at 4° C in a desiccator and was considered to be stable for up to five months.

2-Thiobarbituric Acid

2-Thiobarbituric acid (2-TBA), supplied by J. T. Baker Co., Phillipsburg, New Jersey, was prepared in the following manner: 800 mg of 2-Thiobarbituric acid was dissolved in 10 ml of concentrated sodium hydroxide. Distilled water (50 ml) was added and concentrated perchloric acid was added as necessary to obtain a pH of 7.4. The solution was then diluted to 100 ml, and 50 ml of 7% perchloric acid was added. The reagent was stored at room temperature for a maximum of 2 weeks.

Anticoagulant

A balanced citrate solution (Ware's anticoagulant) was prepared by mixing 6 parts of 0.1 M sodium citrate and 4 parts of 0.1 M citric acid. One part anticoagulant was mixed with 9 parts of whole blood.

B. PROCEDURE

Informed consent was obtained from each patient. The risks and inconveniences of the procedure were explained fully so that the patient understood what was expected. Patients could withdraw from the study at any time. (Appendix A)

1. Obtaining the Blood Sample

For the determination of platelet malonaldehyde production, hematocrit and platelet count, 20 ml of blood were drawn from an anticubital vein, using plastic disposable syringes and disposable 20 gauge needles. Nine ml of blood was gently mixed with 1 ml of citrate anticoagulant in each of 2 polypropylene tubes. The remaining 2 ml of blood were added to a "Vacutainer" (Beckton-Dickinson) tube containing 7.5 mg of disodium edetate (EDTA) for peripheral platelet count and hematocrit.

2. Platelet Counts

Whole blood and platelet counts on platelet rich plasma (PRP) were performed using an electronic particle counter, (Coulter Model F_n) and the procedure described by Bull (12). Whole blood platelet counts were corrected for both coincidence passage and hematocrit. Platelet counts from PRP were corrected only for coincidence passage.

3. Measurement of Platelet Malonaldehyde Production

Lipid peroxidation of platelet membranes was quantitated by spectrophotometrically measuring the production of malonaldehyde following its reaction with 2-TBA. Malonaldehyde is a by-product of platelet lipid peroxidation (44, 45).

PRP was obtained by centrifuging fresh anticoagulated blood at 150 g for 15 minutes at room temperature. Centrifugation was carried

out at room temperature since centrifugation at 4° C gave decreased malonaldehyde values. Not more than 90 minutes elapsed between the collection of the blood specimen and the first centrifugation.

PRP (2 ml) was removed and transferred to glass tubes. After thorough mixing by gently inverting the tubes, an aliquot was removed and platelet counts performed. The PRP was then centrifuged at 600 g for 15 minutes to concentrate the platelets. Red cell contamination of the platelet button was not seen. The platelet poor plasma (PPP) was decanted and the tubes were inverted on filter paper and drained for 3 minutes. If insufficient PRP was obtained after the first centrifugation, a second centrifugation at 150 g for 15 minutes was performed and the PRP removed. If the volume of PRP still did not total 2 ml the solution was centrifuged at 600 g for 10 minutes to obtain platelet poor plasma which was used to dilute the PRP to a total volume of 2.0 ml.

NEM (2 ml of a 1 mM solution) was added to stimulate lipid peroxidation of the platelet membranes. The platelets were thoroughly resuspended in the NEM solution by repeatedly aspirating and expelling the solution through a Pasteur pipette. Additionally, the platelet solution was mixed for 20-30 seconds or until no clumps were visible using a Vortex mixer, (Lab-Line Instruments, Inc.). The platelet-NEM suspension was incubated at 37° C for 60 minutes with continuous gentle circular mixing.

Following incubation, 2 ml of 2-TBA was added to react with any malonaldehyde that was present. Again the suspension was mixed using the Vortex mixer.

After mixing, the solution was heated in a boiling water bath for 15 minutes to develop the pink color indicating a reaction of

malonaldehyde with 2-TBA. After centrifugation for 3 minutes at 600 g to remove the platelet precipitate, the optical density of the supernatant containing the pink chromagen was read at 532 nanometers in a spectrophotometer (Beckman DU, Fullerton, California).

Once the 2 ml aliquot of PRP was obtained, a specimen was processed continuously until after the addition of 2-TBA. A maximum of 30 minutes was allowed before the solutions were boiled and the optical density of the supernatants determined.

C. CALCULATIONS

Malonaldehyde produced following lipid peroxidation of the platelet membranes was calculated using the formula:

$$A = Ebc$$

A = absorptivity (O.D.)

E = molar absorptivity (1.37×10^5)

c = moles per liter

b = length of light path in cm; 10mm = 1cm

transposing: $c = \frac{A}{Eb}$

or

$$c = \frac{\text{O.D.}}{1.37 \times 10^5 \times 1} = \text{nm/ml of malonaldehyde}$$

example: Using O.D. = 0.190

$$c = \frac{.190}{1.37 \times 10^5} = 1.387 \text{ nm of malonaldehyde}$$

An adjustment for the platelet count of the PRP was made and the result was reported as nmoles of malonaldehyde/10^9 platelets.

To adjust for the platelet count, e.g.:

PRP count = 443,800/mm^3 x 2 ml = 8.8 x 10^8 = 0.9 x 10^9

1.387/0.9 x 10^9 = 1.541 nm of malonaldehyde/10^9 platelets

D. DETERMINATION OF PLATELET SURVIVAL CURVES

The quantity of malonaldehyde produced by lipid peroxidation of the platelet membranes was used to estimate platelet survival. The subjects were instructed to take neither aspirin nor aspirin-containing drugs 10 or more days before the study. An initial baseline value of malonaldehyde production was determined. Two aspirin tablets (600 mg) were administered and serial malonaldehyde determinations were performed daily for 10 days or until malonaldehyde production approximated the baseline value. Each subject's survival curve was plotted on linear and semilogarithmic paper by plotting percent of initial lipid peroxidation on the ordinate and time in hours on the abcissa.

Two normal subjects were studied in a unique manner. As described before, initial baseline values for malonaldehyde production were determined before aspirin ingestion. Instead of giving only the test dose of aspirin (600 mg), these subjects were instructed to take aspirin three times daily for three days. The test dose of aspirin was then administered in the same manner as with previous subjects and the 24 hour post aspirin malonaldehyde level was determined. Serial malonaldehyde values were obtained through the tenth day. (Aspirin intake was restricted following the test dose.) A survival curve was drawn and a T_{50} computed for each subject.

Additional malonaldehyde levels were determined on the 11th and 12th days following ingestion of the test dose of aspirin. The platelets labeled by the aspirin had been removed from the circulation so that these malonaldehyde levels were comparable to the initial malonaldehyde levels determined before aspirin ingestion. A second T_{50} was computed for each subject using the malonaldehyde levels obtained on days 11 and 12 as the baseline level. A comparison between the two T_{50}'s obtained for each subject showed virtually no difference. Thus the baseline value for malonaldehyde production could be determined 10 days after aspirin ingestion as well as before aspirin ingestion. This could prove useful if one wished to study a patient who routinely ingested aspirin. Instead of waiting 10 days to determine the initial level of malonaldehyde production, the test dose of aspirin could be administered and serial malonaldehyde determinations could be performed until a stable value was obtained. This then would be considered the baseline value to be used to compute the T_{50}.

Subjects were excluded if day to day variation of the peripheral platelet count was excessive (greater than 25%) since accurate survival curves could not be obtained.

In order to determine if the platelet survival curves best approximated an exponential or linear function, a computerized curve fitting program was utilized. The correlation coefficient of 'R' value for an exponential curve was 0.98 while the 'R' value for a linear curve was 0.92 when the group of 20 normal curves were analyzed. Thus, an exponential curve was chosen to analyze the raw data for the survival curve (39, 42).

A programmable calculator (Wang Instruments, Tewksbury, Mass.) was used to perform least squares analysis of the survival curves. The formula for the exponential curve was $Y = A^{eBx}$. (A and B are constants and Y is the time in days. By substituting 50 for X and using the given value for A and B, Y or the T_{50} in days was obtained for each subject.

Other statistical methods such as standard deviation and the Student "t" test were performed using the Wang programmable calculator and standard statistical methods (17).

E. SELECTION OF THE NORMAL SUBJECTS

Normal subjects including medical students and laboratory personnel were utilized to obtain normal ranges of platelet survival. Included in this group were 17 males and 3 females with ages ranging from 22 to 39 years. The fact that there were more males than females should not affect the results since it has been shown that no significant difference in platelet survival exists between the sexes (56). All but 3 subjects remained healthy during the study. These subjects contracted upper respiratory infections. One took erythromycin on day 9 of the study. Non-aspirin analgesics were allowed.

