THE ROLE OF PLATELETS IN RESTENOSIS FOLLOWING CAROTID ENDARTERECTOMY

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ABSTRACT

Platelet accumulation on the arterial wall has been studied after intimal trauma in rabbits and following endarterectomy in patients. Physical and pharmacological factors affecting deposition at the site of arterial damage were investigated and quantified as thrombogenicity. This has been related to the degree of luminal narrowing measured in patients by non-invasive ultrasound, one year after surgery.

A model of intimal trauma was developed in the carotid artery of rabbits to simulate endarterectomy. Radiolabelled platelet accumulation was consistently higher in the damaged artery when compared to control vessels. Platelet deposition at the site of injury was significantly reduced by platelet inhibitory therapy.

Autologous ^{111}Indium labelled platelet uptake was measured over 3 days in patients following carotid endarterectomy and expressed as Thrombogenicity Index. Postoperative platelet accumulation was reliably demonstrated by gamma camera imaging but was not identified by probe counting. Platelet uptake over the endarterectomy had resolved by 2 months after surgery which suggests endothelial healing.

Factors influencing platelet kinetics were investigated following endarterectomy. The rate of carotid platelet accumulation was significantly reduced in patients receiving platelet inhibitory therapy, compared to untreated controls. Patch repair of the endarterectomy statistically increased postoperative thrombogenicity in contrast to direct suture of the arteriotomy.

In a retrospective study the incidence of restenosis following carotid endarterectomy was assessed using non-invasive Doppler ultrasound. Significant restenosis, of greater than 50% diameter reduction, was identified in 13% of patients, however, only one sixth of these developed recurrent symptoms of cerebrovascular insufficiency. Predisposing factors to restenosis were not identified.

Early postoperative carotid platelet accumulation was correlated to the subsequent development of luminal narrowing and restenosis in a prospective study. The relationship between platelet deposition and subsequent restenosis was established. Luminal narrowing was significantly greater in patients with high postoperative carotid thrombogenicity.

STATEMENT OF ORIGINALITY

This work was completed between May 1985 and January 1988 while I was a Research Fellow and subsequently Lecturer in the Department of Surgery at Charing Cross Hospital, London.

Although I initially required considerable help, I performed all the experimental techniques involved in this thesis, with the exception of electron microscopy. I examined all the patients, labelled platelets from both rabbits and humans and carried out the animal experiments. I measured radioactivity by probe counting and gamma camera and calculated the results of Thrombogenicity.

After instruction, I carried out Duplex evaluation of the carotid artery and measured the degree of luminal narrowing.

I recorded all the data, calculated the results and performed the statistical analysis.

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SECTION I INTRODUCTION

Chapter 1

Carotid Endarterectomy in the Treatment of Extra-cranial Arterial Disease

1.1 Atheroma in the Carotid Artery

The clinical and pathological features of carotid artery atherosclerosis have been recognised for more than one hundred years. In 1856, William Savory described the case of a 22 year old woman who suffered monocular visual disturbances with episodic contralateral transient hemiparesis. She was found at post mortem to have both upper limb and cerebral arterial insufficiency (Savory 1856). Following this discovery, Penzoldt reported a case of sudden right sided blindness followed by dense left hemiplegia. Autopsy revealed complete thrombotic occlusion of the right internal carotid artery with right sided cerebral softening (Penzoldt 1881).

Further understanding of the pathophysiology of cerebro-vascular disease came from Chiari, who demonstrated non-occlusive thrombus on atherosclerotic plaques in the carotid artery, in seven cases out of a series of 400 consecutive postmortem examinations (Chiari 1905). He identified emboli in the cerebral vasculature causing encephalomalacia and noted that in the absence of significant atheromatous changes in the aorta and arch vessels, the carotid bifurcation was a likely source of these thrombi (Gunning et al 1964).

In 1914, Ramsay Hunt described 2 cases of traumatic carotid thrombosis with hemiparesis and related the reduction in the pulsation of the carotid artery in the neck to the ipsilateral "softening of the brain". In further observations on cerebrovascular

disease he described areas of cerebral infarction corresponding to the distal portion of the arterial tree, "where the circulation is weakest", but also identified isolated lesions in the region of the central ganglia which he attributed to embolisation (Hunt 1914).

Reports of post mortem findings of internal carotid artery occlusion continued until 1937, when Moniz described this phenomenon in 4 patients using the new technique of cerebral arteriography. This brought the condition to the attention of the clinicians (Moniz, Lima & de Lacerda 1937). By 1943, Andrell had collected reports of 23 cases of carotid thrombosis, diagnosed by angiography, and added 9 cases of his own (Andrell 1943). A year earlier Hultquist had published a definitive monograph on the pathological findings in the carotid system in 1400 post mortem examinations. He found evidence of thrombo-embolic disease in 3% and described in detail the location of the carotid arterial lesions showing the resultant changes in the brain. He concluded that embolisation was more common than indicated by the sporadic reports of the time (Hultquist 1942).

It was Fisher's review of 1951 which stimulated most interest in the subject of thromboembolism. Disregarding the theory that thrombosis of the middle cerebral artery accounted for the majority of cases of stroke, he examined more proximal structures. After excluding emboli from the pulmonary veins, left heart, and ascending aorta, he concentrated on the carotid arteries (Fisher 1951). He suggested that internal carotid artery occlusion was far more common

than was previously thought and that the underlying process was atherosclerosis. He described hemiplegia, preceded by transient paralysis, aphasia and blindness, and finally forged the link between cerebral embolism and the "thrombotic material lying in the carotid sinus". He not only described the pathological process on which the subsequent surgical treatment of extra cranial carotid arterial disease would be based but suggested; "It is even conceivable that some day vascular surgery will find a way to bypass the occluded portion of the artery during the period of ominous fleeting symptoms. Anastamosis of the external carotid artery or one of its branches with the internal carotid artery above the area of narrowing should be feasible".

Subsequently Fisher stressed the importance of carotid atheroma in the aetiology of transient cerebral ischaemia and made the distinction between carotid territory and vertebro-basilar attacks (Fisher & Cameron 1953). In 1951, Denny-Brown had suggested that transient ischaemic attacks were caused by episodic hypotension associated with carotid disease, however Millikan, and eventually Fisher, supported the theory of embolisation from mural thrombus on atheromatous plaque in the carotid arteries (Denny-Brown 1951; Millikan, Siekert & Shick 1955; Fisher 1959). Platelet thrombi and fragments of atheromatous plaque were visualised in the retinal arteries (Russell 1961; Russell 1963) and it was confirmed in a pathological study that these emboli were made up of platelets (McBrien et al 1963).

1.2 Endarterectomy

The technique of thromboendarterectomy was first reported by Cid dos Santos in 1947 although arterial thrombectomy had previously been described by Delbet (dos Santos 1947; Delbet 1906). It was dos Santos who realised that the success of this procedure required that the plane of dissection be in the media of the arterial wall, removing both the overlying thrombus and the atheromatous tissue in the intimal layer. It was first applied to the abdominal aorta and involved extensive exposure of the aorto-iliac and femoral arteries with excision of the occlusive lesion (Leriche & Kunlin 1947). Five years later Wylie reported a series of 25 patients in whom successful aorto-iliac or aorto-femoral endarterectomy had been performed so proving that disobliteration of the major arteries was possible (Wylie 1952).

1.3 Carotid Endarterectomy

The first recorded operations on the carotid artery are those of ligation and John Abernathy is thought to have performed one of the earliest of these procedures for haemorrhage in 1798 (Hamby 1952). Sir Astley Cooper ligated the common carotid artery for aneurysm in 1805 (Cooper 1836), Travers tied the carotid artery for carotid-cavernous fistula in 1809 and Horsley performed ligation for intracranial aneurysm in 1855 (Thompson 1983).

By 1951 when Fisher published his report on internal carotid artery atheroma, no effective procedure had been accepted in the

treatment of patients with thromboembolic disease. Sporadic reports of excision of the carotid artery with ligation had not been successful (Krayenbuhl & Weber 1944). Although it had been suggested that periarterial or cervical sympathectomy had improved cases of cerebral endarteritis obliterans, surgery had justifiably not been accepted (Foerster & Guttman 1933). Fisher had implied that prolonged anticoagulation might allow the diseased artery to undergo re-endothelialisation, however he also suggested that surgery might be the treatment of choice (Fisher 1951).

1.3.i The Development of Carotid Endarterectomy

After successful thromboendarterectomy in the aorto-iliac segment and following Fisher's suggestions, there followed a series of reports on carotid artery surgery. In 1951, Carrea performed the operation first suggested by Fisher, anastomosing the external carotid to distal internal carotid, so bypassing the site of thrombosis (Carrea, Molins & Murphy 1955). In 1953, Strully reported an unsuccessful endarterectomy in a patient with carotid occlusion (Strully, Herwitt & Blackenburg 1953) however the first successful carotid thromboendarterectomy was performed by DeBakey on 7th August 1953, but was not reported until 20 years later (DeBakey 1975). This was carried out on a 53 year old man who presented with recurrent weakness of the arm and leg and was found to have complete occlusion of the internal carotid artery. The following year, Eastcott, Pickering and Robb gave greatest impetus to the development of carotid surgery when they described the successful treatment of a 66

year old woman, with intermittent attacks of hemiplegia. By excising a partially thrombosed segment of common and internal carotid artery, they effected repair by end to end anastomosis (Eastcott, Pickering & Rob 1954).

Although thromboendarterectomy of the origin of the internal carotid artery is no longer performed in patients with complete carotid thrombosis, it has come to be regarded as the operation of choice in the surgical treatment of non-occlusive carotid atherosclerosis.

1.3.ii Carotid Endarterectomy: Current Techniques

In the 30 years that have elapsed since these early procedures, there have been considerable advances in operative techniques, instrumentation and suture materials. However the principle of the operation remains the same, to remove the atheromatous plaque which is causing either haemodynamically significant stenosis or is the source of emboli. In this respect little has changed since the first carotid thromboendarterectomy was performed in 1953 and the operative technique has been described in detail (Thompson & Talkington 1982; Greenhalgh 1984).

Under general anaesthesia an oblique incision is made over the anterior border of sternomastoid and the carotid bifurcation is exposed. The common carotid artery is mobilised proximally to the level of the omohyoid and the internal carotid distally to the

posterior belly of digastric, which can be divided for additional exposure. Care is taken not to damage the hypoglossal, vagus, superior laryngeal or glossopharyngeal nerves and during mobilisation the artery must be handled gently, to avoid embolisation of loose atheromatous material or thrombus. Each of the three vessels is controlled by slings and after heparinisation the arteries are occluded with vascular clamps. An arteriotomy is made from the common carotid into the internal carotid reaching distally beyond the the plaque to visualise the entire endarterectomy segment. Depending on the measurement of the internal carotid stump pressure, an intraluminal shunt may then be placed to maintain cerebral blood flow for the duration of carotid clamping. Endarterectomy is performed in the appropriate plane of the media starting in the common carotid artery and the plaque is divided transversely at the proximal extent of the dissection (Plate 1.1). The plane is developed to the origin of the external carotid artery and into the internal carotid where the distal margin of the plaque usually tears off smoothly to leave a satisfactory endpoint (Plate 1.2). It is imperative to see this endpoint clearly, as the intima may have to be secured with tacking sutures to prevent dissection. After removing any debris from the arterial wall, the arteriotomy is closed with a 6.0 polypropolene suture and the shunt removed before the final few stitches are placed. The vessels are flushed and the internal carotid allowed to back bleed to remove loose thrombus before reclamping. Blood flow is first restored to the external carotid, preventing any air from

embolising to the brain and finally the internal carotid clamp is released.

In order to ensure a satisfactory endarterectomy, operative arteriography may be performed to visualise technical defects of intimal flap or dissection (Rosental, Gaspar & Movius 1973). Alternatively the proximal and distal limits of the endarterectomy may be visualised directly by angioscopy prior to final closure of the arteriotomy (Towne & Bernhard 1977).

In those cases where the internal carotid artery is narrow and direct suture may produce stenosis, the arteriotomy can be closed by patch angioplasty using either autogenous vein or prosthetic graft (Katz et al 1987; Deriu et al 1984).

Cerebral protection during carotid cross-clamping has been practiced since the earliest days of carotid surgery and originally systemic hypothermia was used to prevent ischaemic damage (Eastcott et al 1954). This has since been replaced by intra luminal shunting which can be inserted routinely (Javid et al 1979; Browse & Ross Russell 1984) or selectively in cases where there is risk of cerebral ischaemia, on the grounds of poor collateral circulation around the circle of Willis (Moore, Yee & Hall 1973).

After satisfactory haemostasis the wound is closed in layers with suction drainage for the first 24 hours. The patient is

returned to the intensive care unit for close monitoring of neurological and cardiovascular status and can be transferred to the ward after 24 or 48 hours.

1.3.iii Carotid Endarterectomy: Current Applications

Cerebrovascular disease affects approximately one third of the adult population and stroke is the third commonest cause of death in the western world. This accounts for 11% of all adult mortality in Great Britain (Haberman, Capildeo & Clifford Rose 1981). In the United States, stroke has been estimated to cost $7 billion annually in medical treatment and time lost from work (Powers & Raichle 1984). Stroke is commonly caused by cerebral infarction due to either haemorrhage or thrombosis and up to 70% of patients are found to have significant carotid artery disease (Hass et al 1968). Better control of hypertension has lowered the incidence of cerebral haemorrhage therefore raising the proportion of strokes produced by thromboembolism (Garaway, Whisnant & Drury 1983). Despite improved understanding of the pathophysiology there has been little change in the prognosis of patients who suffer completed stroke. There is a 50% mortality in patients admitted to hospital with stroke and over 60% of the 4 week survivors will die during the next 5 years from either ischaemic heart disease or recurrent stroke (Marquardsen 1976). Of those survivors, 25% will be invalided and require nursing care, 60% will have either moderate or mild permenant disability and only 15% will recover to the degree that they can return to their pre-stroke activities or employment (Marquardsen 1983).

The role of extra-cranial atherosclerosis as a cause of stroke has been increasingly recognised and the value of carotid surgery in preventing stroke has been assessed in three groups of patients. These are asymptomatic carotid disease, those with symtoms of transient ischaemic attacks and patients who have already suffered a completed stroke.

Asymptomatic carotid disease

Studies of patients with asymptomatic cervical bruit indicated an increased risk of stroke at 14% over 6 years, compared to 3.4% in a similar group without bruit (Heyman et al 1980). There was no correlation between the side of the bruit and the area of subsequent cerebral infarction and cervical bruit could only be regarded as a sign of severe widespread atherosclerosis (Wolf et al 1981).

Whether or not stroke is sufficiently more common in patients with asymptomatic carotid disease to justify any form of treatment remains contraversial (Fields 1978; Mohr 1982). Proponents of prophylactic endarterectomy recommend this as a low risk procedure and a study, with follow up to 10 years, compared an overall perioperative and postoperative stroke rate of 5% in patients treated by endarterectomy, to 17.4% in a group of unoperated controls (Thompson, Patman & Talkington 1978). Similarly Burke reported a low combined perioperative and postoperative stroke rate of 4%, but found a high incidence of postoperative cardiac events (40%) in patients with significant cardiac risk factors (Burke, Callow & O'Donnell

1982). This implied that significant cardiac morbidity may outweigh the long term benefit of stroke prophylaxis in this group of patients.

Stroke prophylaxis in asymptomatic carotid disease was examined by Chambers & Norris (1984). Assuming a two thirds reduction in stroke risk reported by the Arterial Occlusion Joint Study (Fields et al 1970) and a perioperative stroke rate of 1.5%, a break even point between surgically and medically treated patients is reached at 13 months. This is true when stroke is considered as the only endpoint but if stroke and death are considered together, the benefit of surgery is not seen until 28 months. This rationale depends on a combined perioperative stroke rate and mortality of 3% in asymptomatic patients (Thompson et al 1978). In other centres perioperative complication rates have been quoted at 11% and 21% (Brott & Thalinger 1983; Easton & Sherman 1977). This alters stroke prophylaxis such that a 15% perioperative morbidity would increase the interval to the break even point to more than 4 years before the benefits of surgery would be apparent (Chambers & Norris 1984).

These results advocate a conservative approach to asymptomatic carotid disease and patients with cervical bruit, endorsed by Ellis & Greenhalgh (1987). In a study comparing bruit patients with age and sex matched controls, they recorded a significantly higher rate of transient ischaemic attacks at 26.5% in bruit patients compared to 6% in controls over 5 years. The rate of stroke, without anticedent

TIA, was 9% in bruit patients and 8% in controls. This would suggest that it is appropriate to wait for the onset of symptoms before considering surgery.

Transient ischaemic attacks

The case for surgery is more widely accepted in patients suffering transient ischaemic attacks (TIA) as the risk of stroke is far higer than in those with asymptomatic disease. The overall incidence of stroke following TIA has been reported by various authors at 36%, 22% and 12% at 3 years after the onset of symptoms (Siekert, Whisnant & Millikan 1963; Baker, Ramseyer & Schwartz 1968; Fields et al 1970). The Mayo Clinic reported a stroke rate of 45% over 5 years in untreated TIA patients and although this improved to 24% with anticoagulants, it never approached the 5% stroke rate in an age matched population over the same period (Whisnant, Matsumoto & Elveback 1973). In a further study, anticoagulants appeared to reduce the recurrence of TIA but did not affect the incidence of stroke recorded at 5% per year (Toole et al 1975).

The results of medical therapy were comparable to the early results of surgery when endarterectomy produced a significant rate of postoperative neurological deficit. In a series of patients who had carotid surgery between 1958 and 1963, combined perioperative and postoperative complications were identified in 26% of patients followed up for 5 to 9 years (Edwards, Wilson & Bennett 1968). However more recent series have been reported with an operative

morbidity and mortality of less than 5 percent (Javid et al 1979; Thompson 1980; Browse & Ross Russell 1984). In order to compare medical and surgical treatments the incidence of stroke in the years following surgery must be considered with the perioperative neurological deficits. At 5.5 years the overall stroke rate was 12%, or 2% per year in operated patients presenting with TIA (Stewart, Ross Russell & Browse 1986), compared with 5% per year in medically treated patients (Whisnant et al 1973).

Established stroke and progressing stroke

Carotid endarterectomy is performed less frequently in patients with completed stroke than for TIA according to a survey of British Vascular Surgeons (Murie & Morris 1986). Ninety-six percent stated that they would perform carotid surgery "often" or "sometimes" for TIA but only 56% of surgeons would operate for minor stroke and 23% for established stroke. This reflects the view that a completed ischaemic event has occured and will not be improved by revascularisation. Carotid endarterectomy is performed primarily for the relief of symptoms, usually TIA, and as prophylaxis against stroke. Of all patients with atherosclerotic carotid disease surviving their initial stroke, 50% will suffer a second stroke within 8 years, a rate of 9% per year (Walton 1977; Laugton-Hewer 1983). The risk of further stroke seems to be inversely related to the severity of the original stroke. Recurrence is rarely seen in patients with major neurological damage and carotid surgery is only appropriate for patients with mild or moderate deficits (Marquardsen

1983). This group of stroke patients has a higher operative mortality of 6.1%, compared to 1.1% in TIA patients, but this may reflect of the severity of their vascular disease (Thompson, Austin & Patman 1970). A combined operative morbidity and mortality of 4.5% and stroke rate of 1.4% per year over 5 years compares favourably with medical management (Rubin et al 1986).

Early endarterectomy for acute major stroke had the highest operative mortality as ischaemic infarcts were thought to be converted to haemorrhagic lesions following revascularisation (Wylie, Hein & Adams 1964). However those few patients with either cresendo TIA or progressing stroke represent a sub group which may benefit from surgery. Medical treatment failed to arrest the progress of stroke in 74% of patients with progressing stroke and 14% died (Millikan 1973). In uncontrolled reports of surgically treated patients, stroke progression was stopped or neurological status improved in 70%, 91% and 100% after emergency endarterectomy. (Mentzer et al 1981; Greenhalgh et al 1985; Goldstone & Moore 1978). Surgery may therefore be appropriate in this group which otherwise has a poor prognosis.

1.4 Complications Specific to Carotid Endarterectomy

1.4.i Neurological

The most serious complication of carotid endarterectomy is the occurrence or exacerbation of neurological deficits at the time of surgery or in the early postoperative period. These can be either of

a transient nature, resolving completely within 24 hours, or permanent defects which indicate irreversible cerebral damage (Browse & Ross Russell 1984). The rates of perioperative stroke vary considerably between different series. Prioleau reported 34 strokes following 317 operations and Easton described 10 strokes after 68 procedures to give overall stroke rates of 10.7% and 14.7% respectively (Prioleau, Alken & Hairston 1977; Easton & Sherman 1977). De Weese identified perioperative stroke in 6% of patients with a further 15% suffering transient deficits in the 5 days after surgery (De Weese et al 1973). Prospective clinical studies have shown the lowest rates of permanent operative deficits to be approximately 1%, with 2% for transient defects (Rich & Hobson 1975; Javid et al 1979; Browse & Ross Russell 1984).

The endpoints defined in these series were all based on clinically detected stroke and did not include the asymptomatic or silent cerebral infarct. In a study of TIA patients, who had pre and postoperative computerised tomographic brain scans (CT), a clinical morbidity was identified in 4% of patients (Berguer et al 1986). In each of these cases a new defect was seen on the second CT scan. In addition radiological evidence of cerebral infarction was seen in a further 8% of otherwise asymptomatic patients, giving an overall operative morbidity of 12% on CT assessment.

Perioperative neurological deficits are caused by two distinct mechanisms; thromboembolism and cerebral ischaemia. In order to

avoid embolism of platelet thrombi or loose debris from necrotic atheromatous plaque, careful mobilisation of the carotid artery is necessary. Platelet aggregates may also form during the closure of the arteriotomy and these can be removed by back bleeding and flushing of the endarterectomy before flow is reestablished to the internal carotid artery (Thompson 1980). Cerebral ischaemia will occur if the collateral blood flow is insufficient during the period of carotid occlusion and the regional cerebral blood flow is less than 18-23 ml/100g/min (Sundt et al 1974). Although induced hypertension and hypocapnia have been used for cerebral protection, these methods have been replaced by the insertion of an intraluminal shunt (Ehrenfeld et al 1970; Hays, Levinson & Wylie 1972).

The assessment of collateral cerebral blood flow and the neccessity for an intraluminal shunt is measured by the response of certain parameters to temporary carotid occlusion. Changes in neurological status in the conscious patient, a reduction in internal carotid artery stump pressure or alteration of the electro-encephalogram have all been assessed as indicators of insufficient cerebral blood flow (Yao & Bergen 1979). However potential cerebral ischaemia is identified in varying proportions of patients by each of these methods. In a series of 359 endarterectomies performed under local anaesthesia, only 4% required an intraluminal shunt on the grounds of neurological deterioration following carotid occlusion for 60 seconds (Steed et al 1982). Using a carotid artery stump pressure of less than 50mm Hg as the critical value for shunt insertion, 100

out of 175 cases (57%) required intraluminal shunts (Hertzer et al 1978). However assessment of cerebral ischaemia by EEG monitoring suggested that shunts were required in 23 out of 213 cases (11%) where ischaemic changes were seen on EEG tracings during carotid occlusion (Baker et al 1975).

Despite varying shunt policies, perioperative stroke has not been seen more frequently in large series of unshunted patients compared to those where shunts are inserted routinely (Baker, Dorner & Barnes 1977; Javid et al 1979). This implies that the majority of neurological deficits related to surgery are caused by thrombo-embolism rather than cerebral ischaemia. In 345 endarterectomies performed without a shunt, under local anaesthetic, the timing of onset of deficit was determined. Of the 21 operative neurological defects (6%) only 1 (0.3%) occurred during carotid occlusion and was related to cerebral ischaemia, while the other 20 were detected at the time of arterial mobilisation, clamp release or in the first 5 postoperative days and were attributed to embolism or reperfusion injury (Steed et al 1982). A subgroup which would benefit from cerebral protection was seen in a review of 940 unshunted carotid procedures. Patients with a carotid stump pressure of less than 50mm Hg and a contralateral carotid occlusion were 11 times more likely to suffer peri-operative neurological deficit than patients with a normal pressure and a patent carotid on the opposite side (Baker et al 1984). This combination of contralateral occlusion and low stump

pressure may represent a high risk subgroup which requires intra-luminal bypass to avoid cerebral infarction.

1.4.ii Thrombosis

Although thrombosis following vascular reconstruction is always serious it may be catastrophic after carotid endarterectomy. Acute thrombosis with carotid occlusion often results in extensive cerebral infarction with irreversible hemiplegia. This was seen after 5 out of 326 endarterectomies (1.5%) where occlusion was confirmed at reoperation (Novick, Millili & Nemir 1985).

The incidence of asymptomatic thrombosis is more difficult to assess. Nineteen of 300 patients (6%) were thought to have postoperative thrombosis on routine postoperative oculo-plethysmography and 3 of these suffered fatal strokes with internal carotid occlusion substantiated at post mortem (Ortega et al 1981). Occlusion was confirmed in only 7 of the remaining 16 by either angiography or reoperation and all of these were asymptomatic. In a study of operative angiography, 2 out of 260 patients (0.8%) were found to have complete internal carotid occlusion after closure of the arteriotomy and 5 more (2%) had non-occlusive thrombosis. In each case thrombectomy was performed and none of these patients suffered a neurological deficit (Rosental et al 1973). This would suggest that thrombotic occlusion causes stroke in 1% of patients after endarterectomy with asymptomatic occlusion occurring in a further 2-5%. Immediate post-operative thrombosis will account for a

proportion of the 6% of patients found with total occlusion in late studies of restenosis (Glover et al 1985).

Data on the pathological appearances of the arterial wall in acute carotid thrombosis is scarce due to the few instances of this complication. French examined 10 arteries from patients who died in the early postoperative period, finding 5 thrombosed and 5 patent vessels. The three consistent histological features reported in both thrombosed and patent vessels were the acute inflammatory reaction, necrosis of the media of the arterial wall and adherent mural thrombus. More extensive inflammation and medial necrosis was seen in those arteries with occlusive thrombus but the most intense inflammatory response was observed in arteries where the greater part of the media had been removed, leaving only a thin medial layer on the adventitia (French & Rewcastle 1974).

1.4.iii Cardiovascular

Myocardial infarction is the major cause of morbidity and mortality following carotid endarterectomy and is more frequent than stroke in patients with asymptomatic carotid disease (Burke et al 1982). This reflects the eldery population undergoing this procedure with 80% of patients having previous history of heart disease, hypertension or diabetes (Whisnant, Cartlidge & Elveback 1978). Overall, myocardial infarction was associated with carotid surgery in 2.3% of 683 patients, but was confirmed in 4.9% of 284 patients with evidence of ischaemic heart disease. This contrasted with an

infarction rate of 0.5% in the remainder (Riles, Kopelman & Imperato 1979).

Postoperative hypertension is common following carotid endarterectomy and is a significant cause of morbidity (Caplan, Skillman & Ojemann 1978). Pre-operative hypertension is found in more than 50% of all patients and a significant rise in systolic pressure, to more than 220mm Hg, was detected in 19% of patients (Bove, Fry & Gross 1979). The aetiology of postoperative hypertension has been attributed to carotid sinus stimulation and correlated with postoperative neurological complications (Angell-James & Lumley 1974). Neurological deficit was detected in 10.2% of patients with postoperative hypertension compared to 3.4% in the normotensive group (Towne & Bernhard 1980).

1.4.iv Wound and cranial nerve

Haemorrhagic complications occur in 3% of cases following carotid surgery although less than half will involve the arterial suture line (Nunn 1975). The skin has a rich dermal blood supply which results in minor postoperative bleeding and may be compounded by the administration of platelet inhibitory therapy (Towne 1980). The dissection requires the division of several large veins, most notably the anterior facial vein, and since coughing or valsalva may cause a ligature to slip, transfixion of these vessels is recommended (Browse & Ross Russell 1984).

Operative damage to the cranial nerves and their branches is recognised and can be detected in 4-17% of cases (Towne 1980). Division of the greater auricular or superficial transverse cervical nerves is more frequent and paraesthesia is seen in up to 45% of patients. An inappropriately high incision can endanger the mandibular or cervical branches of the facial nerve (VII) causing labial asymmetry (Dehn & Taylor 1983).

Injury to the hypoglossal nerve (XII) has been reported to occur in up to 17% of patients undergoing carotid endarterectomy and can result in tongue deviation, dysarthria and difficult with mastication (Hertzer et al 1980; De Weese et al 1973). The XII nerve is exposed as it crosses antero-lateral to both the external and internal carotid arteries above the bifurcation. In order to achieve sufficient distal exposure it may be neccessary to mobilise this nerve. This is facilitated by dividing the descendens hypoglossi and the sternomastoid branch of the external carotid artery which tethers the nerve laterally (Verta et al 1976). The possibility of XII nerve damage should always be considered when contemplating bilateral operation, either synchronously or as a staged procedure (Bageant, Tondini & Lysons 1975).

Damage to the vagus nerve (X) is less common, however postoperative voice changes and vocal chord palsies may result from injuries to the superior and recurrent laryngeal nerves (Evans, Mendelowitz & Liapis 1982). The posterolateral relationship of the

vagus nerve to the carotid artery makes it vulnerable to damage from arterial clamps, particularly during the insertion of an intra-luminal shunt (Dehn & Taylor 1983).

1.4.v Restenosis

Before the developement of non invasive techniques of carotid assessment, recurrent stenosis was identified almost exclusively in patients with recurrence of either neurological symptoms or cervical briut (Thompson 1970). The published series of angiographic follow up after carotid surgery were small with selected patients studied and few of these were asymptomatic (Blaisdell, Lim & Hall 1967; Schutz, Fleming & Awerbuck 1970).

Restenosis was first examined in a review of 1654 carotid endarterectomies reported by Stoney and String in 1976. They identified 32 stenoses in 29 patients of whom 27 were symptomatic, between 5 months and 13 years after surgery. Histology of the resected lesions showed intimal fibrosis in those seen less than 2 years after surgery but later restenosis was caused by recurrent atherosclerosis. It was this paper which prompted comments from Imparato, Callow and Kartchner who all suggested that symptomatic lesions could represent only a small proportion of all restenoses and that the true incidence of restenosis was unknown (Stoney & String 1976). At that time reoperation was being performed in approximately 4% of patients and half of these were for asymptomatic restenosis. Recurrent symptomatic restenosis was found in less than 2% of

patients following carotid endarterectomy (Cossman et al 1978; Hertzer, Martinez & Bevan 1979).

The impact of non-invasive assessment was first noticed in a study of oculoplethysmography (OPG) following endarterectomy. This identified restenosis in 12% of patients although only 1.7% were symptomatic (Kremen et al 1979). This prompted further reports on this subject using increasingly sensitive non-invasive techniques. Restenosis was found in 29 out of 199 arteries (14.6%) examined by OPG and phonoangiography (Cantelmo et al 1981). Doppler spectral analysis initially indicated restenosis in 36% of patient studied in the year after surgery (Zierler et al 1982), however this high rate was not confirmed at follow up beyond 12 months and may represent either regression of disease or error in the technique.

