

Platelet Thrombosis in Cardiac-valve Prostheses

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## SUMMARY

The contribution of platelets and clotting factors in thrombosis on cardiovascular prostheses had been quantified with several tracers. Thrombus formation in vivo could be measured semiquantitatively in animal models and patients with indium-111, Technetium-99m labeled platelets, iodine-123, iodine-131 labeled fibrinogen, and In-111 and Tc-99m labeled antibody to the fibrinogen-receptor on the platelet-membrane, or fibrin. The early studies demonstrated that certain platelet-inhibitors, e.g. sulfinpyrazone, aspirin or aspirin-persantine increased platelet survival time with mechanical valves implanted in the baboon model and patients. Thrombus localization by imaging is possible for large thrombus on thrombogenic surface of prosthesis in the acute phase. The majority of thrombus was found in the sewing ring (Dacron) in the acute phase in both the mechanical and tissue valves. The amount of retained thrombus in both mechanical and tissue valves in our one-day study in the dog model was similar (< 1% of injected In-111 platelets = 5 billion platelets). As the fibrous ingrowth covered the sewing ring, the thrombus formation decreased significantly. Only a small amount of thrombus was found on the leaflets at one month in both the dog and calf models. Small size of the adherent platelet-thrombus could not be imaged in patients due to the high background radioactivity present in blood-pool. This technique has been applied for imaging the site of thrombus formation in bacterial endocarditis of mechanical and tissue valve prostheses in patients without success. The dynamics of the process and the absolute amount of thrombus formed depend on the size of the annulus and the velocity of blood flow. In addition, in vitro quantification permits platelet-, fibrin-density, and determination of the number of fibrin-monomer/platelet in the sewing ring or leaflet-adherent thrombus. The role of low-dose aspirin, aspirin-persantine and diphosphonate on platelet-thrombosis has been evaluated in mechanical and pericardial tissue-valves implanted in dogs, calves, baboon and patients. These

studies indicate that although these drugs reduce platelet deposition in thrombus, they are not very effective in complete platelet-inhibition. These tracer techniques thus provided invaluable information about platelet-fibrin deposition, their organization, dissolution and development of less thrombogenic surface for use in cardiovascular prostheses. In addition, we observed that platelet-thrombus calcifies; the reduction of platelet-thrombosis indirectly decreases the kinetics of calcification of tissue-valves. We also observed encouraging results of spontaneous endothelial cell coverage of pericardial tissue-valve in the calf model with the techniques of detergent processing and diphosphonate immobilization.

The multiple factors responsible for complications resulting from the use of cardiovascular prostheses are described below:

**A. Platelet-thrombosis in cardiovascular prostheses, the role of platelet-scintigraphy and quantitation of regional platelet-density**

The recognition of the various factors controlling normal hemostasis and the role of platelets and clotting factors in thromboembolic diseases, post-implantation of cardiovascular prosthesis and the development of new imaging techniques have stimulated investigations in the evaluation of thrombus formation, embolization, and the evaluation of various prophylactic and therapeutic interventions (1-20). In vitro studies of platelet adhesion to biomaterials of catheters (polyurethane, silicone, polyvinyl chloride), synthetic vascular grafts (Dacron,teflon), arterio-venous shunts, conduits, mechanical/tissue valves, ventricular assist devices and artificial hearts (mechanical valves and polyurethane diaphragms) involve the use of light or electron microscopy and/or Cr-51 or In-111 labeled platelets (21-38). Chromium-51 appears to be a marker of platelet activation and In-111 appears to be a marker of platelet consumption. The neutral complexes of In-111, i.e. In-111 oxine or In-111 tropolone or Tc-99m HMPAO penetrate cell membrane due to high lipid solubility. These tracers after intracellular dissociation bind mainly to

soluble proteins, organelles, and membrane proteins (16, 17, 20, 26, 32). Although platelets had been labeled with Technetium-99m, the radionuclide washes off continuously from the platelets after administration, the radioactivity in the kidneys and bladder makes image interpretation difficult in the abdomen. Recently, lipid soluble 99mTc-complex ( $^{99m}\text{Tc}$ -hexamethylpropyleneamine oxime) had been used for granulocyte and platelet labeling. We also developed high-efficiency platelet-labeling technique via a lipid-soluble Sn(II)-MPO complex. The Tc-99m labeled platelets may be useful for repeated measurements of platelet thrombosis and surface passivation of a variety of prostheses.

Platelet survival study representing global platelet consumption is not a sensitive technique for the measurement of the thrombogenicity of cardiovascular prostheses, whereas imaging study allows estimation of regional platelet consumption. The present techniques of diagnoses of adherent-thrombosis or vegetations of cardiac valves and valvular prostheses involve echocardiography (1-13).

Imaging thrombus was possible in the case of large thrombus in the acute phase for annulus with deep injury or thrombogenic surface of prostheses of sewing ring. In the chronic phase, the deposition of the labeled platelet is relatively small with respect to the circulating radioactivity in the blood; and thrombus imaging is not possible; although in vitro technique is suitable for regional quantitation of the thrombus on the components of valve-prostheses. These studies also permitted the evaluation of platelet-inhibitors, aspirin, persantine, motrin and calcification-inhibitors. Decrease (minimal to moderate) in platelet deposition on prosthetic surface due to medical intervention had been observed by noninvasive imaging with In-111 platelets in animal models and patients. The nature of the platelet deposition depends on thrombogenicity of the surface, the amount of blood flow, the diameter and configuration of the prosthesis, and the time-interval after prosthesis implantation. The amount of platelet deposition

also changes with time post-implantation. At least in two types of prostheses, we had the opportunity of quantifying platelet thrombosis in the acute and chronic phases. For valvular prosthesis, similar techniques could be used for quantification of total number of adherent platelet deposition over a period of several hours. In addition, the effects of other medications were evaluated in the tissue valve and mechanical valve implanted in calves, dogs and patients.

#### A.(i). Mechanism of thrombosis on cardiovascular prosthesis

The exposure of blood to artificial surfaces leads to a complex series of interrelated reactions resulting in the activation of platelets, white cells, the blood coagulation/fibrinolytic system and the complement system. These reactions are followed by progressive passivation of the surfaces by the adsorption/desorption of adhesive proteins (fibrinogen, fibronectin and von Willebrand factor) and other plasma proteins. The platelets and coagulation system are activated by conditions of flow (trapping of platelet-releasate and aggregate in disturbed flow or vortex), accelerated platelet transport to the surface, intensification and prolongation of platelet-surface interaction, properties of surface (hydrophilicity, area of surface exposed to blood and low C-H on polymer surface increase platelet consumption), fluid shear-induced platelet interaction, pressure gradient across the prosthesis and orifice diameter. Excessive activation of platelets and coagulation system leads to regional thrombus formation, thromboembolic complications, consumption coagulopathy and local or generalized bleeding (exhausted platelet-granules and procoagulants). Figure 1 summarizes the nature of complications of the two types of valvular prostheses.

It has been suggested that in platelet adhesion, activation and thrombus formation on the prosthetic surface, several platelet membrane glycoproteins (Ia, Ib, IIb, IIIa, IV and V) participate along with adhesive proteins e.g. fibrinogen, fibronectin, von Willebrand (vWd) and thrombospondin; both the vWd factor and

fibronectin appear to function as a glue between circulating platelets and leaflet-collagen. The nature of the binding of vWd factor and fibronectin to platelet glycoproteins might be dependent on the shear rates, the type of surfaces, and the concentration of these adhesive proteins present at the site of interaction. In valve-prostheses, platelets interact with the injured collagen and other extracellular proteins at the annulus and biomaterials of pyrolytic carbon, suture materials and stainless steel of housing or cross-linked fibers of collagen in tissue valves. With labeled platelets and proteins, we were able to identify the molecular and cellular composition of adherent thrombus as the platelet/fibrin ratio on prosthetic surface of grafts and valves implanted in calves and dogs at different sites; they appear similar for adherent thrombus in the acute and chronic phases. In-111 platelets provide a direct technique for quantification of regional platelet thrombus formation; in addition it can provide indirect information about the process of embolization. The embolization is usually indicated by the increase in the tissue/blood radioactivity ratio in the distal tissue bed (Tables 2-5). In general, it has been demonstrated that if the amount of thrombus formation is higher, the sequential events of embolization from prosthesis and occlusion also should be higher. Thrombus formation decreases with time post-implantation as the surface of the prostheses undergoes organizational changes leading to passivation.

#### A.(ii). Scintigraphy with Indium-111 Labeled Platelets

The half-life of 2.8 days, physical decay by electron capture process and high photon abundance of Indium-111 radionuclide (90 percent of 171 keV and 96 percent of 245 keV) make this radionuclide ideal for noninvasive imaging. The gamma camera head used for scintigraphic studies consist of a disk of thallium-doped sodium iodide crystal (25-54 cm in diameter, 6.5-12.7 mm in thickness) fitted with several photomultiplier tubes. The thinner disc (6.4mm) in the portable gamma camera can be fitted with a specially designed collimator for bedside studies, although the

detection efficiency is lower. In general, for most of the scintigraphic studies a large field of view gamma camera with a 12.7 mm thick crystal fitted with a medium energy parallel hole collimator is used. The spectrometer windows (20% of photopeak energy) are adjusted to include the 171 and 245 keV peaks. Gamma rays of 320- and 140-keV are used for in vitro radioactivity measurements of Cr-51 and Tc-99m radionuclides with a gamma counter. In spite of limited spatial resolution, the validation of in vivo distribution studies in animal models, the use of test objects with computerized gamma cameras, and in vitro measurements of isolated organs and components with gamma counters provide acceptable quantitative information of In-111 platelet distribution by scintigraphy. Since only a small percentage of labeled platelets participates in thrombosis on the wall of the injured vessel or prostheses, and platelet survival time is relatively long, a high blood-background radioactivity is always present in the early phase. Ideal imaging time is usually two to five days post-injection of In-111 platelets.