F. SELECTION OF BALL VALVE PATIENTS

Fourteen patients with prosthetic heart valves were studied in the same manner as the normal subjects. This group included patients with variable prosthetic valve function and single as well as multiple valves. Of the 14 subjects, 3 were outpatients. The remaining 11 were patients from either the Portland Veterans Administration Hospital or the University of Oregon Health Sciences Center. Eight females

and six males ranging in age from 45-72 years were studied. Six of the patients had multiple valve replacements while eight had single valve replacements (Table 1).

The patients were receiving a variety of drugs during the study (Table 2 and 3). All but one patient was taking warfarin. None of the patients had taken aspirin for two or more weeks. In fact, most denied taking aspirin at any time after receiving their prosthesis. Seven of the hospitalized patients had cardiac catheterization within one year of the study to evaluate valvular function. Three of the five were found to have abnormal prosthetic function and subsequently underwent surgery to replace the defective valve. Bilirubin and lactic acid dehydrogenase (LDH) were obtained in 12 patients. A reticulocyte count was available in 5 of the 12. These tests were performed in the clinical laboratory by standard methods.

G. SELECTION OF DRUG CONTROL PATIENTS

Three patients who were receiving a variety of medications but who did not have cardiac valve replacement were studied to see if these drugs interferred with the aspirin method for determining platelet survival.

The effect of dipyridamole was studied in 2 patients. One subject was taking a combination of aspirin and dipyridamole but discontinued his aspirin 2 weeks prior to the study. A second patient was taking dipyridamole as well as diazapam, corticosteroids, digoxin, potassium chloride supplement and terbutaline. Neither had prosthetic heart valves.

A third patient was taking multiple drugs during the study which included digoxin, potassium chloride supplement, hydrochlorothiazide and insulin.

RESULTS

A. NORMALS

Twenty volunteers were studied to establish a normal range for peripheral platelet counts, platelet survival and malonaldehyde production before and after aspirin ingestion.

The mean daily platelet counts for the entire group varied between 213,000/mm^3 - 232,000/mm^3 (Figure 1). A variation greater than that of the group as a whole was seen in some individuals. The minimum variation above and below the mean 10 day platelet count was 8% and the maximum was 25%. The greatest variation was found in 3 individuals who contracted an upper respiratory infection during the study. If the peripheral platelet count varied excessively (more than 25%) the data was excluded from analysis. The daily variation in the peripheral platelet count of one normal individual is shown (Figure 2). The mean platelet count varied from 174,000 - 217,000/mm^3 (i.e., 11.5%).

Mean baseline platelet malonaldehyde production in normals after stimulation with NEM was 1.672 nm of malonaldehyde/10^9 platelets. Using the Student "t" test the reduction in malonaldehyde production from the initial level of malonaldehyde to the 24 hour post aspirin ingestion is highly significant ($p < .001$).

As serial lipid peroxidation studies were performed, malonaldehyde production gradually rose until the pre-aspirin value determined 10 days earlier was approached.

Each assay for malonaldehyde was performed in duplicate. The sample was divided at the time the blood was obtained. Duplicate readings agreed to within 10% and an average of the 2 values was used in the calculations. A normal individual's daily malonaldehyde production after aspirin is shown in figure 3 and table 4.

Mean daily platelet production of malonaldehyde from 20 normal subjects before and after aspirin ingestion is shown in figure 4 and table 5. Variation about the mean is greater during days 6-10 than during days 1-5.

Analysis using a curve fitting program showed that the data best fit an exponential curve (correlation coefficient 0.98). Figures 5 and 6 show the computer-assisted least squares derived curve and a semi-logarithmic plot of raw data respectively for the normal group.

The mean derived T_{50} (time in days for 50% production of malonaldehyde) for the entire group of 20 normals was 4.0 $\pm$ 0.7 (1 S.D.) days with a range of 3.0 to 5.6 days. A T_{50} less than 3.0 days was considered to be abnormal.

In order to learn if the time and number of blood samples needed to perform this test could be reduced, a separate analysis was made using only the data from the first five days. Good correlation (with 2 exceptions) between the T_{50} derived from the five day data and from the 10 day data was found (Table 6 and Figure 7). The mean T_{50} for the five day curve was 4.3 $\pm$ 1.1 (1 S.D.) days as compared to a T_{50} for a full curve of

4.0 $\pm$ 0.7 (1 S.D.) days. No significant difference was found ($p > 0.2$). (Two patients were excluded who produced less malonaldehyde on day 5 than on the previous day, giving excessively long T_{50} values.)

Since many people habitually take aspirin, 2 normal subjects were studied by drawing a pre-aspirin baseline sample, giving 600 mg of aspirin three times daily for three days and then administering the test dose of aspirin and carrying out the survival curve. (Use of aspirin was restricted following the ingestion of the test dose.) After malonaldehyde production became stable (on days 10, 11, and 12) an average of these values were substituted for the baseline measurement, curves plotted and the T_{50} calculated (Figure 8).

B. BALL VALVE PATIENTS

The patients with artificial cardiac valves could be divided into two groups based on cardiac catheterization, clinical and laboratory data. Group A included 9 patients with presumably normal artificial valve function (Table 7). Four of the patients in this group had cardiac catheterizations performed within a year of hospitalization. Results showed normal prosthetic function. Indirect bilirubin was normal in 6 out of 7 patients. LDH was less than 800 units in each case (8/8). The 5 patients who did not undergo catheterization were doing well clinically with no evidence of valve dysfunction.

The second group of patients, Group B, had evidence of valve malfunction (Table 8). Included in this group were 5 patients, three of whom had cardiac catheterization results indicating the need for valve replacement. Subsequently, all three went to surgery and received new valves. On patient refused catheterization but other parameters

including markedly elevated LDH (1661), bilirubin (4.2/1.4) and reticulocyte count (2.4%) supported the clinical impression of valve dysfunction.

Although all but one initial peripheral platelet count was within the normal range, a distinction between the 2 groups of valve patients could be made on the basis of the mean peripheral platelet count. The means of the peripheral platelet counts for the 10 day study period for Group B (valve dysfunction) were significantly lower than the group of normals ($p < .001$) (Table 9). In contrast, the mean of the daily peripheral platelet counts for Group A or Group A plus Group B were not significantly different from the group of normals.

Analysis and comparison of initial malonaldehyde production of Group A valves, Group B valves and all valves with the normal group shows no statistically significant difference. Malonaldehyde production 24 hours post aspirin ingestion did differ for Group B (valve dysfunction) and was significantly higher when compared to the normal ($p < .001$). No significant difference was detectable between Group A or groups A & B.

The most useful parameter to differentiate between Group A and Group B proved to be the T_{50}. A significant difference was found between the mean T_{50} of the Group A and the normals. The mean T_{50} of Group A valves was significantly lower than the normals ($p < .01$). However a highly significant difference was found between the mean T_{50} for Group B and the normals ($p < .001$) and between the overall mean for all the valves and the normal group ($p < .001$) (Table 9). Curves obtained by least squares analysis for Group A, Group B and all valves are shown in figure 9.

Evaluation of individual results (Table 10) shows good correlation between decreased platelet survival and suspected valve dysfunction. Three of the five patients who required valve replacements had platelet survival times of 2.5, 2.6 and 2.8 days, respectively. The remaining two patients who were not catheterized but who had clinically suspected valve dysfunction had a T_{50} of 2.8 and 2.6 days, respectively.

One other patient had a platelet survival of less than 3 days. The patient with subacute bacterial endocarditis had a T_{50} of 2.9 days although valve dysfunction was not suspected clinically. Clinical findings in this patient included a septic joint, anemia and thrombocytosis.

The T_{50} calculated using data from days 1-5 was compared in Group A, Group B and all valves. No significant difference was found between the T_{50} obtained from a full curve (10 days) or the T_{50} obtained from a curve using 5 days (Table 11).

C. DRUG CONTROLS

Comparison of the mean (10 day) peripheral platelet counts for the 3 drug controls and the normals shows that the platelet count for drug controls was significantly lower than the normal group (Table 12).

No statistically significant difference was found when comparing the groups of drugs with the normals for malonaldehyde production, either initially or 24 hours post aspirin ingestion. Further, the T_{50} obtained from either a five day curve or a 10 day curve was not significantly different (Table 12).

DISCUSSION

The main objective of this study has been to evaluate a new method of platelet survival utilizing aspirin as a label for determination. Currently the method of choice being used to determine platelet life-span is ^{51}Cr. Although ^{51}Cr is felt to be accurate, the expense and time required to perform an assay have prevented it from becoming a more useful clinical procedure. In comparison, the aspirin labeling technique uses relatively inexpensive equipment and reagents and involves a minimum amount of technologist time. A single malonaldehyde determination can be performed in 3 hours. Moreover, actual working time required is less than one hour since a majority of the three hours involves incubation and centrifugation. In addition, multiple samples (e.g. 6-8) can be run concurrently. Conversely ^{51}Cr, requires at least 4-6 hours to phlebotomize one patient and prepare the platelets for reinfusion. Multiple blood samples must then be drawn at 30 minutes, 2 hours, 4 hours and 8 hours to determine the amount of radioactivity present in the peripheral blood. Both ^{51}Cr assay and the aspirin technique require daily venipunctures for approximately 10 days. A total of 100 ml of blood are needed for the serial 10 day malonaldehyde determination unless a 5 day curve is used (then 50 ml of blood is required).