Using the more accurate technique of Duplex scanning, restenosis was identified in 15% of patients, although this was judged to be greater than 50% of the luminal diameter in only 9% (Thomas et al 1984). Subsequently restenosis rates of between 6.7% and 22% were reported from major series, using either Duplex ultrasound or digital subtraction angiography and all confirmed the previous supposition that recurrent symptomatic restenosis occured in only 1-2% of patients (Table 1.1). In contrast to the original reports of symptomatic restenosis with follow up extending to 13 years (Thompson 1982), follow up in these non-invasive studies ranged from only 6 months to 4 years with a mean of less than 24 months and represented

	Patients	Restenosis %
Edwards et al 1968	93	3.2
DeWeese et al 1968	227	2.7
Schutz et al 1970	39	5
Stoney & String 1976	1654	1.5
Cossman et al 1978	360	3.6
Hertzer et al 1979	1250	1.04
Thomson et al 1982	1286	0.86
Thomas et al 1984	257	9
Pierce et al 1984	139	6.7
Keagy et al 1985	122	22
Colgan et al 1984	80	12.5
Nicholls et al 1985	145	22
Glover et al 1985	155	16
Ackroyd et al 1986	292	15

Table 1.1 Reported incidence of restenosis after carotid endarterectomy. Prior to the use of non-invasive assessment, cases of restenosis were only identified in patients with recurrent symptoms of cerebrovascular insufficiency or cervical bruit.

early postoperative restenosis (Pierce et al 1984; Colgan, Kingston & Shanik 1984; Keagy et al 1984; Glover et al 1985; Nicholls et al 1985; Ackroyd, Lane & Appelberg 1986).

Histologically, carotid restenosis may be due to recurrent atheroma or intimal hyperplasia but the pathogenesis of either process remains obscure (French & Rewcastle 1977). Technical factors may be responsible in some cases and clamp damage, intimal flaps and failure to remove the distal tongue of plaque have all been implicated (DePalma et al 1977; Edwards et al 1968; Javid et al 1970).

The macroscopic and histological differentiation between intimal hyperplasia and recurrent atheroma has been described in detail (Cossman et al 1978). In the postoperative period and up to 24 months after surgery the lesion is smooth, fibrous and without ulceration. Microscopy reveals spindle shaped nucleated cells in a fibromyxomatous matrix, without the neovascularisation or lipid deposition characteristic of recurrent atheroma. Whether or not the earlier lesion of intimal hyperplasia is a precursor of the later recurrent atheroma remains unanswered. There is no histological similarity between the fibrous stroma of hyperplasia and the ulcerating lipid lesion of atheroma (Callow 1982) and until a transitional stage linking the two separate pathological appearances is identified, they must be regarded as separate entities.

The role of platelets in restenosis has not been defined although platelet hyperaggregability was suggested as a predisposing factor (Stoney & String 1976). Similarly the effect of aggregating platelets on the exposed media of the arterial wall may be responsible for the hyperplastic reaction which causes intimal thickening and restenosis (Thomas et al 1984; Das et al 1985)

1.5 Measurement of Restenosis

The measurement of restenosis of the carotid artery after endarterectomy fortunately may utilise the same imaging techniques used to define carotid disease.

1.5.i Arteriography

Before 1980, assessment of arterial stenosis was limited to the only effective method of radiological imaging, contrast angiography. This was performed either by direct carotid puncture or the technique of retrograde cannulation (Steiner et al 1984; Seldinger 1953). Despite the excellent images obtained, major complications were seen in approximately 1% of cases (Plate 1.3). Haemorrhage, luminal thrombosis and false aneurysm occurred at the site of arterial puncture and 1% of patients may have an anaphalactic reaction to the contrast medium. In the case of cerebral angiography, stroke may result from the catheterisation of the arch vessels (Maini, Eisenberg & McDonald 1978). This morbidity restricted these imaging procedures to those patients who were likely to undergo surgery and there could

be no justification to repeat conventional angiography in routine follow up of postoperative patients.

Digital Subtraction Angiography

With the exception of allergic response to radioopaque dye, which may occur with the injection of any contrast medium, the complications of angiography are attributable to the introduction, manupulation and positioning of the intra-arterial catheter (Cronquist, Efsing & Palacios 1970). Injection of the contrast by an intravenous route avoids the arterial puncture but by the time that the bolus reaches the arterial phase it is too dilute to give adequate definition on a standard radiograph (Cebul & Paulus 1986). This problem has recently been overcome by the use of Digital Subtraction Angiography (DSA) in which a radiological image without contrast is subtracted from one with contrast and the resulting angiogram improved by digital enhancement (Butler 1986). Although such dilute contrast may provide satisfactory arterial images in some cases, definition sufficient for surgery may not be always be achieved in the carotid arteries (Anderson & Fischer 1985; Crocker, Tutton & Bowen 1986). In this situation intra-arterial contrast can be introduced into the ascending aorta through a fine 5 French gauge catheter. An arch flush DSA may be performed without the morbidity associated with individual catheterisation of the common carotid arteries (Plate 1.4, 1.5) (Sumner et al 1985). This technique has been found to have an accuracy of 94%, which is comparable with conventional angiography, without its complications, and is superior

Plate 1

1.1 Endarterectomy in progress. With the intraluminal shunt in place, the atheromatous tissue is removed with the plane of dissection through the media.

1.2 After endarterectomy the vessel wall appears smooth, but in the absence of vascular endothelium this surface will cause an intense thrombotic reaction. Once the endarterectomy is closed a layer of thrombus forms which persists until healing occurs with the ingrowth of intima.

1.3 Selective carotid arteriograph: a catheter is introduced into the origin of the common carotid bifurcation and its branches. This patient has a tight internal carotid artery stenosis which was causing transient ischaemic attacks.

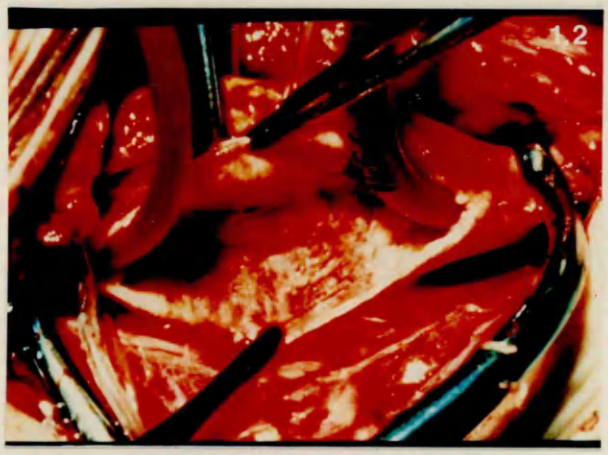
1.4 Digital subtraction angiography obtains comparable images without the morbidity of standard arteriography. This patient has severe bilateral internal carotid artery stenosis.

1.5 Intra-arterial DSA shows different patterns of disease with a deep atheromatous ulcer at the origin of the right internal carotid artery and 2 eccentric stenoses of the proximal left ICA. This patient had right carotid territory symptoms.

1.6 A composite image of Duplex scanning. B-mode imaging shows the common and internal carotid arteries with stenosis at the origin of the ICA. Doppler frequency analysis is displayed in the lower part of the screen confirming a haemodynamically significant lesion.


1.1


1.3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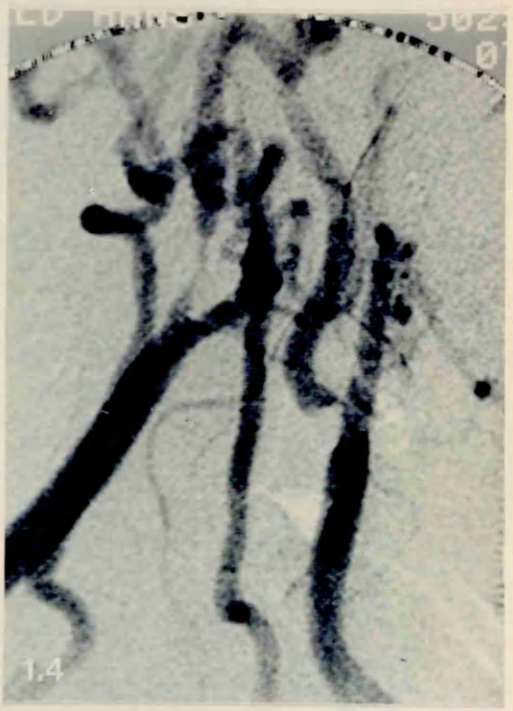
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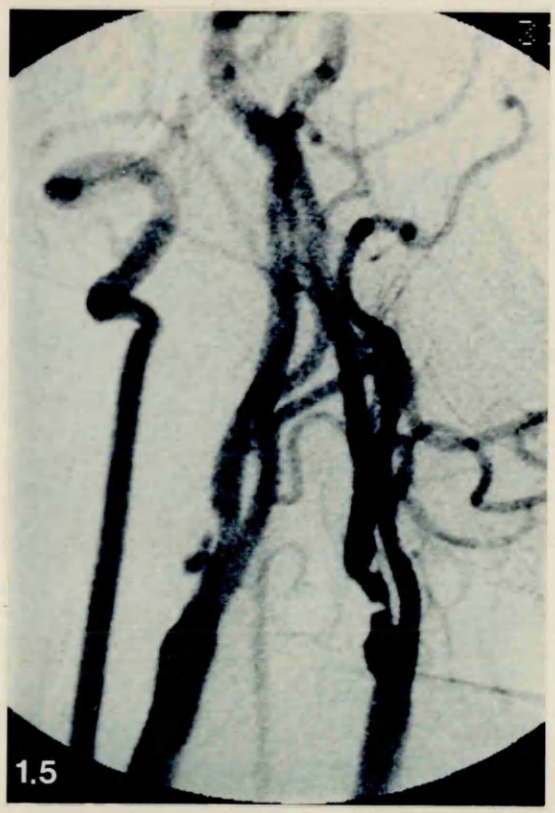
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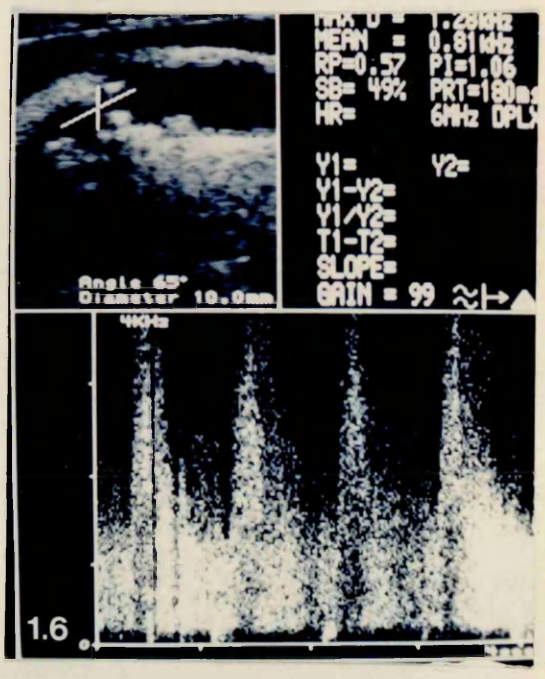
MEAN =
RP=0.57
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HR=
Y1=
Y2=
Y1-Y2=
Y1/Y2=
T1-T2=
SLOPE=
GAIN = 99
Angle 65°
Diameter 10.0mm
1.6

to intravenous DSA in both accuracy and the volume of contrast injected (Reilly, Ehrenfeld & Stoney 1984).

1.5.ii Non invasive assessment of restenosis

Despite the refinements in technique and improvements in radiological imaging, arteriography remains an invasive procedure with an appreciable morbidity of 4% for intravenous and 7% for intra-arterial DSA (Reilly et al 1984). Assessment of the carotid bifurcation is required in patients with atypical symptoms pre-operatively and for sequential follow up in the postoperative period. This has lead to the development of various non-invasive techniques which assess carotid artery disease. These investigations utilise both pulsed Doppler which measures blood flow and B-mode ultrasound which visualises arterial stenoses and atherosclerotic plaque in the vessel wall (Strandness 1985).

A combination of these methods is presently available as Duplex Ultrasound (Hames et al 1985). In these instruments real-time, B-mode ultrasound and pulsed Doppler transducers are combined in the same probe. The pulsed Doppler beam is positioned under imaging control and frequency spectral analysis measured at the site of stenosis (Plate 1.6) (Roederer et al 1983). Duplex scanning is particularly appropriate for the assessment of patients after carotid endarterectomy, in that it has none of the complications of angiography and can be used repeatedly in the follow up period (Nicholls et al 1985). Its overall accuracy of 80% and the ability

to detect a diameter reduction of greater than 50% in 94% of cases justifies its clinical use in the assessment of carotid restenosis (Roederer et al 1983).

1.6 Summary

Carotid endarterectomy has been performed for more than thirty years in the treatment of patients with carotid atherosclerosis. In centres with low operative morbidity and mortality, the risks of surgery are probably acceptable relative to the benefits of symptomatic relief and improvement in stroke free survival, although a definitive controlled trial has yet to be performed (Warlow 1984).

Restenosis occurs in approximately 15% of patients in the first 2 years after surgery, however less than 2% develop recurrent neurological symptoms on the side of recurrent narrowing. Either conventional or digital subtraction arteriography carries too great a morbidity to justify its use for sequential follow up of these patients and is only indicated when detailed anatomical information is required before operation.

In order to investigate the incidence of restenosis, Duplex scanning offers an appropriate alternative for postoperative assessment of the carotid bifurcation. The combination of continuous wave and B-mode ultrasound has no recorded morbidity and gives information on blood flow, the anatomical structure of the arterial wall and plaque morphology.

Chapter 2

Platelets, Thrombosis and Intimal Hyperplasia

2.1 Platelets and the arterial wall

The function and morphology of platelets and their relationship with the structure of the arterial wall is considered before examining the interaction of platelets with the carotid artery following endarterectomy.

2.1.i The Platelet

Platelets are derived from megakaryocytes by endomitosis and cleaving of the cytoplasm (Penington, Streatfield & Roxburgh 1976). In man the platelet count is normally maintained between 250 and 450 x 10^9/l and at any time approximately 20% of the platelet pool is held within the spleen, where it is thought that the more active and older platelets are removed from the circulation (Penny, Rozenberg & Firkin 1966).

The platelet is disc shaped, measures 2-4um in diameter and is approximately 1um in thickness. Both granules and vesicles within the cytoplasm have been demonstrated by electron microscopy. The vesicles contain liposomal hydrolytic enzymes (Bentfeld & Bainton 1975) and the granules can be divided into dense bodies and alpha granules (White 1974; Gordon & Milner 1976). Adenosine diphosphate, adenosine triphosphate, serotonin and calcium have been identified in the dense bodies (Weiss 1975a; Weiss 1975b) whilst alpha granules are thought to store platelet factor 4, beta-thromboglobulin and platelet derived growth factor (Weiss et al 1977).

The two major functions attributed to the platelet are to support the integrity and physiology of the vessel wall and to participate in thrombosis and haemostasis (Firkin 1984).

2.1.ii Arterial wall

Arteries are made up of the three concentric layers of intima, media and adventitia (Fig 2.1). Separating these layers are the internal elastic lamina between the intima and media, and the external elastic lamina between the media and adventitia. The intima consists of two layers, endothelium and subendothelium which contain smooth muscle cells (French 1971). The media is composed of smooth muscle, collagen and elastic fibres which respond to the mechanical demands made on the vessel and allow a uniform distribution of tension throughout the wall (Wolinsky & Glagov 1964). The adventitia consists of a loose arrangement of connective tissue containing vasa vasorum, lymphatics and nerve fibres which are in contact with the surrounding tissues (Woolf 1982).

The endothelium lining the intima constitutes the first site of contact between blood and the vessel wall. This monolayer of flattened cells has been described as the guardian of the smooth muscle cells of the intima (McMillan 1978). Arterial endothelium is selectively permeable to allow diffusion of nutrients (Landis 1927) but must also maintain a non-thrombogenic surface in order to prevent platelet acculumation (Mason et al 1977).

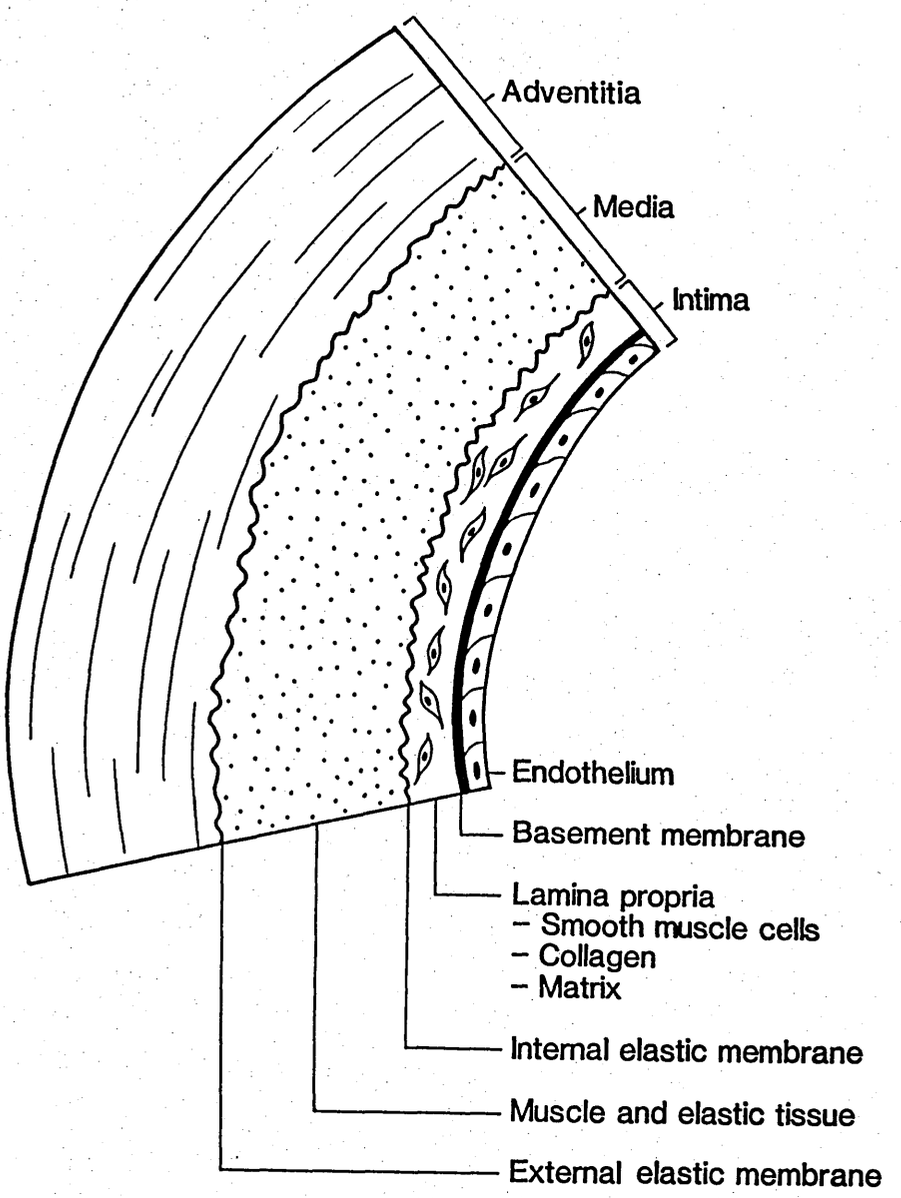


Figure 2.1 The arterial wall consists of concentric layers of connective tissue and smooth muscle cells which are separated from blood by the vascular endothelium.

Although both endothelial cell membrane glycoproteins and cell wall surface charge may play a passive role in the repulsion of platelets, it is the secretion of locally active hormones that are thought to be responsible for antithrombotic activity (Risberg, Peterson & Zettergren 1975). These include plasminogen activator and thrombin binding substance (Aubrey, Hoak & Owen 1979), however the pulmonary endothelium removes many of these vasoactive substances from the circulation (Vane 1969). The platelet inhibitory activity of the vascular wall is thought to be mainly effected by the release of prostacyclin (PGI_2), which was recognised in 1976 (Moncada et al 1976; Moncada 1982).

2.1.iii Arachidonic acid metabolism

Arachidonic acid (Eicosa - 5,8,11,14 - tetraenoic acid) is a polyunsaturated fatty acid. It is the precursor of all binenoic prostaglandins and is liberated from membrane phospholipids by the action of phospholipases (Vonkeman & van Dorp 1968). The enzynme cyclo-oxygenase (prostaglandin synthetase) metabolises arachidonic acid into the prostaglandin endoperoxide PGG_2 and subsequently to the stable compounds PGE_2, PGF_{2a} and PGD_2 via the intermediate PHG_2 (Fig 2.2) (Hamberg & Sanuelson 1974).

However in platelets an unstable intermediate product, thromboxane A_2 was identified (Hamberg, Svensson & Samuelsson 1975) which was found to be identical to aorta contractile substance (Vane 1969). Thromboxane A_2 (TxA_2), which has a half life of only 30

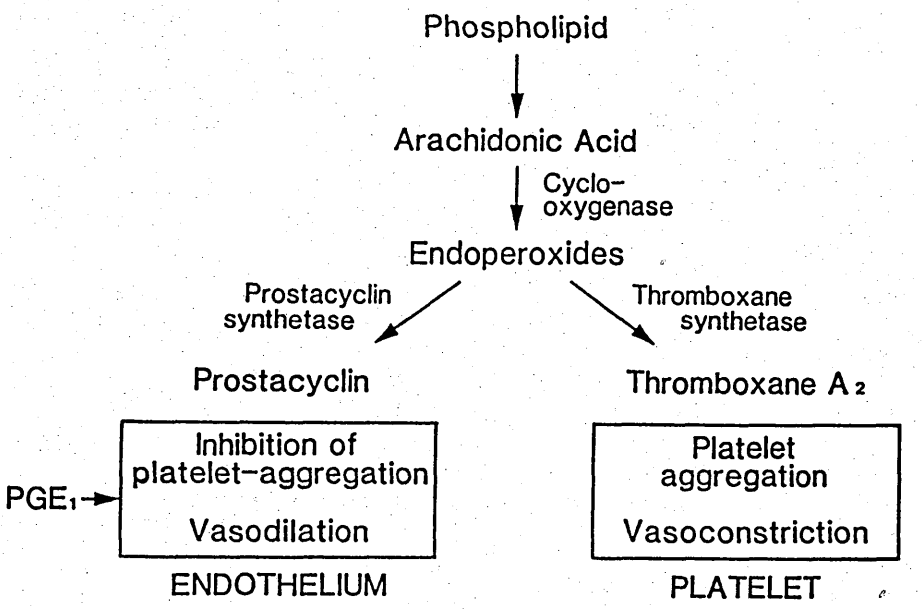


Figure 2.2 Arachidomic acid metabolism in vascular endothelium and platelets.

seconds, is a potent vasoconstrictor and platelet activating agent (Gorman et al 1977) and is converted to the stable thromboxane B_2 (TxB_2), which may be assayed in serum and urine (Hamberg et al 1975).

In the vascular endothelium, prostacyclin synthetase converts anti-aggregatory PGH_2 into the unstable product PGI_2, which is known as prostacyclin (Moncada et al 1976). This conversion can occur from both endogenous and exogenous platelet derived endoperoxide (Moncada & Vane 1978). Alternatively the prostaglandin endoperoxides may be converted to eicosanoids via the 5- or 12- lipoxygenases (Moncada 1982), or into leukotrienes which are found in leucocytes (Murphy, Hammarstrom & Samuelsson 1979). Eicosanoids may be related to slow reacting substance detected in immediate hypersensitivity reactions (Austen 1978).

While cyclo-oxygenase is found in all cells except erythrocytes, lipoxygenases have been identified only in platelets, lungs, white cells, blood vessels and epicardium. However prostacyclin synthetase is thought to be present solely in vascular endothelium (Vane, Bunting & Moncada 1982; Moncada et al 1976). Prostacyclin has a half life of about 3 minutes and is the most potent endogenous platelet inhibitor known (Whittle, Moncada & Vane 1978; Vane 1982).

2.2 Platelet Involvement in Thrombosis and Coagulation

Normal haemostasis depends on interaction between the vessel wall, platelets and clotting factors (Johnson 1971). Vessel wall injury releases tissue thromboplastin which activates prothrombin. This synthesises thrombin which causes platelet activation and adhesion to the damaged vessel wall (Mustard, Packham & Kinlough-Rathbone 1981). Platelet activation is associated with rapid extension of pseudopodia and a change from disc to a spherical shape (Schick 1979).

All aggregating agents in adequate concentrations can cause the platelet to release arachidonic acid by the action of phospholipase A on phospholipid in the platelet membrane. In the presence of cyclo-oxygenase this leads to the formation of thromboxane A_2 (Marcus 1982). Different concentrations of activating agents produce two distinct phases of aggregation. In response to low concentrations of ADP, phase I aggregation occurs without granule secretion, effected by fibrinogen cross-linking of platelets (Polley et al 1981). This is reversible although platelets become refractory to subsequent exposure to ADP (Mills 1981). Phase II aggregation occurs with higher concentrations of aggregants and follows the release of the aggregatory products ADP, 5-HT (serotonin) and von Willebrand factor from the dense bodies (Zucker & Nachmian 1985). In these circumstances aggregation is irreversible and degranulation occurs with the release of ADP and TxA_2 which perpetuates the process (Mustard et al 1981).

The release reaction is effected by high levels of platelet cytoplasmic calcium in a response to TxA_2, which stimulates calcium ion transport across membranes and increases calcium flux from the dense tubular system into the cytoplasm (Gerrard, Peterson & White 1981). Platelet activation is accompanied by change in the intracellular distribution of calcium and a rise in cyclic GMP but not 3-5-cyclic adenosine monophosphate (cyclic AMP) (Gerrard et al 1977). Conversely, the prostaglandins which inhibit platelet aggregation activate adenyl cyclase with increased production of intracellular cyclic AMP (Holmson & Karpatkin 1983).

Although thrombus normally consists of fibrin, platelets and enmeshed red and white cells, blood coagulation may proceed in the absence of platelets. Phospholipids in intact platelet membranes have been found to accelerate coagulation due to platelet factor-3 activity (Marcus 1967) and the rate of conversion of prothrombin to thrombin is increased in the presence of a platelet membrane receptor for an activated form of factor V (Tracy et al 1980). Clot retraction occurs only in the presence of platelets, possibly involving the interaction of platelet membranes with fibrin starch and the platelet actinomyosin contractile mechanism (Cohen et al 1979). Other pathways have been proposed, including ADP-induced factor XII activation, but the physiological significance of these in vitro observations remains to be determined (Walsh 1979).

2.2.i Hormonal regulation of platelet activity

The positive feedback effect of ADP causing further platelet aggregation would continue if unopposed. Vascular endothelium synthesises prostacyclin from its own endogenous precursors but may also use platelet released prostaglandin endoperoxides to produce PGI_2 (Marcus et al 1980). In a similar manner the endothelium may convert platelet released ADP to adenosine which inhibits further platelet aggregation (Walsh 1979). Whilst locally secreted PGI_2 is effective, dilution by the blood stream ensures that under basal conditions plasma levels are insufficiently high to inhibit platelet function (McIntyre 1981).

Prostacyclin inhibits platelet aggregation at much lower concentrations than those needed to inhibit platelet adhesion to collagen. Platelet attachment to vascular endothelium may produce sufficient endoperoxide substrates to raise local levels of PGI_2 then preventing further aggregation (Higgs et al 1978).

Prostacyclin inhibits platelet aggregation by stimulating adenyl cyclase which leads to increased cyclic AMP levels in platelets (Gorman et al 1977). This directly opposes the action of Thomboxane A_2 which induces platelet aggregation but the mechanism which controls the balance of haemostasis is not yet clear (Moncada & Vane 1978). Platelet aggregation in response to TxA_2 may be mediated by increased cytoplasmic concentrations of calcium following its release

from vesicles and consequent inhibition of cyclic AMP (Holmson & Karpatkin 1983).

2.2.ii Physical factors influencing platelet involvement in thrombosis

Although thromboxane and prostacyclin determine the biochemical basis for thrombosis, certain physical factors may produce the conditions which predispose to platelet interaction with the vessel wall. Increased blood velocity will reduce the time available for a platelet to adhere to an aggregating surface. Arterial branch points, stenoses and surgical anastomoses create turbulence which increases the collision rate of platelets with the intima in selected areas. In a model measuring platelet deposition in rabbit aortas, reduced flow was associated with less platelet adhesion (Baumgertner 1973) and subsequently higher blood velocity and arterial wall shear stress were found to increase platelet deposition. Above a critical value of shear stress, platelet deposition decreased due either to the removal of platelets by high shear or inadequate time for platelets to bond to the endothelial surface (Turitto, Muggli & Baumgartner 1977).

Local occurence of atherosclerosis may be linked to the differential flow patterns and shear stress at the carotid bifurcation. The carotid bulb has been examined by hydrogen bubble visualisation in a glass model (Zarins, Giddens & Glagov 1982). Rapid laminar flow with high shear was seen on the inner wall of the

carotid bulb while on the outer wall there was an area of flow separation with complex secondary and tertiary flow patterns. Although stasis was not observed, this area of low shear was found to correlate with the preference of atherosclerotic plaque for this region.

Electrostatic charge may also prevent platelet adhesion as the intact vascular intima is covered with negatively charged proteoglycans. Platelets carry a similar negative charge but the importance of this electrostatic repulsion between endothelium and platelets has never been adequately investigated (Wight 1980).

2.3 Platelet Inhibitory Compounds

Most platelet inhibitors act on the arachidonic acid pathway or on ionophore production.

2.3.i Agents acting on arachidonic acid metabolism

Drugs interfering with arachidonic acid metabolism prevent the production of TxA_2. They do not influence primary collagen or thrombin mediated aggregation but inhibit the secondary aggregation caused by the release of TxA_2 from the alpha granules (Packham & Mustard 1980; Kelton & Hirsh 1981).

Aspirin irreversibly inhibits platelet cyclo-oxygenase by acetylation of the amino-terminal serine residue and this lasts for the life of the platelet (Burch, Stanford & Majerus 1978).

Cyclo-oxygenase inhibition in the endothelium reduces the production of prostacyclin (Smith & Willis 1971) and may diminish the platelet inhibitory effect when the compound is used to prevent platelet-intimal interaction on autologous vein grafts and following endarterectomy (Wu et al 1981). This may be reflected in the differing effects of high and low dose aspirin. The increased bleeding time seen with low dose aspirin (3-6 mg/kg) in both rabbits and humans is reversed by high doses (40-80 mg/kg) and may manifest a dose related inhibition of endothelial cyclo-oxygenase with reduced prostacyclin production (O'Grady & Moncada 1978).

Cyclo-oxygenase is also irreversibly inhibited by other non-steroidal anti-inflammatory drugs such as indomethacin, mefanoic acid and phenylbutazone while sulfinpyrazone is a competitive and reversible inhibitor of platelet cyclo-oxygenase (Packham and Mustard 1980; Kelton & Hirsh 1981).

The combined effect of cyclo-oxygenase inhibition on thromboxane A_2 production in the platelet and prostacyclin synthesis in the endothelium has lead to the investigation of drugs which act specifically on thromboxane (Needleman et al 1977). Dazoxiben, which blocks the conversion of cyclic endoperoxides to TxA_2 does not block the synthesis of PGI_2 in endothelial cells (Marcus et al 1980), and a specific thromboxane A_2 antagonist has been shown to be effective in reducing platelet aggregation in vitro to a thromboxane mimetic (Coleman et al 1981).

2.3.ii Compounds that increase cyclic AMP levels

Prostaglandins increase the concentration of PGI_2, E_1 or D_2 and cause platelet inhibition by stimulating adenylate cyclase. This produces cyclic AMP which lowers the concentration of calcium in the cytoplasm (Gorman & Marcus 1981). In contrast to aspirin, which inhibits secondary platelet aggregation via arachidonic acid metabolism, increased cyclic AMP levels inhibit primary platelet adhesion and aggregation induced by ADP, adrenalin, thrombin and collagen.

Dipyridamole, one of the pyrimido-pyrimidine group, inhibits phosphodiesterase and maintains high cyclic AMP levels (Mills & Smith 1971). However the exact mechanism of action is not clear and inhibition of thromboxane synthetase has also been proposed (Ally et al 1977). These compounds inhibit both primary and secondary platelet aggregation and high concentrations of dipyridamole inhibit platelet adhesion to injured vessels (Eliasson & Bygdeman 1969; Groves et al 1982). These effects can be produced by a lower dose of dipyridamole when used in combination with Aspirin (Harker and Slichter 1972).