The estimated radiation dose for 500 microcuries of In-111 platelet administration is 1.13 rad to liver, 0.70 rad to lung, 16.75 rad to spleen, 1.16 rad to kidneys, 0.27 rad to red marrow, 0.17 rad to ovaries, 0.07 rad to testes, and 0.30 rad to the whole body. Due to high radiation dose to the spleen, most of the clinical studies with In-111 labeled platelets were performed with 500 microcuries requiring longer imaging times (5-15 minutes per view of approximately 100,000 counts). For most of the platelet deposition studies on injured vessel wall and cardiovascular prostheses, the peak time of In-111 platelet deposition had been obtained in experimental and clinical studies. The time course of platelet deposition depends on the diameter of the vessel lumen, texture of surface, blood flow, shear rate, types of biomaterials used in prostheses, presence of drugs, and platelet reactivity.

A.(iii). Limitations of thrombus imaging on cardiovascular prostheses with In-111 labeled platelets

The site of platelet thrombus formation could be imaged only for reasonably large thrombus in valvular prostheses and vascular grafts. An imaging technique is successful most of the time in the acute phase post-injury or after prostheses implantation. Noninvasive imaging without background subtraction is not a very sensitive technique for detection of platelet deposition in patients. Six patients with bacterial endocarditis were scanned for the evaluation of platelet deposition without success. Three of these patients were treated with antibiotics. Platelet deposition on the sewing ring of mechanical and tissue valve prostheses could not be imaged in several patients at one to five years post-valve prostheses implantation; at this period the sewing ring is covered with fibroblasts and small amount of the platelet-thrombus forms on the disc or the leaflets.

B.(i). Quantitation of platelet thrombosis in valvular prostheses in animal models.

A variety of mechanical and tissue prosthetic valves are used in patients with diseases of cardiac valves (1-13). The major limitation of the former is propensity of thrombus formation, embolization and that of the latter is calcification. The nature of collagen fiber arrangement in the pericardium and porcine valve are different (34). Patients undergoing valve replacement with tissue-valve benefit from improved performance and have higher quality of life for an extended period of time. The hemodynamic performance is superior to that of mechanical valves, except in small sizes; hemolysis is low. It occurs from perivalvular leaks or stenosis. The failures result from perivalvular leaks, thromboembolic complications, stenosis, infective endocarditis or collagen degeneration. The overall failure rate in adult patients resulting in reoperation or death is 3-5% per year (Table 1) and 12-20% in pediatric patients. The bovine pericardial valve-prostheses had higher failure than porcine valve counterparts. The cuspal calcification leading to stiffening or structural defects account for the clinical symptoms of stenosis or regurgitation or

both. Schoen et al(34) indicated that 79% of tissue-valve explants had cuspal defects, 67% of which had regurgitation through tears and 20% had stenosis. The nodular grey-white matter of calcific deposits are predominant at the cuspal commissures and basal attachments and originate at the attached thrombus, residual connective tissue cells and collagen.

The following sections will describe our experience of In-111 labeled platelets for noninvasive imaging and quantification of platelet deposition on valvular prostheses, conduits, and that of Zenger and DeVries (31) on artificial heart.

With In-111 labeled platelets, Dewanjee et al.(21-24) did systematic studies for the imaging of platelet deposition and quantitation of platelet thrombus on the components of valves in the acute phase on both the mechanical and tissue valve prostheses implanted in dogs and calves. These valves were implanted in the mitral annulus and platelet deposition was followed for a one-day to three month period. In addition, in vitro studies with gamma counter permitted quantitation of the number of adherent platelets on the components of the mitral valve prostheses; higher tissue to blood radioactivity ratio in skeletal muscle and kidneys was observed due to embolization. Figure 3B shows the dynamic process of platelet incorporation in the mitral annulus of bovine pericardial tissue valve prostheses in calves. The 24 hour platelet deposition showed a consistent decrease on all components of the tissue valve prostheses at 1, 14, 30 and 90 days post implantation. The 4 zones of leaflet for regional measurements of platelets, calcium and fibrinogen are shown in Figure 4A. These results of quantitative study of platelet density and deposition of total platelets, fibrin-fibrinogen, fibrin/platelet ratios are shown in Figures 5A, 5B, 6A, 6B respectively. Our studies also indicate that platelet vesicles provide a nucleation site for calcification in collagenous valvular prostheses and synthetic polymers (24).

The initial site of thrombus formation and embolization post-

tissue valve replacement is at the annulus of the host-valve interface. The most thrombogenic component is the sewing ring made of Dacron fibers. This provides the rationale for early temporary therapy with platelet-inhibitors or anti-coagulation therapy. Other complicating factors are platelet-activation during extracorporeal circulation with the oxygenator and arterial filter, atrial fibrillation, large atrium, low cardiac output and inadequate anticoagulation therapy. The tissue growth in the sewing ring confers thromboresistance, the late thrombosis occurs on the cusps of the valves. The age of the thrombus could only be determined by the labeled platelets. The atrial fibrillation and large size increase the risk of thrombotic complications in the mitral than aortic positions and manifest as acute neurologic deficits, acute ischemia of extremity infarction of abdominal viscera. The risks of thromboembolism (fatal, non-fatal or local thrombosis) for porcine valves without anticoagulation, are less than that for contemporary mechanical valves in anticoagulated patients. Comparing the clinical findings from several studies, Schoen et al. (34) demonstrated that freedom from thrombosis or thromboembolism at 9 years from porcine valves was 85% (aortic and mitral) compared with 83% (aortic) and 77% (mitral) with the tilting-disc valves. Majority of the patients had only mild and transient sequelae; a small fraction sustained permanent neurologic damage or death. The patients should be adequately medicated for prevention of ventricular thrombus and arrhythmia and tested for functioning of prosthetic valves by two-dimensional echocardiography. After two episodes of thromboembolism, the old valve should be replaced.

B. (ii). Effect of pyrolytic carbon-coating of Dacron-sewing ring on platelet-thrombosis of mechanical valves

The pyrolytic carbon prostheses (tilting disc) provide a non-adherent surface to platelets and plasma proteins. We tested the effect of pyrolytic-carbon coating of Dacron-fibers of the sewing ring on the thrombogenicity. In five dogs, the 25-mm Bjork-Shiley tilting disk valves were implanted, 24 hours post-injection of In-

111 labeled platelets. The In-radioactivity in the components of the valves was measured with the ionization chamber and gamma counter. The percent of injected radioactivity in the sewing ring was similar (0.5%) in the control and treated valve prostheses.

C(i). The effect of low-dose aspirin and aspirin-persantine on platelet thrombosis and calcification of pericardial tissue valves

Our experimental study also indicated that the platelet thrombus provides a nucleation site for calcification in the tissue valve. Treatment with aspirin-persantine reduces both platelet thrombosis and calcification (Figures 7A, 7B).

C.(ii). Platelet deposition on extracardiac porcine valved-conduits in dog model and clinical studies

We measured platelet-deposition in valved Dacron conduits in the dog model in the thoracic aorta and in young patients with pulmonary atresia. In the dog, these conduits were occluded at 24 hours post-implantation and 12-15% of injected In-111 radioactivity was found in the conduits; the majority of the radioactive thrombus was around the valve prosthesis.

The use of porcine valved extracardiac conduits (Dacron tube 10-20 cm long, 1.2-2.6 cm external diameter, with or without porcine valve) in several congenital cardiac lesions, e.g. pulmonary atresia, had permitted corrective surgery. We have measured platelet deposition on conduits (six with valves and three without) with In-111 labeled platelets in nine young patients (19). In this study, In-111 platelets (290-483 micro-curies) were administered either immediately or on the fifth to eighth post operative day and imaging was performed between one and six days. The highest platelet deposition on conduit was observed at three to four days post injection. Treatment with aspirin (75 to 325 milligrams) administered orally three times a day caused no recognizable changes. Three patterns of platelet deposition (no uptake, diffuse uptake and focal uptakes at anastomoses and valves) were observed.

C.(iii). Effect of aspirin-persantine and diphosphate on infection of tissue-valve prosthesis

Although we observed a reduction of platelet thrombus on the sewing and leaflets in the aspirin and diphosphonate-bonded pericardial tissue valves in the calf model, we also observed higher level of infection. The staining and transmission electron microscopic (TEM) studies of the thickened leaflets demonstrated the presence of pockets of bacterial colonies in all the leaflets. These studies suggest that suppression of inflammatory response, monocyte and granulocyte infiltration by aspirin and diphosphonate had probably affected wound-healing and facilitated infection of tissue valves. In two pericardial tissue valves implanted in calves, we observed pericardial leaflet thickening due to bacterial infection; all the three leaflets were infected. No infection was found in the 30 calves in the control group. The transmission electron micrograph of the infected leaflets are shown in Figure 8.

C.(iv). Identification of new collagen formation in valve-leaflet with I-125 labeled anti-collagen antibody

We wanted to test the hypothesis whether failure of pericardial tissue valve in young patients is due to residual antigenicity of fixed pericardium! Polyclonal antibody (IgG) against type I collagen was made in rabbits and purified by protein A affinity column. This IgG was labeled with I-125 iodide by the Iodogen-transfer method (32); 250 microcuries of I-125 IgG was administered to calves at 1, 14, 30 and 90 days post-valve implantation and sacrificed 4 hours post-injection. Each leaflet from the explanted valve was cut into 4 sections and the radioactivity of these sections were compared to that of a control aortic valve. The leaflets retained 5-10 times more radioactivity than the control aortic valves suggesting that the ingrowing fibroblast is layering new collagen on the pericardial collagen leaflets.