The use of the aspirin labeling technique presents a minimum amount of risk to the patient compared with the ^{51}Cr assay.

No exposure to a source of radioactivity is required. Potentially, ingestion of aspirin could involve dangers such as an allergic response to acetylsalicylic acid, bleeding or gastritis, although the incidence of these are minimal with a single aspirin dose of 600 mg. Conceivably, the aspirin-labeling technique for determination of platelet survival could be performed on a patient who could not withstand the stress of removal of a unit of blood and reinfusion of radioactively labeled cells required for a ^{51}Cr study.

The aspirin-labeling technique, however, has potential drawbacks. Platelet replacement rather than platelet survival is being measured. One ingestion of 600 mg of aspirin labels all the platelets present in the peripheral circulation and the label persists until the platelets are removed from the peripheral circulation by the reticuloendothelial system. As the labeled platelets are removed, new platelets are released from the bone marrow. The rate of platelet replacement is indicated by the gradual rise in malonaldehyde production until the initial value is again approximated. For this reason the peripheral platelet count must be relatively stable throughout the study indicating that the rate of production equals the rate of destruction. Therefore, the existence of a steady state is necessary for the method to be accurate. Rapid changes in the peripheral platelet count may lead to error in the estimation of platelet survival.

A second potential source of error is the possibility that aspirin itself could lengthen decreased platelet survival. The effect of aspirin on platelet function can be measured *in vitro*. Theoretically, aspirin could interfere with utilization of platelet *in vivo* allowing the

biochemically labeled platelets to persist for longer than usual, leading to prolongation of a shortened survival curve. This effect is not likely to be great in view of the data presented by Harker showing that aspirin did not lengthen shortened survival in heart valve patients unless it was given in combination with dipyridamole (30). A second study showed that aspirin did not lengthen platelet survival in patients with mitral and aortic prosthesis (60). Moreover, even if aspirin does lengthen platelet survival results indicating shortened platelet survival would be of even greater significance.

The mechanism that leads to platelet loss from the circulation has not been elucidated. It is not known whether platelet loss is the result of an age-dependent process (senescence) or a process independent of age (randomness). Senescent destruction should give a linear survival curve, while random destruction would result in an exponential curve. Some investigators feel a curvilinear plot should be constructed since cell death might be attributed to a combination of randomness and senescence (39, 42). For practical purposes an exponential curve is used to determine cell survival. In addition, the T_{50} is usually used to represent the length of cell survival (39). In this study, it was found that the curves closely resemble an exponential function ($R = 0.98$). Therefore it was decided to use an exponential function and to compute the T_{50} for cell survival determination.

The results of this and other studies of platelet survival in normal indicate excellent agreement between the ^{51}Cr technique and the aspirin labeling technique. The normal T_{50} range established by this study using the aspirin method was 3.0 to 5.6 days with a mean of 4.0 days. Stuart et al, reported a normal T_{50} range of 2.9 to 5.9 days with a mean of

4.4 days using the aspirin method (59). Stuart also performed ^{51}Cr platelet survival assays and determined the normal T_{50} range to be 3.2 to 5.5 days.

A variation of the original method which has potential utility is the determination of the "initial" malonaldehyde level on days 10-12 after aspirin ingestion. Two normal subjects were studied in this manner. No differences in platelet survivals calculated either from the curve determined by the pre-aspirin malonaldehyde baseline or from the curve determined by the 10-12 day post-aspirin malonaldehyde baseline were noted in either subject. Theoretically, then, patients who had ingested aspirin can still be studied without a 2 week delay.

Recipients of artificial cardiac valves were chosen as a study group for several reasons. Although platelet survival in this group has not been previously studied by the lipid peroxidation technique a variety of test results would be expected. Potentially, the method could also provide useful information regarding the functional status of the values.

The results indicate decreased mean survival for the entire group of valve patients. However the group with poor valve function had markedly shortened survival. One additional patient also had decreased platelet survival.

The ninth patient in Group A had received aortic and mitral valve replacements in 1967. He was admitted to the University Hospital with a septic right glenohumeral joint following a cortisone injection. Subacute bacterial endocarditis was suspected but could not be substantiated by culture. The patient did not have any clinical evidence of prosthetic dysfunction or overt heart failure. Bilirubin was very

slightly above normal (1.4/0.4) and the LDH was below 800 (483). The only positive finding in this patient was a septic joint with secondary anemia (Hct 30.5%) and thrombocytosis (peripheral platelet counts ranged from 425,000 to 500,000/cu mm). The decreased platelet survival, 2.9 days, can probably be attributed to the presence of an acute infection.

The mechanism responsible for decreased platelet survival in patients with artificial cardiac valves has not been elucidated. Actual physical destruction of the platelets by the ball valves may occur. Alternatively, the platelets may be activated by the presence of a foreign surface and consumed in the vascular system. A third possibility is that the platelet membrane may be slightly damaged leading to premature removal of the platelet from the peripheral circulation by the reticuloendothelial system.

Various groups of cardiac patients could benefit from the information provided by platelet survival determinations. For example, some patients develop emboli while taking anticoagulants such as warfarin. If these patients had shortened platelet survival and valve replacement were risky, then antiplatelet therapy could be of benefit. In addition, if the technique of platelet survival were more readily available the effect of antiplatelet drugs could be monitored by determining platelet survival both before and after administration of a therapeutic agent. This information has not been available without a great amount of time, expense and discomfort to the patient.

The test may be able to predict prosthetic heart valve dysfunction due to clot, apron tears, or ball variance. Another predictive function might be to determine platelet survival in patients pre-operatively to

estimate the likelihood of the occurrence of thromboemboli. Patients with shortened survival before surgery might prove to be at a higher risk for developing emboli post-operatively.

It has been shown in this study that the method is useful even when various drugs are being taken by the subject. Dipyridamole does not interfere with the method. However, other antiplatelet drugs such as sulfinpyrazone and clofibrate need to be evaluated.

The aspirin labeling technique is significant because it is a relatively simple but accurate method which compares well with ^{51}Cr. It has been successful in identifying valve dysfunction and may prove potentially useful in identifying other individual patients and groups of patients with abnormal platelet function. Such potential groups include patients with hyperlipidemia, saphenous vein bypass grafts, compensated ITP, congenital platelet abnormalities, homocysteinuria, and patients with prosthetic devices other than ball valves such as arterio-venous shunts and artificial blood vessels.

SUMMARY

This study was designed to evaluate a relatively new technique for detecting platelet survival. A normal range was established which agrees closely with the accepted ^{51}Cr method used to evaluate platelet survival.

The aspirin labeling technique was then used to evaluate patients with artificial cardiac valves to see if the method was sensitive enough to detect altered platelet survival. A good correlation between patients with valve dysfunction and shortened survival was obtained.

In addition, 3 drug controls were studied. Preliminary results show that none of the drugs affected the use of the aspirin method to detect survival. However, further evaluation of therapeutic agents is needed.

APPENDIX A

CONSENT FORM

I,_____herewith
(Last Name) (Middle Initial) (Last Name)

agree to participate in an investigation entitled "Aspirin Platelet Survival" under the supervision of Dr. Scott H. Goodnight. This investigation is aimed at evaluating a new method for determining platelet life span without the need of radioisotopes. Platelets are blood cells which take part in the blood clotting mechanism.

The procedures to which I will be subjected are as follows:

1. A little more than 1 tablespoon of blood (18 ml) will be drawn from a vein. Two aspirin tablets (600 mgs) will be given and a little more than 1 tablespoon of blood will be taken each day thereafter for 5 to 10 days.

2. I realize that I cannot take aspirin or aspirin containing compounds such as Anacin, Empirin, Contact or Midol during the course of the study without invalidating the investigation. However, Tylenol or Datril can be taken.

The risks for me are as follows:

1. There is an extraordinarily small risk that there may be a reaction to the aspirin. This however, would be very unusual.

2. I may lose up to 200 ml of blood during the course of the study. This amounts to less than ½ of the amount usually donated to the Blood Bank.

Although I probably will receive no direct benefits from this procedure, the information may lead to the adoption of a safer test of platelet survival.

Dr. Scott H. Goodnight or other members of his staff have offered to answer any questions about these procedures. I understand that I am free to refuse to participate or withdraw from participation in this study at any time and it will in no way affect my relationship with the University of Oregon Health Sciences Center or Veterans Administration Hospital. I have read the foregoing information.