2.3.iii Compounds that inhibit membrane phopholipase activity

Reduction in the mobilisation of membrane phospholipids may be achieved by inhibition of the membrane associated phospholipase complex. High doses of corticosteroids and mepacrine act in this way and thus block TxA_2 synthesis (Packham & Mustard 1980).

2.3.iv Side effects of platelet inhibitory therapy

The combination of aspirin plus dipyridamole or aspirin alone form the basis for most regimens in clinical practice as there is continuing debate as to the efficacy of dipyridamole alone (Eicher 1984). Aspirin is associated with marked gastrointestinal side effects as seen in a prospective trial of antiplatelet therapy in 2026 patients (Persantin-Aspirin Reinfarction Study 1980). Eighteen percent of patients treated with aspirin alone (1gm/day) reported symptoms suggestive of peptic ulcer or gastritis and this increased to 20% in those receiving aspirin and dipyridamole. Serious side effects of proven gastro-intestinal bleeding were seen in 6.4% of the treated patients. As the majority of patients require platelet inhibitory therapy indefinitely there is clearly a need for further safe compounds to be developed and assessed.

2.4 Platelets in Atherogenesis

Early atherosclerosis has the appearance of fatty sub-intimal streaks which mature to the classical lesion of raised fibriolipid plaques, covered by large amounts of connective tissue and smooth muscle cells (Woolf 1982). The migration of smooth muscle cells from the media and their proliferation in the subintimal layers is thought to initiate atherogenesis. This may be controlled by a mitotic factor which is released from the alpha granules of activated platelets (Ross & Vogel 1978). This protein, now known as platelet derived growth factor (PDGF), is released following aggregation induced by all stimuli including collagen, arachidonic acid and

thrombin (Ross 1980; Witte et al 1978). Macroscopic or microscopic endothelial damage exposes the thrombogenic subendothelium which results in platelet adhesion, release of platelet derived growth factor and subsequently the proliferation of smooth muscle. Repeated cycles of endothelial damage may therefore result in smooth muscle proliferation and the development of atherosclerosis (Ross & Glomset 1973; Ross, Glomset & Harker 1977).

2.5 Platelet Inhibition in Vascular Surgery

Platelet inhibitory therapy is indicated in patients where arterial thrombosis can be prevented by inhibition of platelet function (Harker & Slichter 1972). Clinically, platelet inhibitory therapy has been shown to be of value in valvular heart disease and patients with prosthetic heart valves. In a prospective randomised double blind trial the number of distal emboli originating from replaced cardiac valves was reduced by platelet inhibitory therapy (Sullivan, Harker & Gorlin 1968).

2.5.i Cerebrovascular and cardiovascular disease

The influence of platelet inhibitory therapy on transient cerebral ischaemic attacks and stroke was examined in the Canadian Cooperative Study Group (1978). Aspirin reduced the recurrence of stroke or death in men to 20% compared to 35% in those receiving placebo (Persantin-Aspirin Reinfarction Study 1980).

In a prospective multicentre trial involving 2026 patients following myocardial infarction, aspirin alone or in combination with dipyridamole reduced the incidence of non fatal re-infarction by 24% and cardiovascular mortality by 18% compared to placebo. The difference in mortality failed to acheive significance. However if this trial is pooled with 5 similar, large, prospective, randomised trials (Elwood etal 1974; Coronary Drug Project Group 1976; Elwood & Sweetnam 1979; Breddin et al 1979; Aspirin Myocardial Infarction Study Reseach Group 1980) aspirin significantly reduces the coronary mortality and the rate of non fatal reinfarction (Eichner 1984).

2.5.ii Arterial bypass

It is within the realms of vascular surgery that the manipulation of platelets by inhibitory therapy may be of most value. The three options in vascular reconstruction involve either the implantation of prosthetic grafts, the use of autologous vein or the disobliteration of occlusive disease by endarterectomy. The present preference for autologous tissue in vascular reconstruction is due to the unsatisfactory results obtained with existing prosthetic materials (Weisel et al 1981). Poor long term results in femoro-popliteal bypass with polytetra-fluoroethylene (PTFE) or glutaraldehyde treated umbilical vein have encouraged the use of saphenous vein, in either the reversed or in situ position (Veith, Gupta & Daly 1980; Dardik et al 1982; Chapman & Charlesworth 1983).

Despite poorer flow characteristics, graft patency is improved with vein and failure of prosthetic materials has been attributed to the absence of prostacyclin secretion by the luminal surface (Clowes 1986). The platelet deposition on venous grafts is less intense than on Dacron or PTFE (Goldman et al 1982a). Although prosthetic grafts are well incorporated into the surrounding tissue, the luminal surface remains thrombogenic and a pseudointima of platelets with red cells enmeshed in fibrin is formed (Yates et al 1973). These surfaces readily accumulate circulating platelets and continued thrombogenicity has been demonstrated, using 111-Indium labelled platelets, up to 10 years after implantation of aorto-bifemoral grafts (Goldman et al 1982b).

Platelet inhibitory therapy has been shown to reduce platelet deposition and improve graft patency in Dacron vascular grafts in baboon carotid arteries (Mackey et al 1984). Intimal hyperplasia, which may result from platelet deposition, was reduced by platelet inhibitory therapy in vein grafts implanted into the iliac arteries of rhesus monkeys (McCann, Hagen & 1980).

Aspirin and dipyridamole improved patency in 2 placebo controlled trials of coronary artery bypass grafts (Cheseboro et al 1984; Lorenz, Schacky, Weber 1984). However there was no benefit from platelet inhibition in a third trial where aspirin, dipyridamole and warfarin were compared to warfarin alone (Brooks et al 1984).

Improved patency with platelet inhibitory therapy in femoro-popliteal grafts in patients (Green, Roedersheimer & DeWeese 1982) may be due to reduced platelet deposition on the luminal surface (Goldman et al 1983a). This may prevent the anastomotic intimal hyperplasia which has been identified in both prosthetic and venous grafts (Echave et al 1979; Kohler et al 1984).

2.5.iii Endarterectomy

Endarterectomy is usually reserved for short vascular reconstructions. In this procedure the intima and atheroma are removed therefore exposing the arterial media to the circulating blood. The smooth muscle and collagen of the media are both potent activators of platelet aggregation and promote an intense thrombotic reaction similar to that seen with a prosthesis (Thakur et al 1976). However unlike prosthetic pseudointima, endarterectomy heals with ingrowth of fibroblasts and smooth muscle cells to form a neointima. This surface appears to develop some secretory function, although true endothelium has not been found to regenerate (Reidy, Clowes & Schwartz 1983).

Despite the empirical use of platelet inhibitory therapy at the time of carotid endarterectomy to prevent stroke (Edwards et al 1985) and avoid restenosis (Ackroyd et al 19865), platelet kinetics have not been investigated during the early postoperative period. It is at this stage that platelet accumulation on the endarterectomy segment is likely to be most intense.

2.6 Pathogenesis of Intimal Hyperplasia

Intimal hyperplasia has been described as intimal fibrosis (Stoney & String 1976), myointimal fibroplasia (Hertzer et al 1979; Callow 1982) and myointimal hyperplasia (Thomas et al 1984). I shall use the term intimal hyperplasia to encompass all of those above and to describe the early lesion of restenosis after endarterectomy which is distinct from recurrent atheroma (Stoney & String 1976). This should not be confused with the pseudo-intimal lining of prosthetic grafts which is a layer of thrombus consisting of fibrin, platelets and red blood cells (Yates et al 1973).

The pathogenesis of intimal hyperplasia remains obscure. It occurs to a degree in all arterial vein grafts and has been implicated in at least one third of all late vein graft failures (Imperato et al 1972). It was originally thought to be due to a process of arterialisation (Brody et al 1977), however this hypothesis became untenable with the discovery of hyperplasia in radial artery used for aorto-coronary bypass (Curtis, Stoney & Alford 1975). Ischaemia in the wall of the venous graft (Brody et al 1977) and turbulence causing abnormal shear in the arterial wall were also suggested as possible mechanisms in the development of this proliferative lesion (Bond et al 1976).

Intimal hyperplasia has commonly been found at the site of the distal anastamosis and the localised nature of this lesion suggests that haemodynamic disturbances may be responsible (Crawshaw et al

1980). Endothelial injury, which occurs to some degree throughout vein grafts, whether in the reversed or in-situ position, is especially severe at the site of anastomosis. Each of these factors may contribute to intimal hyperplasia mediated by platelet adhesion and release of PDGF (Bush et al 1986).

Experimental endothelial damage leads to platelet adherence to the exposed sub-intimal layers and degranulation (Crowely & Pierce 1981). Repeated injury causes smooth muscle proliferation and intimal hyperplasia (Stemerman & Ross 1972; Fisherman, Ryan & Karnovsky 1975; Carson, Equivel & French 1981). The involvement of platelets in this process is suggested by prevention of intimal proliferation by platelet inhibition using antiplatelet serum (Moore et al 1976) or drug therapy (Harker, Ross & Glomset 1974; McCann et al 1980).

In carotid endarterectomy the plane of dissection involves the outer media with more than half of the thickness of the arterial wall being removed. This leaves a circumferential surface of collagen and smooth muscle on which platelets will accumulate. Platelets adherence continues until a new endothelial layer is generated and healing occurs (Dirrenberger & Sundt 1978). This is difficult to measure in humans (French & Rewcastle 1974), however rat and rabbit models show that this process is probably complete by 30 days (Reidy et al 1983). Platelet aggregation will continue to stimulate smooth muscle proliferation until this process is complete. Interference

with this repair process either by platelet inhibition or repeated injury could result in failure of the neointimal ingrowth, persistent platelet deposition and promote further intimal hyperplasia.

2.6.i Platelet derived growth factor

The growth promoting properties of serum were first identified by Carrel (1912) in experiments on nutrients required for cell growth. In later studies comparing plasma and serum as culture media, cell proliferation was inhibited in media prepared from cell free plasma but accelerated growth rates were found following the addition of serum (Balk et al 1973).

Restoration of cellular growth, following the addition of platelet extract to plasma, confirmed that these factors were derived from platelets and subsequently it was shown that release occured on degranulation (Ross et al 1974). This inability of cells to replicate in plasma, without the addition of either whole blood serum or platelet extract, led to the investigation and identification of growth factors. It was demonstrated that the antigenic properties of whole blood serum growth factor (Antoniades, Stathakos & Scher 1975) and platelet derived growth factor were similar (Antoniades & Scher 1977).

Rutherford and Ross (1976) showed that other constituents present in plasma were important for cell growth, in addition to the growth factors derived from platelets. Despite optimal levels of

platelet factors, the process of cell growth in culture progressed only as far as a single cell division if low concentrations of plasma were used (Ross & Vogel 1978).

Platelet derived growth factor (PDGF) is thought to be a heat stable protein, of approximately 30,000 molecular weight, which can be isolated by radio-immuno assay (Antoniades & Scher 1977). More recently other growth factors, such as epidermal growth factor and transforming growth factor, have been localised in platelets although these are thought to be inactive on the endothelial cell (Bowen-Pope, Ross & Seifert 1985). Vascular endothelial cell proliferating factor (VEPF) has also been described as a separate polypeptide which stimulates endothelial cell growth (Miyazono et al 1985) but further investigation is required to evaluate the effects of these substances in isolation and combination. For the purpose of this thesis it is appropriate to regard platelet derived growth factor activity to be due to a collection of PDGF like molecules as suggested by Bowen-Pope et al (1985).

Burke & Ross (1977) investigated the effect of PDGF on the connective tissues of the arterial wall. On exposure to this substance there was an increase in protein synthesis with stimulation of collagen production in arterial smooth muscle cells. This effect follows the binding of PDGF to a cell surface glycoprotein which triggers a sequence of cellular events (Ross et al 1984). Binding occurs only to connective tissue, including vascular smooth muscle

cells and not to epithelial, skeletal muscle or nerve cells (Bowen-Pope et al 1985). Initially the receptor undergoes phosphorylation which results in DNA synthesis leading to mitosis. Other detectable effects of PDGF binding include increased protein and RNA synthesis (Ross et al 1984).

The extrapolation of experimental work on PDGF performed in cell culture to in vivo evaluation remains unsatisfactory (Ross & Vogel 1978). Serum is a pathological rather than a physiological substance and its use in cell culture is probably analogous to the in vivo conditions seen in tissue injury or thrombosis. Normally vascular endothelial cells are exposed only to interstitial fluid or plasma. Radiolabelling of PDGF with ^{125}Iodine has improved understanding of these polypeptides by demonstrating receptor sites in cell culture and the technique may be applicable to in vivo investigation (Bowen-Pope et al 1985).

The response to injury with cell proliferation, connective tissue formation and scarring may in some degree be mediated by the substances found in serum. Similarly there are indications that cellular division in the reparative response to injury may be controlled by platelet derived growth factors. This would encourage speculation that platelets are involved in the control of cell growth and and could be a factor in the aetiology of atherogenesis (Kinlough-Rathbone, Packham & Mustard 1983).

In summary, platelet derived growth factor has been identified as a low molecular weight polypeptide which is released from platelets on aggregation. In the presence of plasma this factor stimulates cellular growth by DNA and protein synthesis and causes arterial smooth muscle proliferation. In order to assess the effect of platelet deposition on areas of damaged arterial endothelium in patients, a simple, low morbidity method of identifying local platelet activity is required.

2.7 The Measurement of Local Platelet Deposition

Initially platelets labelled with Chromium^{51} were used to measure platelet survival (Aas & Gardiner 1958), however the low gamma emissions precluded the use of ^{51}Cr labelled platelets for external scintographic detection. When the method of labelling platelets with ^{111}Indium was described, a new field of radio-isotope imaging was opened (Thakur et al 1976). Experimental models were developed to assess the use of ^{111}In platelets in localising thrombotic processes and were applied first to pulmonary emboli and infective endocarditis (McIlmoyle et al 1977; Dewanjee et al 1978). Subsequently localised platelet uptake was diagnosed by scintigraphic imaging in 3 patients with atherosclerosis and venous thrombosis (Davis et al 1978).

These gamma camera techniques are applicable to the investigation of platelet accumulation in vascular grafts. Following aortoiliac replacement, postoperative platelet deposition was found

to be high initially but decreased as further studies were performed up to a year after implantation (Goldman et al 1982b). Even mature grafts have been found to accumulate radiolabelled platelets on gamma scans 10 years after implantation, with greatest thrombogenicity at the sites of arterial anastamosis (Stratton, Theile & Ritchie 1982; Goldman et al 1982b).

Similarly prosthetic femoro-popliteal bypass grafts showed an intense luminal radioactivity following the injection of radio-labelled platelets, but minimal deposition was detected in vein bypasses (Goldman et al 1982a). The use of radiolabelled platelets has also been established in assessing the efficacy of platelet inhibitory drugs in preventing graft platelet interaction in laboratory experiments or in animals (McCollum et al 1980). A reduction in platelet accumulation on 10 year old mature Dacron aorto-bifemoral grafts occured in patients treated with a thromboxane antagonist (Lane et al 1985).

Carotid artery atherosclerosis was first identified by isotope imaging of platelets in 1978 (Davis et al 1978). In contrast to angiography which demonstrates the arterial lumen, scintography detects the site of platelet accumulation on atherosclerotic plaque which may be a source of thrombotic emboli (Davis et al 1980, Powers 1984). The direct correlation between gamma imaging and ^{111}In platelet deposition was demonstrated by excision of the carotid plaque (Goldman et al 1983b). Ex vivo measurement of ^{111}In platelets

on the carotid plaque showed that areas of atheromatous ulceration represent the most thrombogenic lesions and accumulate sufficient labelled platelets to be detected by external imaging.

Radiolabelled platelet accumulation following endarterectomy has been described in dogs and was related to intimal healing (Lusby et al 1983). In patients postoperative accumulation, identified by gamma camera imaging following carotid endarterectomy, was found to be significantly higher after endarterectomy than after other similar surgical dissections (Stratton, Zieler & Kazmers 1987). This technique may provide information on the duration of platelet accumulation, reflecting healing, and provide a direct measurement of the effect of platelet inhibitory drugs on platelet thrombus in patients.

Scintigraphic imaging with ^{111}In labelled platelets in the neck is often poor because of high background activity from blood pool. Dual isotope subtraction techniques have been tried but numbers studied remain small (Kessler, Reuther & Rosch 1984; Isaka et al 1984).

2.8 Summary

Platelets are known to play a major role in the mechanisms of normal thrombosis and haemostasis. The activation of this mechanism depends on the balance of thromboxane and prostacyclin, both of which are derived from the same precursor, arachidonic acid. Platelet

inhibition effectively improves the patency of vascular grafts and lowers morbidity from cardiovascular disease.

There is evidence which suggests that platelet interaction with the tissues in the subendothelium may be a factor in the development of the smooth muscle proliferation which causes intimal hyperplasia. The identification of platelet derived growth factors support this theory and futhermore these substances may be involved in the pathogenesis of atherosclerosis.

To investigate the role of platelets in carotid restenosis a reliable method of measuring in vivo platelet accumulation is required such that the intensity, duration and response to therapeutic manipulations can be measured. The labelling of autologous platelets with ^{111}Indium and gamma camera imaging has detected platelet deposition on atherosclerotic plaque and localised platelet accumulation after endarterectomy. These techniques may be applicable to the study of platelets in the response to endarterectomy and to investigate the relationship between platelet accumulation and the development of intimal hyperplasia.

Chapter 3

Hypothesis and Aims

3.1 Hypothesis

The hypothesis which will be investigated in this thesis is that platelets accumulate on the arterial wall at the site of endothelial damage in rabbits or surgical endarterectomy in patients. This deposition of platelets may be identified and quantified by the external detection of autologous platelets labelled with ^{111}Indium oxine. Futhermore these techniques may detect changes in the intensity of platelet deposition in response to pharmacological inhibition of platelet function or changes in the arterial configuration following vascular repair. Platelet-vessel wall interaction may be a factor in the development of intimal hyperplasia and the measurement of postoperative radiolabelled platelet deposition may be used to investigate the relationship between platelet accumulation and the subsequent development of restenosis.

3.2 Aims

1. To identify and measure platelet uptake following carotid endarterectomy and to determine the duration of platelet accumulation on the endarterectomised segment.

2. To identify the factors which influence the rate or extent of platelet deposition following carotid endarterectomy.

3. To study the incidence of restenosis following carotid endarterectomy by non-invasive methods.

4. To determine whether platelet uptake following carotid endarterectomy influences the rate of subsequent restenosis.

3.3. Summary of experiments

Radiolabelled platelet deposition was initially studied following intimal trauma on the carotid arteries of rabbits. The deposition of ^{111}In labelled platelets was measured and the effects of platelet inhibitory therapy assessed.

^{111}In platelet uptake was then measured in human carotid arteries both in the early postoperative period following endarterectomy and at 2 months to assess the time taken to achieve intimal repiar. Two methods of measuring platelet accumulation were considered and the efficacy of probe counting and gamma camera imaging compared.

Postoperative platelet deposition was measured in patients receiving aspirin plus dipyridamole, to assess the effects of platelet inhibitory therapy, and compared to untreated controls. Subsequently platelet uptake following patch angioplasty repair of the arteriotomy was compared to that after standard closure.

In order to determine the overall incidence of carotid restenosis following endarterectomy, a retrospective study of a consecutive series of patients was carried out. Non-invasive methods were used to assess the degree of carotid artery restenosis following

surgery. The relationship between carotid restenosis and the recurrence of symptoms of cerebrovascular insufficiency was investigated.

The rate of radiolabelled platelet deposition on the endarterectomised carotid artery, measured as Thrombogenicity Index, was then compared to the degree of luminal narrowing assessed by Duplex ultrasound, 12 months after surgery.

SECTION II RADIOLABELLED PLATELET UPTAKE IN THE CAROTID ARTERY AND FACTORS INFLUENCING THROMBOGENICITY

Chapter 4

^{111}In-platelet Methods for Measuring Platelet Uptake following Carotid Endarterectomy

4.1 Introduction

The method of labelling human platelets with ^{111}Indium oxine is applicable to several of the studies in this thesis. These techniques are described in detail in this chapter whereas experimental methods appropriate to a single study will be found in the relevant section.

4.2 Development of Radiolabelled Platelet Methods

Platelets have been labelled in vivo with ^{35}S-Sodium sulphate, ^{75}Se-selenomethionine, ^{35}S-methionine, ^{14}C-seratonin and ^{32}P-di-isopropylfluorophosphate, however these are non-specific markers (Thakur et al 1976). It was not until 1958 that reliable radionucleotide labelling with ^{51}Cr-sodium chromate was performed allowing in vivo platelet life span to be calculated (Aas & Gardner 1958). These techniques were restrictive as poor labelling efficiencies with ^{51}Cr required a large volume of blood to harvest sufficient platelets (Abrahamsen 1968). The major disadvantage however was the low power gamma emmission with ^{51}Cr of 9% at 320kev which prevented the use of external imaging devices such as the gamma camera. In 1976, Thakur described a method of labelling human platelets using ^{111}Indium chelated with 8-hydroxyquinalone (oxine) and also demonstrated that the in vitro collagen induced aggregation of ^{111}In labelled platelets was unaffected by radiolabelling (Thakur et al 1976). This contrasts to ^{51}Cr labelling which adversely altered platelet function (Kattlove & Spaet 1970). Additioally ^{111}Indium is ideal for short term in vivo platelet studies, with a

half life of 2.81 days and the high gamma photon energies of 84% at 173 kev and 94% at 247 kev allow external detection of labelled platelets.

Joist confirmed that the ^{111}Indium label in humans was bound to non-releasable components of platelets and less than 4% of the label was lost from the cells on exposure to aggregating agents (Joist et al 1978). Radionucleotide uptake was far superior using ^{111}Indium with 90% of the label taken up by the platelets in 15 minutes incubation whereas ^{51}Cr reached only 15% uptake by 30 minutes. The method of isolation of platelets was critical as the isotope was bound avidly by both red cells and transferrin and the presence of small amounts of either of these markedly reduced the labelling efficiency of ^{111}In oxine onto platelets (Thakur, Coleman & Welch 1977). Refinement of this technique leading to improved platelet yield confirmed that linear platelet survival was compatible with values achieved using ^{51}Cr labelling. In vivo distribution by whole body counting and scintillation camera showed marked uptake in the liver and spleen as sequestration of platelets occured, but low radioactivity in the limbs suggested that platelets did not accumulate or interact with the peripheral vasculature in normal subjects (Heyns et al 1980).

In view of the large volume of blood required and the poor labelling efficiencies of Thakur's method, Hawker developed a technique in which platelets were labelled whilst suspended in

Tyrode's solution (Hawker, Hawker & Wilkinson 1980). Successful ^{111}In labelling was effected with platelets from 26mls of blood with efficiencies of 90% after only 60 seconds incubation. Platelet aggregation responses in healthy volunteers were compatible with previously reported values using ^{51}Cr. This method has been used for all ^{111}In-platelet labelling procedures described in this thesis.

4.3 Labelling Platelets with ^{111}Indium Oxine

4.3.i Chemical reagents

All reagents were prepared in the Pharmacy, Charing Cross Hospital. Chemicals were obtained from British Drug Houses (Poole, Dorset, UK) unless otherwise stated. Plastic containers were used for handling all specimens.

Acid sodium citrate

Individual 3ml vials of a solution of 2.5g trisodium citrate $2H_2O$ and 1.49g citric acid H_2O in 100mls of water were prepared. The resultant pH of the anticoagulant was measured to be 6.5.

Sodium citrate

3.85g trisodium citrate $2H_2O$ were dissolved in 100ml water and divided into 1ml aliquots.

Calcium-free Tyrodes solution (buffer)

The following were dissolved in 1 litre of water:

Sodium chloride 8g

Potassium chloride 0.2g

Sodium bicarbonate 1.0g

Sodium dihydrogen orthophosphate $2H_2O$ 50mg

Magnesium chloride $6H_2O$ 406.6mg

D-glucose 1g

Sodium heparinate 25000 units

Carbon dioxide was bubbled into the solution until the pH recorded was 6.5. Ampoules containing 20cc of Tyrode's buffer solution were prepared.

Prostaglandin E1 (PGE_1)

One milligram of PGE_1 (Sigma Chemical Company Ltd, Poole, England) was dissolved in Tyrodes buffer to produce a concentration of 300 nanograms/ml. Aliquots of 0.5mls were freeze dried in a dessicator (Edwards EF03, Crawley, Sussex).

4.3.ii Method

Twenty six millilitres of blood was obtained by venepuncture using an 18 gauge needle and plastic syringe (Fig 4.1). All subsequent manipulations were performed in a class II unidirectional laminar down flow cabinet (20229, Microflow, Dent & Hellyer Ltd., Andover, Hampshire).

a. Seventeen millilitres of blood were added to 3ml of acid citrate in a sterile universal container (Sterilin 128/B, Teddington,

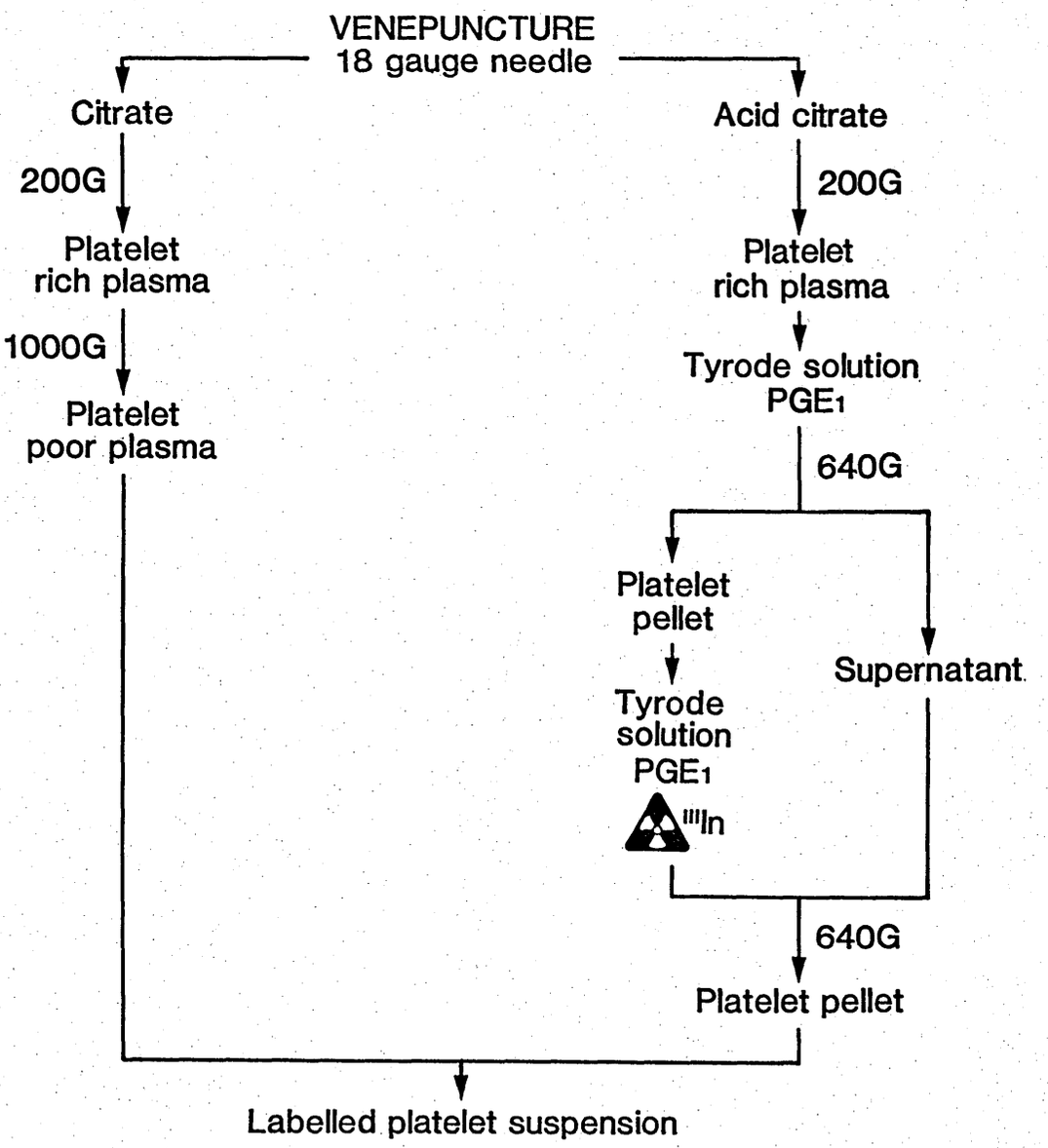


Figure 4.1 Method of labelling human platelets with ^{111}Indium Oxine.

Middx). The remaining 9ml of blood were added to 1ml of trisodium citrate in a sterile round bottomed tube (Sterilin 142/AS).

b. Following mixing, both tubes were centrifuged at 200g for ten minutes to generate platelet rich plasma (PRP).

c. A sample of citrated PRP was retained for aggregation studies and the remainder centrifuged at 1000g for ten minutes to produce citrated platelet poor plasma (PPP).

d. Using a Pasteur pippette, the supernatant acid citrated platelet rich plasma was transferred to a round bottomed tube. One freeze dried aliquot of PGE_1 was dissolved in 1ml of water and 400ul mixed with 20ml of buffer at $37^{o}C$. This PGE_1/buffer solution was passed through a 0.22um Millex filter unit (Millipore SLGV 025BS, 67 Molsheim-France) and added to the acid citrated PRP to bring the volume to ten millilitres.

e. The platelet suspension was centrifuged at 640g for ten minutes producing a platelet pellet and the supernatant was retained. The pellet was then washed twice with 2.5mls PGE_1/buffer and the platelets were resuspended in a further 2.5mls of PGE1/buffer solution.

f. A mixture of 200uCi of ^{111}Indium oxine in saline and 0.2mls of 0.2M Tris buffer (ph 8.0, Mallinckrodt Diagnostica BV, Petten,

Holland) was added to the platelet suspension and incubated at 37°C for 60 seconds. The labelling reaction was stopped after one minute by the addition of 7.5mls of the previously obtained diluted supernatant. The remaining free Indium oxine is instantly bound to the plasma transferrin.

g. The mixture was centrifuged at 640g for 10 minutes and the supernatant removed. The radiolabelled platelets were resuspended in 4mls citrated platelet poor plasma and the radioactivity of the ^{111}Indium labelled platelets and supernatant assessed in a well crystal and ratemeter (Nuclear Enterprises SR-7 ratemeter/DMI-2 scintillation counter). Labelling efficiency was expressed as the ratio ofthe radioactivity of platelets compared to that of the supernatant and platelets. Preparations with labelling efficiencies of less than 90% were discarded.

Prior to reinjection of the resuspended platelets two 100ul samples were separated for aerobic and anaerobic bacteriological culture and 0.5ml for aggregation assessment. Only platelets which demonstrated aggregation in response to ADP were reinjected into the patient. The radiolabelling procedure, from venesection until reinjection, takes approximately 60 minutes.

4.3.iii Ethical Consideration

At a dose of 200uCi of ^{111}Indium oxine, the total radiation to the critical organ, the spleen, is approximately 2.7 rads and within

the guidelines for category 2 research projects involving irradiation of human beings, as specified by the World Health Organisation Expert Committee (1977). Written consent was obtained from all patients involved in the studies as recommended by the Department of Health and Social Security (Health Circular 1979) and the experimental design was approved by Charing Cross Hosptial Ethical Subcommittee.

4.4 ^{111}In-platelet Function in Vitro

In vitro platelet function was assessed by aggregation in a platelet suspension stimulated by adenosine diphosphate (ADP) measured by light transmission aggregometry (Born 1962). In principle the proportion of light transmitted through the platelet preparation increases with aggregation and this change was detected by a photoelectric cell and potentiometer (Weiss 1976).