C.(v). Higher survival of Ionescu-Shiley tissue valve-implanted calves with aspirin-treatment

We observed reduced level of post-operative (open-heart surgery) mortality in the dogs and calves in the aspirin-treated groups than the control groups. This suggests that the nonsteroidal anti-inflammatory drugs and diphosphonate may affect the nerve cells in the myocardium and reduce arrhythmia-induced death in the treated groups; further studies are necessary to explore this interesting lead.

C.(vi). Spontaneous endothelial cell coverage of tissue-valve with detergent treatment and immobilization with amino-diphosphonate

The problem of thrombosis and calcification of tissue-valve could be solved, if collagenous matrix of porcine valve or pericardium could be modified in such a way that endothelial cells spontaneously cover the leaflets as in natural valve-cusp. We have developed a new technique of mixed-detergent treatment to remove cell-debris from pericardium and reduce antigenicity (38). In addition, we immobilized aminodiphosphonate (Henkel Corporation, Dusseldorf, West Germany) to pericardial collagen by Schiff-base reaction (Figure 9A). We believe the diphosphonate immobilization reduces the foreign body response further. The invading PMN granulocyte and monocyte may kill the ingrowing endothelial cells without this protection. This hospitable host matrix of smooth ADP-treated collagen-fiber promoted endothelial-cell like coverage of 20-30% of this modified tissue-valves in calves(Figure 9B). On the other hand, if fibroblast covers the glutaraldehyde-fixed collagen-surface, the new collagen fibers laid down by the regenerating fibroblast will provide a thrombogenic surface. The thrombus in turn will calcify, specially in young patients, repeating the vicious cycle of thrombosis and calcification.

D. Thrombosis and calcification of teflon and polyurethane valves

We measured platelet-thrombus in plastic valve-prostheses of teflon-, and polyurethane leaflets in the calf model. The explanted valve prostheses at 1, 14, 30 and 90 days were monitored for adherent thrombus by In-111 platelets and calcium by atomic absorption spectrometry. The thrombus formation, embolization as

measured by renal infarct, kidney radioactivity and calcification were slightly higher than the tri-leaflet pericardial tissue valve prostheses. The calcification of trilaminar teflon-leaflets resulted in separation of lamina at the free edge of the leaflets at 30-90 days.

#### E. Platelet thrombosis in total artificial heart (TAH)

The Jarvik-7 TAH (Symbion) consists of two chambers of four-layered polyurethane diaphragms and four Medtronic-Hall mechanical valves (Medtronic Inc., Minneapolis) of titanium-housed pyrolytic carbon disks. Zenger and DeVries (31) studied two TAH-implanted patients with In-111 labeled platelets; they used 210 microcuries at 21 post-operative day and imaged the patient at 6 and 68 hours. In the second patient, 395 microcuries of In-111 labeled platelets were injected at 110 post-operative days; in spite of repeated episodes of thrombotic complications in these patients, they could not visualize the TAH-induced thrombus and embolus in the scintiphotos. Higher amount of In-111, heterologous platelets and repeated imaging may have permitted the thrombus-visualization in the TAH prostheses. The exhausted platelets incapable of hemostatic capacity, were responsible for the repeated episodes of hemorrhage. In general, due to low incorporation of platelets in embolus, they were always difficult to detect, if labeled platelets were injected after prosthesis-induced embolization.

#### F. Type of oxygenator/arterial filter used during extracorporeal circulation on post-surgical thromboembolic and hemorrhagic complications

During extracorporeal circulation (ECC) with oxygenator (bubble, membrane and hollow-fiber) and arterial filter (Dacron screen, porosity > 20-25 micrometers) and artificial pumps (roller or centrifugal), platelets are activated and consumed in thrombus formation and embolization, resulting in post-surgical complications of hemorrhage requiring platelet-transfusion (12,25). With In-111 labeled autologous platelets administered 24 hours before ECC, we measured platelet platelet-trapped in the three

types of oxygenators and arterial filters in the dog and pig models. We observed that 20-25%, 10-12% and 1-4% of platelets are consumed in the bubble (Bentley Inc., polyurethane; Sci-Med. Inc., silicone and Bentley Inc., polypropylene hollow fiber) oxygenators. Heparin-bonding to the hollow-fiber and arterial filter reduced platelet to 1-2% in the oxygenators and 0.2-0.8% in the arterial filters. The microemboli trapped in the lungs accounted 8-10% of total radioactivity; this was two times higher than normal values in control unoperated pigs. The infusion of prostacyclin or its analog (Iloprost) along with systemic low-dose heparin also reduced platelet activation/exhaustion and consumption during extracorporeal circulation.

We observed the highest level of intra-platelet free calcium during the fluid-shear induced platelet activation with the oxygenator/arterial filter. The free calcium was monitored by chlorotetracycline fluorometry, before, during and after ECC in the pig model; 3-4 fold sudden increase of free calcium was observed at 15 minutes of oxygenation, followed by a decline at 60 minutes of ECC. This early increase in free calcium and shear-induced platelet-activation may be minimized by chelating agents and calcium-channel blockers.

The labeled platelets and clotting factors thus provided very important markers for quantitative studies of thrombosis on cardiovascular prostheses in animal models, and semi-quantitative studies by imaging in animal models and patients.

## REFERENCES

1. Rahimtoola SH: Valvular heart disease: a perspective. *J Am Coll Cardiol* 1:199, 1983.
2. Selzer A: Recent status of prosthetic cardiac valves. *Arch Intern Med* 143:1965, 1983.
3. Edmunds LH: Thromboembolic complications of current cardiac valvular prostheses. *Am Thoracic Surg* 34: 96, 1982.
4. Acar J, Enriquez-Sarano M, Farah E, et al: Recurrent systemic embolic events with valve prostheses. *Eur Heart J* 5 (Suppl D) 33:1984.
5. Venugopal P, Kaul U, Iyer KS, et al : Fate of thrombectomized Bjork-Shiley valves. A long-term cinefluoroscopic, echocardiographic and hemodynamic evaluation. *J Thoracic Cardiovasc Surg* 91:168, 1986.
6. Harris RL, Wilson WR, Williams TW: Infections associated with prosthetic heart valves. In "Infections Associated with Prosthetic Devices". Eds. Sugarman B, Young EJ. CRC Press, Boca Reton, FL 1984.
7. Ionescu MI: Tissue Heart Valves. Butterworths, Boston, 1979, pp 1-373.
8. Cohn LH, Gallucci V. Cardiac Bioprostheses, Yorke Medical Books, New York, 1-591.
9. Bodnar E, Yacoub MH: Biological and Bioprosthetic Valves, Yorke Medical Books, New York, 1986, pp 1-374.
10. Thiene J, Laborde F, Valente M, et al: Experimental evaluations of porcine-valved conduits processed with a calcium-retarding agent (T6). *J Thoracic Cardiovasc Surg* 91:215, 1986.
11. Magilligan DJ. Choice of heart valves. *ASAIO Transactions* 33:90-95, 1987.
12. Salzman EW, Merrill EW: Interaction of blood with artificial surfaces, in Coleman RW, Hirsh J, Marder VJ, Salzman EW (eds), Hemostasis and Thrombosis, Philadelphia, JB Lippincott Co., 1987, pp 1335-1347.

13. Goldsmith HL, Turitto VT: Rheologic aspects of thrombosis and hemostasis: basic principles and applications. *Throm Haemost* 55: 415-435, 1986.
14. Chenoweth DE: Complement activation produced by biomaterials. *Trans ASAIO* 32:226-232, 1986.
15. George JN: The role of membrane glycoproteins in platelet function. *Transfusion Med Rev* 1:34-46, 1987.
16. Dewanjee MK, Rao SA, Didisheim P: Indium-111-tropolone, a new high-affinity platelet label: preparation and evaluation of labeling parameters. *J Nucl Med* 22:981-987, 1981.
17. Dewanjee MK, Robinson RP, Hellman R, Serafini AN, Sfakianakis GN: A new efficient platelet labeling method with Tc-99m via neutral and lipid-soluble Sn(II)-complex and comparative evaluation with Tc-99m HMPAO labeled platelets. *J Nucl Med* 30, 1989.
18. Didisheim P, Dewanjee K, Frisk CS, Kaye MP: Animal models for predicting clinical performance of biomaterials for cardiovascular use. In: *Contemporary Biomaterials. Material and Host Response, Clinical Applications, New Technology and Legal Aspects*. J.W. Boretos (ed). *M. Eden Noyse Publications*. Park Ridge, NJ, pp 132-179, 1984.
19. Agarwal KC, Wahner HW, Dewanjee MK, Fuster V, Puga FJ, Danielson GK, Chesebro JH, Feldt RH: Imaging of platelets in right-sided extracardiac conduits in humans. *J. Nucl. Med.* 23:342-347, 1982.
20. Harker LA, Slichter SJ: Studies of platelet and fibrinogen kinetics in patients with prosthetic heart valves. *N. Engl. J. Med.* 283:1302-1305, 1970.
21. Dewanjee MK, Trastek VF, Tago M, Torianni M, Kaye MP: Non-invasive radioisotopic technique for detection of platelet deposition on bovine pericardial mitral valve prosthesis and in vitro quantification of visceral microemboli in dogs. *Trans. Am. Soc. Artif. Intern. Organ* 29:188-193, 1983.