_____ _____
Date and Time Subject Signature

_____ _____
Date and Time Witness Signature

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TABLE 1. SINGLE VALVE REPLACEMENTS

Patient By Number	Site of Valve Replacement	Year of Valve Replacement
1	Mitral	1967
2	Aortic	1969
3	Mitral	1969
4	Mitral	1970
5	Aortic	1971
6	Mitral	1972
7	Aortic	1975
8	Mitral	1976
	MULTIPLE VALVE REPLACEMENTS	
9	Aortic & Mitral	1967
10	Mitral & Tricuspid	1970
11	Mitral & Tricuspid	1970
12	Mitral & Aortic	1971
13	Mitral & Aortic	1975
14	Mitral & Aortic	1975

TABLE 2.

DRUGS RECEIVED DURING THE PLATELET SURVIVAL STUDY BY PATIENTS WITH SINGLE VALVE REPLACEMENTS (Patient Number Correspondes With Table 1.)

Number of Patient	Drugs
1	Digoxin, furosimide, potassium chloride supplement, warfarin, zyloprim
2	Amitriptyline HCL, digoxin, tolbutamine, warfarin
3	Acetominophen, digoxin, ferrous sulfate, furosimide, warfarin
4	Digoxin, hydrochlorothiazide, potassium supplement, warfarin
5	Acetominophen, digoxin, hydrochlorothiazide, potassium supplement
6	Digoxin, conjugated estrogens, warfarin
7	Digoxin, warfarin
8	Digoxin, hydrochlorothiazide, potassium supplement, warfarin

TABLE 3.

DRUGS RECEIVED DURING THE PLATELET SURVIVAL STUDY BY PATIENTS WITH MULTIPLE VALVE REPLACEMENT (Patient Number Corresponds With Table 1.)

Number of Patient	Drugs
9	Alpha methyl dopa, digoxin, gentamicin sulfate, hydrochlorothiazide, meperidine, morphine, sodium nafcillin, potassium supplement, zyloprim, warfarin
10	Furosimide, potassium chloride, warfarin
11	Digoxin, warfarin
12	Warfarin
13	Digoxin, furosimide, hydrochlorothiazide, lidocaine, potassium suppliment, procaine amide, quinidine sulfate, warfarin
14	Ferrous sulfate, furosimide, potassium chloride supplement, warfarin

TABLE 4.

DAILY MALONALDEHYDE PRODUCTION FOR 1 NORMAL SUBJECT

Time (Days)	NM of Malonaldehyde/ 10^9 Platelets		Percent of Initial Lipid Peroxidation	
	Tube #1	Tube #2	Tube #1	Tube #2
1	2.190	2.190	100	100
2	.638	.638	29.13	29.13
3	.738	.770	33.70	35.16
6	1.460	1.460	66.67	66.67
7	1.569	1.569	71.64	71.64
8	1.643	1.643	75.02	75.02
9	1.779	1.779	81.23	81.23
10	1.743	1.743	80.00	80.00

TABLE 5.

MALONALDEHYDE VALUES FOR COMPOSITE CURVE NORMALS

Time (Hours)	Mean Amount of Malonaldehyde/ 10^9 Plt. ± 1 S.D.	Mean % of Initial Lipid Peroxidation ± 1 S.D.
0	1.672 ± .498	100
24	0.396 ± .128	24.33 ± 6.15
48	0.568 ± .159	33.90 ± 7.49
72	0.706 ± .200	46.92 ± 10.54
96	0.955 ± .311	57.97 ± 11.62
120	1.064 ± .278	77.43 ± 9.96
144	1.167 ± .249	79.14 ± 16.66
168	1.314 ± .363	80.50 ± 12.40
192	1.482 ± .446	89.97 ± 15.12
216	1.537 ± .417	89.76 ± 13.96

TABLE 6.

COMPARISON OF T_{50} USING DATA FROM DAYS 1-10 AND FROM DAYS 1-5 POST ASPIRIN

Subject	T_{50} Days 1-10	T_{50} Days 1-5
1	3.03	3.05
2	3.15	3.22
3	3.30	3.25
4	3.34	3.48
5	3.38	3.44
6	3.45	3.73
7	3.56	3.44
8	3.70	4.69
9	3.75	4.93
10	3.82	4.20
11	3.85	3.74
12	3.98	3.93
13	4.16	(9.90)*
14	4.33	5.53
15	4.33	5.70
16	4.39	4.88
17	4.61	4.35
18	4.63	5.61
19	5.08	(8.92)*
20	5.55	6.75
Mean	4.0 $\pm$ 0.7 days	4.3 $\pm$ 1.1 days

$p > 0.2$ (N.S.)

*Values in parentheses were excluded from analysis (see text).

TABLE 7. GROUP A: LABORATORY AND CARDIAC CATHETERIZATION RESULTS

Patient	Cath. Results	LDH(I.U.) Normal <225	Hct. (%) Normal >38%	Ind. Bili.(mg%) Normal <0.9	Retic. Ct.(%) Normal 1-2%	Init. Plt. Ct. (/mm^3) Normal 150,000-350,000
1	Normal prosthetic function (1976)	502	45	0.8	ND*	196,000
2	ND	393	48	0.4	ND	182,000
3	ND	649	30	0.8	ND	230,000
4	ND	ND	40	ND	ND	215,000
5	Normal prosthetic function (1976)	792	40	0.8	1.6	194,000
7	Normal prosthetic function (1975)	525	50	0.7	ND	205,000
9	ND	483	30.5	ND	ND	470,000
13	ND	531	31.6	0.7	2.4	200,000
14	Normal prosthetic function (1975	586	47	1.9	ND	172,000
AVERAGE		558	40.2	0.9	2.0	229,000

*Not Done

TABLE 8. GROUP B: LABORATORY AND CARDIAC CATHETERIZATION RESULTS

Patient	Cath. Results	LDH(I.U.) Normal <225	Hct. (%) Normal >38%	Ind. Bili.(mg%) Normal <0.9	Retic. Ct.(%) Normal 1-2%	Init. Plt. Ct. ($/mm^3$) Normal 150,000-350,000
6	Cath. indicated need for valve replacement	744	37	0.5	1.0	178,000
8	Cath. indicated need for valve replacement	748	35	4.8	0.9	240,000
10	ND*	1371	41	ND	1.3	132,000
11	Cath. indicated need for valve replacement	494	38	ND	0.6	161,000
12	Patient refused	1661	40	2.7	2.8	180,000
AVERAGE		1003.6	38.2	2.7	1.3	178,000

*Not Done

TABLE 9. PLATELET COUNTS, MALONALDEHYDE PRODUCTION & T_{50} IN NORMAL PATIENTS AND THOSE WITH CARDIAC VALVES

	All Cardiac Valves	Group A	Group B	Normal Subjects
Average Platelet Count (10 days) Range Mean ± 1 S.D.	207-232,000/mm^3 215,000 ± 12.3 NS	195-249,000/mm^3 221,000 ± 28.6 NS	156-199,000/mm^3 182,000 ± 16.2 *$p < .001$	213-232,000/mm^3 221,000 ± 5.5
Mean ± 1 S.D. Initial Malonaldehyde level (nmole/10^9 plts)	1.763 ± .212 NS	1.804 ± .196 NS	1.688 ± .230 NS	1.672 ± .498
Malonaldehyde level 24 hr post aspirin ingestion nmoles/10^9 plts.	0.501 ± .188 NS	.428 ± .157 NS	.621 ± .190 $p < .001$	0.396 ± .128
T_{50} Days Range Mean ± 1 S.D.	2.5 - 3.9 3.1 ± 0.4 $p < .001$	2.9 - 3.9 3.3 ± 0.3 $p < .01$	2.5 - 2.8 2.7 ± 0.1 $p < .001$	3.0 - 5.6 4.0 ± 0.7

*Values refer to a comparison of the normal mean with the mean for each group.
NS = Not Significant

TABLE 10.

COMPARISON OF CARDIAC CATHETERIZATION RESULTS AND T_{50} FOR ALL CARDIAC VALVE PATIENTS

Patient	Cath Results	T_{50} (Days 1-10)
1	Normal prosthetic function (1976)	3.87
2	ND*	3.42
3	ND	3.25
4	ND	3.75
5	Normal prosthetic function (1976)	3.05
6	Cath indicated need for valve replacement	2.54
7	Normal prosthetic function (1976)	3.10
8	Cath indicated need for valve replacement	2.84
9	ND	2.86
10	ND	2.63
11	Cath indicated need for valve replacement	2.62
12	Patient refused (valve dysfunction suspected)	2.78
13	ND	3.46
14	ND	3.06

*Not Done

TABLE 11.

COMPARISON OF THE T_{50} OBTAINED FROM DAYS 1-10 WITH THE T_{50} OBTAINED FROM DAYS 1-5

GROUP A, GROUP B, GROUP A & B

Group A	T_{50} Days 1-10	T_{50} Days 1-5	p value-comparison of mean of each group NS = Not Significant
1	3.87	4.98	
2	3.42	3.44	
3	3.25	3.43	
4	3.75	3.94	
5	3.05	3.06	
7	3.10	3.60	
9	2.86	2.82	
13	3.46	3.46	
14	3.06	3.22	
Mean ± 1 S.D.	3.31±.34	3.55±.63	NS
Group B			
6	2.54	2.54	
8	2.84	2.63	
10	2.63	2.78	
11	2.62	2.62	
12	2.78	2.97	
Mean ± 1 S.D.	2.68±.12	2.71±.17	NS
A & B			
Mean ± 1 S.D.	3.09±.42	3.25±.65	NS

TABLE 12.