Twenty-five microlitres of a 1:100,000 solution of ADP was added to 250ul of platelet rich plasma in a light transmission aggregometer (Payton Single Channel 300B/5, Payton Associates Ltd, Scarborough, Canada) connected to a potentiometer chart recorder (Serbo Scribe 1S, Smiths Industries) running at 60mm/minute. The temperature of the platelet suspension was maintained at 37^{o}C. The maximum aggregation response and the rate of aggregation can be calculated for each sample from the trace obtained (Fig 4.2).

In these studies platelet aggregation reponses were used to ensure the viablity of labelled platelets by comparing them to

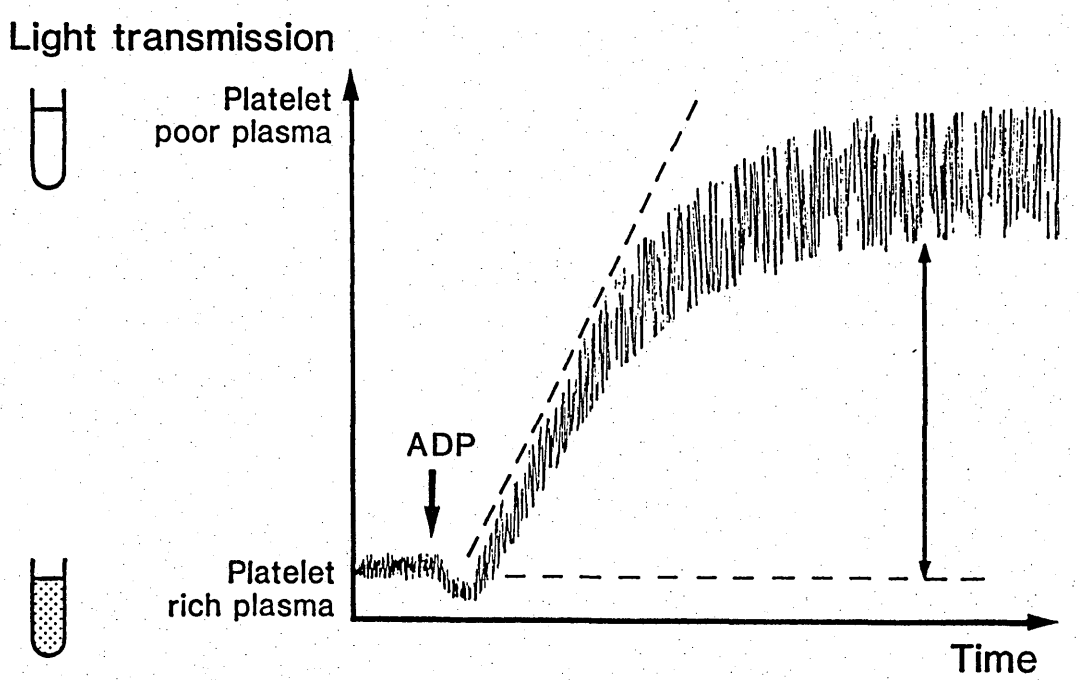


Figure 4.2 Light transmission aggregometry. Following the addition of ADP, platelets undergo aggregation and clumping so light transmission through the suspension is improved.

unlabelled platelets. Platelet preparations showing inadequate ADP aggregation responses were discarded.

4.5 Platelet Survival

Platelet survival may be estimated by linear regression, exponential analysis or multiple hit methods depending on the theory of platelet destruction (Mustard, Rowsell & Murphy 1966). This can be either age related (linear), indisciminate (exponential) or a process of cumulative ageing (multiple hit) (Murphy & Francis 1971). Calculation of the mean platelet life span is based on the measurement of the rate of decrease of blood radioactivity, after correction for radioisotope decay as suggested by the Panel on Diagnostic Application of Radioisotopes in Haematology (1977).

4.6 Measurement of Radioactivity in Carotid Arteries

In vivo tissue and vessel radioactivity may be assessed by either scintillation probe counting or gamma camera imaging. Radiolabelled platelet uptake was assessed by each of these methods 2 hours following the injection of radiolabelled platelets and subsequently at 24 and 48 hours.

4.6.i Scintillation probe counting

A sodium iodide crystal (Eberline Instrument Co. RD-19 Crawley, U.K.) was used to assess gamma counts, collimated with a lead sheath of 6mm thickness to reduce uptake of scattered radioactivity from the background. In order to focus the sodium iodide crystal on the

carotid artery, approxiamtely 2cm deep to the skin, a lead collimator was fitted to reduce the penumbra with a skin contact probe to ensure the crystal was situated 10cms from the artery (Fig 4.3). This counter was connected to a rate meter (Erbline Instrument Company SAM-2, Crawley U.K.), which was gated to count both peaks of ^{111}Indium activity at 173 and 247 kev. In each patient, prior to the assessment of radioactivity the surface markings of the carotid bifurcation and the internal carotid artery were mapped by Doppler ultrasound. The probe position was marked on the first day to ensure identical probe geometry for each subsequent daily measurement. Patients were seated in a specially designed chair with the head held steady by a head rest and restraining leather strap (Plate 2.1). Counting was performed in the upright position to minimise the radioactivity from circulating platelets in venous blood, pooling in the great veins of the neck. Comparative reference readings were taken from the contralateral carotid artery and in addition from the forearm.

Initially 2 measurements of radioactivity were taken from each probe position over a 30 second period and were recorded. If the difference between these 2 readings was greater than 10 percent, 2 further measurements were recorded. The average counts over 30 seconds was calculated from these measurements and following subtraction of background activity, used for calculation of Carotid Uptake Ratio and Thrombogenicity Index.

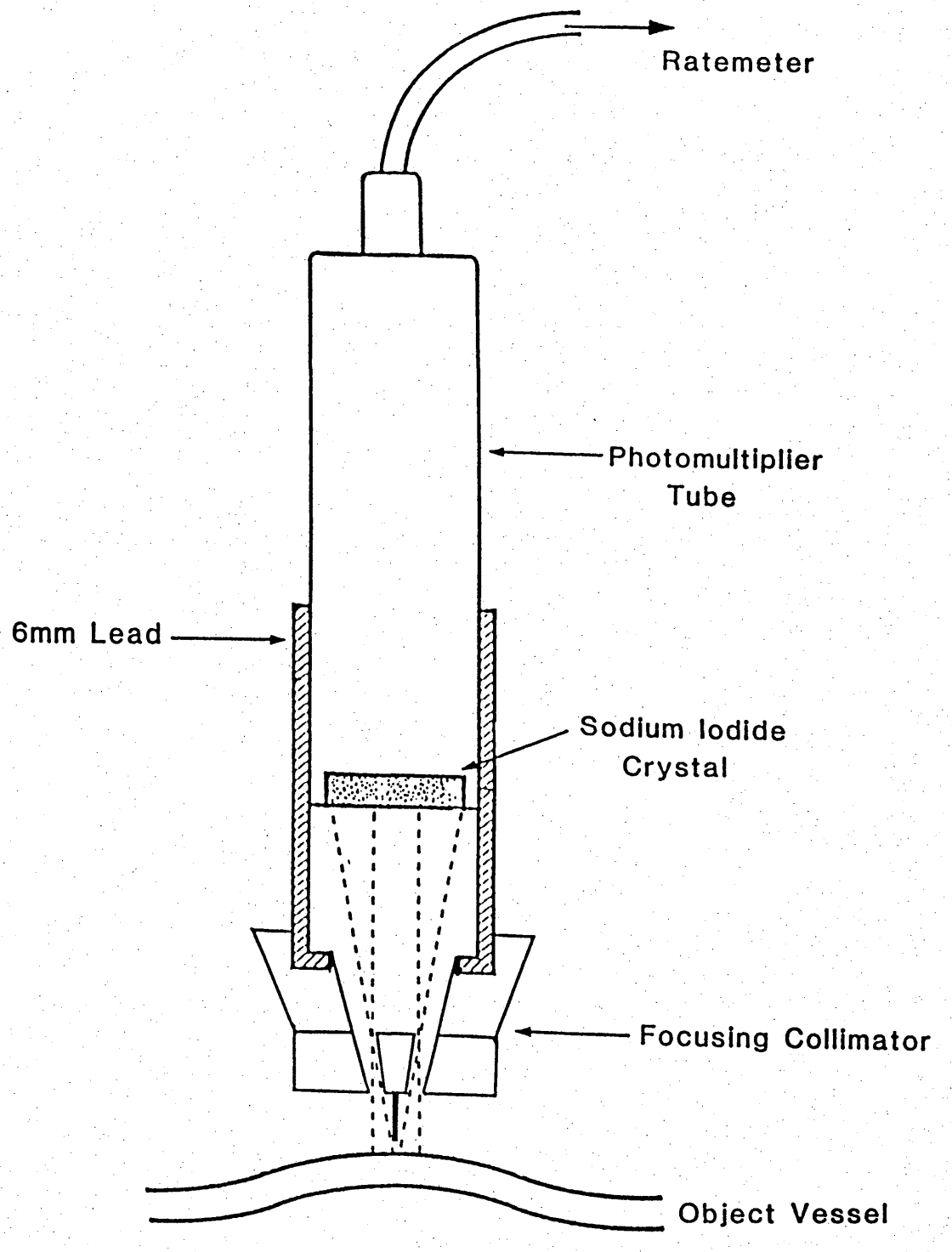


Figure 4.3 The sodium iodide crystal was fitted with a focusing collimator to reduce background activity recorded from the surrounding tissue.

4.6.ii Gamma Camera Imaging

Immediately following scintilation probe counting, patients were transfered for gamma camera imaging. A Scintronix LFOV (large field of view) gamma camera was fitted with a medium energy parallel hole collimator and connected to a computer, gated to record both peaks of Indium activity. Patients were scanned for 10 minutes, again sitting upright, with the head and neck immobilised by a restraining strap (Plate 2.2).

Gamma images were stored by computer and the data retrieved for analysis (Plate 2.3). Computed areas of interest were outlined over the operated artery comprising between 12 and 24 gamma camera cells and the radioactivity from this area was expressed as counts per gamma camera cell or pixcel. A similar area of interest was outlined over the non operated carotid and was used as a reference for subsequent calculation of carotid platelet accumulation (Plate 2.4).

4.7 ^{111}In-platelet Accumulation in Carotid Arteries

Following the injection of radiolabelled platelets the decline in blood radioactivity will depend on 2 factors, the decay constant of the isotope used and the rate of elimination of platelets from the circulation. A site of platelet accumulation may, in absolute value, become less radioactive, however the radioactivity will appear to increase when compared a reference site which represents predominantly blood pool. This is the theory on which the determination of accumulation of platelets in blood vessels is based

Plate 2

2.1 Probe counting using a sodium iodide crystal and rate meter. Each carotid and reference area is counted over 30 seconds to measure radioactivity. Surface markings of the carotid bifurcation are mapped out and used at each daily measurement to ensure identical probe geometry.

2.2. Gamma images were obtained on three consecutive days. Each scan takes 10 minutes to accrue and therefore the patient's head is supported by a headrest and strap.

2.3 Gamma scan following the injection of ^{111}In labelled platelets. This demonstrates the head and neck with radioactivity seen in the regions of the aortic arch and cavernous sinus. Increased uptake is noted on the left side of the neck which corresponds to the carotid endarterectomy performed 72 hours previously.

2.4 Areas of interest are outlined over the site of endarterectomy and over the contralateral carotid artery. The radioactivity measured in this area, expressed as counts per gamma camera cell, is used to calculate Carotid Uptake Ratio and Thrombogenicity Index.

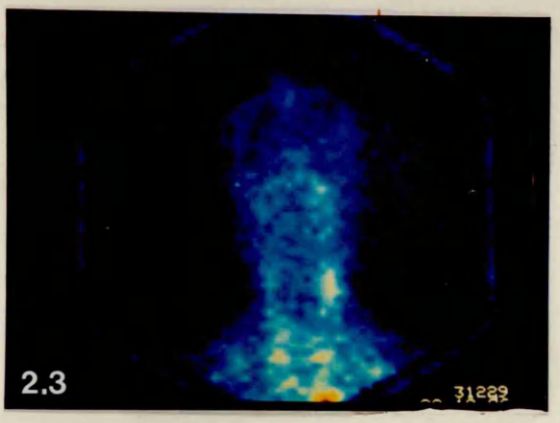
2.5 Gamma image of a patient taken 2 months after surgery. Increased radioactivity is seen over the right carotid artery which corresponds to a 50% stenosis of the ICA. This patient had undergone a left carotid endarterectomy.

2.6 B-mode ultrasound image of the common carotid artery after endarterectomy. This vessel is widely patent without evidence of intimal hyperplasia.

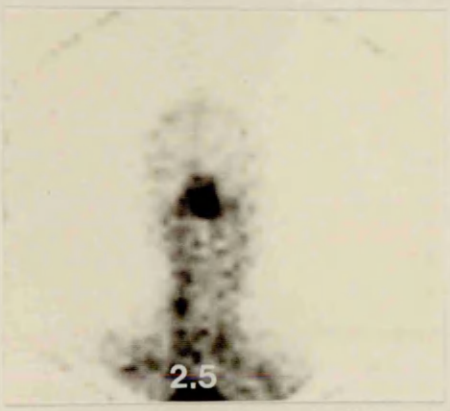
2.7 Considerable thickening of the intima is seen in this common carotid artery following surgery. This represents mixed echoes but there is no acoustic shadowing which would suggest calcification.


2.1


2.2


2.3


2.4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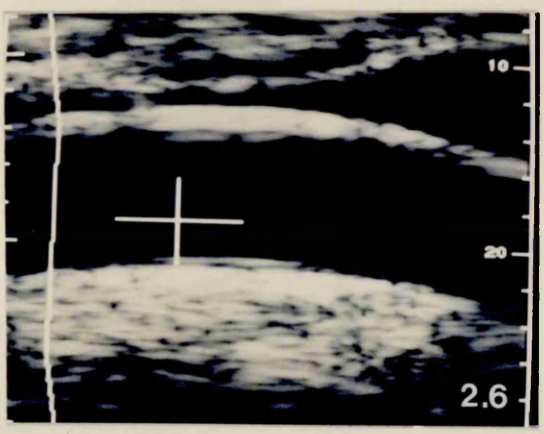
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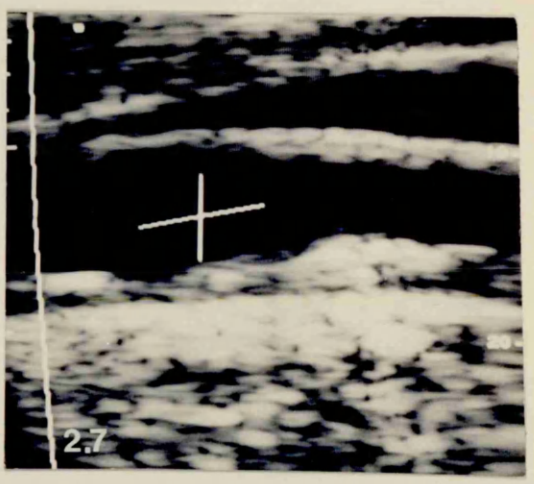

2.7

and was initially described to assess platelet uptake in prosthetic femoro-popliteal grafts by deriving Thrombogenicity Index (Goldman et al 1982a).

4.7.i Carotid Uptake Ratio

Platelet accumulation measured by either probe counting or gamma camera imaging is expressed as counts per second or counts per pixel respectively. A ratio is calcualted by dividing the counts from a site of platelet accumulation with the counts from the reference area. In this work the radioactivity from the endarterectomised carotid is compared to the contralateral reference artery to derive the Carotid Uptake Ratio (CUR). Carotid Uptake Ratio values of greater than 1.0 demonstrate increased radioactivity in that area of interest over the operated carotid and represent the deposition of radiolabelled platelets at that site. All values of CUR quoted in this thesis are calculated from data recorded 24 hours after the infusion of radiolabelled platelets.

4.7.ii Thombogenicity Index

Carotid Uptake Ratio, calculated from counts derived from gamma camera or probe counting, represents a static comparison of the radioactivity from the operated to reference artery at either 2, 24 or 48 hours after the injection of radiolabelled platelets. Early scans reflect predominently blood pool, but as platelets accumulate at the site of endarterectomy, radioactivity in this region becomes progressively greater compared to reference. By plotting the ratio,

recorded on each day of the study, against time the line of best fit through these points can be calculated by linear regression. The slope of this regression line, which represents the increase in this ratio over the 3 days of each study, reflects the rate of platelet accumulation has been termed Thrombogenicity Index (TI). Because this index uses the contralateral artery as a reference, the exponential component of isotope decay is eliminated from this calculation (Goldman et al 1982a).

Chapter 5

Intimal Platelet Deposition on the Traumatised Rabbit Carotid Artery

5.1 Introduction

Rabbits have provided successful models for the investigation of atherogenesis and have proved effective in evaluating the interaction between platelets and vessel wall (Moore 1973). Although there are quantitative variations between different species, platelets from humans, rats, rabbits, guinea pigs, horses and dogs all aggregate in the presence of ADP (Sinakos & Caen 1967). As ADP induced aggregation is inhibited by adenosine monophosphate (AMP) in humans and this effect is also seen in dog and rabbit platelets, these species are appropriate for comparative studies on platelet involvement in arterial trauma. This recommends rabbits in cardiovascular research as a cheaper alternative to more expensive canine or primate models.

Effective methods of radiolabelling rabbit platelets with ^{111}Indium oxine have been described with minimal damage to the labelled cells, such that successful localisation of platelet thrombus in rabbits has been achieved (Wistow et al 1978; Schmidt & Rasmussen 1979). The infusion of intravenous iron particles induced venous thrombosis and the presence of ^{111}In platelet thrombus was confirmed by both radiographic and isotope scans (Grossman et al 1978). Similarly radiolabelled platelet thrombus was detected by gamma camera on areas of exposed subendothelium following the passage of a balloon catheter. These lesions were not localised by indium labelled red cells or plasma proteins (Finklestein et al 1982).

Various methods have been used to inflict a standard and reproducible injury to the arterial endothelium in rabbits. Isolation of the carotid artery produced an ischaemic injury (Buchanan & Hirsch 1984) and an electric current from a luminal electrode caused platelet uptake in the central artery of the ear (Grossman et al 1978). Balloon catheter damage has been used in the rabbit aorta (Groves et al 1982; Finklestein et al 1982) and carotid artery (Reidy et al 1983). The constant infusion of homocystine caused endothelial damage (Harker et al 1976) as did dietary induced hypercholesterolaemia (Armstrong et al 1980). Carotid interposition vein grafting produced a model of intimal hyperplasia but had a high operative mortality (Murday et al 1983).

Platelet inhibition by aspirin has been demonstrated to reduce thrombus in the jugular veins of rabbits in a model using ^{125}I labelled fibrinogen. Low dose aspirin (10mg/kg) proved effective as an antithrombotic agent, but higher doses (200mg/kg) augmented thrombus formation, possibly by inhibition of prostacyclin production in the endothelium (Kelton et al 1978). As low dose aspirin has been shown to cause maximal inhibition of both TXA_2 and PGI_2, the reversal of the antithrombotic effect with high dose aspirin may not be soley due to the inhibition of PGI_2 synthesis (Buchanan & Hirsh 1984).

It is the aim of this study to develop a rabbit model of carotid intimal trauma using a standardised surgical injury. ^{111}In-labelled platelet deposition measures the intensity of platelet interaction

with the damaged arterial wall can evaluate the effect of low dose aspirin and dipyridamole.

5.2 Materials and Methods

Twenty young adult New Zealand white rabbits (2.0-2.5kg) were randomised to receive either placebo or aspirin (ASA) 5mg/kg daily plus dipyridamole (DPM) 5mg/kg twice daily. The drugs, prepared in powder form by Charing Cross Hospital Pharmacy, were disolved in 5mls of water and administered by oro-gastric tube. Treatment was started 48 hours prior to operation and continued until completion of the study. At operation the carotid artery was damaged by a standardised method and 24 hours later platelets labelled with ^{111}Indium oxine were injected into the rabbits. After a further 24 hours the animals were sacrificed, the carotid arteries excised and radiolabelled platelet uptake was assessed.

5.2.i Operative technique

Anaesthesia was induced by mask inhalation of a 70:30 mixture of nitrous oxide and oxygen with 4% Halothane and maintained with 2% Halothane. An anterior mid-line incision was made in the neck and the strap muscles were reflected laterally. Both carotid arteries were identified and mobilised over a distance of 2 cms (Plate 3.1). On one side, the carotid artery was crushed over a distance of 1cm for a 3 minute period using non-toothed dissecting forceps mounted on a G clamp with a compression screw providing a standardised trauma (Plate 3.2). The artery on the contralateral side was mobilised by

the same method but left undamaged and used as a control. The skin wounds were closed with continuous 5/0 Ethilon (W6669 Ethicon, Edinburgh, Scotland) and the animals were allowed to recover.

5.2.ii ^{111}In platelet labelling

Platelet labelling was performed by a technique modified from the 'button-saline' method descibed by Wistow et al (1978). Platelets for each labelling procedure were obtained from a single animal, anaesthetised using 4% Halothane, nitrous oxide and oxygen. Thirty millilitres of blood were obtained by cardiac puncture and the animal was sacrificed with intravenous sodium barbiturate. The blood was anticoagulated with 4mls of 3.4% sodium acid citrate and centrifuged at 200G for 10 minutes to obtain platelet rich plasma (Fig 5.1). This suspension was then centrifuged again at 640G to produce a platelet pellet and the resulting supernatant was retained. The platelet pellet was washed and resuspended in 5mls of calcium free Tyrode's solution with 70ng of PGE_1. The washed platelets were again isolated, resuspended and incubated with 250uCi of ^{111}indium oxine for 60 seconds and the radiolabelling reaction stopped by the addition of the supernatant. Free nucleotide remaining in solution was bound to plasma proteins in the supernatant. Radiolabelled platelets were separated from the supernatant and residual plasma-bound isotope by centrifugation at 640G for 10 minutes and resuspended in 10mls of Tyrode's solution. Labelling efficiency was assessed by expressing the activity bound to labelled platelets as a percentage of the total activity in the platelets and supernatant.

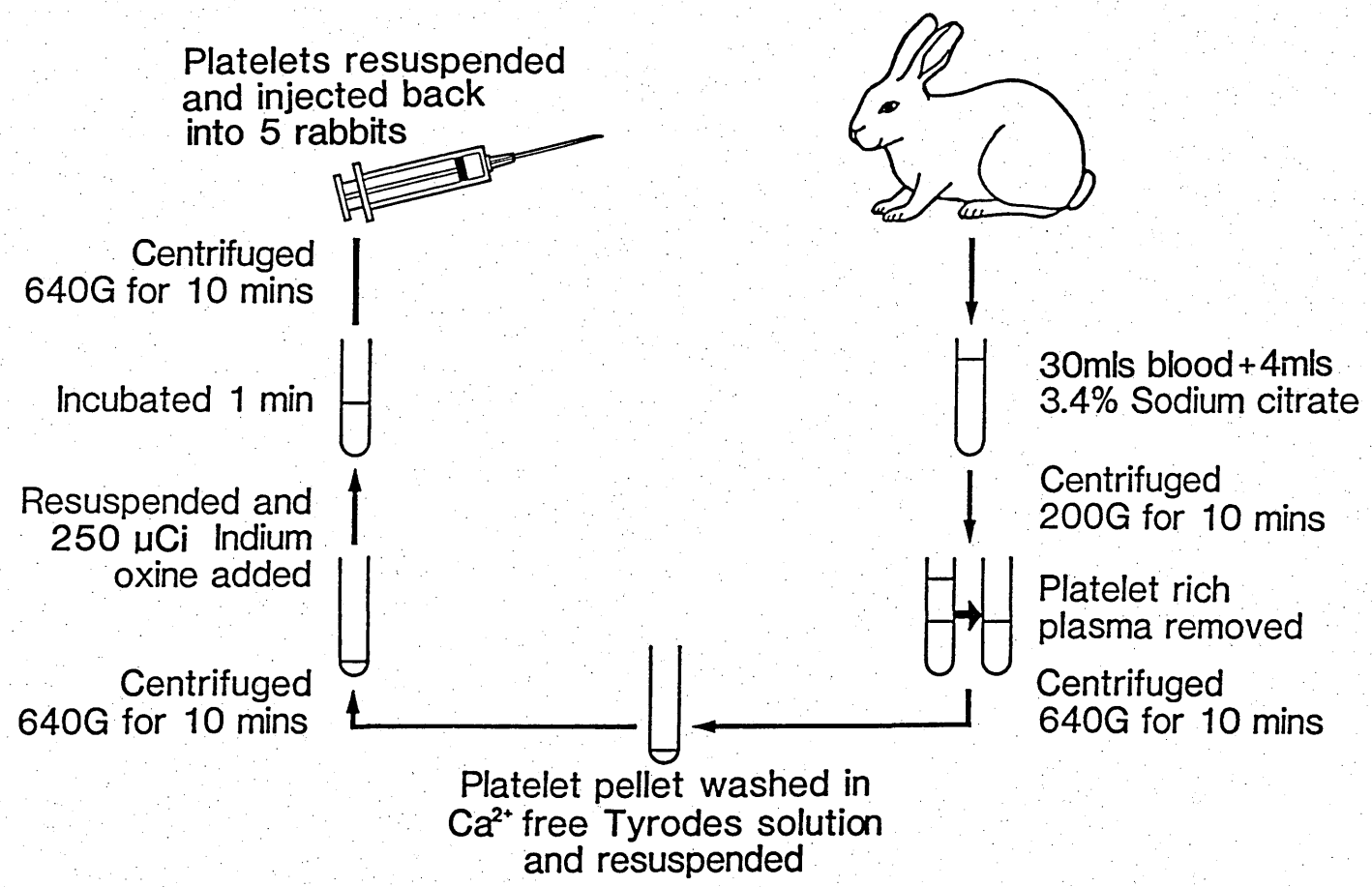


Figure 5.1 Method of radiolabelling platelets in rabbits.

Each rabbit was then reinjected with 2 mls of ^{111}In platelet suspension via the ear vein. Sufficient platelets were harvested from one donor to label five experimental animals.

5.2.iii Measurement of radiolabelled platelet uptake

Twenty four hours after injection of radiolabelled platelets, the animals were reanaesthetised and both carotid arteries excised. A 5ml blood sample was withdrawn to measure circulating blood activity and the animals were sacrificed with intravenous sodium barbiturate. The excised arteries were irrigated for 60 seconds with 0.9% saline, introduced through a 20 gauge intravenous infusion catheter (Abbocath-T 4535-20, Abbott Ireland Ltd). This procedure removed blood remaining in the lumen and adherent tissue was dissected from the adventitia of the vessel (Plate 3.3). Two centimetre lengths of vessel were isolated with a 5mm section of normal artery proximal and distal to the traumatised segment. The contralateral artery was removed, irrigated and prepared in an identical fashion and used as a reference. Radioactivity was measured in a well crystal and ratemeter (Nuclear Enterprises SR-7 ratemeter/DMI-2 scintillation counter) and the specimens weighed. The endothelial appearances of the traumatised and control arteries were examined by scanning electron microscopy (Plate 3.4, 3.5).

Specimen radioactivity counts were measured over a 10 minute period. Carotid platelet uptake was estimated by expressing the radioactivity counts from each specimen, per gram of tissue, as a

Plate 3

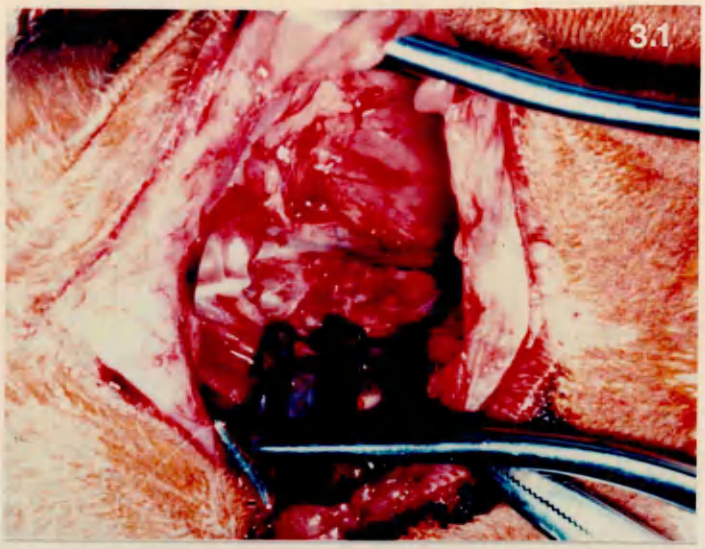
3.1 The left carotid bifurcation in the rabbit. The common carotid artery is seen on the right of the exposure with the bifurcation to the left at the level of the thyroid cartilage.

3.2 After mobilisation the common carotid artery is crushed using the jaws of a series of non toothed dissecting forceps, mounted on a G clamp. The compression injury is sustained for 3 minutes and then released.

3.3 Traumatised and control rabbit carotid arteries. The endothelial damage caused by external compression can be seen with the naked eye. Before measuring radioactivity in this specimen all post mortem thrombus and arterial adventitia is removed.

3.4 Low power scanning electron micrograph (x306) of the vascular endothelium following carotid trauma in rabbits. Thrombotic material is seen obscuring the normal folds of the arterial intima.

3.5 High power scanning electron micrograph (x2420). A network of platelets and fibrin make up luminal thrombus which is adherent to the damaged endothelial cells.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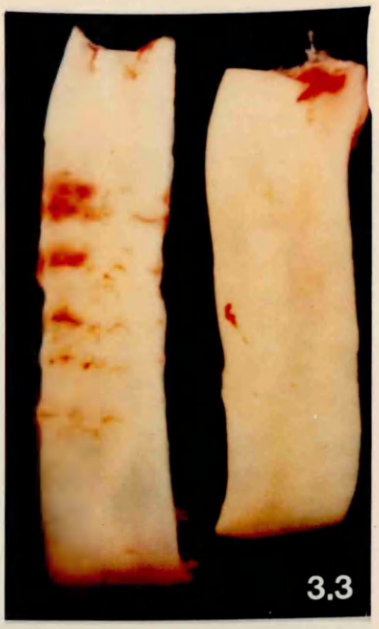
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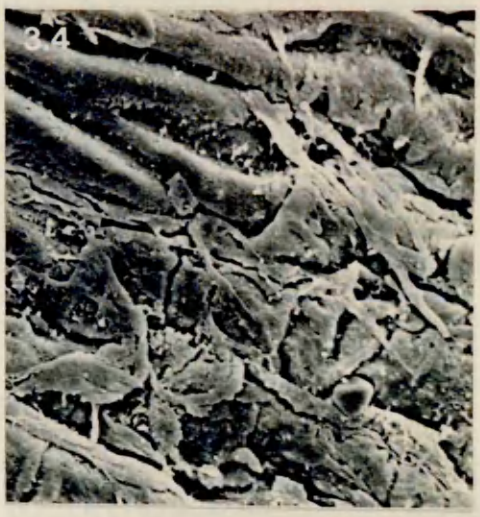
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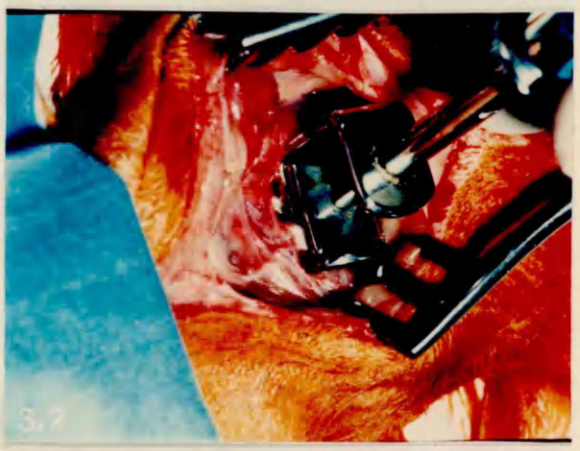

3.2


3.5

percentage of the counts from 1ml of circulating blood. Carotid Uptake Ratio was calculated by expressing the carotid platelet uptake of the traumatised artery as a ratio of that from the contralateral reference artery.