22. Dewanjee MK, Fuster V, Rao SA, Forshaw PL, Kaye MP: Non-invasive radioisotopic technique for detection of platelet deposition in mitral valve prostheses and quantitation of visceral microembolism in dogs. Mayo Clinic Proc. 58:307-314, 1983.
23. Dewanjee MK, Didisheim P, Kaye MP, Solis E, Zollman PE, Francis MD, Torianni M, Trastek VS, Tago M, Edwards WD: Platelet deposition on and calcification of bovine pericardial valve. Eur. Heart J. 5(Suppl D):1-5, 1984.
24. Dewanjee MK, Solis E, MacKey ST, Lenker J, Didisheim P, Chesebro JH, Zollman PE, Kaye MP: Quantification of regional platelet and calcium deposition on pericardial tissue valve prosthesis in calves and effect of hydroxyethylene diphosphonate. J. Thorac. Cardiovasc. Surg. 92:337-348, 1986.
25. Peterson KA, Dewanjee MK, Kaye MP: Fate of indium-111-labeled platelets during cardiopulmonary bypass performed with membrane and bubble oxygenators. J. Thorac. Cardiovasc. Surg. 84:39-43, 1982.
26. Heyns ADP, Badenhorst PN, Lotter MG: Platelet kinetics and imaging. Vol. 1. Techniques and Normal Platelet Kinetics. Vol. 2. Clinical Applications. CRC Press, Boca Raton, Florida, 1985.
27. Waller BF: Evaluations of operatively excised cardiac valves. Chapter 10, Contemporary Issues in Cardiovascular Pathology, Cardiovascular Clinics, ED. Brest AN, FA Davis Co., 1988, Philadelphia, PA, pp 203-246.
28. Silver MD, Butany J: Complications of mechanical heart valve prostheses. Chapter 12, *ibid*, pp 273-288.
29. Dewanjee MK, Solis E, Lenker J, Tidwell C, Mackey S, Didisheim P, Kaye MP. Quantitation of platelet and fibrinogen-fibrin deposition on components of tissue valves (Ionescu-Shiley) in calves. Trans ASAIO, 32:591-596, 1986.

30. Dewanjee MK, Mackey ST, Solis E, Kaye MP: Effect of aspirin and low-dose aspirin-Persantine treatment on calcification of pericardial tissue valves in calves (In Press).
31. Zenger ZH, DeVries WC: The role of nuclear medicine in three permanent total artificial heart recipients. *Sem Nucl Med* 28:241-245, 1988.
32. Dewanjee MK, Singh SK, Wooley PH, Mackey ST, Solis E, Kaye MP: Identification of new collagen formation with 125-Iodine labeled antibody in bovine pericardial tissue valves implanted in calves. *Nuc Med Biol, Int J Radiat Appl Instrum, Part B*, 13(4):413-422, 1986.
33. Dewanjee MK: Noninvasive imaging of dystrophic calcification. Chapter 74, In *Calcium in Biological Systems*. Rubin RP, Weiss GB, Putney JW, Eds. Plenum Publishing Corp. Philadelphia, PA, 1985, pp 677-683.
34. Schoen FJ, Kujovich JL, Levy RJ, Sutton MSJ.: Bioprosthetic valve failure. Chapter 13, *ibid*, pp 289-317.
35. Dewanjee MK: In-111 platelets in bypass grafts: experimental and clinical applications. NATO Symposium "Radiolabeled Cellular Elements of Blood: Thakur ML, Hardeman M, Ezekowitz MD, Eds." Plenum Press, NY, 1985 pp 229-263.
36. Dewanjee MK: Cardiac and vascular imaging with labeled platelets and leukocytes. Semin Nucl Med 13(3):154-187, 1984.
37. Dewanjee MK: Methods of assessments of thrombosis in vivo. Blood in contact with natural and artificial surfaces. Vol. 516, Theme 4, Part one. Eds. Leonard EF, Vroman L, Turitto VT. New York Academy of Science, New York, N.Y. 1987, pp. 541-571.
38. Dewanjee MK, Solis E, Lanker J, Mackey ST, Lombardo GM, Tidwell C, Ellefson RD, Kaye MP: Effect of diphosphonate binding to collagen upon inhibition of calcification and promotion of spontaneous endothelial cell coverage on tissue valve prostheses. *Trans Amer Soc Artif Int Org XXXII*: 24-29, 1986.

## FIGURE CAPTIONS

Figure 1. Categories of failure of mechanical and porcine valves removed at operation. Courtesy of Schoen FJ (34).

Figure 2. Scintiphoto (5000 counts) of platelet deposition on components of Bjork-Shiley mechanical valve prostheses 24 hours post-injection of In-111 labeled platelets and photographs of the same components in the same orientation. A. Scintiphoto (25 hours after injection of In-111 labeled platelets in a dog and 24 hours after valve implantation in the mitral annulus) of sewing ring (left), perivalvular tissue (right), and thrombus (arrow, bottom). The disk and housing of the valve had few adherent platelets (top). (B) Photograph of the Bjork-Shiley mechanical valve-explant from a dog in the same orientation. The thrombus at the bottom was removed from the sewing ring. Dewanjee MK (22).

Figure 3a. Schematics of measurement of regional density of calcium, platelet, fibrinogen-fibrin and fibrin/platelet ratios in tissue-valve in the calf model.

Figure 3b. Dynamics of platelet deposition on components of Ionescu-Shiley (25-mm) tissue-valve explant from calves. Note peak time of platelet deposition on all components is about 4-8 hours post-injection. Dewanjee MK (21,23,24).

Figure 3c. Top: (A) Scintiphoto (5000 counts) of isolated components of Ionescu-Shiley valve prosthesis explanted from a dog at 25 hours post-implantation and 24 hours post-injection of In-111 labeled platelets. White arrow indicates most intense radioactivity, which comes from adherent platelet-thrombus (black arrow) on sewing ring. (B) Photograph of the same components: sewing ring with three leaflets around it (upper left); perivalvular tissue (upper right); stent supports, thrombus from sewing ring and suture (from left to right) in small trays below. Thrombus from sewing ring amounted to 31.44 mg (wet weight).

Bottom: (A) In a similar scintophoto at 30 days, most of the platelet deposition occurs at in the leaflets; the sewing ring

covered with fibroblast remains nonthrombogenic. (B) Photograph of components of the tissue valve-explant from dog at 30 days. Dewanjee MK (21,24).

Figure 4A. Regions of pericardial leaflet for the quantitation of platelet and calcium in tissue-valve explant. Figure 4B. Time course of exponential growth of regional calcification and total calcium level (4C) in components of tissue valve. The calcification of thrombus parallels that of collagen fibril in pericardial leaflet. Dewanjee MK (24). Reproduced with permission from the publisher.

Figure 5A. The platelet deposition expressed as total number of adherent platelets (mean  $\pm$  SD) on leaflet components at 1, 14, 30, and 90 days post-implantation of 25-mm Ionescu-Shiley valves in calves. The platelets were allowed to accumulate for 24 hours on tissue-valves before sacrifice. (A) Four zones of leaflets and (B) Components of tissue-valve explants. Dewanjee MK (24).

Figure 6A. Regional fibrin density on components of tissue valve at 1, 14, and 30 days post-implantation; 6B. Regional distribution of fibrin monomer per platelet on components of tissue-valve prostheses. Although the fibrin density decreases, the fibrin/platelet ratio tends to increase for outer sewing ring, attachment and central zone of tissue-valve leaflet. Dewanjee MK (29).

Figure 7A. Regional platelet density in aspirin and low-dose aspirin persantine treated calves. (B). Total platelets in different regions of tissue-valves in treated calves.

Figure 8. TEM of aspirin-treated infected tissue-valve. Note pockets of bacteria at lower and higher magnification (A) and (B). Similar infection was also observed in one diphosphonate treated valve.

Figure 9A. Principle of immobilization of aminodiphosphonate to pericardial tissue by Schiff-base reaction. (B). Detergent treatment and aminodiphosphonate immobilization of pericardial

tissue. (C) Tissue valve explant at 60 days post-implantation. Note smooth surface of valve. (D) Spontaneous endothelial cell coverage of diphosphonate-immobilized tissue-valve explant (SEM).

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1. Complications of valve-prostheses.
2. Biodistribution of In-111 labeled platelets in mitral-valve prostheses (Bjork-Shiley) implanted dogs.
3. Distribution of In-111 labeled platelets at 24 hours post-injection in the components of Bjork-Shiley valve prostheses implanted in dogs.
4. Biodistribution of In-111 labeled platelets in Ionescu-Shiley tissue-valve implanted dogs at 1, 14 and 30 days.
5. Distribution of In-111 labeled platelets in components of Ionescu-Shiley valves implanted in dogs.

5/15/89

TABLE 1

EFFECTIVE ORIFICE AREA (EOA) AND INCIDENCE  
OF THROMBOEMBOLISM (PT/YR) % OF PROSTHETIC  
MITRAL VALVES

<u>VALVE</u>	<u>EOA (cm<sup>2</sup>)*</u>	<u>(PT/YR) %</u>
STARR-EDWARDS (1960)	1.1	5.9
IONESCU-SHILEY (1966)	1.8	2.9
HANCOCK (1969)	1.6	2.8
ST. JUDE (1977)	2.1	2.9
HALL-MEDTRONIC (1977)	1.6	2.8

\* 21 mm VALVE

Table 2

—Biodistribution (Mean  $\pm$  SD) of Administered Dose (Percent) of  $^{111}\text{In}$ -Labeled Platelets in Unoperated Normal, Sham-Operated, Björk-Shiley Mitral Valve Prosthesis (BSMVP)-Implanted, and Prosthesis-Implanted and Dipyridamole- and Aspirin-Treated Dogs 24 Hours After Intravenous Administration and 25 Hours After Operation