PLATELET COUNTS, MALONALDEHYDE PRODUCTION & T_{50} IN NORMAL SUBJECTS & DRUG CONTROLS

	Drug Controls	Normal Subjects
Average Platelet Count (10 days)		
Range	175-233,000/mm^3	213-232,000/mm^3
Mean ± 1 S.D.	190,000 ± 21.8	221,000 ± 5.5
	$p < .001$	
Mean ± 1 S.D. Initial Malonaldehyde Level (nmoles/10^9 plt)	1.550 ± .287	1.672 ± .498
	NS*	
Malonaldehyde level 24 hr post aspirin ingestion (nmoles/10^9 plt)	0.397 ± .100	0.396 ± .128
	NS	
T_{50} Days 1-10		
Range	3.3 - 4.0	3.0 - 5.6
Mean ± 1 S.D.	3.6 ± .4	4.0 ± 0.7
	NS	
T_{50} Days 1-5		
Range	3.3 - 3.9	
Mean ± 1 S.D.	3.6 ± 0.4	
	NS	

*NS = Not Significant

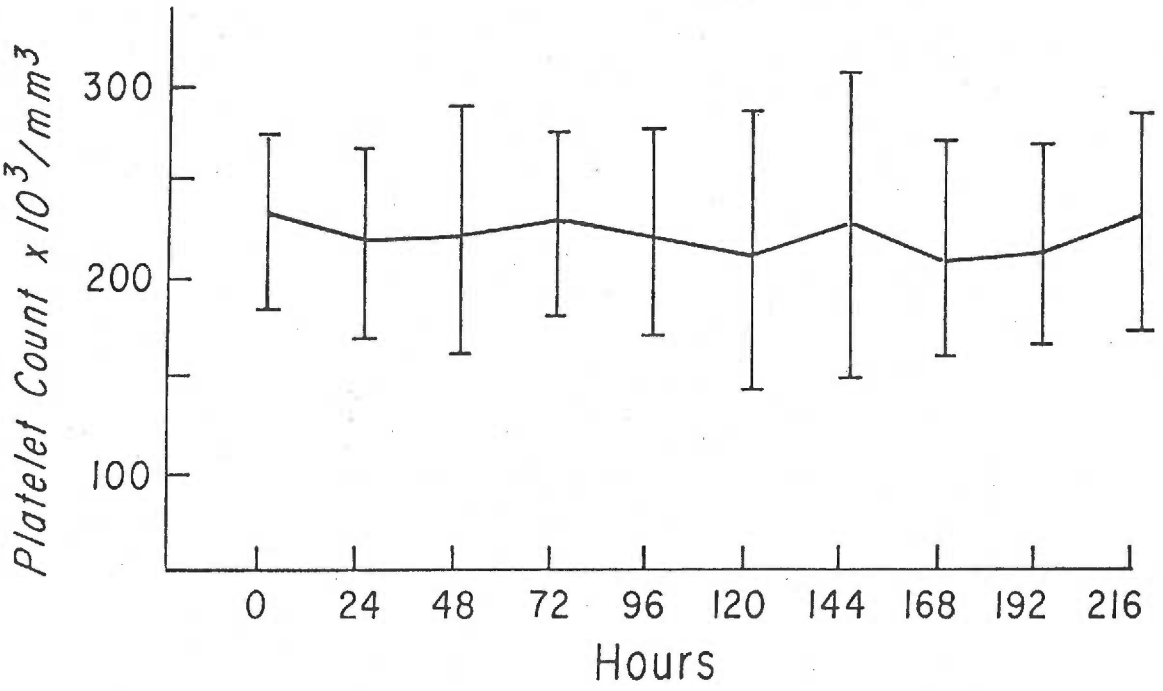


FIGURE 1. Mean Daily Platelet Counts (± 1 S.D.) for 20 Normal Subjects

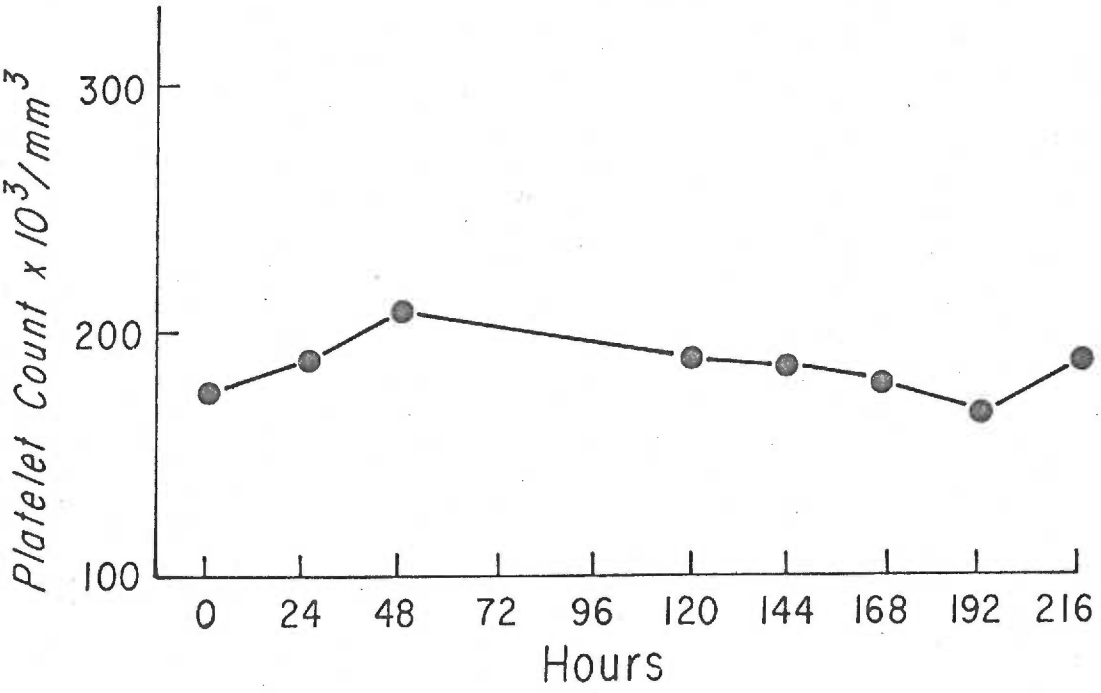