Specimen radioactivity in the traumatised and reference arteries was compared by the Student's t test for paired data. The comparison of carotid platelet uptake between traumatised and reference vessels was made by the Wilcoxon signed-rank test for paired data. Carotid uptake ratio in the treated and placebo groups were compared by the Mann Whitney U test for non parametric data.

5.3 Results

Mean labelling efficiency was 87.2 $\pm$ 4.2 percent and all radiolabelled platelets showed a normal aggregatory response to ADP.

5.3.i Specimen radioactivity

Specimen radioactivity in the reference artery of 3.2 $\pm$ 0.3 x 10^3 counts was marginally higher than background at 2.0 $\pm$ 0.07 x 10^3 (Fig 5.2) but was much lower than those from the traumatised artery at 5.7 $\pm$ 0.7 x 10^3 ($p<0.001$). Specimen radioactivity, per gram of artery, were consistently higher from the traumatised side at 4.79 $\pm$ 0.39 x 10^5 counts/gram compared to 3.11 $\pm$ 0.37 x 10^5 from the reference artery ($p<0.001$).

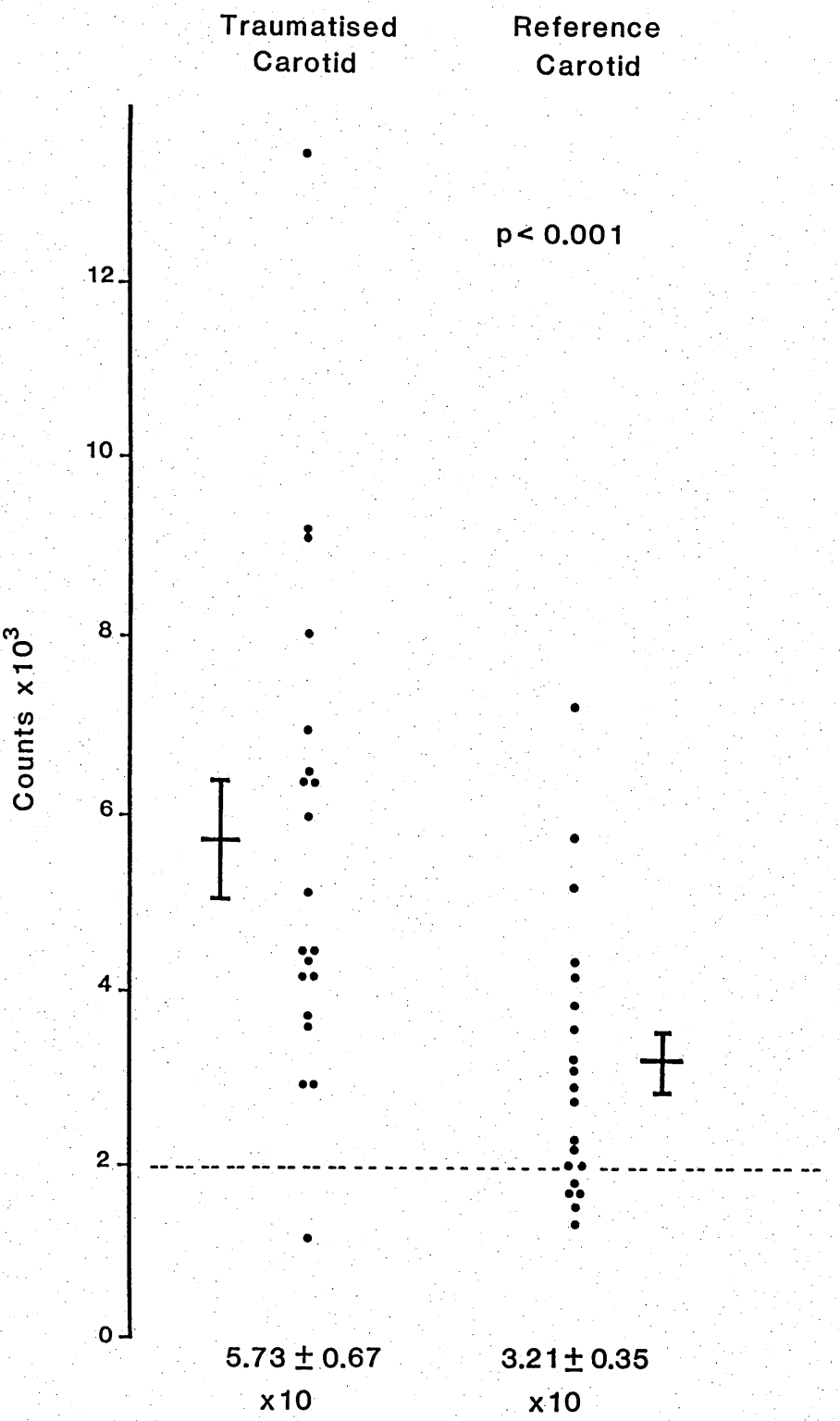


Figure 5.2 Radioactivity in traumatised and contralateral carotid arteries.

5.3.ii Carotid platelet uptake

Carotid platelet uptake, which expresses the counts per gram of artery as a percentage of the blood activity, showed consistently higher platelet accumulation on the traumatised artery of 55.1 $\pm$ 9.8 percent compared to 28.6 $\pm$ 6.3 percent from the reference side in the placebo group ($p<0.01$) (Fig 5.3). This difference in carotid platelet uptake was seen to a lesser extent in the animals treated with aspirin and dipyridamole with carotid platelet uptake of 43.7 $\pm$ 6.0 on the crushed vessels and 34.5 $\pm$ 5.3 percent on the reference arteries ($p<0.05$) (Fig 5.4). There was no difference between the platelet acivity form the reference arteries in the treated and control groups.

5.3.iii Carotid Uptake Ratio

Carotid Uptake Ratio was significantly reduced in those animals treated with ASA+DPM at 1.35 $\pm$ 0.13 compared to 2.2 $\pm$ 0.29 in the placebo group ($p<0.02$) (Fig 5.5).

5.4 Discussion

This model of intimal injury identifies radiolabelled platelet deposition on areas of damaged endothelium in rabbit carotid arteries. Platelet deposition was significantly higher on the injured arteries compared to the reference vessels which were mobilised in the same fashion. Radioactivity in the control vessels was similar to background which suggests that there was no appreciable platelet accumulation on the arterial adventitia or the

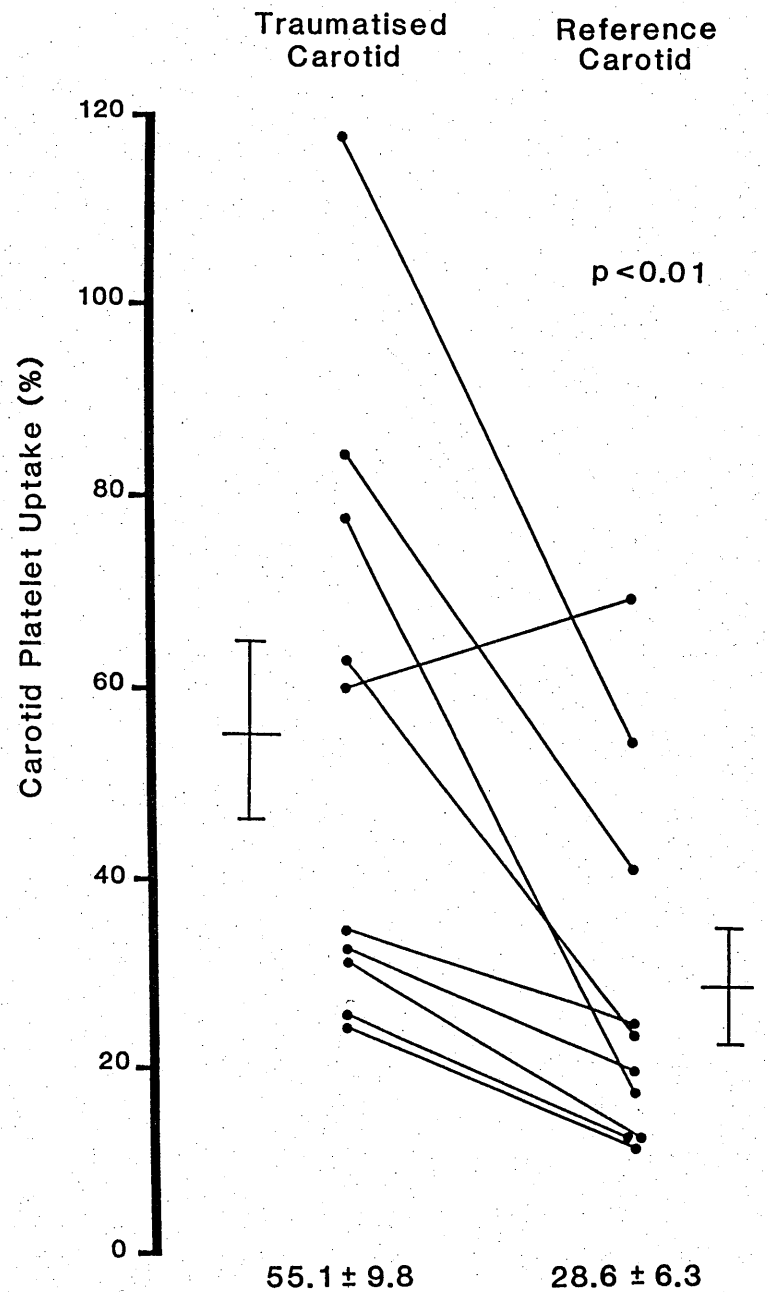


Figure 5.3 Carotid platelet uptake expressed the specimen radioactivity from the traumatised and reference arteries as a percentage of the activity in 1ml of blood.

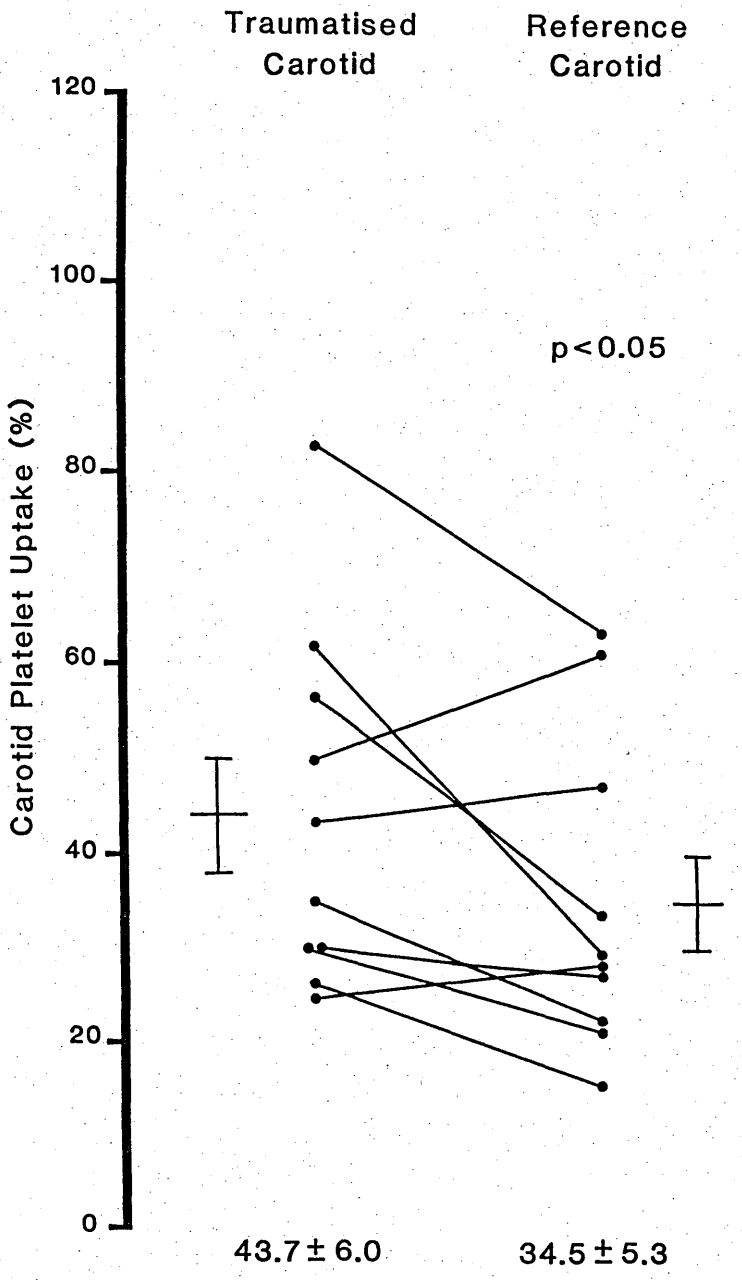


Figure 5.4 The difference in carotid platelet uptake between the traumatised and reference arteries is less marked in those animals receiving ASA and DPM.

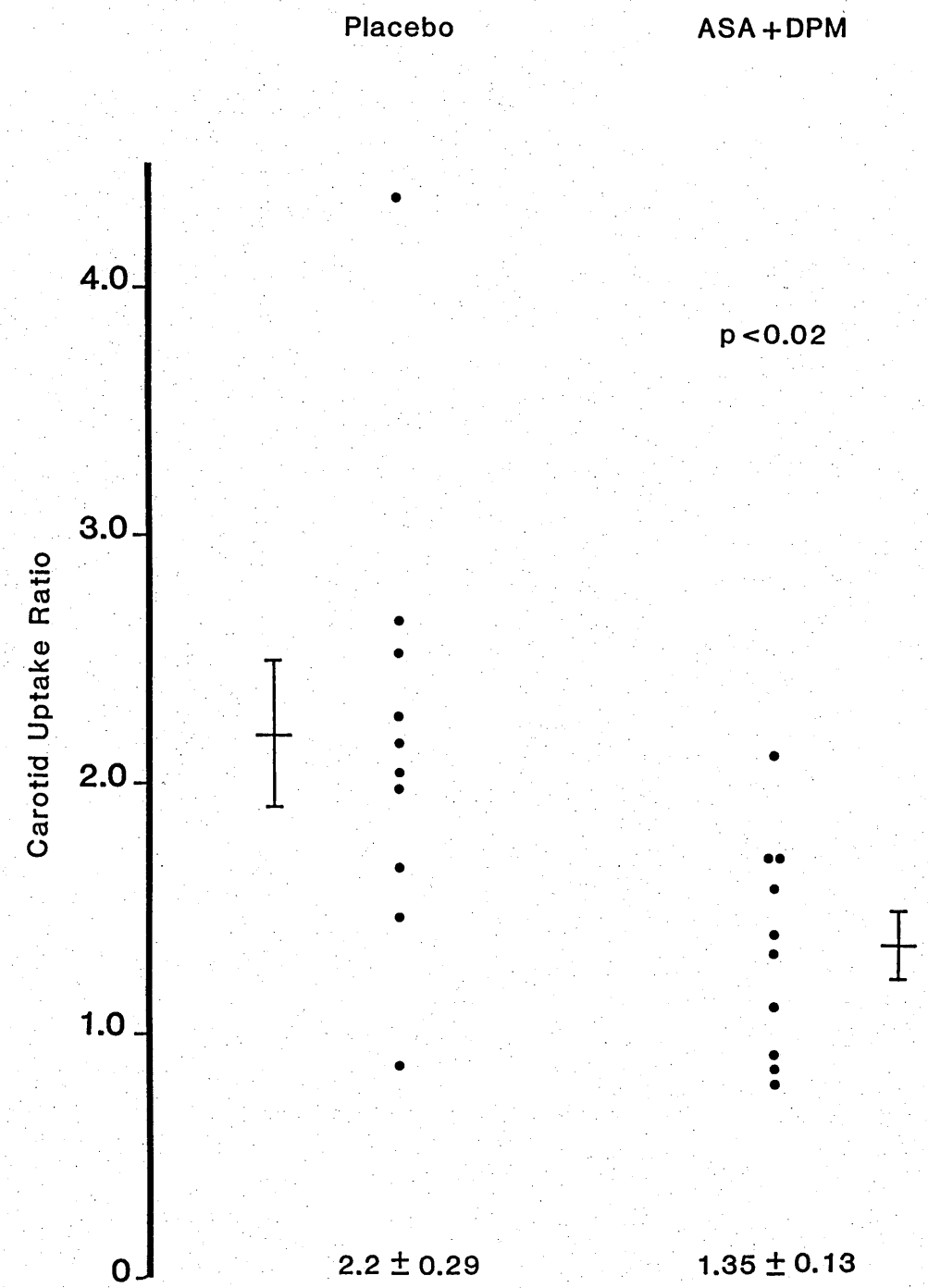


Figure 5.5 Carotid Uptake Ratio is significantly reduced in rabbits receiving platelet inhibitory therapy.

tissues of the wound. Using aspirin and dipyridamole the platelet interaction with the subendothelium was significantly reduced in this model.

Successful radiolabelling of rabbit platelets with ^{111}In oxine was achieved with efficiencies comparable to other series (Finklestein et al 1982: Schmidt, Rasmussen & Lorentzen 1982). The use of donor platelets confirmed that heterologous cells are effective in identifying endarterial damage (Grossman et al 1978). A proportion of the isotope is localised in the dense bodies and to a lesser extent the alpha granules of platelets, unlike human platelets which carry the radionucleotide in the cytoplasm of the cell (Baker et al 1982). This might theoretically result in isotope loss following the release reaction in platelet aggregation, but did not affect the assessment of arterial platelet deposition in this model. Although a proportion of the isotope may be lost from the alpha granules on aggregation, this activity may be retained in the arterial wall, in a similar way that platelet factor 4 is retained within the vessel wall after platelet deposition (Goldberg, Stemerman & Haudin 1980).

The degree of trauma inflicted in this model should be regarded as less severe than formal endarterectomy. Platelet uptake is more intense following endarterectomy than after endothelial stripping by balloon catheter (Crowley & Pierce 1981). This model measures the intense platelet deposition which occurs on the arterial wall in the

48 hours after trauma. Subsequent intraluminal platelet uptake will continue until intimal regeneration is complete after approximately 2 weeks (Reidy et al 1983) but has not been investigated in this experiment.

True endothelial regeneration occurs only over small areas of intimal loss. Large defects in the endothelium are replaced by a neointima of vascular smooth muscle which retains anti-aggregatory properties but does not reform a true endothelium (Reidy et al 1983). The interface between smooth muscle cells and endothelium is seen as a transitional zone where the endothelial cells are not aligned with flow. Whether endothelial cell repair is limited by an inherant inability to regenerate or is inhibited by smooth muscle remains unclear (Groves et al 1979).

Platelet deposition on damaged smooth muscle cells and its effect on endothelial repair has yet to be identified. A single endothelial injury causes less platelet aggregation than repeated trauma inflicted on the smooth muscle cells of neointima (Groves et al 1982). This increased thrombus formation after repeated damage is reduced by heparin which has no effect on platelet accumulation after a single injury (Kinlough-Rathbone et al 1983). The assessment of prostacylcin synthesis in damaged endothelium and reparative neointima will require further study.

5.5 Conclusions

This rabbit model has proved reliable for the investigation of radiolabelled platlet uptake in the carotid artery following standardised arterial injury. The efficacy of low dose aspirin and dipyridamole in reducing platelet deposition on the damaged arterial wall was confirmed. These methods can now be applied to patients where a comparable arterial injury is inflicted by carotid endarterectomy.

Chapter 6

Carotid Platelet Uptake Following Endarterectomy

6.1 Introduction

Since the problems of early postoperative thrombosis and restenosis following carotid endarterectomy were recognised, it has been suggested that platelets are involved in the pathophysiology of these processes (Stoney & String 1976; Ortega et al 1981). However this concept has defied further study as in vivo platelet accumulation had not previously been detectable in patients. In recent years, ^{111}Indium platelet labelling has greatly improved imaging of in vivo thrombus and these techniques have been applied to measurement of platelet uptake on prosthetic grafts in dogs and in patients (Mergerman et al 1982; Goldman et al 1982a; Goldman et al 1982b).

Platelet accumulation on the carotid artery was measured in patients to assess the thrombogenic characteristics of atherosclerotic lesions (Davis et al 1980; Goldman et al 1983b) but only recently has this technique been used to evaluate the accumulation of thrombus on the arterial wall following carotid endarterectomy (Lusby et al 1983; Stratton et al 1987).

This experiment investigates platelet deposition on the carotid artery in patients following endarterectomy. This has been evaluated both in the early post operative period and later at 2 months and compares gamma camera imaging with probe counting, the two principle methods by which radioactivity is assessed.

6.2 Patients and Methods

Ten patients undergoing unilateral carotid endarterectomy were included in this study. There were 6 men and 4 women with a mean age of 66.7 years (range 47-79 years). Standard endarterectomy was performed under general anaesthesia and heparin 5000 units was administered prior to cross clamping of the common carotid artery. Longitudinal arteriotomy was performed and an intraluminal shunt was introducted if cerebral perfusion was inadequate. In this group, the arteriotomy was closed by direct suture, without the use of patch angioplasty, with continuous 6/0 Prolene (W8706 Ethicon Ltd, Edinburgh, Scotland).

On the second postoperative day autologous platelets from 26mls of blood were prepared by the method described in chapter 4 and labelled with approximately 200uCi ^{111}Indium oxine (Hawker et al 1980). Two hours following reinjection, carotid platelet uptake was measured by both gamma camera imaging and probe counting with repeated measurements taken at 24 and 48 hours after injection. Prior to assessment of carotid radioactivity, the surface markings of the carotid bifurcation were identified on both sides of the neck by continuous wave ultrasound with a 4MHz probe (Vasoscan, Sonicaid 8220-6901, Sonicaid Ltd., Chichester, England). This ensured accurate probe geometry and confirmed internal carotid patency.

Counts from the operated artery were compared to those form non operated side using both methods of radioactivity assessment. The

counts, from the operated artery over reference, were expressed as the Carotid Uptake Ratio (CUR) and the daily increase in this ratio, over the three days of this study, was termed Thrombogenicity Index (TI). This procedure was repeated 8 weeks later, when after a further injection of autologous labelled platelets, Carotid Uptake Ratio and Thrombogenicity Index were again calculated to assess late postoperative platelet uptake. In addition the gamma images were examined visually by an indepentent observer asessing increased platelet uptake over the carotid arteries.

Statistical comparison of the counts from the operated and reference carotid arteries was performed by the Wilcoxon signed-rank test for paired data as was the comparison between early and late results of CUR and TI. The correlation between CUR and TI, derived from gamma camera and probe counting, was calculated by linear regression.

6.3 Results

All patients recovered from anaesthesia without neurological deficity and non-invasive carotid assessment at 48 hours demonstrated wide patency in all 10 operated arteries. Mean ($\pm$ sem) labelling efficiency in all 10 patients, 2 days after surgery, was 93.6 $\pm$ 0.8 percent with injected activity of 258 $\pm$ 16.4 uCi. These were similar to the results of 94.5 $\pm$ 0.5 percent and 222.3 $\pm$ 13.9 uCi respectively obtained from subsequent labelling procedures at 2 months after operation. In all cases labelled platelets showed a

satisfactory response to ADP and were sterile to both aerobic and anaerobic culture. All platelet preparations had labelling efficiencies in excess of 90 percent. Values of CUR and TI calculated from gamma images are recorded in Table 6.1. Comparative results of probe counting are seen in Table 6.2.

6.3.i Gamma camera imaging

Three days after surgery, mean counts per gamma camera cell of 46.3 $\pm$ 4.3, calculated from an area of interest over the operated artery were significantly higher than 38.6 $\pm$ 3.9 counts per pixcel recorded on the non operated side ($p<0.001$) (Fig 6.1). At two months the counts per pixel over the operated artery had fallen to 38.9 $\pm$ 3.2 and were similar to the reference counts of 39.1 $\pm$ 3.1 (Fig 6.2). By expressing the radioactivity from the operated artery as a ratio of the reference, mean CUR was calculated at 1.22 $\pm$ 0.04 in the early postoperative period. This initial CUR was significantly higher than that of 1.01 $\pm$ 0.06, measured at 2 months ($p<0.01$) (Fig 6.3).

An independent observer correctly identified the side of endarterectomy in 8 of the 10 early scans and judged the remaining 2 equivocal. Visual analysis of gamma scans from the later examination correctly identified the side of operation in two patients, judged 7 equivocal and was incorrect in one patient.

Thrombogenicity Index, reflecting the rate of platelet deposition, was initially raised at 0.11 $\pm$ 0.01 in the early post

Patient no	CUR^1	CUR^2	TI^1	TI^2
1	1.18	0.78	0.14	-0.05
2	1.15	1.12	0.08	0.11
3	1.19	1.29	0.08	0.03
4	1.48	0.98	0.13	-0.03
5	1.10	1.18	0.07	0.04
6	1.26	0.94	0.04	-0.01
7	0.96	0.69	0.15	0.09
8	1.30	1.13	0.21	0.05
9	1.28	1.08	0.03	0
10	1.34	0.88	0.11	-0.05

Table 6.1 **Tabulated values of Carotid Uptake Ratio and Thrombogenicity Index measured in the early[1] and later[2] postoperative periods using gamma camera assessment of radioactivity.**

Patient no	CUR^1	CUR^2	TI^1	TI^2
1	0.96	0.94	-0.4	0.02
2	0.95	0.78	-0.28	-0.30
3	1.30	1.31	-0.12	0.09
4	1.46	1.15	0.13	-0.09
5	0.84	1.33	0.05	-0.17
6	0.76	0.88	0.05	-0.09
7	1.29	0.95	-0.10	-0.02
8	0.74	0.77	0.06	0.04
9	1.16	0.97	0.02	-0.08
10	0.84	1.03	-0.11	0.11

Table 6.2 **Values of early and late CUR and TI calculated from data obtained from probe counting.**

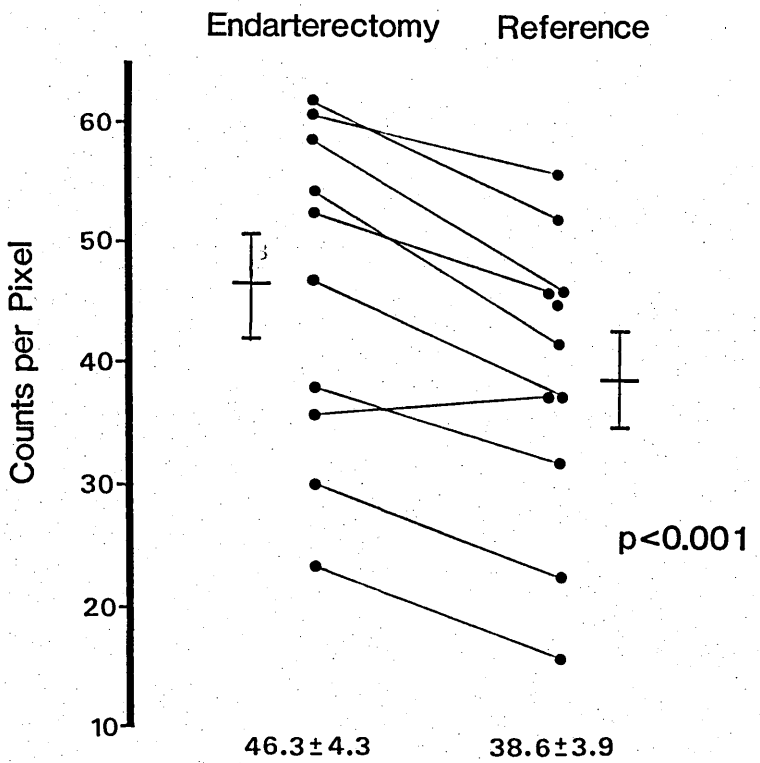


Figure 6.1 Counts per gamma camera cell measured over the operated and reference carotid arteries in 10 patients, 3 days after endarterectomy.

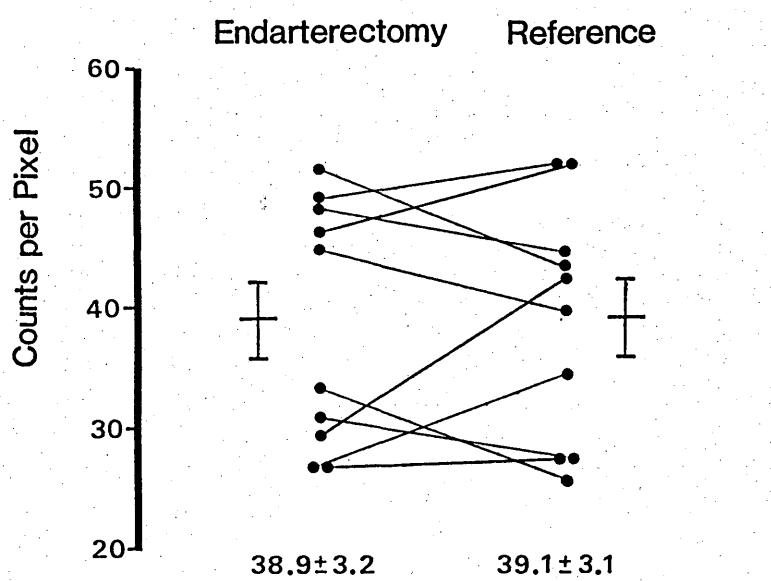


Figure 6.2 Comparison of gamma camera radioactivity from the endarterectomy and reference, 2 months following surgery.

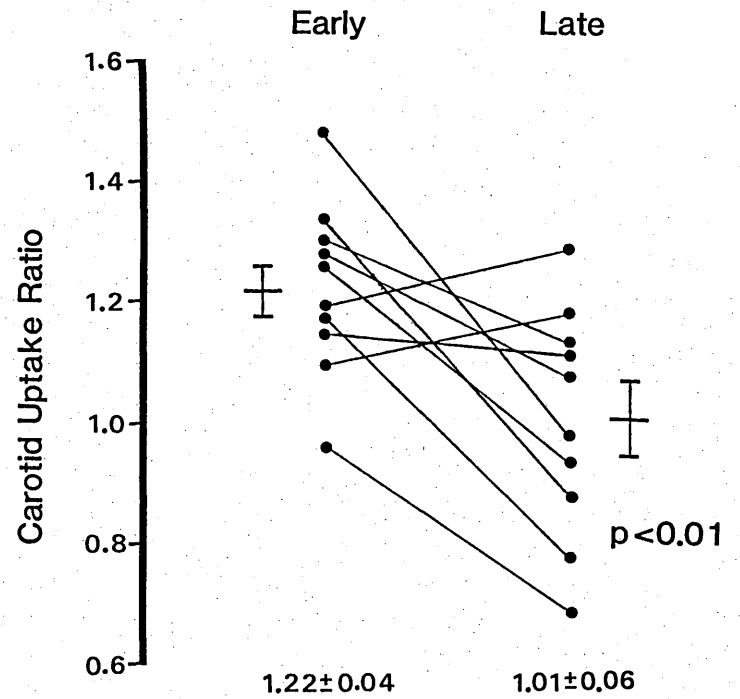


Figure 6.3 Carotid Uptake Ratio derived from gamma camera at 3 days and 2 months following endarterectomy.