Tissue and components of mitral valve prosthesis	Group 1: Unoperated normal (N = 7)	Group 2: Sham-operated (N = 5)	Group 3: Prosthesis-implanted (N = 5)	Group 4: Prosthesis-implanted, treated (N = 5)
Liver	20.94 $\pm$ 12.38	16.53 $\pm$ 6.56	18.06 $\pm$ 2.27	18.84 $\pm$ 4.18
Spleen	32.27 $\pm$ 10.78	24.05 $\pm$ 7.17	21.52 $\pm$ 2.68	24.78 $\pm$ 6.38
Lungs	0.98 $\pm$ 0.14	1.54 $\pm$ 0.70	1.64 $\pm$ 0.44	2.45 $\pm$ 1.11
Kidneys	0.38 $\pm$ 0.15	0.95 $\pm$ 0.33	1.60 $\pm$ 0.63	1.31 $\pm$ 0.66
Skeletal muscle	2.47 $\pm$ 0.15	3.06 $\pm$ 1.39	7.93 $\pm$ 0.91 (P<0.01, group 3 vs. 1 and 3 vs. 2)	5.06 $\pm$ 3.44
Blood	42.76 $\pm$ 9.53	35.41 $\pm$ 8.58	28.66 $\pm$ 6.10 (P<0.01, group 3 vs. 1 and 3 vs. 2)	32.24 $\pm$ 4.62
Total BSMVP	...	...	0.76 $\pm$ 0.12	0.49 $\pm$ 0.16
Cumulative post-surgical consumption (24 h)	...	18.20 $\pm$ 6.70	20.12 $\pm$ 7.82	14.59 $\pm$ 8.23 (P<0.05, group 4 vs. 3)

Table 2.—Distribution (Mean  $\pm$  SD) of Administered Dose (Percent) of  $^{111}\text{In}$ -Labeled Platelets in Components of Björk-Shiley Mitral Valve Prosthesis-Implanted and Prosthesis-Implanted and Dipyridamole- and Aspirin-Treated Dogs 24 Hours After Implantation and 25 Hours After Operation

Components	% administered $^{111}\text{In}$ -labeled platelets	
	Group 3: Implanted	Group 4: Implanted, treated
Pyrolytic carbon disk	0.0031 $\pm$ 0.0003	0.0009 $\pm$ 0.0002 (P<0.01, group 4 vs. 3)
Valve housing	0.0033 $\pm$ 0.0004	0.0020 $\pm$ 0.0003
Sewing ring	0.30 $\pm$ 0.11	0.21 $\pm$ 0.10
Thrombus on sewing ring	0.26 $\pm$ 0.04	0.20 $\pm$ 0.08
Perivalvular damaged cardiac tissue	0.19 $\pm$ 0.11	0.09 $\pm$ 0.03
Total (% injected dose)	0.76 $\pm$ 0.12	0.49 $\pm$ 0.18 (P<0.05, group 4 vs. 3)

Table 3

Biodistribution of Administered Dose of  $^{111}\text{In}$ -Labeled Platelets (Mean Percentages  $\pm$  SD) in Dogs With and Without Mitral-Valve Prostheses\*

	Group I	Group II	Group III (implantation) sacrifice at:		
	(no operation) n = 7	(sham) n = 5	Day 1 n = 3	Day 14 n = 3	Day 30 n = 5
Liver	20.94 $\pm$ 12.38	16.53 $\pm$ 6.56	19.49 $\pm$ 3.46	31.96 $\pm$ 5.01	22.36 $\pm$ 8.67
Spleen	32.27 $\pm$ 10.78	24.05 $\pm$ 7.17	22.56 $\pm$ 7.26	17.42 $\pm$ 6.57	28.33 $\pm$ 10.15
Lung	0.98 $\pm$ 0.14	1.54 $\pm$ 0.70	1.98 $\pm$ 0.67	0.92 $\pm$ 0.47	2.70 $\pm$ 0.41
Kidney	0.38 $\pm$ 0.15	0.95 $\pm$ 0.33	1.52 $\pm$ 0.51	0.64 $\pm$ 0.27	0.96 $\pm$ 0.38
Skeletal muscle	2.47 $\pm$ 0.15	3.06 $\pm$ 1.39	6.19 $\pm$ 0.84	4.53 $\pm$ 0.95	3.13 $\pm$ 0.79
Blood	42.76 $\pm$ 9.53	35.41 $\pm$ 8.58	29.89 $\pm$ 7.52	41.88 $\pm$ 14.30	43.18 $\pm$ 9.95
Prosthesis,* total	—	—	0.79 $\pm$ 0.22	0.18 $\pm$ 0.09	0.04 $\pm$ 0.015
Platelet consumption within first 24 hours postop	—	18.2 $\pm$ 6.70	17.73 $\pm$ 6.96	—	—

\*Bovine pericardial-tissue mitral-valve prosthesis (Ionescu-Shiley).

Table 4.

Distribution of  $^{111}\text{In}$ -Labeled Platelets (Mean Percentages  $\pm$  SD) on and About Mitral-Valve Prosthesis\* in Dogs, at 24 Hours After Injection

	Group III (implantation) sacrifice at:		
	Day 1 (n = 3)	Day 14 (n = 3)	Day 30 (n = 5)
Leaflets (3)	0.154 $\pm$ 0.018	0.114 $\pm$ 0.092	0.036 $\pm$ 0.023
Sewing ring,	0.549 $\pm$ 0.154	0.044 $\pm$ 0.023	0.002 $\pm$ 0.0136
Thrombus on sewing ring	0.069 $\pm$ 0.057	0.022 $\pm$ 0.012	0.001 $\pm$ 0.0004
Perivalvular tissue	0.112 $\pm$ 0.019	0.018 $\pm$ 0.012	0.001 $\pm$ 0.0009
Leaflets/aortic valve	106 $\pm$ 21	57 $\pm$ 15	36 $\pm$ 30
Thrombus/blood	545 $\pm$ 157	2.35 $\pm$ 1.86	1.34 $\pm$ 1.14

\*Ionescu-Shiley mitral valve prosthesis, with leaflets of bovine pericardial tissue.

Table 5

Figure 1

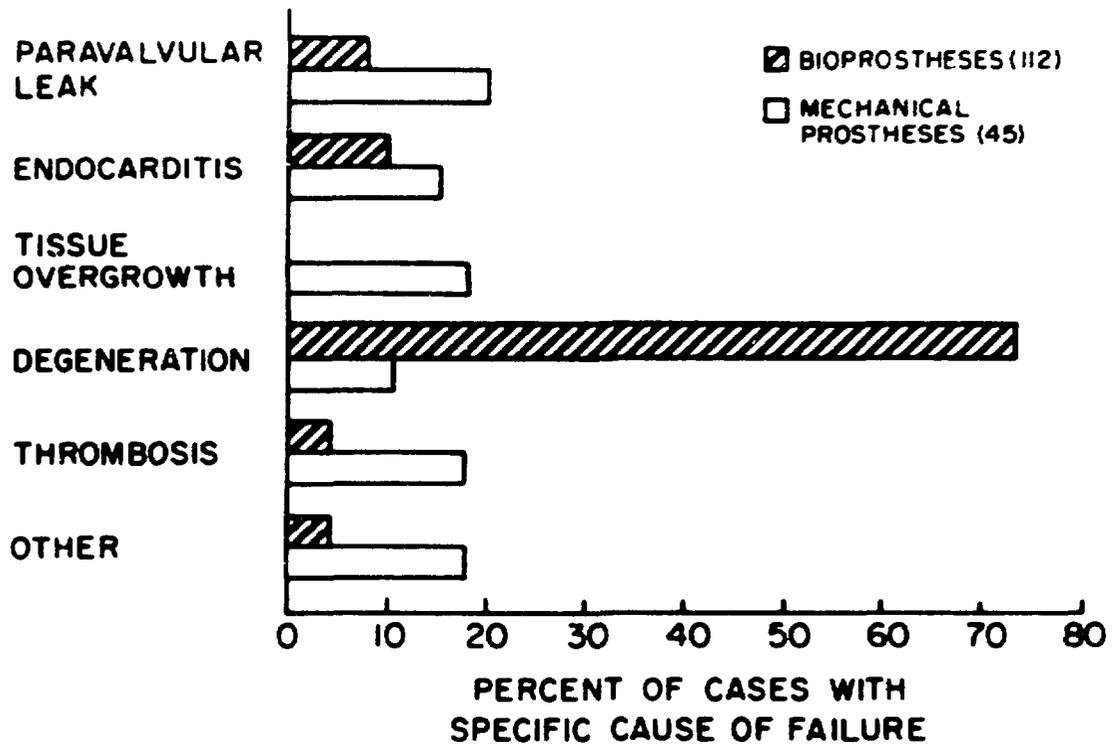
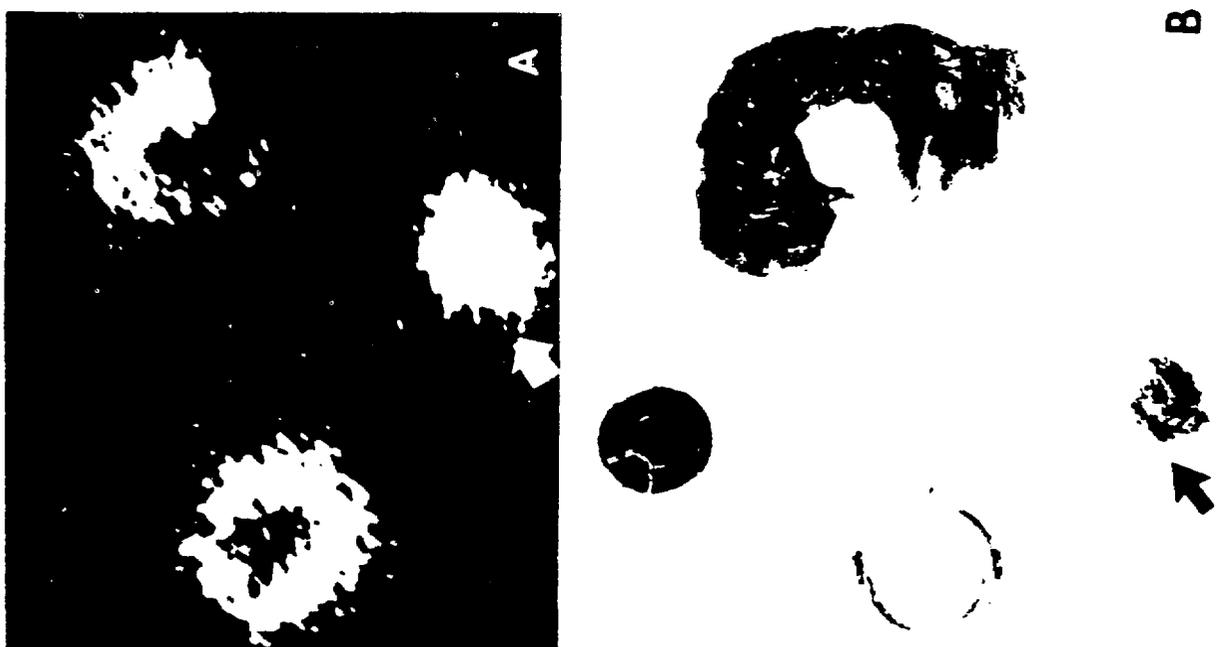