FIGURE 2. Daily Peripheral Platelet Count for 1 Normal Subject

FIGURE 3. Malonaldehyde Production (% Pre-Aspirin Level) for 1 Normal Subject

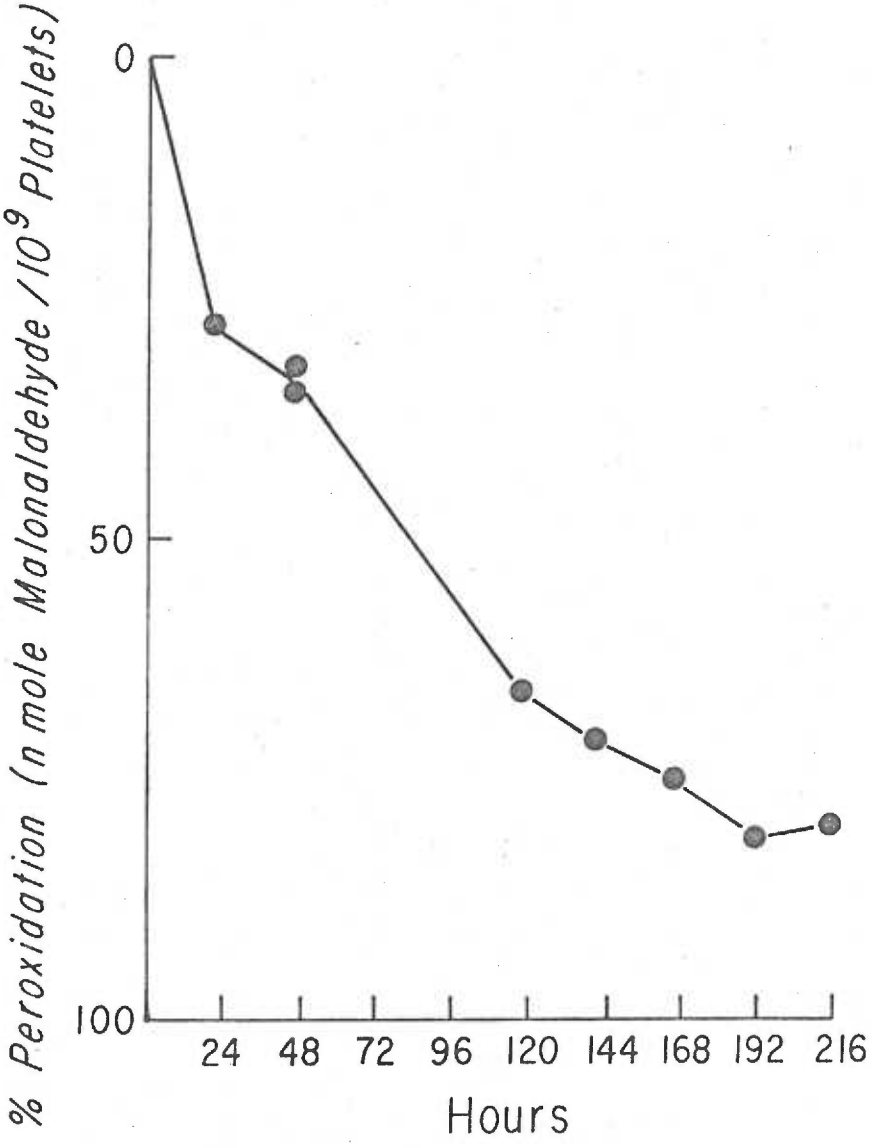

% Peroxidation (n mole Malonaldehyde /10⁹ Platelets)
0
50
100
24
48
72
96
120
144
168
192
216
Hours

FIGURE 4. Malonaldehyde Production (% Pre-Aspirin Level) for 20 Normal Subjects

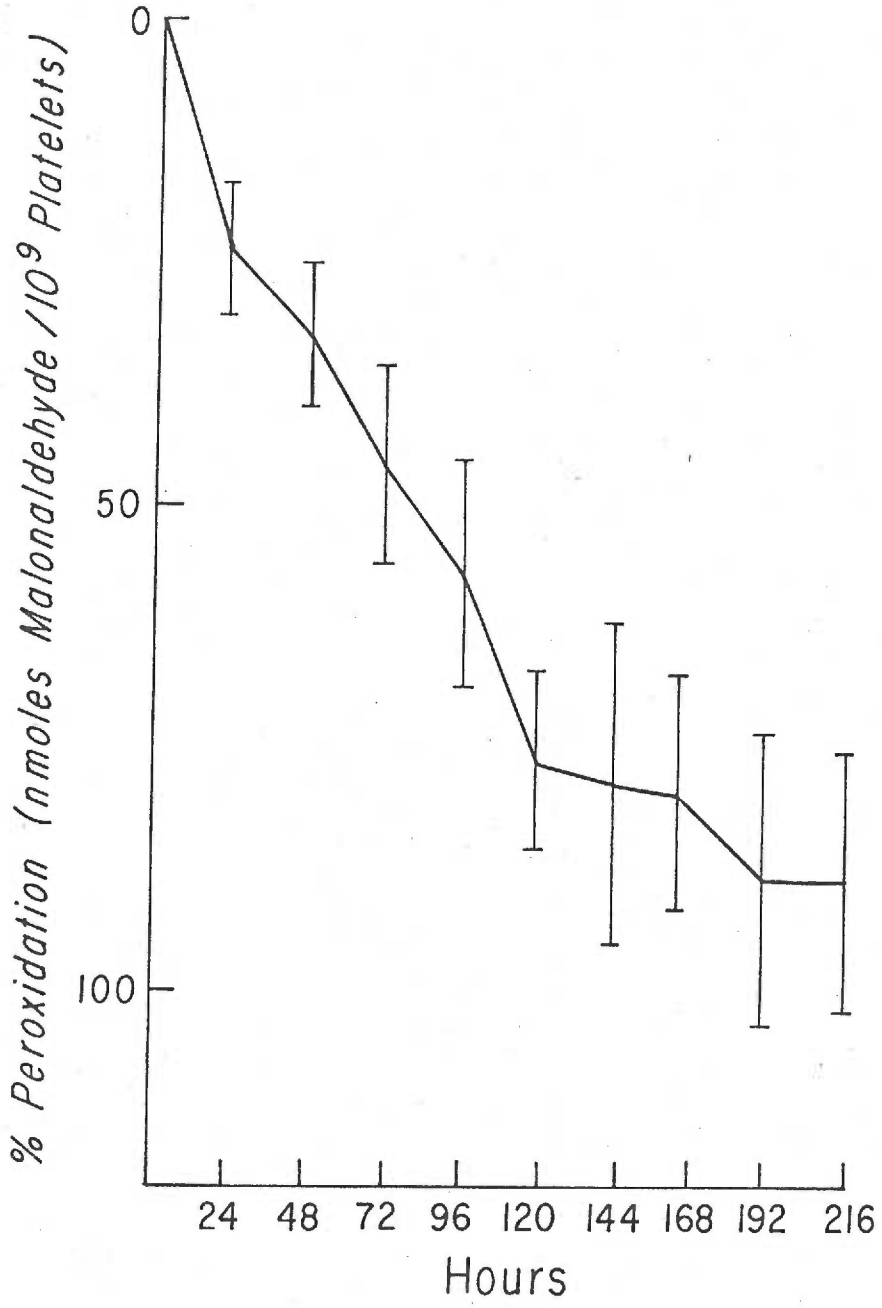

% Peroxidation (nmoles Malonaldehyde / 10^9 Platelets)
0
50
100
24
48
72
96
120
144
168
192
216
Hours

FIGURE 5. Mean Malonaldehyde Production (% Pre-Aspirin Level) for 20 Normal Subjects. Least square analysis was used to obtain the exponential curve

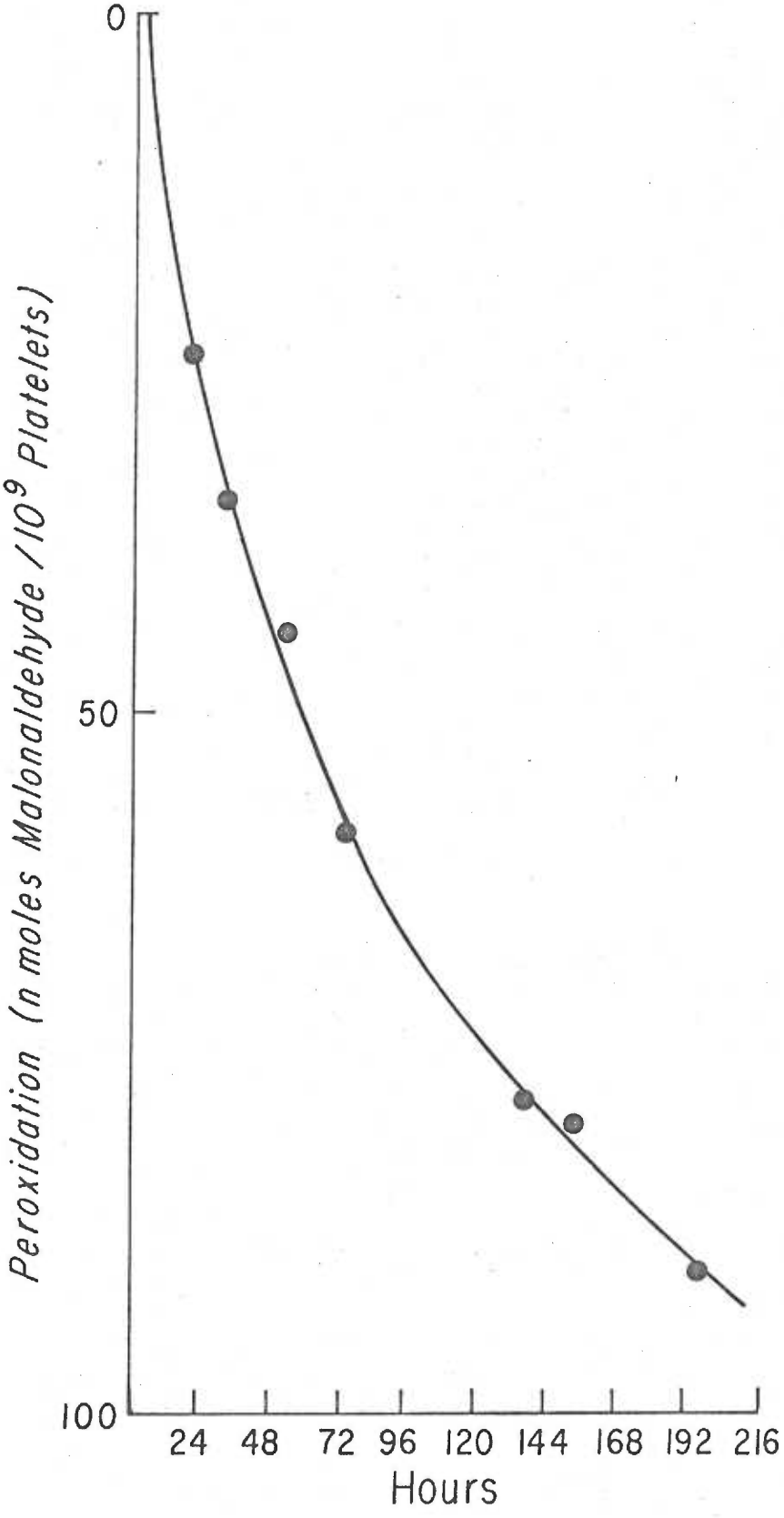

0
50
100
Peroxidation (n moles Malonaldehyde /10⁹ Platelets)
24 48 72 96 120 144 168 192 216
Hours