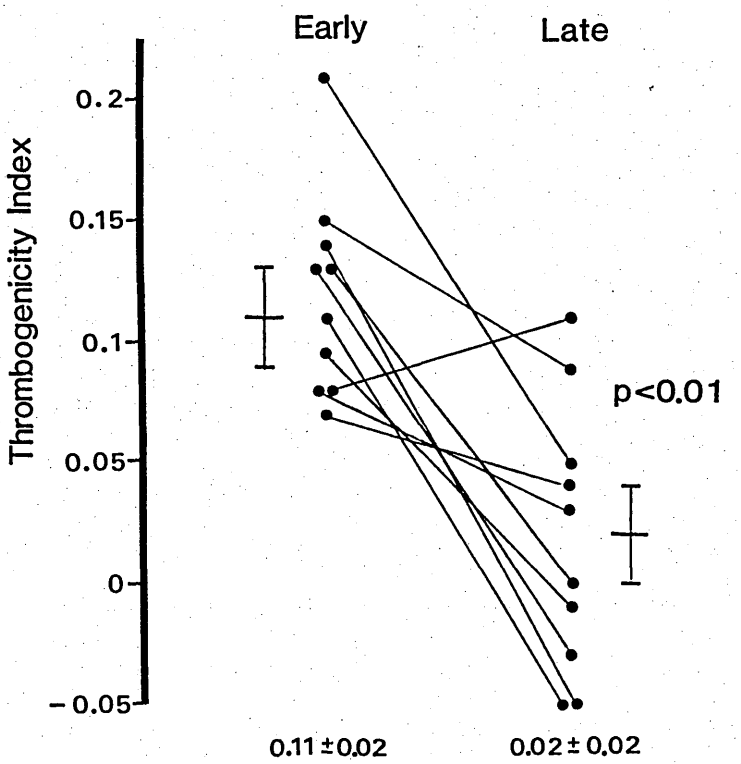


Figure 6.4 Thrombogenicity Index is significantly reduced 2 months after surgery.

operative period, but fell to 0.02 ± 0.02 by 2 months ($p<0.01$) (Fig 6.4).

6.3.ii Probe counting

In the early postoperative study, the counts measured by sodium iodide crystal and rate meter over the operated carotid of 3.0 ± 0.8 x 10^3 were indistinguishable from the contralateral non operated reference artery of 2.9 ± 0.8 x 10^3 (Fig 6.5). At 2 months identical counts of 1.9 ± 0.3 x 10^3 were recorded over both operated and reference arteries (Fig 6.6). There was no significant difference between the early results of CUR at 1.03 ± 0.08, and 1.01 ± 0.06 two months later (Fig 6.7).

Results of Thrombogenicity Index, from the probe counting data, also failed to identify any difference between -0.03 ± 0.03 calculated from the second to fourth postoperative days and -0.05 ± 0.04 over a 3 day period, 2 months later (Fig 6.8).

Alternative reference areas had no greater sensitivity. Using the forearm as a reference, CUR was found to be 1.75 ± 0.3 in the early study but unchanged at 1.71 ± 0.1 at 2 months. These higher readings merely reflect the volume of tissue and blood in the neck compared to the forearm, however the scatter of these counts at 1.8 ± 0.4 x 10^3 was similar to the carotid counts of 3.0 ± 0.8 x 10^3.

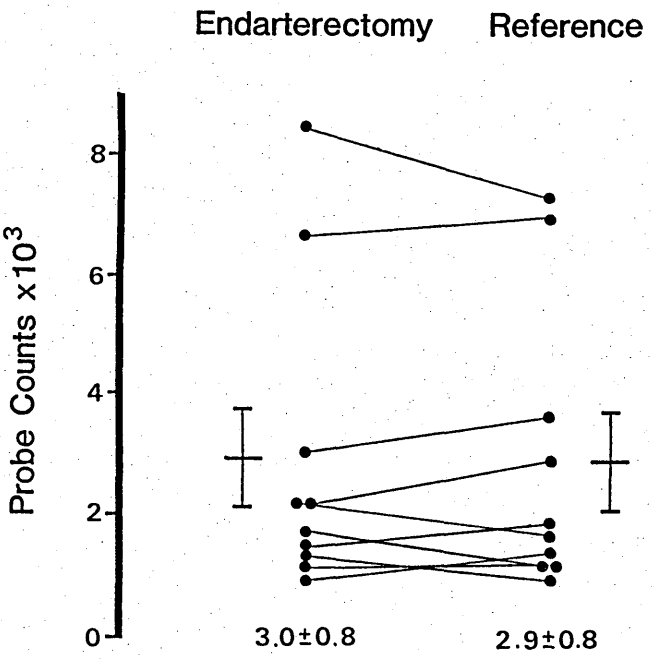


Figure 6.5 Early post-operative probe counting showed similar activity over the operated and reference carotids.

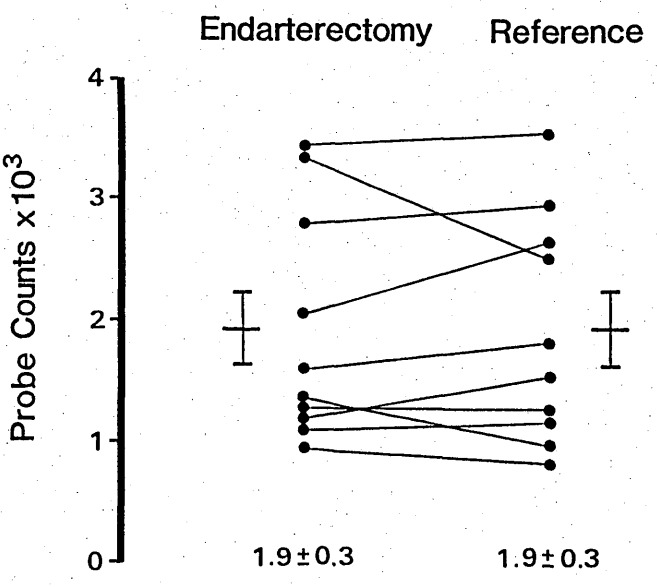


Figure 6.6 Identical results were obtained for radioactivity measured by probe counting 2 months after surgery.

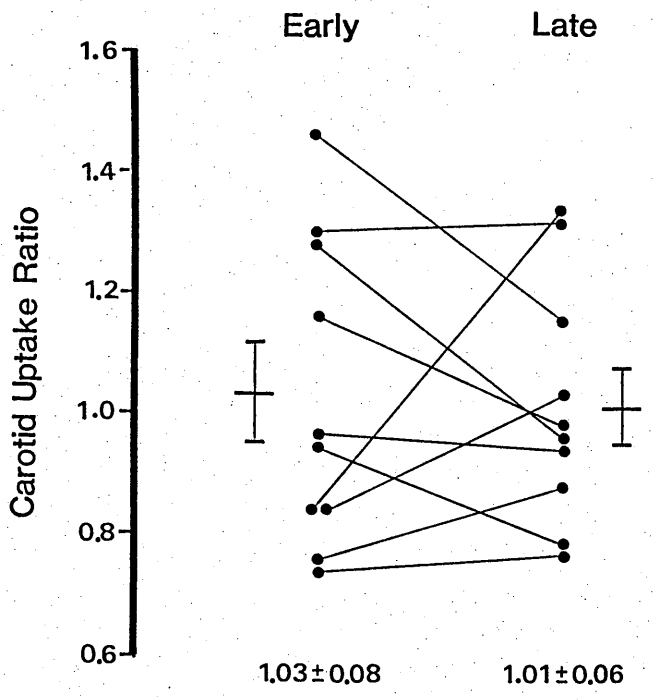


Figure 6.7 Early Carotid Uptake Ratio was indistinguishable from late CUR on probe counting.

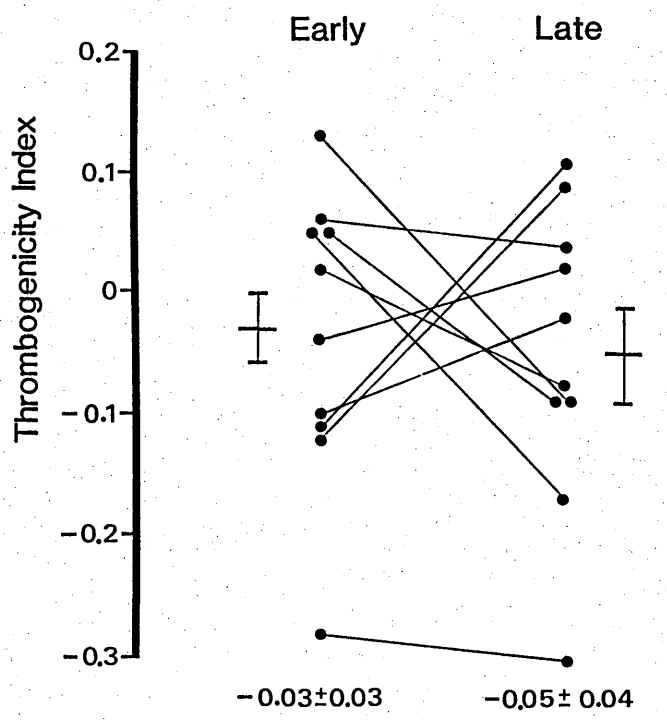


Figure 6.8 Probe counting failed to show any difference between early and late Thrombogenicity Index.

6.3.iii Comparison of methods

There was no significant correlation between the values of CUR and TI measured by the 2 methods of radioactivity assessment. Linear regression comparing probe counting and gamma imaging showed no relationship between Carotid Uptake Ratio ($r=0.06$) (Fig 6.9) and Thrombogenicity Index ($r=0.22$) in the early postoperative period (Fig 6.10). Similarly these indices of platelet uptake were not comparable in the later examination with $r=0.36$ for CUR and an inverse trend seen with TI ($r=-0.49$).

6.4 Discussion

These results demonstrate the efficacy of gamma imaging in the measurement of radiolabelled platelet uptake at the site of carotid endarterectomy. These gamma scans consistently showed detectable levels of radioactivity in the early postoperative period, both visually and on computer analysis. By comparing the operated artery to the contralateral reference and calculating Carotid Uptake Ratio and Thrombogenicity Index, it was posssible to show a significant reduction in platelet accumulation in the later examination. Although this resolution of the platelet-vessel wall interaction at that stage suggests the regeneration of a neointima, this remains unproven without histological evidence.

These data show probe counting to be ineffective in identifying postoperative carotid platelet uptake. Even in patients in whom increased radioactivity was easily visible on gamma camera imaging,

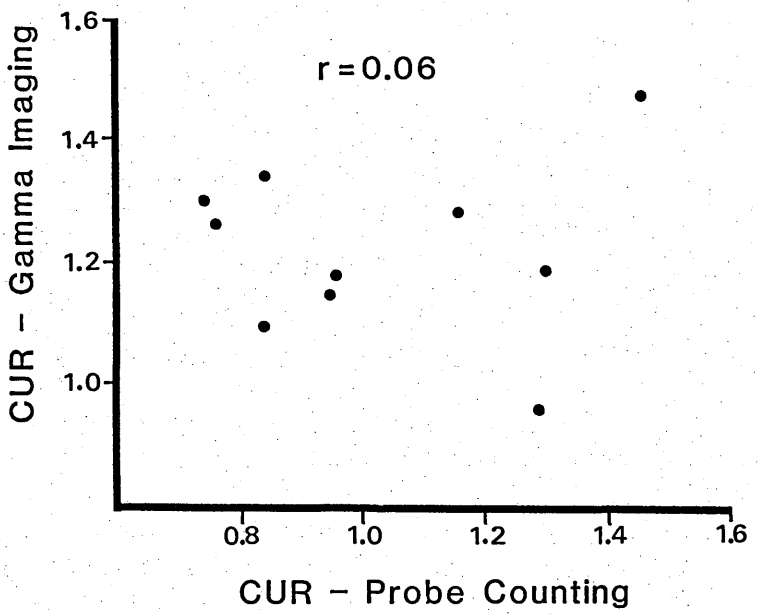


Figure 6.9 Values of early CUR, calculated by probe counting (X-axis) and gamma imaging (y-axis), failed to show any similarity.

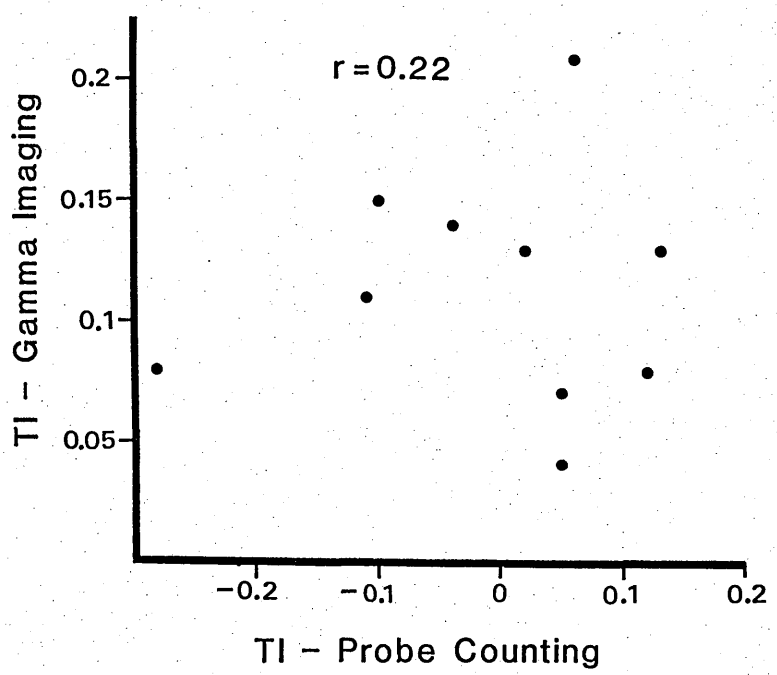


Figure 6.10 Early postoperative TI did not correlate between the 2 methods of radioactivity assessment.

probe counting did not detect increased levels of radioactivity. This contrasts to the studies in patients measuring radiolabelled platelet uptake on femoro-popliteal grafts by probe counting (Goldman et al 1982a). Inaccuracies with this technique may be due to insufficient collimation such that activity is recorded from the other vascular structures in the neck. The proximity of the operated and reference arteries may be important as activity from the endarterectomised carotid may influence counts recorded from the reference artery. The wide spread of the probe counting data, seen with the high standard error of the mean, may reflect the inaccuracies of this method. Even using the forearm reference, counts were still inconsistent and failed to identify increased platelet uptake in the early postoperative period.

The use of the contralateral carotid artery reference for the calculation of CUR and TI may lead to error as platelet accumulation has been identified at the site of carotid atheroma (Goldman et al 1983b). Only one patient in this series had a postoperative CUR of less than 1.0 which would indicate greater platelet activity on the non-operated side. This patient was found to have significant contralateral disease with a 50% internal carotid stenosis on non-invasive assessment. The reference radioactivity was comparatively higher in the later scan where CUR was recorded at 0.69, indicating a reduction in platelet accumulation at the site of endarterectomy as would be expected at this stage (Plate 2.5). Consistently higher

uptake on the reference artery might suggest continuous platelet accumulation on a thrombogenic, atheromatous plaque.

The early postoperative studies were performed from the second to the fourth postoperative day to avoid radiolabelled platelet uptake into wound haematoma. Previous studies, injecting labelled platelets within one hour of endarterectomy, have successfully measured greater activity on the side of operation but made no allowance for wound uptake (Lusby et al 1983). However this consideration may not be important as control studies in patients undergoing inguinal hernia repair have not demonstrated platelet uptake into the tissues of the wound in the immediate postoperative period (Stratton et al 1987).

6.5 Conclusion

Radiolabelling of autologous platelets with ^{111}Indium and gamma camera imaging is a suitable method for measuring platelet uptake in the early postoperative period and has demonstrated a reduction in carotid platelet uptake by 2 months after surgery. Carotid Uptake Ratio and Thrombogenicity Index both appear to be reliable measurements of platelet accumulation.

Results of probe counting have not shown an increase in postoperative CUR or TI and do not correlate with gamma imaging. The high background blood pool in the neck seems to make probe counting inappropriate in these circumstances.

Chapter 7

The Effect of Platelet Inhibitory Therapy on Carotid Thrombogenicity Following Endarterectomy

7.1 Introduction

Although antiplatelet therapy is widely used in the treatment of cardiovascular disease, the measurement of in vivo changes in platelet function and interaction with the arterial wall have proved more difficult to investigate. Clinical studies of aspirin and dipyridamole showed a reduction in the coronary mortality in the myocardial reinfarction (PARIS 1980) and recurrent stroke (Canadian Cooperative Study Group 1978) but these epidemiological surveys did not demonstrate the mechanism by which this morbidity was improved. Similarly although aspirin and dipyridamole were significantly better than placebo in preserving graft patency following aorto-coronary bypass (Chesebro et al 1984), lower platelet activity in these grafts was not evaluated.

The difficulties arise in the detection of platelet uptake at areas of arterial damage or disease. In vitro platelet kinetics are certainly altered by the cyclo-oxygenase blockade of aspirin and phosphodiesterase inhibition with dipyrimadole but the methods of investigating reduced in vivo platelet activity in the blood vessels of patients are unsatisfactory (Eichner 1984). Improved coronary vein graft patency suggests that platelet inhibition prevents early postoperative thrombosis but this is seen only when therapy has been commenced either preoperatively or in the early postoperative period (Chesebro et al 1984; Lorenz et al 1984). There was no demonstrable benefit from therapy started more than 48 hours after surgery (Brooks et al 1985). Long term anti-platelet therapy also reduced the

incidence of late graft occlusion, perhaps inhibiting intimal hyperplasia which may be platelet mediated, and this would encourage the use of therapy for at least a year after surgery (Chesebro et al 1984).

The choice of either high or low doses of aspirin remains a problem yet unsolved by clinical investigation. In high doses inhibition of endothelial cyclo-oxygenase activity may reduce prostacyclin synthesis whereas low doses, while irreversibly blocking platelet cyclo-oxygenase, may have less effect on the arachidonic acid metabolism in vascular endothelium (Clowes 1986). Experimental data suggests that orally administered low dose aspirin may produce a cumulative and optimal acetylation of platelet cyclo-oxygenase in the portal circulation without affecting the systemic vascular endothelium (Pedersen & Fitzgerald 1984). This may be important in vein grafts and following endarterectomy where neointima formed by a combination of arterial smooth muscle and regenerated endothelium retains anti-thrombotic properties (Reidy et al 1983).

At present aspirin, with or without dipypidamole, has been used extensively both before and after carotid surgery. The majority of patients (72%) refered to the Vascular Service at Charing Cross Hospital for treatment of symptomatic carotid artery disease have been prescribed aspirin since the onset of symptoms. In many centres aspirin is used empirically after carotid surgery (Glover et al 1985; Ackroyd et al 1986) but the effects on platelet uptake on the

endarterectomy segment have not been investigated nor has the clinical efficacy of this therapy has not been confirmed. Although a retrospective study suggested that platelet inhibitory therapy reduced the incidence of thrombosis related neurological deficits following carotid endarterectomy (Edwards et al 1985), conclusions should not be drawn from this non randomised study.

The direct effects of inhibitory therapy on platelet uptake on the arterial wall following carotid endarterectomy have not been established. It is the purpose of this study to measure postoperative platelet uptake in patients following carotid endarterectomy and to evaluate the effects of a standard regimen of platelet inhibitory therapy.

7.2 Patients and Methods

Eighteen patients were studied after unilateral carotid endarterectomy for treatment of amaurosis fugax or transient cerebral ischaemia. There were 12 men and 6 women with mean age 63.5 years (range 52-78). Twelve patients who had been taking platelet inhibitory therapy at referal were continued on aspirin (ASA) 300mg daily and dipyridamole (DPM) 100mg three times daily starting pre-operatively and continuing to the end of the study period. Six patients who were refered for surgery and had not been taking platelet inhibitory drugs or other non-steroidal anti-inflamatory drugs for 14 days prior to operation were included for comparison as controls.

Endarterectomy was performed under general anaesthetia, with systemic heparinisation, using a longitudinal arteriotomy from the common carotid extending into the internal carotid artery. Continuous 6.0 Prolene was used to repair the arteriotomy and in these patients patch angioplasty was not required.

7.2.i Assessment of platelet uptake

On the second postoperative day autologous platelets were labelled with ^{111}Indium oxine using the method described in Chapter 4 (Hawker et al 1980). Gamma images were taken 2 hours after infusion of radiolabelled platelets and subsequently at 24 and 48 hours. Carotid Uptake Ratio at 24 hours and Thrombogenicity Index were calculated.

Gamma camera counts were compared by the Wilcoxon signed-rank test for paired data and results of Carotid Uptake Ratio and Thrombogenicity Index were analysed using the Mann Whitney U test for non parametric data.

7.3 Results

Twelve patients in the treatment group tolerated drug therapy without side effects and none of the 18 patients developed postoperative wound haematomas. There were no perioperative or postoperative neurological deficits. Non-invasive assessment of the endarterectomy perfomed 5 days after operation showed all vessels to be widely patent.

7.3.i Carotid platelet uptake

Radiolabelled platelet deposition was identified by an independent observer on 11 of the 18 gamma images taken at 24 hours. In all 18 patients mean (±sem) counts per pixcel of 39.8 ± 4.1 over the operated artery was significantly higher than 32.1 ± 2.7 over the contralateral reference side ($p<0.001$) (Fig 7.1). This difference in gamma camera counts was seen in both those receiving anti-platelet therapy and the untreated controls. Radioactivity over the operated carotids was 35.4 ± 4.6 counts per pixcel in the treated group compared to 31.2 ± 3.4 over the reference side ($p<0.01$). In the untreated patients, counts over the endarterectomy were statistically higher at 48.7 ± 7.4 compared to 33.8 ± 4.9 despite the smaller numbers in this group ($p<0.01$).

7.3.ii Carotid Uptake Ratio

Mean (± sem) CUR in all 18 patients was 1.22 ± 0.04 and was significantly lower in 12 patients receiving ASA and DPM at 1.11 ± 0.04 compared to 1.44 ± 0.03 in 6 untreated patients ($p<0.002$) (Fig 7.2).

7.3.iii Thrombogenicity Index

Overall TI was calculated at 0.15 ± 0.04 and was similarly reduced from 0.34 ± 0.07 in control patients to 0.06 ± 0.02 in those taking ASA and DPM ($p<0.002$) (Fig 7.3).

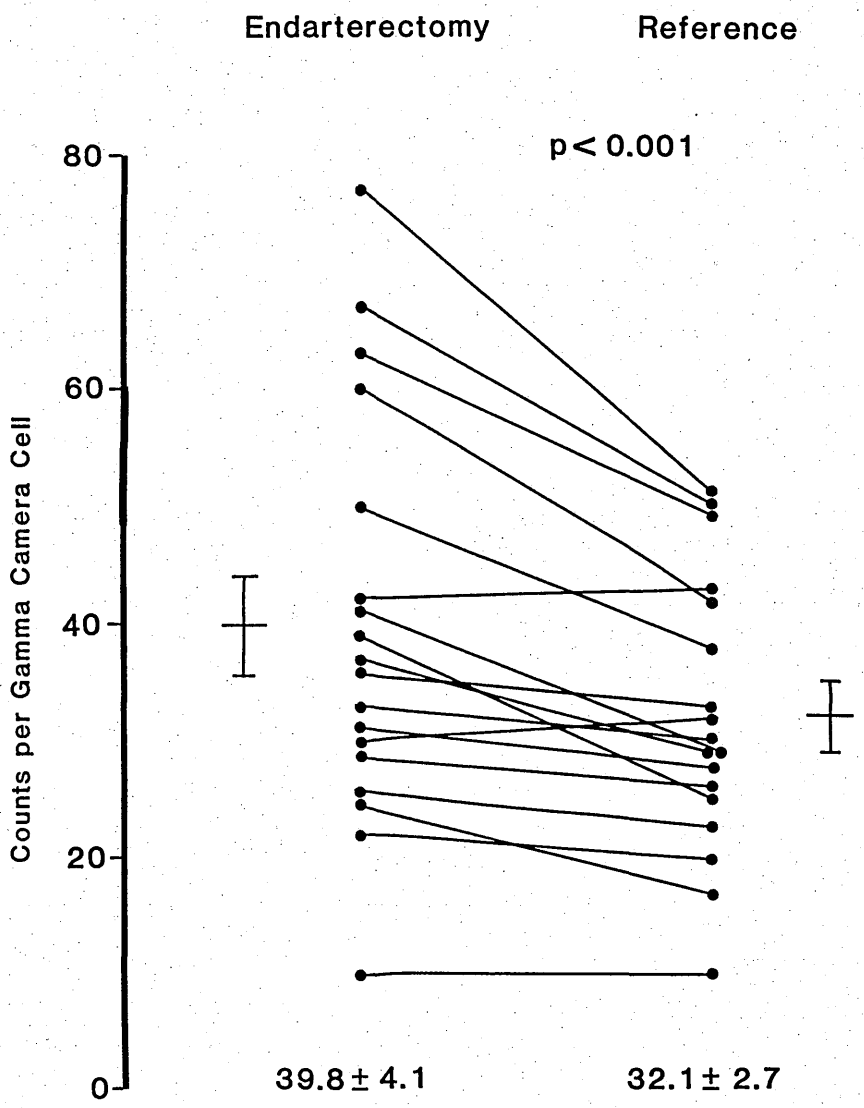


Figure 7.1 Counts per gamma camera cell in areas of interest over the operated and contralateral artery in 18 patients following carotid endarterectomy.

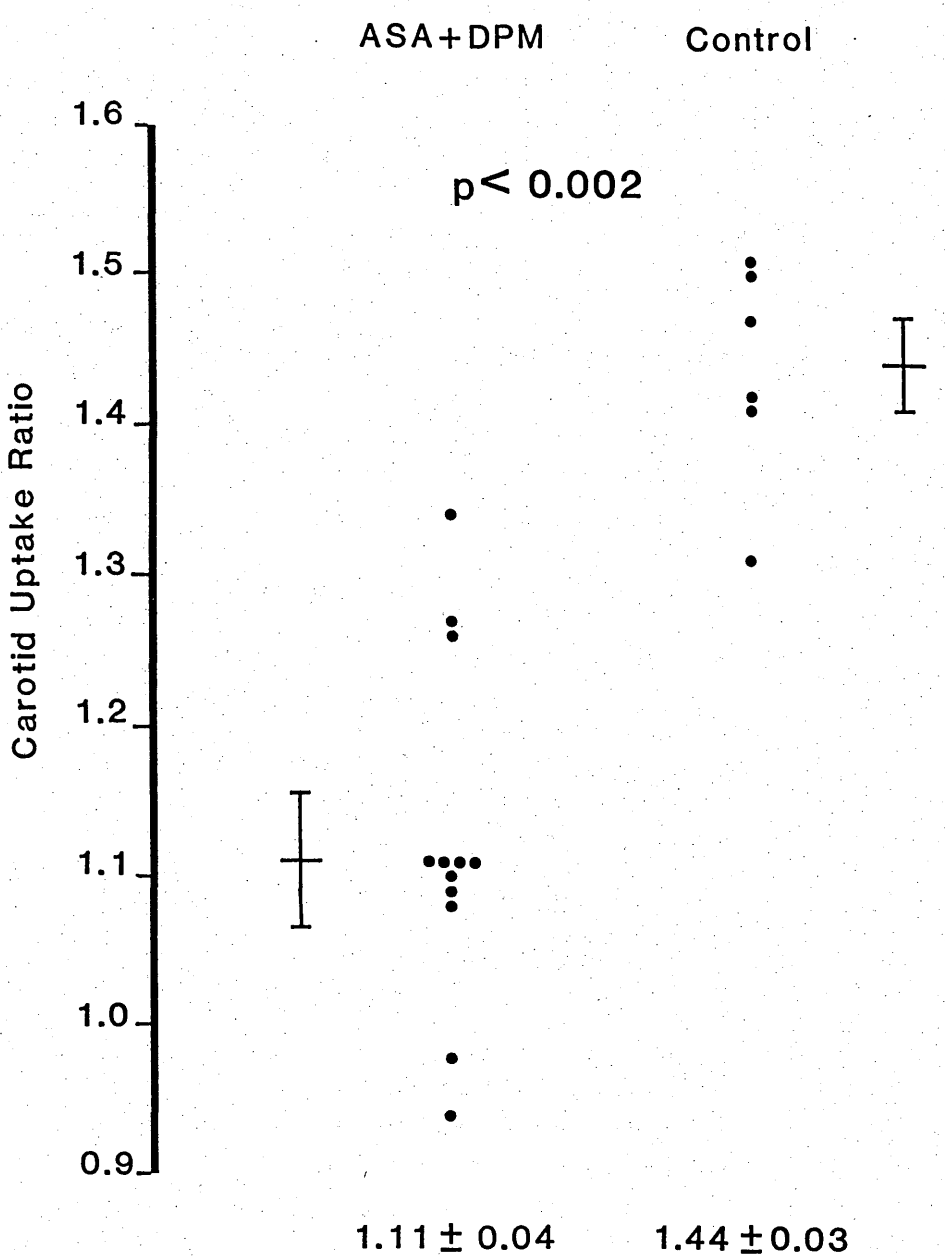


Figure 7.2 Carotid Uptake Ratio in 12 patients receiving platelet inhibitory therapy compared to 6 untreated controls.

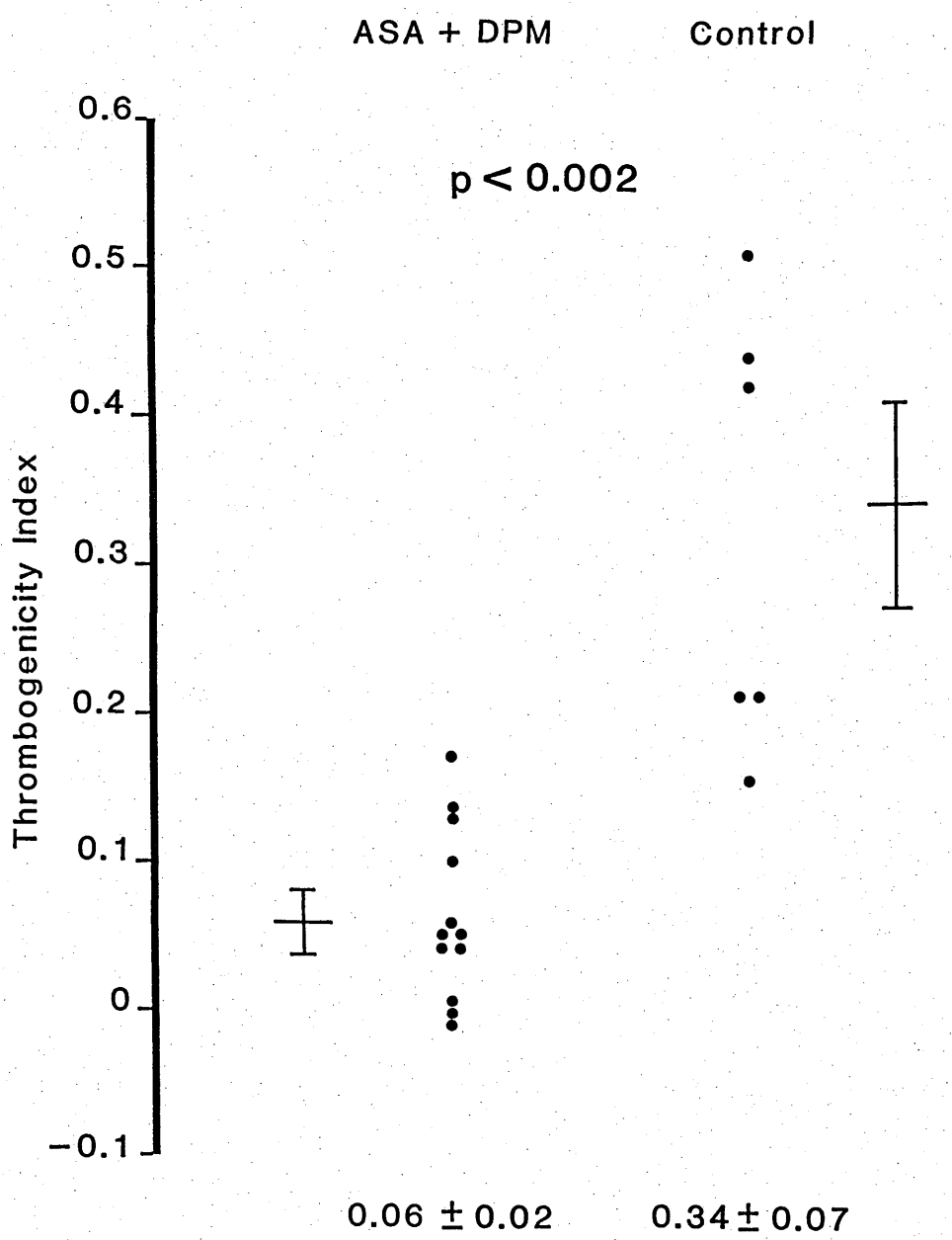


Figure 7.3 Thrombogenicity Index of the endarterectomised carotid in patients receiving platelet inhibitory therapy.