Figure 2



## QUANTIFICATION OF PLATELET DEPOSITION AND CALCIUM IN TISSUE VALVES

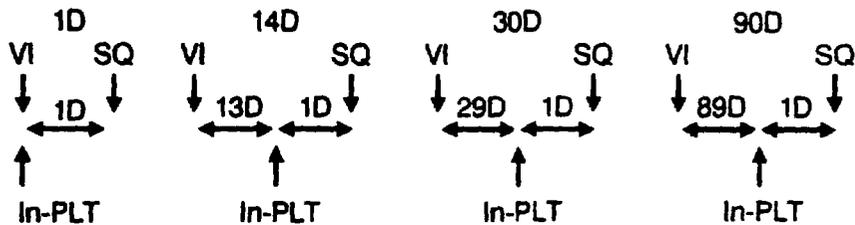


Fig 3A.

In-PLT-; In labeled platelet inj.  
250-350  $\mu$ Ci (autologous)

VI: Valve implantation

SQ = Sacrifice and quantification  
(PLT and Ca)

D = Days post-implantation or  
injection

Fig 3b

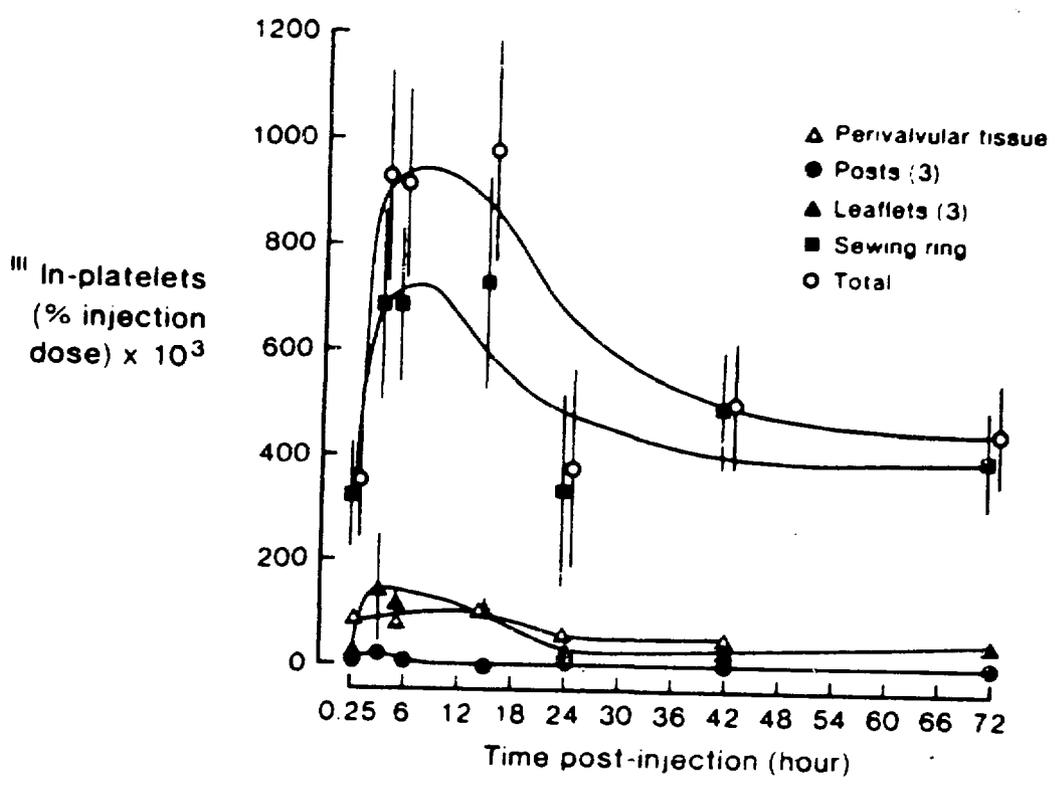
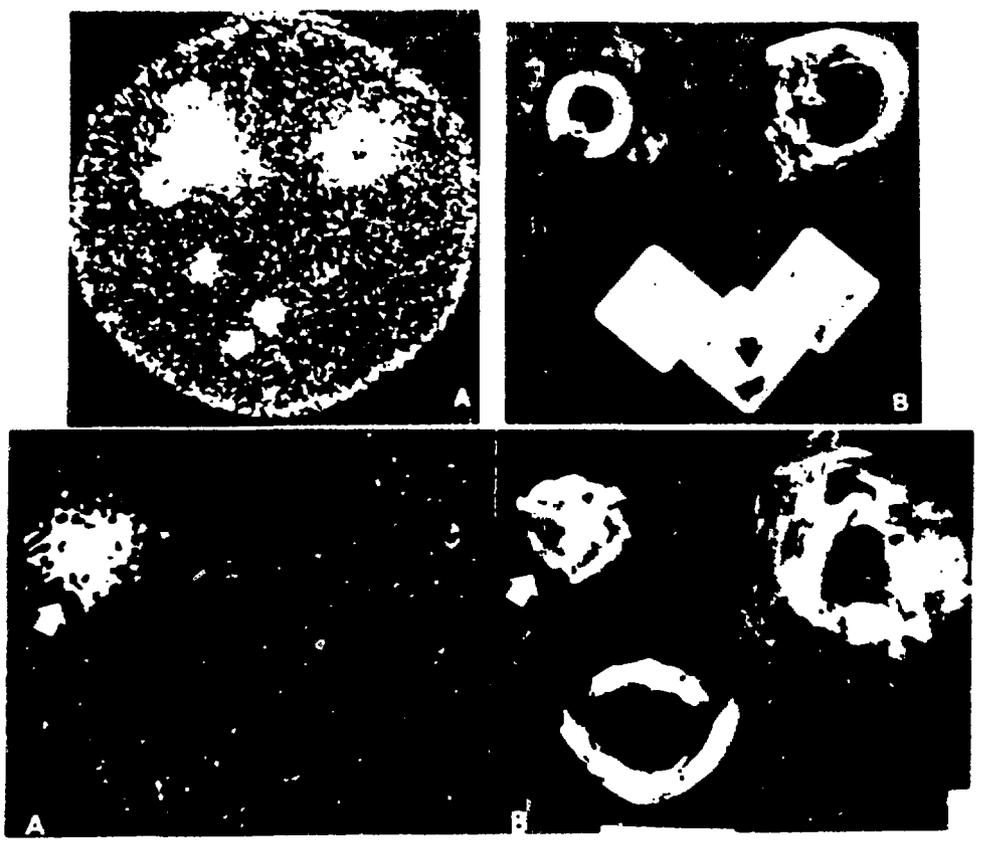