FIGURE 6. Malonaldehyde Production (% Pre-Aspirin Level) for 20 Normal Subjects. Semilogarithmic Plot

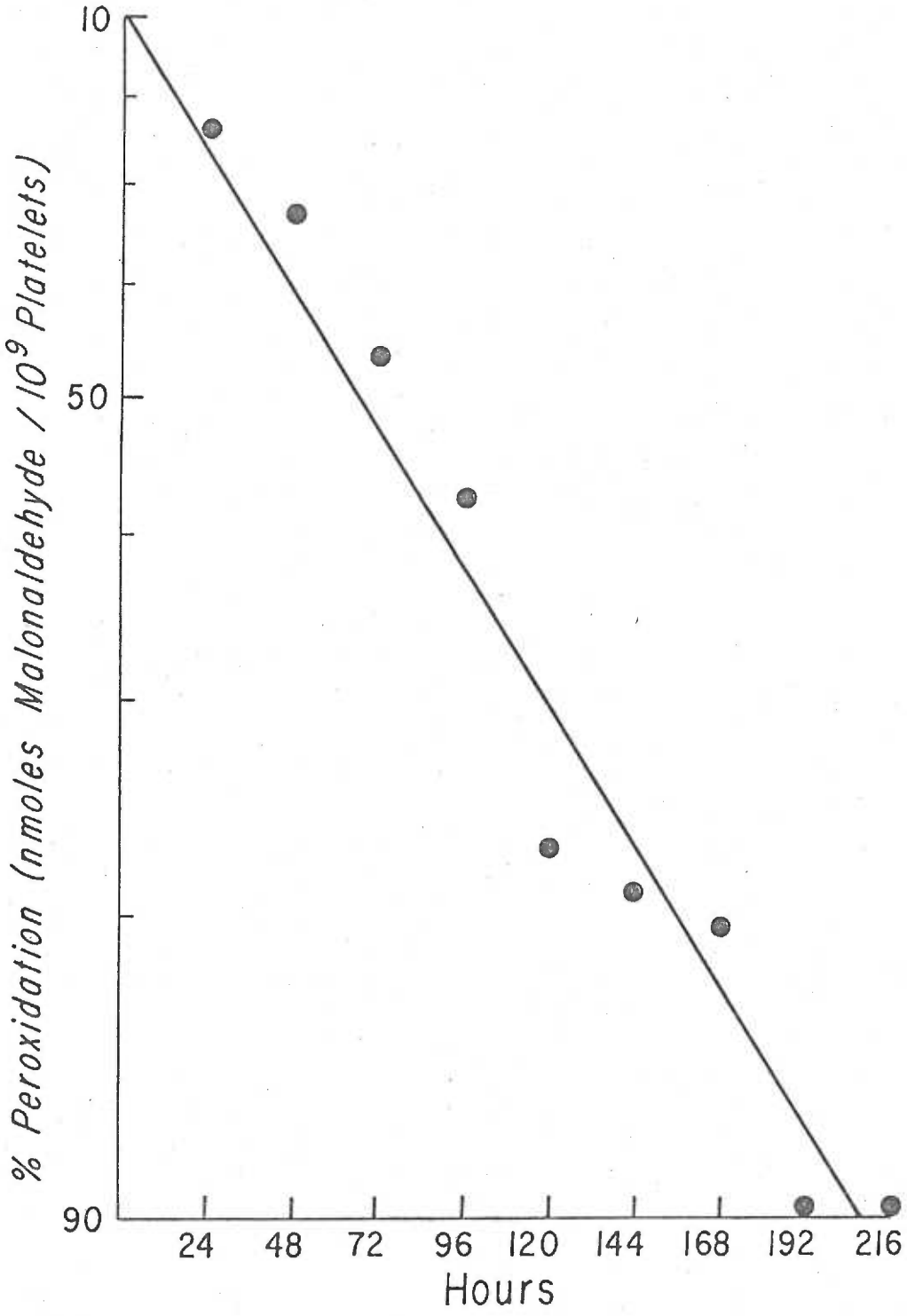

10
50
90
% Peroxidation (nmoles Malonaldehyde / 10^9 Platelets)
24
48
72
96
120
144
168
192
216
Hours

FIGURE 7. Comparison of T_{50} Obtained from Days 1-5 and T_{50} Obtained from Days 1-10

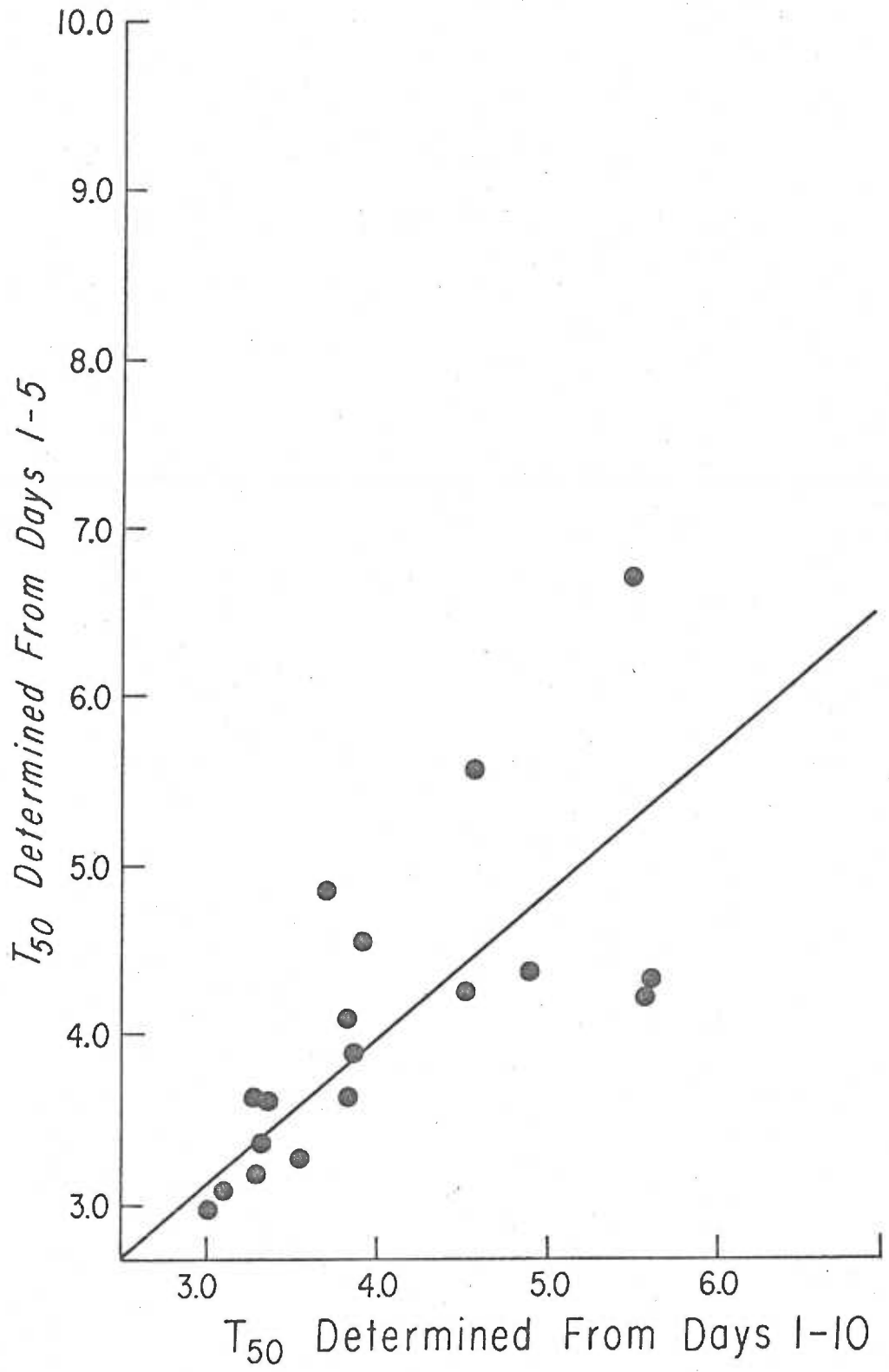

10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
T_{50} Determined From Days 1-5
3.0
4.0
5.0
6.0
T_{50} Determined From Days 1-10