7.4 Discussion

This study demonstrates a significant reduction in radiolabelled platelet uptake on the luminal surface of the vessel wall after endarterectomy in those patients receiving platelet inhibitory therapy. This is seen both in the Carotid Uptake Ratio, comparing the operated to the reference side, and Thrombogenicity Index which reflects the rate of platelet deposition.

The administration of aspirin 300mg is between the accepted low (100mg) and high (1gm) daily doses (Masotti et al 1979). In the patients studied, this dose of aspirin in combination with dipyridamole 100mg tds caused no reported side effects, in particular there was no evidence of gastro-intestinal upset nor of gastro-intestinal haemorrhage. This platelet inhibitory regimen is used consistently in the patients following vascular surgery at Charing Cross Hospital with satisfactory patient compliance and with relatively few of the side effects commonly reported (PARIS 1980; Brooks et al 1985).

Endarterectomy removes the vascular endothelium leaving the connective tissue of the media exposed to the circulating blood. Initial platelet adhesion will form a layer of platelets and fibrin on the luminal suface of the endarterectomy segment regardless of cyclo-oxygenase inhibition as this early aggregation is mediated by collagen. Further platelet deposition on the platelet-fibrin matrix is effected by aggregation as Thromboxane A_2 is released from adherent

platelets. It is this secondary platelet aggregation which may be inhibited by cyclo-oxygenase blockade and reduces the platelet accumulation which is identified at the site of endarterectomy in this experiment (Packham & Mustard 1980).

It is unlikely that endothelial repair would be sufficiently advance that local endothelial prostacyclin production was reestablished in the 5 days taken to complete this study. Therefore, in the absence of an secretory endothelial surface, the theoretical effects of cyclo-oxygenase inhibition of prostacyclin synthesis should not be relevant.

There is, as yet, no evidence to suggest that a reduction in platelet deposition after carotid surgery is clinically beneficial. Total absence of the platelet response to tissue damage would be inappropriate as platelets play a major role in normal haemostasis. In addition the release of platelet growth factors may be required in the cellular response to trauma and be instrumental in tissue proliferation and repair (Ross 1985). Until the mechanism by which platelets contribute to endothelial repair is defined, it is inappropriate to recommend platelet inhibitory therapy in these circumstances.

7.5 Conclusion

The platelet response in the early postoperative period following endarterectomy can be modified with platelet inhibitory

therapy. Using radiolabelled platelets, a standard regimen of aspirin and dipyridamole significantly reduced carotid platelet accumulation in the postoperative period.

Chapter 8

The Effect of Patch Angioplasty on Postoperative Platelet Uptake in the Carotid Artery

8.1 Introduction

Patch angioplasty has been used from the earliest days of carotid surgery, however the number of patients undergoing this procedure with endarterectomy varies widely between published series. De Weese reported a series in which less than 4% of cases required patch repair, and Pierce described 139 consecutive endarterectomies with only a single case of angioplasty (De Weese et al 1968; Pierce et al 1984). In contrast Blaisdell's study of angiographic assessment of restenosis reported that virtually all patients (97%) had patch repair of the arteriotomy, in keeping with Edwards who used patch angioplasty in 70% of cases (Blaisdell et al 1967; Edwards et al 1985).

The indications for patch angioplasty are seldom described and many surgeons patch selectively, simply using this technique if the artery "is small" (Thompson et al 1970). Endarterectomy is usually performed to remove atheromatous stenosis but closure of the arteriotomy may cause localised narrowing. Patch repair remains the standard procedure by which adequate diameter is maintained. Although this may increase the cross sectional area of the artery, the effects on flow are less certain. Flow patterns at the carotid bifurcation have been closely studied, and it has been suggested that turbulence and the resulting changes in shear stress may in some degree, contribute to the pathogenesis of atheroma (Zarins et al 1982). Following endarterectomy, the removal of a haemodynamic stenosis may improve laminar flow but excessive widening with a large

patch may be detrimental. Inappropriate widening of the arterial lumen will interupt the laminar flow causing turbulence and abnormal shear on the arterial wall. Also a vessel repaired by patch angioplasty may effectively become aneurysmal with the deposition of layers of thrombus in regions of low blood flow. Thus it has been stressed that the graft must be fashioned so as to produce a uniform lumen of normal dimensions (Katz et al 1987).

The choice of either prosthetic or autogenous vein has not yet been investigated and although vein with a secretory endothelium should be less thrombogenic, this has not been confirmed for patch angioplasty by in vivo studies. It is known that prosthetic materials implanted as arterial grafts form a pseudo-intima which continue to accumulate circulating platelets more than one year after implantation (Goldman et al 1982b). This may not occur following patch angioplasty where neointima formed from pannus ingrowth probably covers the surface of the relatively small carotid patch.

Prevention of restenosis is the prime indication for patch angioplasty, and although the pathogenesis of restenosis is poorly understood, this technique has been shown to decrease the recurrence of restenosis in two non-randomised reports (Deriu et al 1984, Katz et al 1987). Simply by increasing the cross sectional area of the lumen, other factors such as platelet deposition, turbulence or smooth muscle cell proliferation may become unimportant. However a

change of practice is not indicated until the benefits of patch angioplasty are evaluated in a properly controlled randomised trial.

The comparison of simple suture, vein patch angioplasty and carotid bifurcation advancement have been studied in a non-randomised trial. Restenosis of greater than 50% diameter reduction was found as often in each of the patched groups as in the unpatched controls with an overall restenosis rate of 13.8% in this series (Curley, Edwards & Jacob 1987).

The only indication for patch angioplasty which seems universally agreed is in the surgical treatment of the recurrent stenosis. Cossman described the operative findings of intimal hyperplasia and stated that a plane of dissection between the stenotic lesion and the arterial wall could only be developed by sharp dissection (Cossman et al 1978). In such cases, where the stenosis is smooth and most likely to cause symptoms by haemodynamic effect rather than emboli, patch angioplasty is indicated and if this is not possible, reverse saphenous vein replacement is the preferred treatment (Das et al 1985).

Platelet kinetics have not been studied following patch angioplasty. In this chapter, the results of carotid platelet uptake following this procedure are assessed and compared to a control group of patients treated by simple suture of the arteriotomy.

8.2 Patients and Methods

In a consecutive series of 33 patients undergoing unilateral carotid endarterectomy, 10 required patch angioplasty while the remainder underwent standard suture of the arteriotomy. There were 21 men and 12 women in the series: 10 women were treated by direct suture and only 2 required angioplasty. Mean age at operation was comparable in both groups, at 69.5 $\pm$ 1.9 years in the direct suture group and 64.7 $\pm$ 2.1 years with angioplasty. Criteria for the use of patch angioplasty included an internal carotid artery of small diameter in 6 patients, kinking of the vessel on standard closure in 3 and a suspicion of arteritis in 1 patient. All arteriotomies were closed with continuous 6.0 Prolene and patch angioplasty was performed with Sauvage Dacron (USCI 007828, CR Bard Inc., Billerica, MA 01821, USA) in 6 patients while in the remaining 4 autogenous saphenous vein was used.

On the second postoperative day, autologous platelets from 26mls of blood were labelled with approximately 200uCi ^{111}Indium oxine and reinfused (Chapter 4). Carotid platelet uptake was measured by gamma camera imaging and Carotid Uptake Ratio (CUR) calculated by computer analysis. The rate of platelet deposition was determined by the daily increase in CUR calculated over the 3 days and expressed as Thrombogenicity Index (TI).

Counts from the operated artery were compared statistically to those from the non-operated reference side by the Students t test for

paired data. In the subgroups of patch and standard suture, counts from the operated artery were compared to reference with the Wilcoxon signed-rank test for paired data. CUR and TI for the 2 groups of patients were compared by the Mann Whitney U tests for non parametric data. Visual analysis of gamma camera scans was compared by Fisher's Exact test for small numbers.

8.3 Results

Mean labelling efficiency of 93.8 ± 0.5 in the control group was similar to 92.3 ± 0.7 in the patients after patch angioplasty. All patients had labelling efficiencies in excess of 90%. The activity of ^{111}In injected was similar at 236 ± 13 and 225 ± 15 uCi in the control and patch groups respectively.

8.3.i Gamma camera imaging

Visual analysis of gamma scans by an independent observer detected increased radioactivity over the operated artery in all patients after patch angioplasty (Plate 4.2). However, only 14 of 23 scans were regarded as positive after direct closure ($p<0.05$).

In all 33 patients, mean (±sem) counts from the operated artery of 44.2 ± 2.6 were significantly higher than the contralateral non operated carotid at 36.4 ± 2.1 ($p<0.001$). Comparing patch angioplasty (Fig 8.1) and standard closure (Fig 8.2), reference carotid counts were almost identical at 36.9 ± 3.8 and 36.1 ± 2.5 respectively. Counts from the operated arteries were significantly

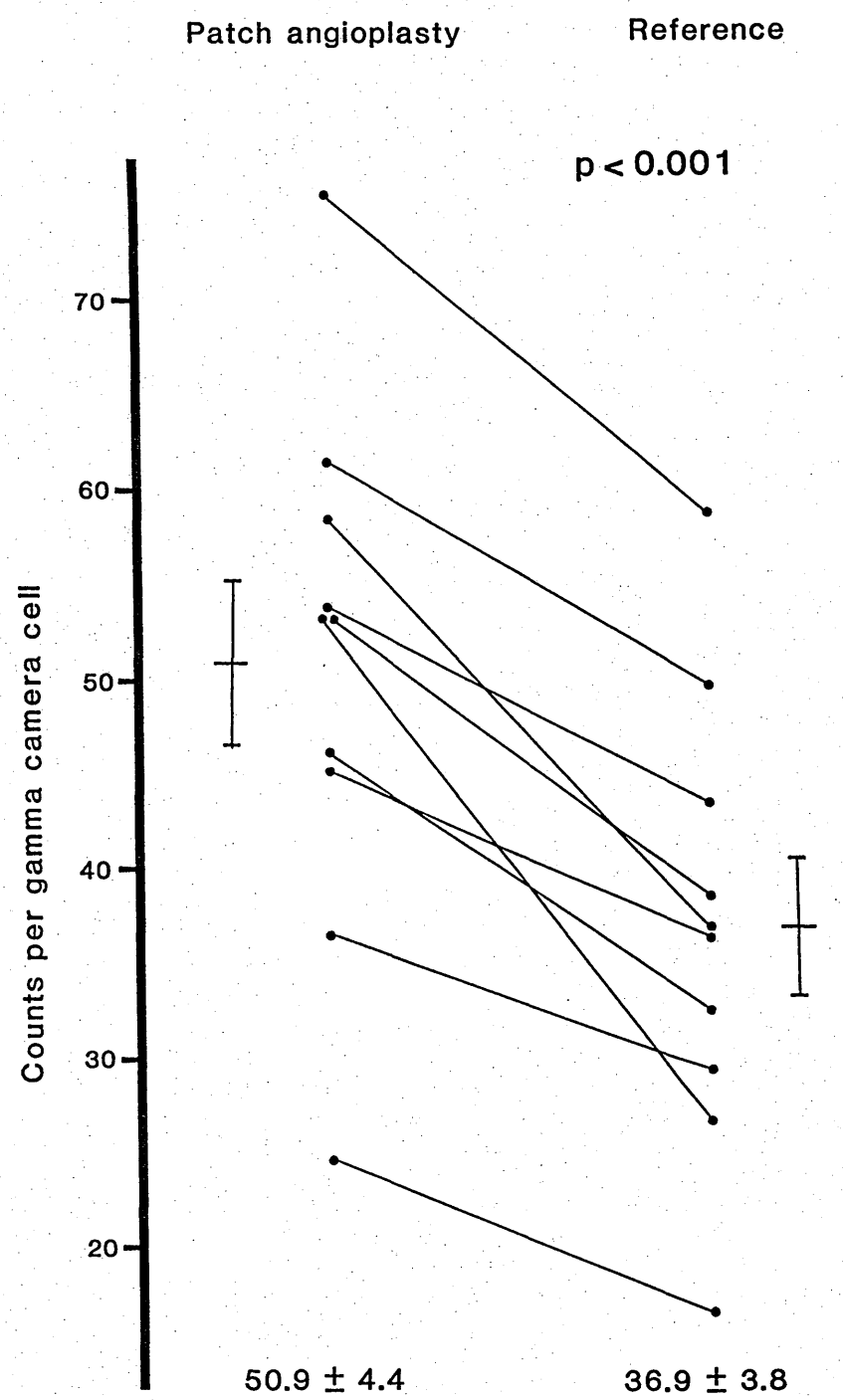


Figure 8.1 Counts per gamma camera cell measured over the carotid arteries repaired by patch angioplasty and the contralateral reference arteries.

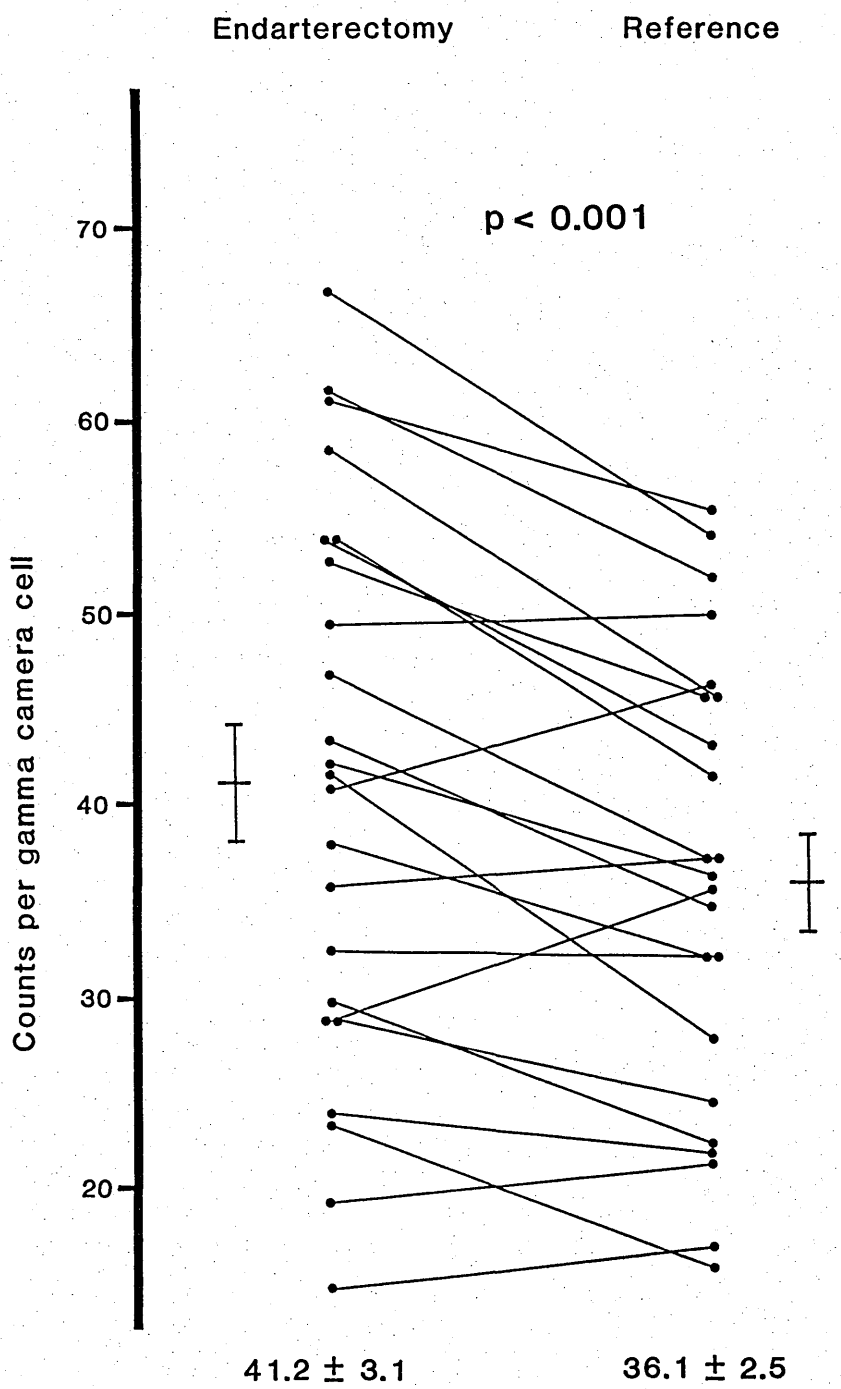


Figure 8.2 Counts per gamma camera cell following carotid endarterectomy with standard arteriotomy closure.

higher in both groups at 50.9 ± 4.4 for patch angioplasty ($p<0.001$) and 41.2 ± 3.1 with direct suture ($p<0.001$).

8.3.ii Carotid Uptake Ratio

Carotid Uptake Ratio, calculated at 1.41 ± 0.07 in 10 patients following patch angioplasty ($p<0.01$), was significantly higher than 1.14 ± 0.04 in 23 patients after standard suture (Fig 8.3). There was no difference between mean CUR of 1.47 ± 0.17 following vein patch in 4 patients and 1.37 ± 0.07 following 6 Dacron patch procedures.

8.3.iii Thrombogenicity Index

Mean TI, the daily rise in CUR which reflects the rate of platelet deposition, was 0.146 ± 0.013 in the 23 patients following simple suture of the arteriotomy. This was significantly higher with patch angioplasty at 0.283 ± 0.021 ($p<0.001$) (Fig 8.4). Surprisingly TI was higher in the 4 patients where autogenous vein was used to patch the arteriotomy at 0.34 ± 0.02 compared to 0.25 ± 0.02 with Dacron ($p<0.05$). In all patients studied TI was greater than zero indicating a higher rate of platelet accumulation over the endarterectomy compared to the reference.

8.4 Discussion

Most vascular surgeons use patch angioplasty occasionally to close the carotid arteriotomy, as in most instances standard closure of the endarterectomy provides an adequate arterial lumen (Thompson

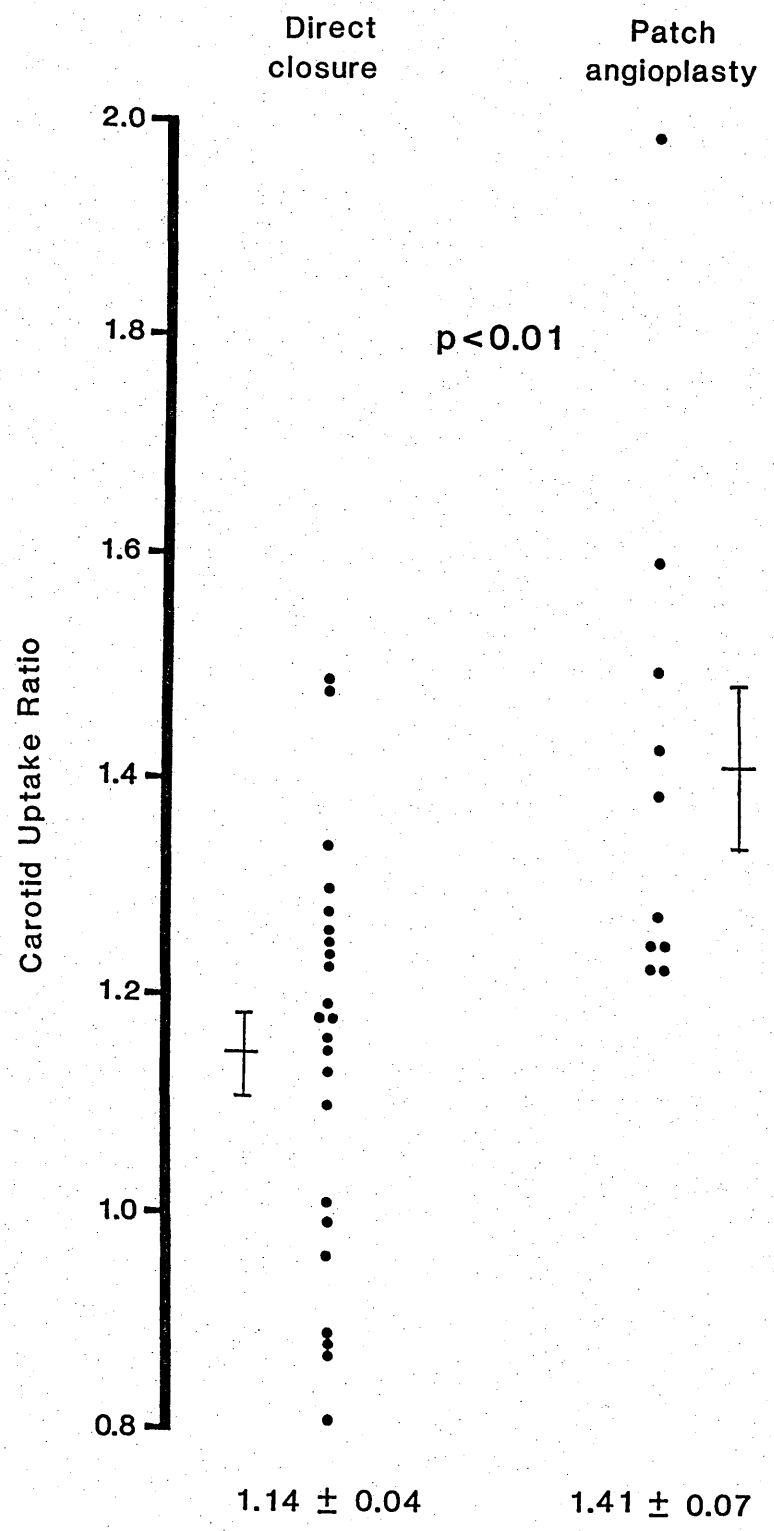


Figure 8.3 Comparison of Carotid Uptake Ratio following direct suture and patch angioplasty.

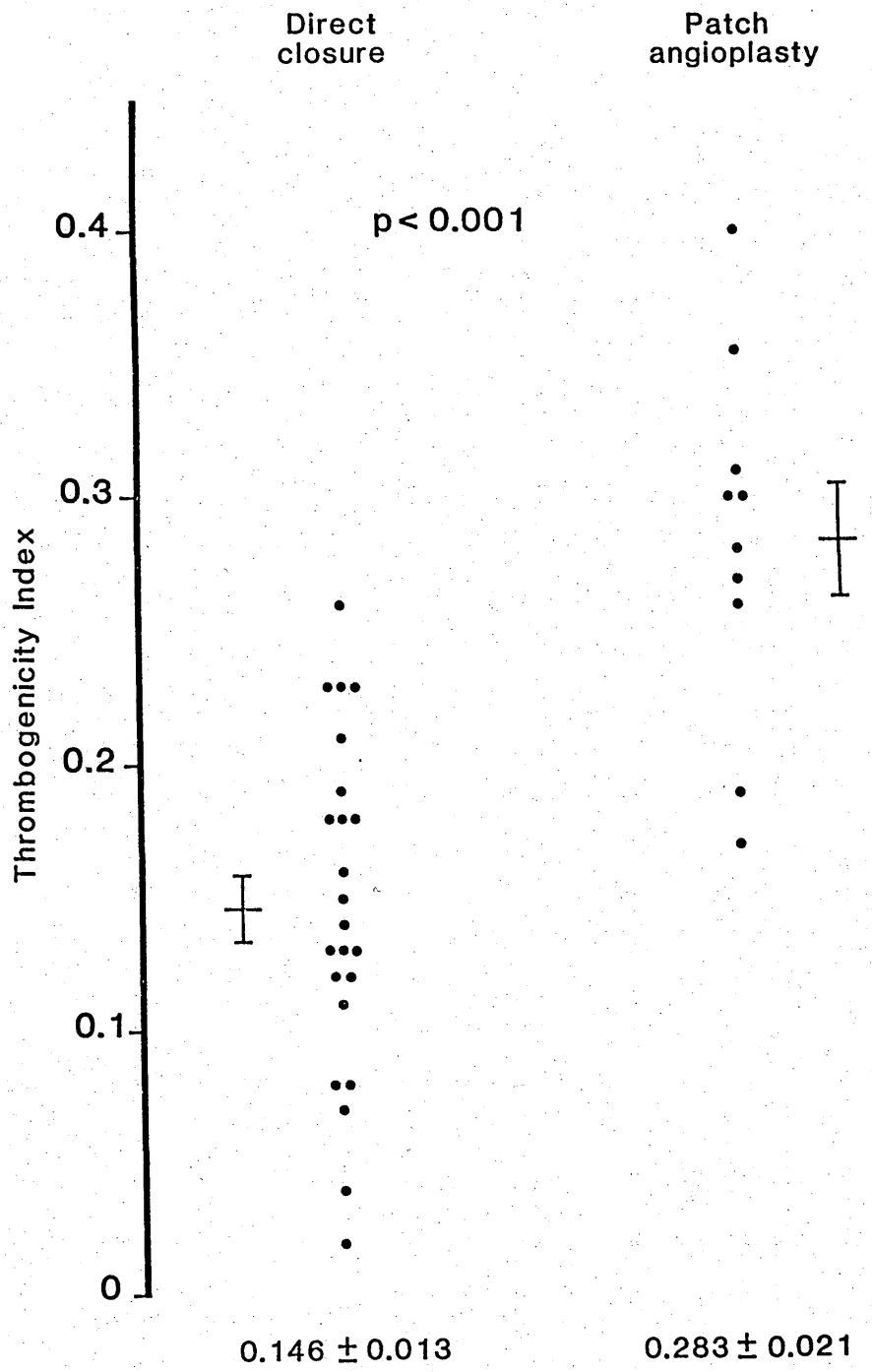


Figure 8.4 Postoperative Thrombogenicity Index is significantly increased following patch repair of the carotid artery.

et al 1970; Baker et al 1983; Schutz et al 1970). Patch angioplasty is therefore reserved for those patients in whom the internal carotid artery is narrow following endarterectomy, or alternatively in whom endarterectomy may be less than satisfactory. The diameter of the internal carotid artery lumen would be expected to be smaller in women, however this was not the case in this series. Fewer women (17%) than men (37%) required patch angioplasty but this did not reach statistical significance.

These data demonstrate greater platelet accumulation at the site of patch angioplasty when compared to closure of the arteriotomy by simple suture. This deposition of radiolabelled platelets may be due to increased adhesion and aggregation from several factors. Platelet deposition may be greater on the patch material during the formation of a pseudo-intima prior to healing or may be related to the longer suture line required with angioplasty. Alternatively increased platelet deposition may result from excessive widening of the vessel which presents a greater luminal surface area for platelet vessel wall interaction.

Extreme widening of the vessel may produce a situation similar to aneurysm formation. Layers of laminated thrombus are commonly seen in atherosclerotic aneurysms and the increased platelet accumulation with patch angioplasty may represent a similar process (Plate 4.1). This could account for the inreased thrombogenicity recorded with vein patches. The numbers studied are too small to

draw conclusions however vein patch angioplasty is more prone to dilatation and rupture (Katz et al 1987). Disproportionate widening may contribute to greater platelet accumulation in this group.

Widening of the vessel by patch closure increases the volume of blood in the arterial lumen. This volume effect may result in higher radioactivity counts on gamma imaging and could be interpreted as vessel wall platelet deposition. Carotid Uptake Ratio is calculated from the radioactivity detected by gamma camera over the operated artery compared to the non operated side. This includes activity from radiolabelled platelets adhering to the arterial wall, in addition to those which remain in the circulation, and CUR may be increased by such a volume effect. Thrombgenicity Index is calculated from the rate of change of CUR and does not reflect an isolated reading. Positive values of TI denote an increase in CUR over the 3 days with a relative increase in gamma counts from the operated side compared to reference. Presuming that any volume effect would remain constant over the three days of each study, platelet accumulation on the arterial wall is detected by Thrombogenicity Index, regardless of the circulating radioactivity. Therefore the greater platelet deposition on the arterial wall after endarterectomy is confirmed by these results (Fig 8.4).

The long term consequences of platelet deposition in relation to patch angioplasty remains obscure. Katz, using autogenous vein, and Deriu with PTFE have both demonstrated that patch angioplasty

compares with standard endarterectomy in respect to the incidence of operative stroke and subsequent freedom from neurological sequelae (Katz et al 1987; Deriu et al 1984). The virtual absence of restenosis claimed in both series contrasts with the 12.5% reported in a study comparing vein patch angioplasty and carotid bifurcation advancement (Curley et al 1987). It is possible that widening of the vessel far outweighs the factors of turbulence or platelet deposition seen following this procedure, however these series have only short follow up to 24 months and do not report the incidence of later stenosis which may be due to recurrent atheroma.

8.5 Conclusions

An increase in luminal platelet deposition has been demonstrated in patients undergoing patch angioplasty closure compared to standard endarterectomy. Published data suggests that widening of the endarterectomy segment with either prosthetic or vein patch reduces the incidence of restenosis. Randomised trials of patch angioplasty and direct suture should be performed before patch closure can be recommended routinely in carotid surgery. Current theories link platelet-vessel wall interaction and intimal hyperplasia, however the clinical importance of postoperative platelet deposition measured by these methods is unknown.

SECTION III RESTENOSIS FOLLOWING CAROTID ENDARTERECTOMY AND THE RELATIONSHIP TO POSTOPERATIVE PLATELET DEPOSITION

Chapter 9

The Incidence of Restenosis Following Carotid Endarterectomy

9.1 Introduction

Before non-invasive methods of carotid assessment were developed restenosis after endarterectomy was seen most often in patients with recurrent symptoms. In view of the morbidity associated with angiography, asymptomatic patients were seldom studied (Maini et al 1978). Recurrent stenosis was identified only in patients who developed symptoms or an audible carotid bruit and based on these criteria very low rates of restenosis were quoted in reviews of carotid endarterectomy (Thompson et al 1970).

Following improvements in non-invasive carotid assessment to determine restenosis, it became practical and ethical to reexamine large numbers of asymptomatic patients following surgery (Roederer et al 1983). This showed that restenosis occurs in significant numbers of asymptomatic patients following endarterectomy, with the reported incidence ranging from 6-22% (Table 1.1). In contrast restenosis associated with recurrent symptoms of cerebrovascular insufficiency is seen in approximately 2% of patients following carotid surgery (Hertzer et al 1979).

It is the aim of this study to determine the incidence of restenosis following carotid surgery using non-invasive methods and to assess the occurence of cerebrovascular symptoms in these patients.

9.2 Non Invasive Assessment of Carotid Stenosis

Non-invasive assessment of the carotid arteries depends on either the indirect measurement of blood flow in the collateral circulation between the internal and external carotid arteries or the direct assessment of blood flow and visualisation of the carotid bifurcation. Carotid stenosis was originally measured indirectly by doppler sonography which assessed the direction of blood flow in the opthalmic arteries but depended on a normal external carotid system (Muller 1972). Skin thermography, used as an index of opthalmic artery perfusion pressure, identified only 57% of patients with internal carotid stenosis of greater than 50% (Capistrant & Gumnit 1973). Oculo-plethysmo-graphy and subsequently oculopneumo-plethysmography used the periorbital arterial circulation to measure the delay in the arrival of the arterial pressure wave (Kartchner, McRae & Morrison 1973; Gee, Mehigan & Wylie 1975). Unfortunately these indirect methods identified only the presence of a haemo-dynamically significant stenosis. They were inaccurate in the presence of contralateral disease and were unable to localise the site of obstruction.