Fig 3c



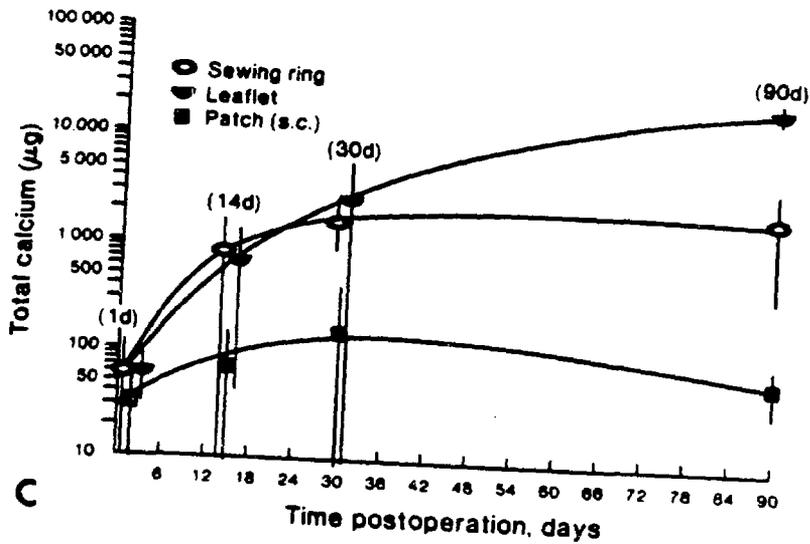
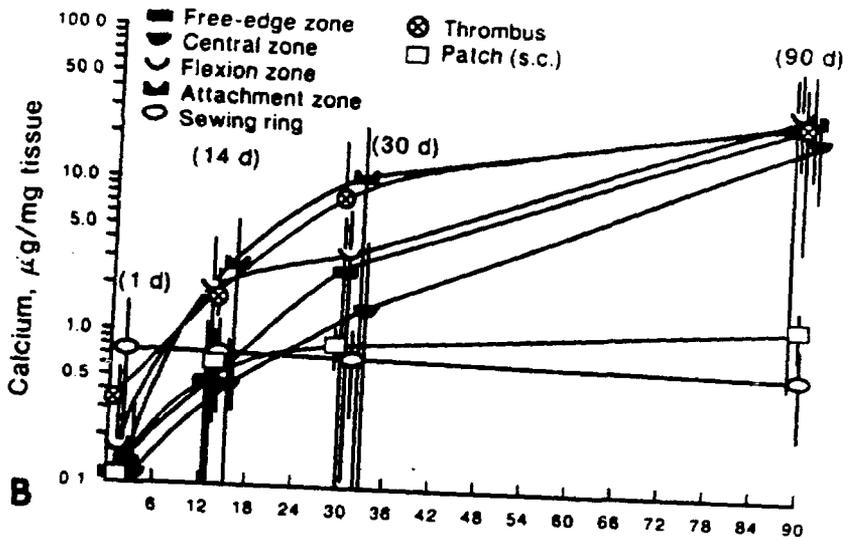
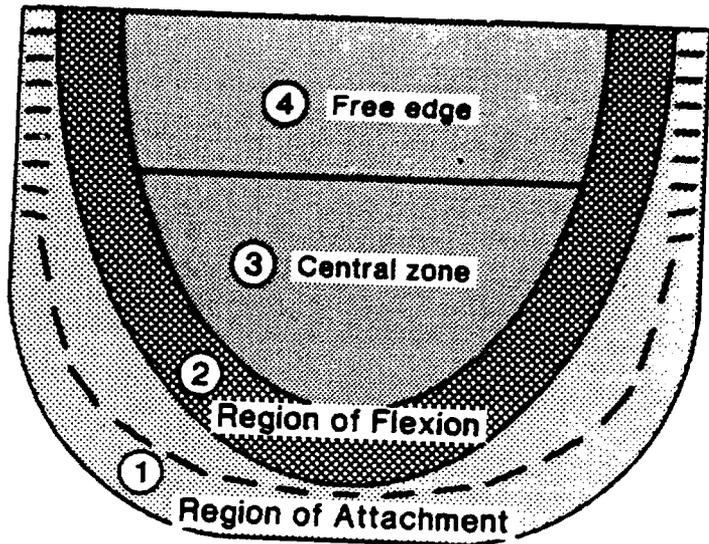


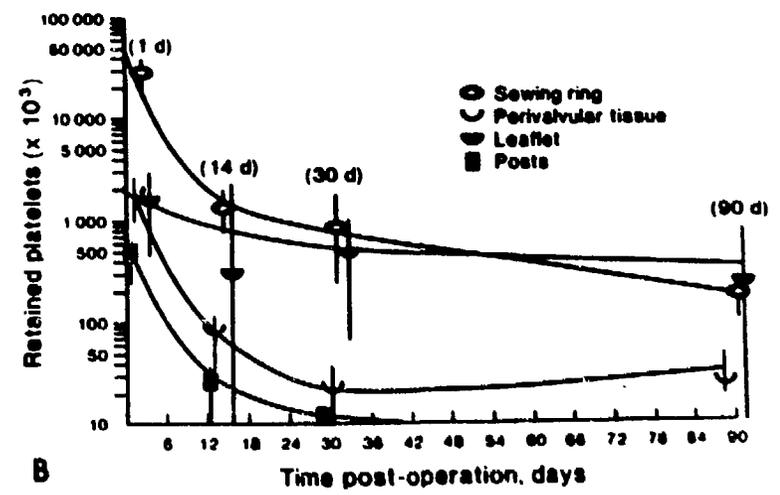
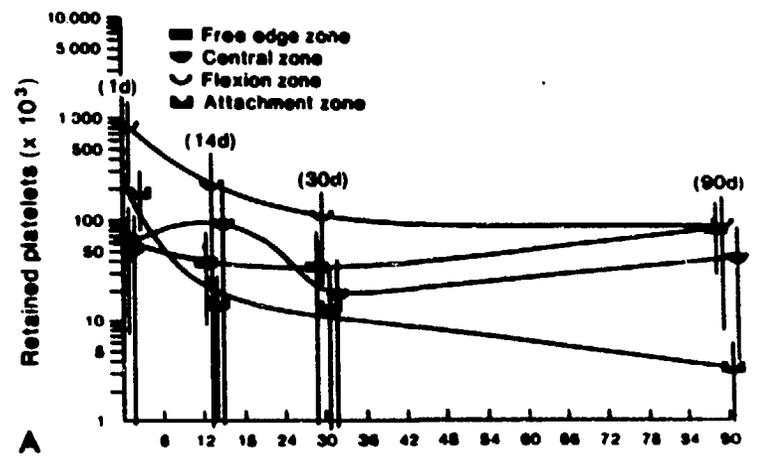
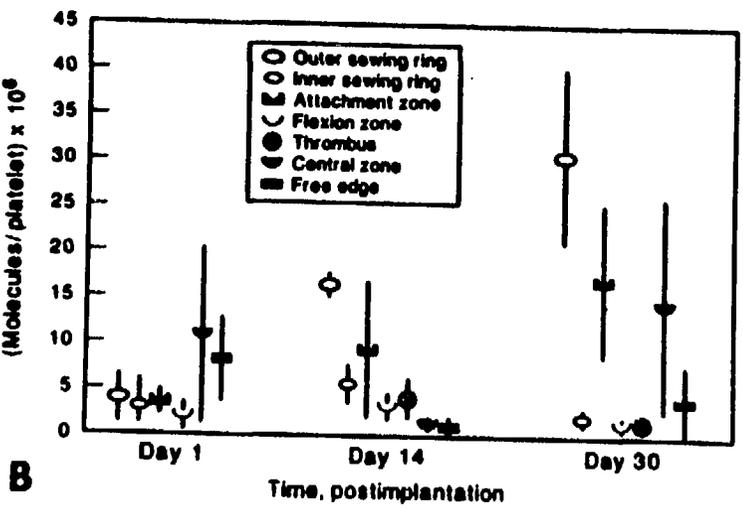
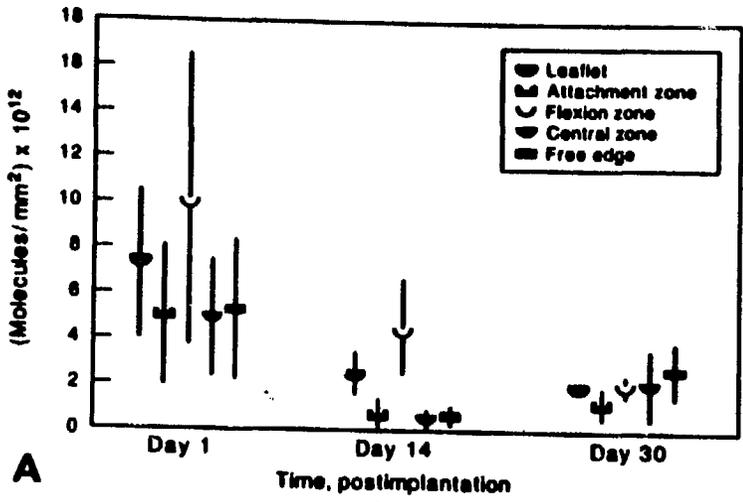
Fig 4. Figure 4