FIGURE 8. Malonaldehyde Production (% Pre-Aspirin Value) for 2 Subjects with Baseline Production Determined Before (○,△) and 10-12 Days after (●,▲) Aspirin Ingestion. Least Squares Analysis

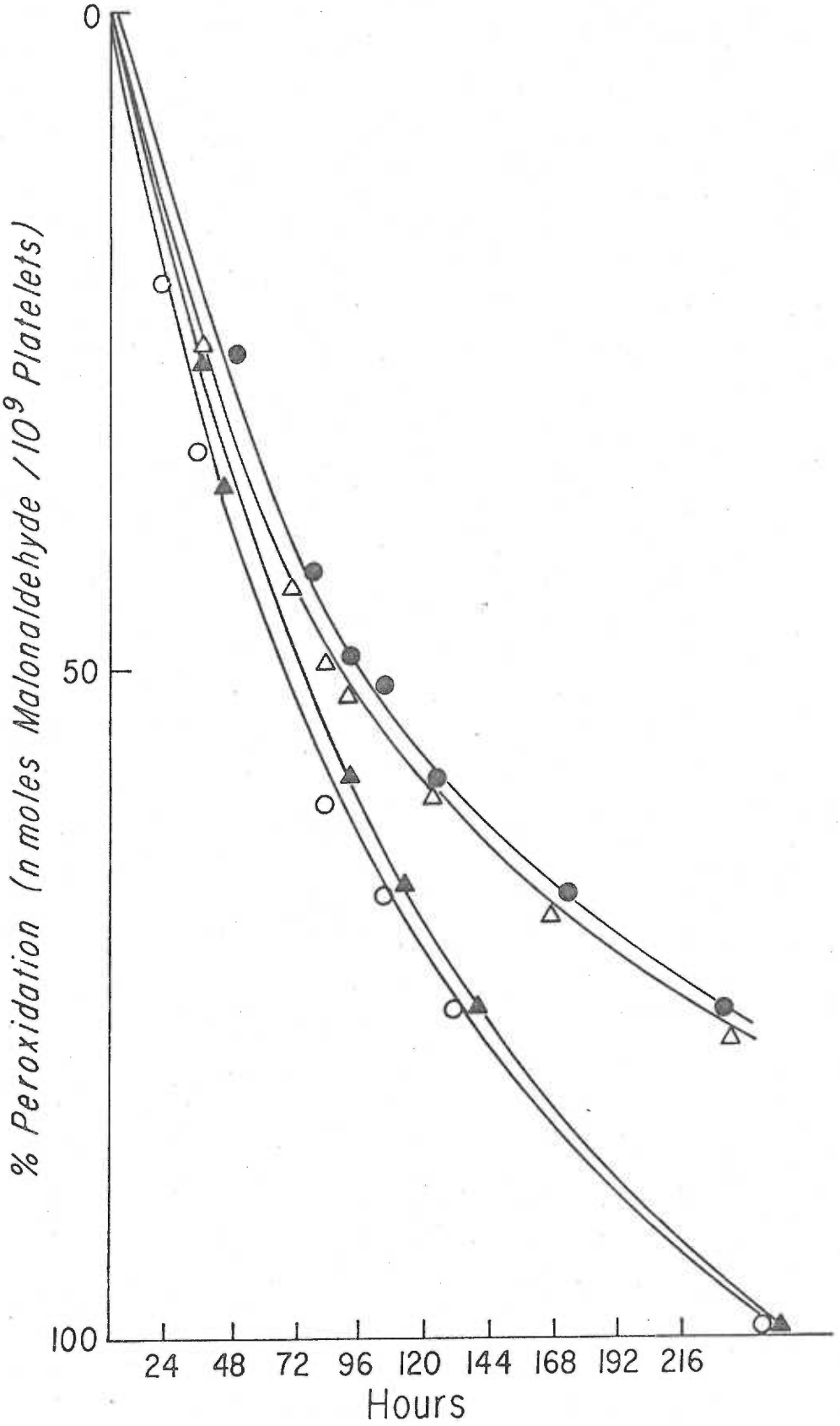
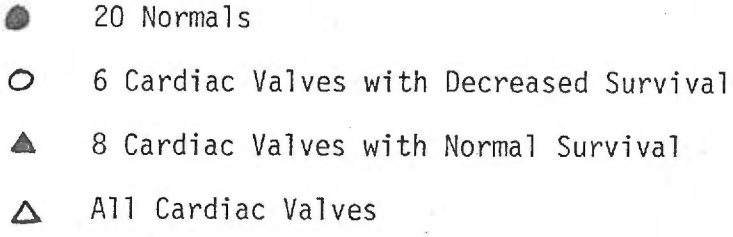
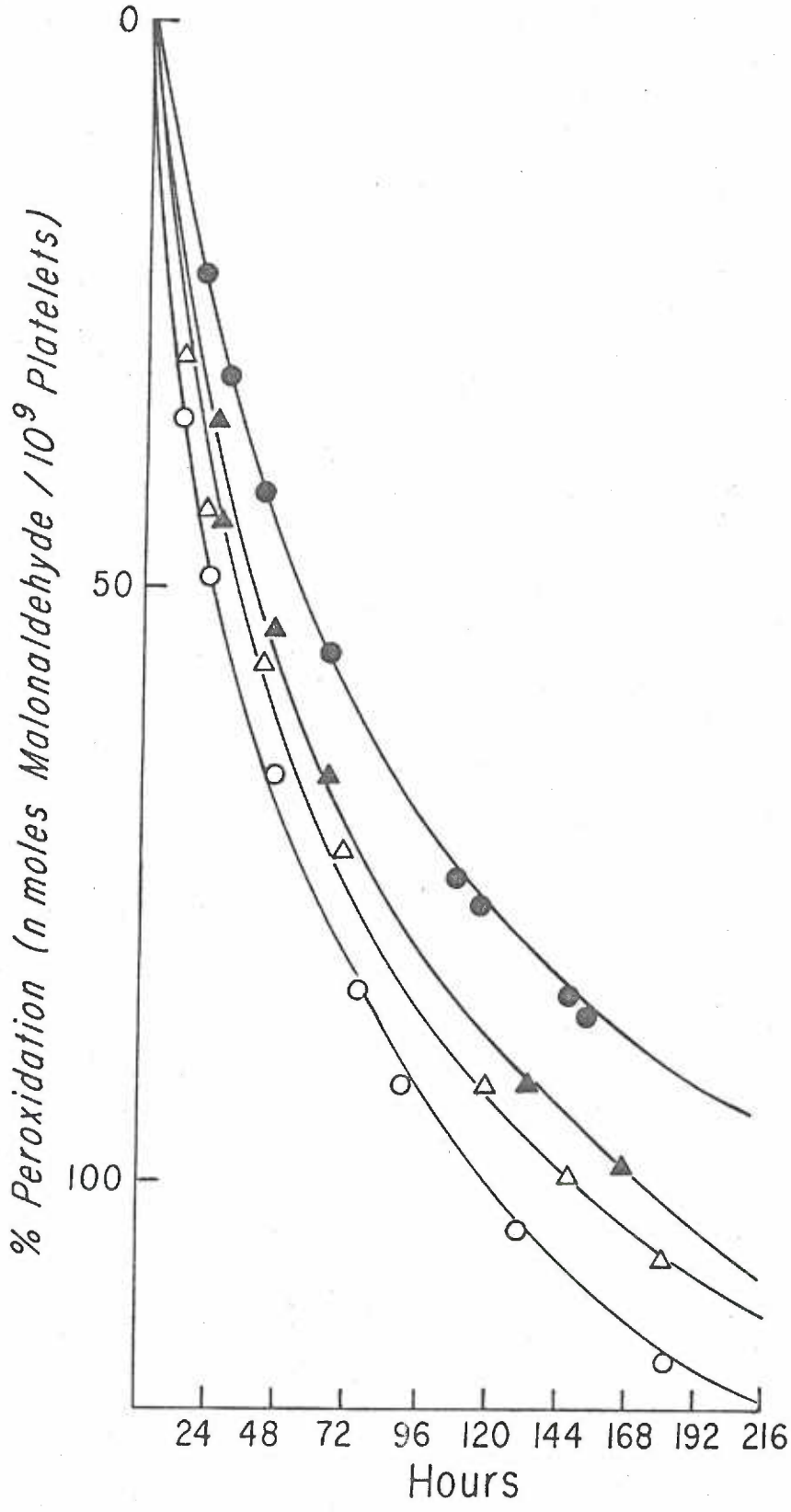

0
50
100
% Peroxidation (n moles Malonaldehyde / 10^9 Platelets)
24
48
72
96
120
144
168
192
216
Hours

FIGURE 9. Least Squares Analysis Comparing the Group of Normals, All Cardiac Valves, Cardiac Valves with Decreased Survival and Cardiac Valves with Normal Survival




0
50
100
% Peroxidation (n moles Malonaldehyde / 10^9 Platelets)
24 48 72 96 120 144 168 192 216
Hours