Fig 5 A, B

Fig 6 A, B

Fig 5 A, B

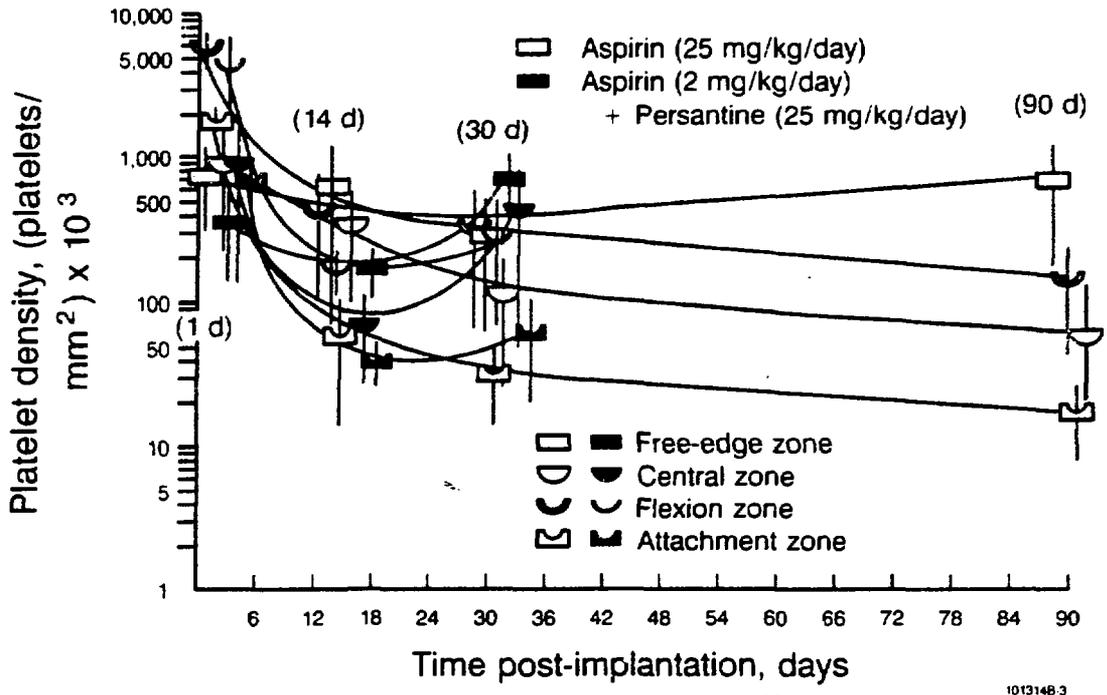
Fig 5 A, B



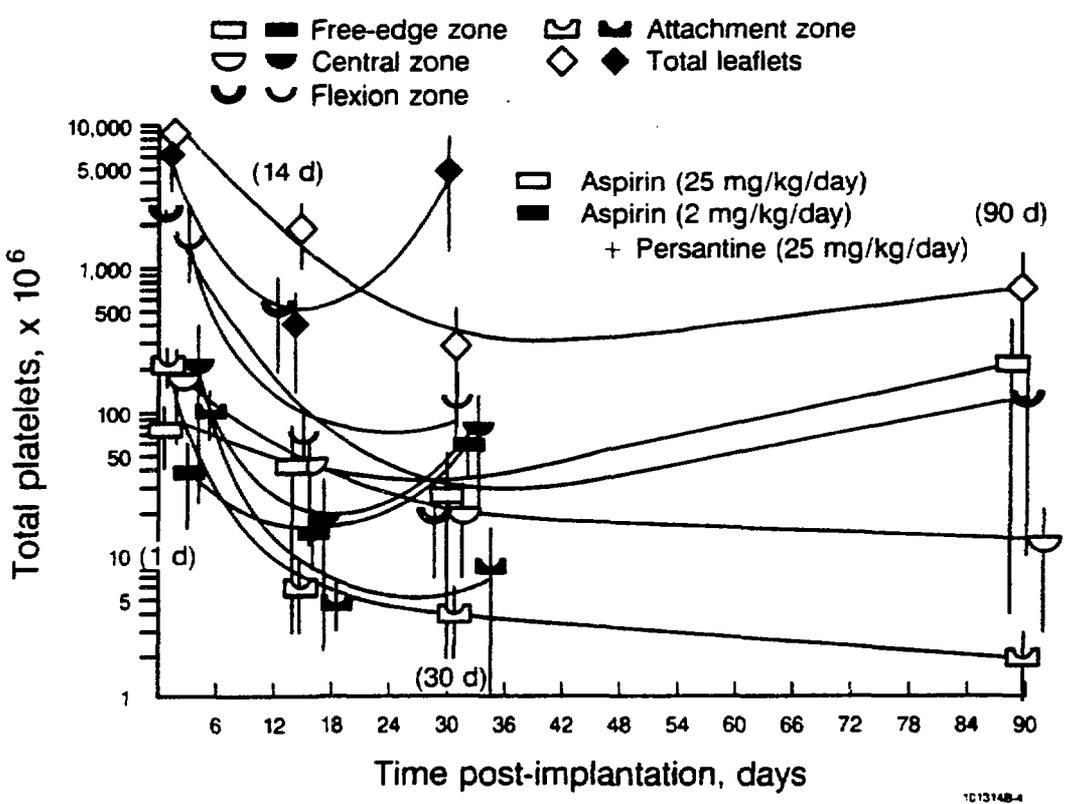
METRIC 1  
2



Fig 7



101314B-3



101314B-4

8A

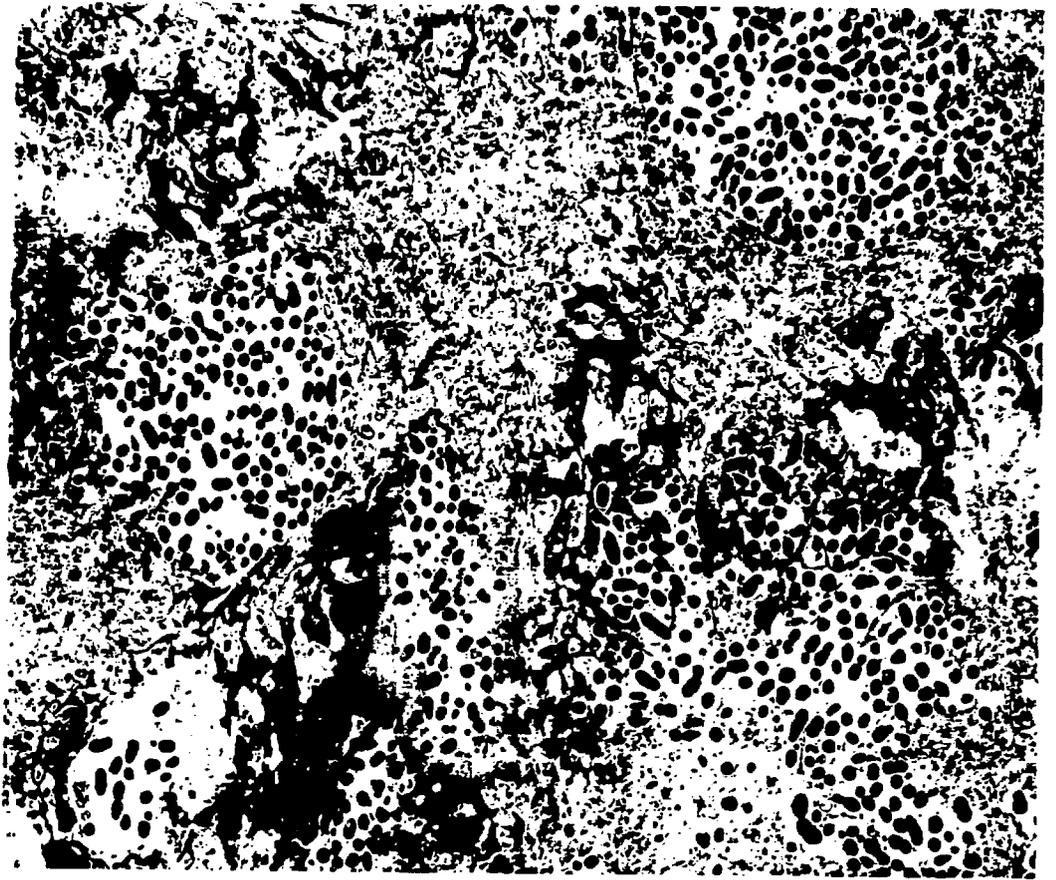
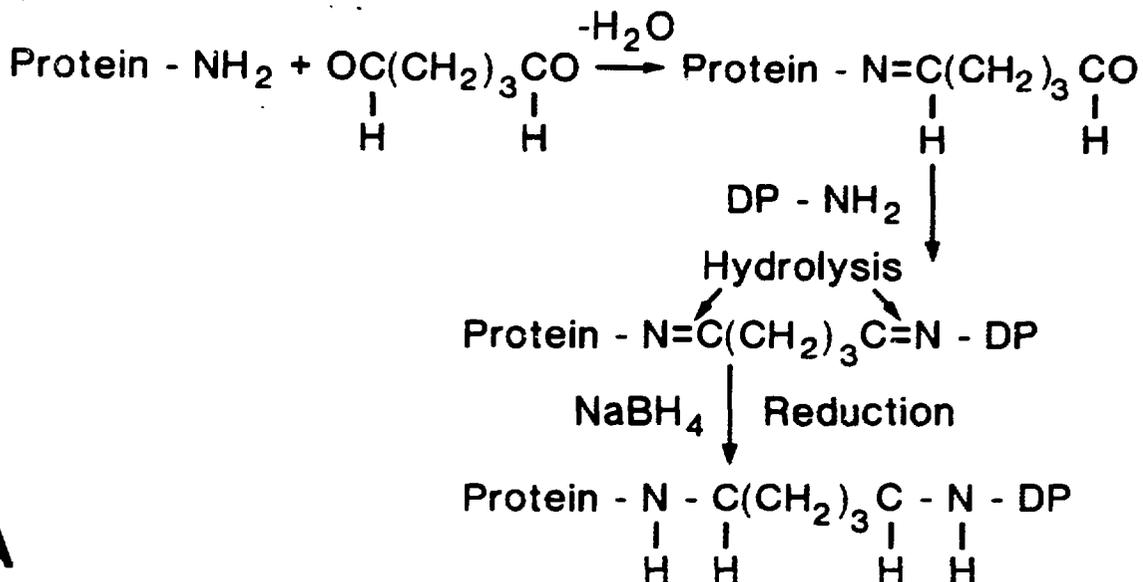


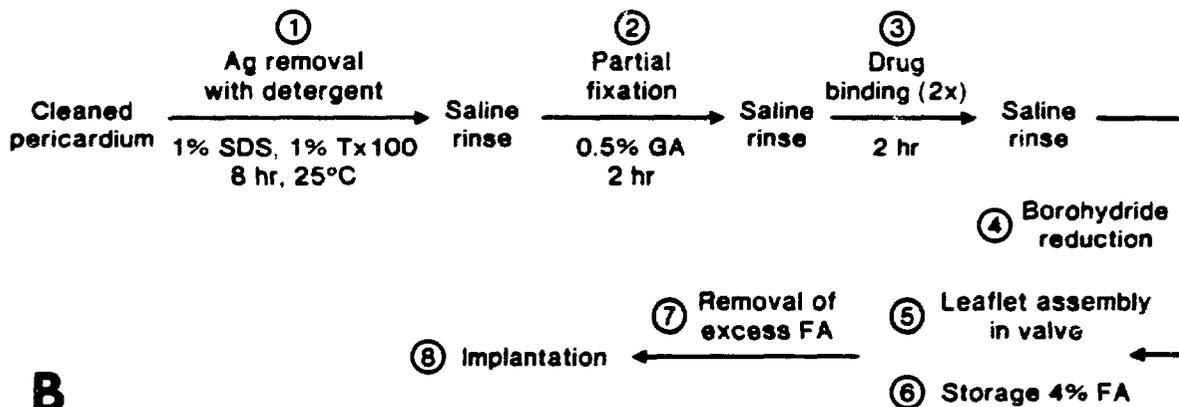
Fig 8B



77



**A**



**B**