Direct assessment of blood flow at the carotid bifurcation initially involved carotid phonoangiography with audiovisual analysis of carotid bruit (Kartchner & McRae 1969). This was superceded by Doppler spectral analysis which measures the reflection of continuous-wave Doppler Ultrasound from red blood cells (Spencer & Reid 1979).

9.2.i Doppler spectrum frequency analysis

Continuous-wave (CW) Doppler Ultrasound uses ultra high frequency sound waves of between 2 and 10 megahertz to insonate the arteries. The ultrasound is reflected back from the moving red blood cells at a slightly different frequency from the emitted ultrasound due to Doppler shift. This change in frequency is proportional to the blood velocity. The relationship between Doppler shift frequency and blood velocity is given by the equation:

$$\text{Doppler shift frequency} = \frac{2 \times F \times V \times \text{Cos}A}{C}$$

where F = Frequency of transmitted ultrasound

V = Velocity of red cells

A = Angle of incident to red cell path

C = Velocity of sound in the medium

With increasingly severe arterial narrowing blood velocity in the stenotic segment rises, changing the Doppler shift frequency (Zweibel et al 1982). Doppler spectral analysis produces a visual display of audiofrequency information and is related to red cell velocity. A normal arterial Doppler spectrum has a peak frequency of less than 4 kHz, with a narrow band of raised frequencies in systole and a clear area beneath the systolic peak known as the spectral window. The first change in the Doppler spectrum, detected with minor degrees of stenosis, is broadening of the systolic peak. With moderate stenosis the spectral broadening increases until the spectral window disappears and progressively more severe lesions

produce an increase in the peak systolic frequencies as well as raising the normally low diastolic frequency (Spencer & Reid 1979).

Distal to stenotic lesions blood flow becomes turbulent and this can be detected by frequency analysis. The narrow band of frequencies found in a normal artery represents the blood velocity seen with laminar flow. In regions distal to stenotic lesions, turbulence is detected as a loss of high frequency definition and dampening of the signal (Sheldon, Murie & Quin 1983). It is therefore possible to use peak systolic freqency and changes in the doppler spectrum to accurately identify the degree of stenosis in the carotid arteries (Langlois et al 1983).

Representative images of the carotid bifurcation have been produced using continuous wave ultrasound. By attaching the CW probe to a position sensing arm, probe movement in any direction is converted by computer onto a visual display. The frequency of the Doppler signals received are colour coded to identify areas of increased blood velocity or turbulence and produce a continuous wave doppler image of the carotid bifurcation (Hames et al 1985). More sophistocated pulsed-wave Doppler devices use a single focused high frequency ultrasound beam which is emmitted in bursts or pulses. The time taken for the reflection of sound waves to travel back from red blood cells can be used to calculate vessel depth. Peak frequency analysis in conjunction with a position sensing arm and computer produces a flow map of the carotid artery (Murie, Sheldon & Quin

1984). The disadvantages of pulsed Doppler imaging are the time taken to build up the image which requires the patient to lie absolutely still for 15 minutes. Poor Doppler signals are obtained if very high frequencies are involved and considerable operator experience is required to obtain reproducible images.

9.2.ii Duplex scanning

One of the variations of ultrasonic assessment is Pulsed Echo Ultrasonography with B-mode Ultrasound. As the ultrasound passes through the tissue planes of different acoustic density, a portion of the signal is reflected back to the transducer at each interface. The time delay between transmission and reception is proportional to the distance from the probe to the tissue interface and can be displayed visually on an oscilloscope. Brightness modulation (B-mode) demonstrates the intensity of the echo at the acoustic interface with the brightness of the oscilloscope image indicating the difference between the densities of the tissues (Blackshear et al 1979).

Real-time B-mode Ultrasound employs echo ultrasonography with a rotating ultrasonic beam which sweeps through an arc, producing a sector scan which is displayed on a video screen (Plate 2.6). This is updated 50 times per second and moving images of whole sections of tissue are obtained, distinguishing the vessel wall and plaque within the lumen (Fell et al 1981). Atherosclerotic plaques can be assessed

for consistency as soft, dense, mixed or calcified and ulceration can be identified (O'Donnell et al 1985) (Plate 2.7).

Doppler spectral analysis assesses only flow and velocity of blood in the vessel. It does not image the vessel wall and will often fail to identify areas of ulceration where flow may be reduced (Spencer & Reid 1979). Echo ultrasonography produces direct images of the arterial anatomy and may be used to assess plaque morphology, but depends on tissue interfaces of different densities. It may not distinguish between blood and thrombus which have similar acoustic densities and therefore fail to demonstrate complete occlusion of the carotid artery by thrombus (Hobson et al 1980).

Real-time B-mode imaging of the arterial wall (Blackshear et al 1979) and flow assessment with Doppler spectral analysis are combined to avoid the shortcomings of each technique and to produce Duplex ultrasound which is now regarded as the optimal imaging technique for the carotid artery in the non-invasive laboratory (Strandness 1984).

9.3 Patients and Methods

Two hundred and eight carotid endarterectomies were performed at Charing Cross Hospital between 1978 and 1986 in 197 patients, 11 requiring bilateral operation. There were 131 men and 66 women with mean age at operation of 62.3 years (39-83 yrs) and 66.2 years (46-80 yrs) respectively.

The indications for surgery is summarised in Table 9.1. Three patients underwent endarterectomy for asymptomatic internal carotid artery stenosis as a prophylactic measure, two prior to coronary artery bypass grafting and one before repair of an abdominal aortic aneurysm. Transient ischaemic attacks (TIA) or amaurosis fugax were the presenting feature in 116 patients (56%) and 66 patients (32%) had suffered a completed stroke. In 23 patients (11%) urgent endarterectomy was performed for either crescendo TIA or stroke in evolution.

In all patients preoperative non-invasive assessment was performed prior to arteriography with standard or digital subtraction techniques. Carotid phonoangiography and oculoplethysmography were used in the early years of the series and latterly these were replaced by continuous wave ultrasound and B-mode imaging.

All surgical procedures were carried out under general anaesthesia with endotracheal intubation and assisted ventilation. Continuous central venous and arterial pressures were monitored and blood pressure was maintained at normal levels during the operative and postoperative period. In patients with an internal carotid stump pressure of less than 50mm Hg or a non pulsatile waveform, an intraluminal Javid shunt was placed to maintain cerebral perfusion. This was required in 53.5% of cases. Endarterectomy was performed through a longditudinal arteriotomy and direct arterial repair performed in the majority. In only 16.3% of operations was the

INDICATION FOR OPERATION

Asymptomatic	3 (1.5%)
TIA	116 (56%)
Establised Stroke	66 (32%)
Progressing Stroke or Crescendo TIA	23 (11%)

Total 208 operations in 197 patients

Table 9.1 Symptoms precipitating carotid surgery in 197 patients. Eleven patients required bilateral procedures.

CAUSE OF DEATH

Stroke	3
Ischaemic Heart Disease	20
Carcinoma	1
Unknown	9
Total	33

Table 9.2 Cause of death following carotid surgery. More than 60% of patients undergoing carotid surgery suffer fatal myocardial infarction.

origin of the internal carotid artery thought to be sufficiently narrow to required vein or dacron patch angioplasty.

All patients were followed at 6 monthly intervals for 2 years and annually thereafter. At each visit the absence or occurence of cerebrovascular symptoms was established and restenosis finally assessed during the period 1985-1986 by continuous wave ultrasound and B-mode Duplex imaging.

9.3.i Method of non-invasive carotid assessment

Doppler spectral analysis was recorded by Vasoscan (8220-6901, Sonicaid Ltd, Chichester, England) using a 4 MHz pencil probe (8200-6941, Sonicaid Ltd) comprising a dual crystal lens-focusing transducer which both transmits and receives ultrasonic waves. Patients were examined lying supine on an examination couch with a pillow under the head, chin up and the head turned slightly away from the side to be examined. A liberal amount of Aquasonic 100 acoustic jelly (0341-0001-08, Sonicaid Ltd) was applied and the probe positioned low in the neck at 60^{o} angle to the body axis, identifying the common carotid artery. The probe is then moved cephalad to examine the bifurcation and then each of the internal and external carotid arteries are insonated.

Following Doppler spectral analysis, Duplex imaging was performed using a Vasoveiw imaging system (8230-8501, Sonicaid Ltd) fitted with a 7.5 MHz Duplex probe (8230-8025, Sonicaid Ltd). Scans

were performed in the longitudinal planes from the common carotid artery in the base of the neck to the most distal point which allowed visualisation of the internal carotid artery. Images were recorded directly from the visual display on polariod photographs (Plate 4.1).

In this study carotid artery stenosis was assessed by continuous wave ultrasound and then confirmed by Duplex imaging. Peak systolic frequencies of less than 4kHz represented insignificant stenosis of less than 50% diameter reduction. Frequencies from 4-6 kHz denoted stenosis of between 50 and 75% and higher recording in excess of 6.0kHz demonstrated stenosis of between 75 and 99%. Absence of a recordable signal was consistant with total occlusion (Spencer & Reid 1979). Confirmation of these findings were established with Duplex imaging expressing the diameter reduction as a percentage of the normal arterial diameter either proximal or distal to the stenosis. The acoustic echoes of stenotic lesions were graded as soft, hard or calcified (O'Donnell et al 1985).

Restenosis in the carotid arteries were therefore classified as insignificant from (0-50%), moderate (50-75%), severe (75-99%) or complete occlusion.

9.4 Results

Of 197 patients in the series, 33 patients (16.7%) died during the follow up period of mean 35.3 months which extended from 1 to 8

years. Only 3 deaths followed stroke (Table 9.2). Twenty deaths were related to myocardial ischaemia and survival, calculated by life table analysis, was 93% at one year, 83% at three years and 62% at five years.

9.4.i Incidence of restenosis

One hundred and thirty three patients were available for study in an 18 month period 1985-1986, allowing non-invasive carotid asessment to be performed on 139 arteries. Eighteen arteries in 17 patients (12.9%) were found to have late restenosis or occlusion, in 11 men and 6 women. One male patient was found to have restenosis on both sides after staged bilateral endarterectomy. There was no difference in age, smoking status, hypertension or presenting symptomatology between patients who developed restenosis and those who remained widely patent. Similarly the incidence of restenosis was no higher in patients who required intraluminal shunting or patch angioplasty. Restenosis was no more common in female patients at 12.5% (6/48) compared to 12.1% (11/91) in males. Of the 11 patients who required bilateral primary carotid endarterectomy, 6 were reexamined, 3 developed restenosis but only one on both sides.

Restenosis in patients requiring carotid surgery for TIA was seen in 10 of the 66 patients (15.1%) and in 6 of the 51 patients presenting with stroke (11.8%). Restenosis was not seen following 23 carotid endarterectomies for stroke in evolution or crescendo TIA's (Table 9.3).

CONDITION AT FOLLOW UP	INDICATIONS FOR SURGERY			
	Asymptomatic	TIA	Established Stroke	Progressing Stroke/ Crescendo TIA
Asympt/Patent	0	54	43	17
Asympt/Restenosis	2	8	5	0
Symptoms/Patent	1	2	2	2
Symptoms/Restenosis	0	2	1	0

Table 9.3 Clinical outcome and occurrence of restenosis in 133 patients following endarterectomy.

9.4.ii Restenosis related to neurological symptoms

One hundred and fourteen patients were found to be asymptomatic at follow-up without evidence of carotid restenosis on non-invasive assessment.

Asymptomatic

Restenosis was found in 15 arteries (10.8%) following endarterectomy without associated neurological symptoms. Three were found to have complete occlusion. One patient, after bilateral procedures, developed a high grade 75-99% stenosis on one side and a moderate 50-74% on the other. The remaining 10 cases had only moderate stenoses of 50-74%.

Symptomatic

Only 3 patients (2.1%) developed neurological symptoms associated with restenosis. A female patient developed recurrent amaurosis fugax 26 months following surgery and despite reoperation with Dacron patch angioplasty, suffered further restenosis associated with amaurosis fugax 9 months after the second procedure. Subsequent symptoms were controlled on platelet inhibitory therapy and she is stroke free 4 years later. A male patient suffered an ipsilateral stroke 33 months after endarterectomy corresponding to a recurrent 75-99% restenosis and contralateral occlusion. At reoperation a good early result was obtained with a reversed saphenous vein graft but after 6 months this had also undergone severe restenosis (75-99%) in association with recurrent ipsilateral TIA's. These have been

successfully controlled with platelet inhibitory therapy. A third symptomatic patient suffered TIA related to a severe restenosis (75-99%), 25 months following surgery. Her symptoms have been contolled by a standard platelet inhibitory regimen of aspirin and dipyridamole without further surgery. Both patients who underwent reoperation for symptomatic restenoses progressed to further, significant, symptomatic narrowing after the second procedure.

Neurological symptoms without restenosis

Seven patients developed late neurological symptoms following endarterectomy without evidence of postoperative restenosis both on non invasive assessment and digital subtraction angiography. Ipsilateral carotid territory TIA occured in 4 patients, 2 of whom had contralateral carotid occlusion and one following Dacron patch angioplasty. Three futher patients demonstrated non-focal vascular events, one with contralateral TIA and two patients suffered contralateral hemispheric stroke at 5 months and 5 years after surgery.

9.5 Discussion

The incidence of recurrent stenosis following carotid endarterectomy in this series is similar to other reports of non-invasive assessment (Colgan et al 1984; Glover et al 1985; Ackroyd et al 1986). The overall rate of restenosis was high at 12.9% however symptomatic restenosis was seen in only 3 patients (2.1%). This may reflect the smooth surface of the artery narrowed by intimal

hyperplasia which is unlikely to give rise to thrombo-embolic events (Cossman et al 1978). Although the 3 patients developed symptomatic restenosis all did so after 24 months, it is not known if these were due to recurrent atheroma as suggested by Stoney and String (1976).

The management of those patients who develop restenosis following carotid surgery remains uncertain. The low incidence of symptomatic recurrence demonstrated in this and other series would suggest that asymptomatic patients should not undergo reoperation (Nicholls et al 1986). Although a proportion of asymptomatic patients with primary atheromatous lesions of the carotid arteries may go on to develop neurological symptoms which require surgical intervention (Humphries et al 1976) this has not been proven in patients with recurrent asymptomatic stenosis (Aldoori & Baird 1987). Operative treatment is technically more difficult and it may be impossible to identify an endarterectomy plane between the vessel wall and the restenotic lesion (Cossman et al 1978). In these circumstances patch angioplasty or interposition vein graft may be required (Das et al 1985).

This study has failed to identify factors predisposing to restenosis. Women have been found more susceptible to restenosis but this was not confirmed in the present series (Nicholls et al 1986). Similarly there was no higher rate of restenosis in smokers, diabetics or hypertensive patients. The prediliction of the individual to develop restenosis has been demonstrated. One patient

had bilateral asymptomatic restenosis and two patients with symptomatic restenosis progressed to further symptomatic narrowing after reoperation.

9.6 Conclusions

This series demonstrates experience similar to that published from other centres. Restenosis following carotid endarterectomy occurs in approximately 13 percent of patients but as yet no predisposing factors have been identified. In the majority of cases restenosis is not associated with recurrent cerebrovascular symptoms and it seems appropriate to treat these patients conservatively. It appears that those few patients who become symptomatic in association with restenosis, are likely to develop further restenosis following corrective surgery.

Duplex scanning is a reliable method of measuring carotid stenosis and may be used in prospective trials investigating the incidence and cause of restenosis following endarterectomy.

Chapter 10

The Relationship between Platelet Deposition and Restenosis following Carotid Endarterectomy

10.1 Introduction

Although several authors have suggested that restenosis following carotid endarterectomy is mediated by platelets, there is little direct evidence to support this theory (Thomas et al 1984; Das et al 1985; Ackroyd et al 1985). However there is experimental data from animal models showing that areas of damaged endothelium are initially covered with platelets and subsequently, the intima becomes thickened (Groves et al 1982). Evidence from in vitro studies showed that substances such as platelet derived growth factors stimulate vascular smooth muscle proliferation in cell culture but platelet adhesion has not been confirmed as the cause of this intimal reaction (Ross 1985). In patients, the rate of radiolabelled platelet deposition was found to predict failure of femoro-popliteal grafts (Goldman et al 1983a) however this may simply reflect the differences in thrombogenicity and patency between venous and prosthetic grafts.

In order to investigate the relationship between platelet deposition and restenosis after carotid endarterectomy, a method of assessing in vivo platelet accumulation is required in humans. The techniques of radiolabelling viable platelets with external detection of thrombus accumulation, seem to be appropriate to this problem and the data reported in previous chapters support this. Accurate assessment of restenosis, by non-invasive ultrasonic methods, makes it ethically possible to reinvestigate large numbers of asymptomatic patients.

This chapter examines the relationship between postoperative platelet accumulation, measured as Carotid Uptake Ratio and Thrombogenecity Index, in the early postopertive period and the subsequent development of restenosis at the site of endarterectomy, one year after surgery.

10.2 Patients and Methods

Forty patients undergoing endarterectomy for symptomatic carotid disease were studied. Endarterectomy was performed under general anaesthesia and 5000 units of sodium heparin were administered prior to clamping the carotid arteries. In 30 patients the arteriotomy was sutured directly and in 10 patients a patch was used to avoid postoperative narrowing.

10.2.i Platelet uptake

On the second postoperative day autologous platelets were labelled with 200uCi Indium oxine, by the method described in chapter 4, and reinjected (Hawker et al 1980). Gamma camera images were obtained on the second, third and fourth postoperative days and platelet deposition calculated as Carotid Uptake Ratio (CUR) and Thrombogenicity Index (TI).

10.2.ii Luminal narrowing

The patients were followed up at 3 monthly intervals until one year after surgery when the carotid artery was examined for restenosis using continuous-wave ultrasound and Duplex imaging (Plate

4.3). Images of the common and internal carotid arteries were obtained in 2 longitudinal planes. Initially the vessels were examined in an antero-posterior view with the probe placed medial to the anterior border of sternomastoid. The arteries were then scanned laterally from the posterior triangle behind the sternomastoid. This view improves visualisation of the internal carotid artery in obese patients (Plate 4.4).

The degree of luminal narrowing was measured by comparison to the normal artery either proximal or distal to a stenosis. The vessel diameter was calculated automatically as the distance between 2 cursors placed on the inner aspect of the arterial wall. Measurements from the normal artery were compared with the luminal diameter at the region of stenosis and expressed as a percentage (Plate 4.5). The average diameter reduction from the anterior and lateral readings were recorded as the percentage luminal narrowing to the nearest 10 percent. All patients were evaluated and classified as 0, 10, 20, 30,...100% luminal narrowing.

10.2.iii Statistical analysis

All results of luminal narrowing, Carotid Uptake Ratio and Thrombogenecity Index in the various groups were compared by the Mann-Whitney U test for non parametric data. The correlation between platelet deposition, measured as CUR and TI, and subsequent narrowing at one year was calculated by linear regression.

Plate 4

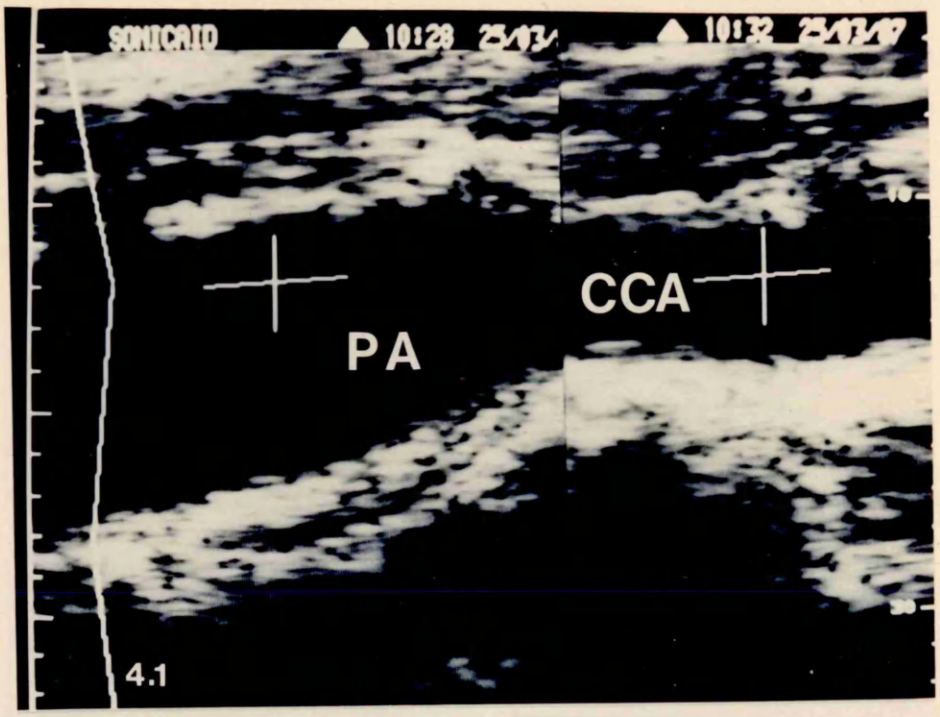
4.1 Composite Duplex image of the common carotid artery following patch angioplasty using autogenous vein. The proximal common carotid artery (CCA) is of normal calibre however distally, at the level of endarterectomy and vein patch repair (PA), the artery has become dilated.

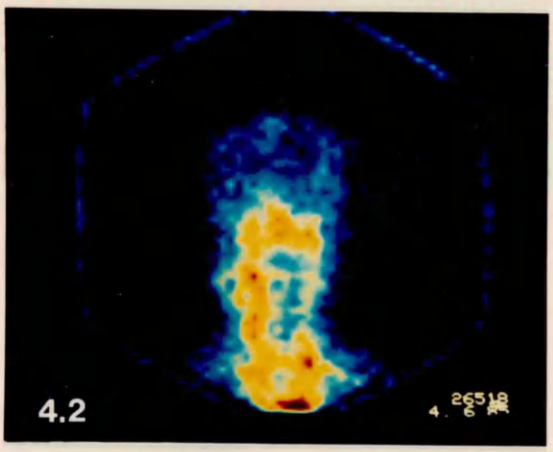
4.2 Gamma image recorded 72 hours after right carotid endarterectomy and patch angioplasty. Intense platelet accumulation is seen at the site of operation.

4.3 Restenosis in the proximal internal carotid artery. This lesion was first detected 6 months after operation and confirmed at one year. Using biplanar Duplex imaging, this was measured at 60% diameter reduction.

4.4 Lateral view of the carotid bifurcation. The cursor is positioned in the proximal internal carotid artery which has been widened by patch angioplasty. The external carotid is visible below the ICA and the normal Doppler frequency analysis confirms a widely patent artery.

4.5 Measurement of arterial diameter. Cursors placed across the width of the common carotid artery (CCA) and internal carotid artery (ICA) measure the diameters at 7.3mm and 6.4mm respectively. Percentage luminal narrowing can be assessed by these techniques.


PA
CCA
4.1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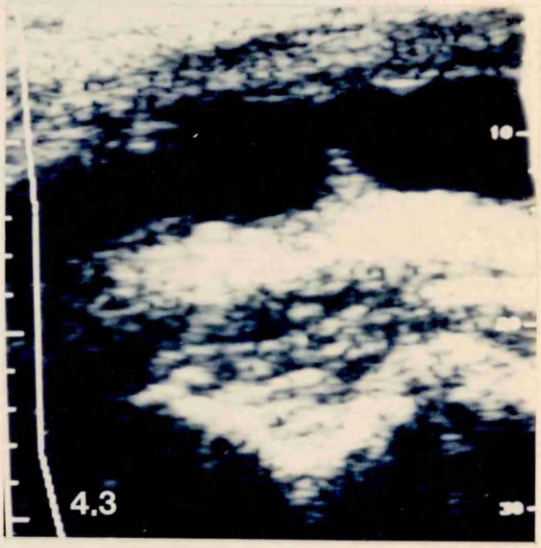
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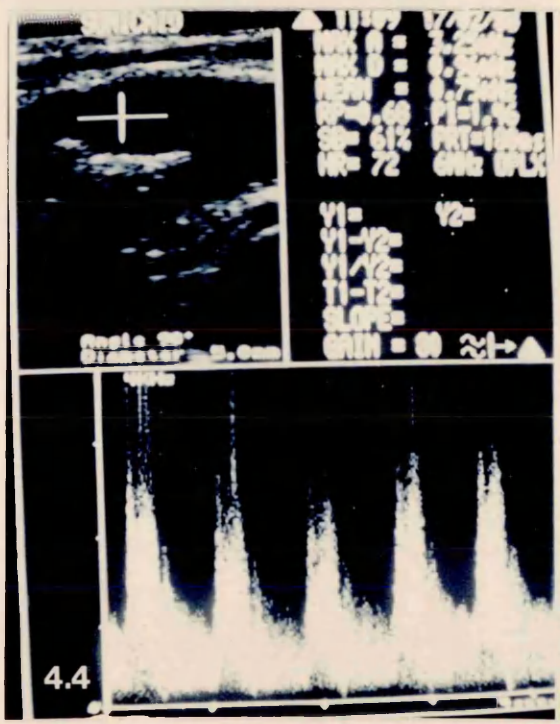
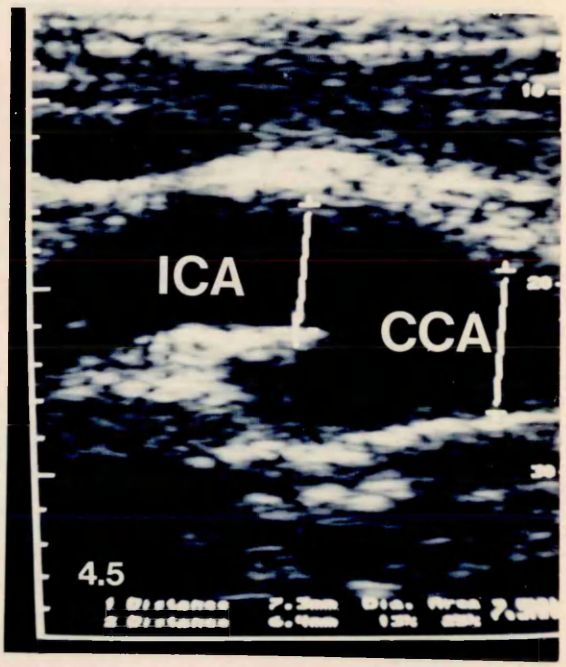

4.4


ICA
CCA
4.5

10.3 Results

10.3.i Carotid platelet accumulation

Mean (± sem) Carotid Uptake Ratio in all 40 patients was 1.25 ± 0.03 (range 0.81 to 1.99) with mean Thrombogenicity Index calculated at 0.19 ± 0.01 (range 0.01 to 0.4). Median CUR and median TI were 1.25 and 0.18 respectively.

10.3.ii Luminal narrowing

Overall mean luminal narrowing was measured at 22.3 ± 3.5 (range 0-90%). Postoperative internal carotid artery occlusion was not seen in this group. Seven patients (17.5%) were found to have carotid restenosis of greater than 50 percent but in only one patient was a severe restenosis of 90% identified. Six of these stenoses were identified in the internal carotid artery and one was seen in the common carotid at the proximal extent of the endarterectomy. Of the 10 patients treated by patch angioplasty, 3 were found to have developed restenosis at one year. In 9 patients the carotid arteries were widely patent without any evidence of restenosis and these were classified at 0 percent. In three of these 9 patients dilatation of the internal carotid artery was found following patch angioplasty and for analysis these were recorded as 0% luminal narrowing. The remaining 22 patients were found to have minor degrees of intimal thickening which were recorded at between 10 and 40% luminal narrowing.

10.3.iii Platelet accumulation related to subsequent luminal narrowing

In those patients where CUR was above the median of 1.25, the subsequent mean luminal narrowing was 29.5 ± 5.9 percent compared to 15.0 ± 3.1% when the ratio was in the lower half of the range. This failed to achieve significance (Fig 10.1). With Thrombogenicity Index however, mean luminal narrowing was 30.0 ± 5.6% in patients above the median of 0.18 and this was statistically higher than 14.5 ± 3.7% from those below the median value ($p<0.05$) (Fig 10.2).

In those 7 patients found to have stenosis of greater than 50% at one year, CUR was significantly higher at 1.47 ± 0.10 compared to 1.20 ± 0.03 in the remaining 33 patients with luminal narrowing of between 0 and 40% ($p<0.02$) (Fig 10.3). Similarly TI, measured in the early postoperative period was increased in the patients with greater than 50% stenosis at 0.27 ± 0.03 compared to 0.17 ± 0.01 in the patients with widely patent vessels ($p<0.01$) (Fig 10.4).

There was a positive correlation ($r=0.53$) between Carotid Uptake Ratio and the degree of subsequent luminal narrowing measured one year after surgery ($p<0.001$) (Fig 10.5). The correlation between TI and subsequent luminal narrowing was similar in all 40 patients with $r=0.41$ ($p<0.02$) despite the wide spread ofthe data (Fig 10.6). This improved to $r=0.45$ when those patients who had required patch angioplasty were excluded ($p<0.02$) (Fig 10.7).

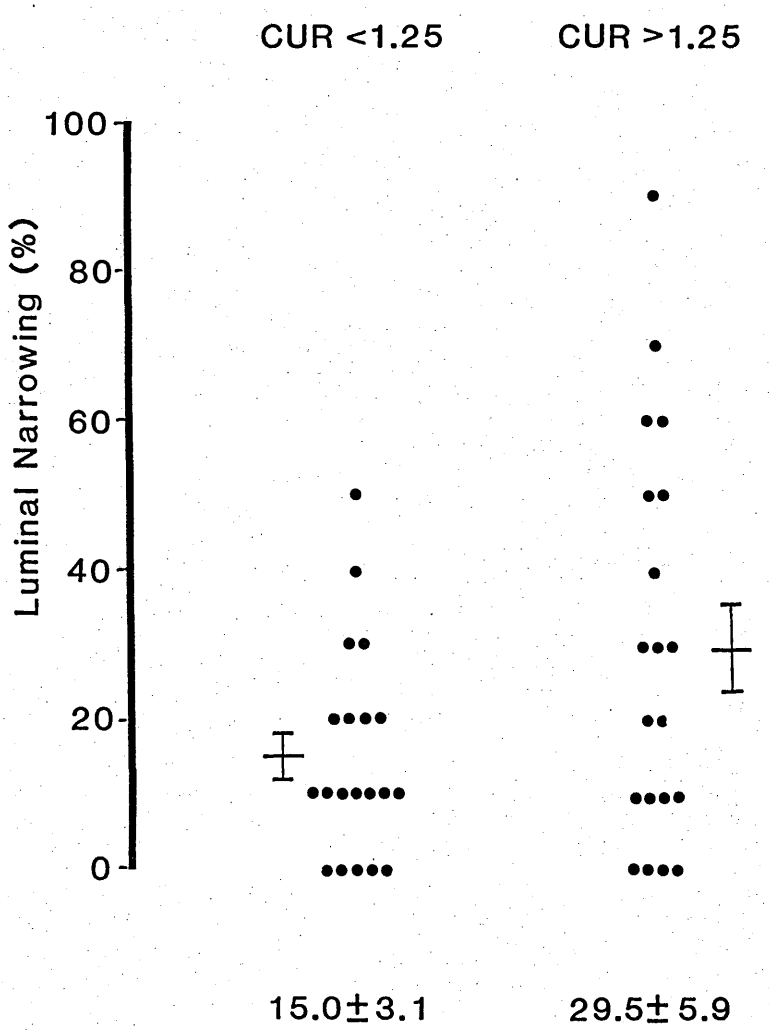


Figure 10.1 There was no statistical difference in luminal narrowing between those patients with CUR results above and below the median of 1.25.

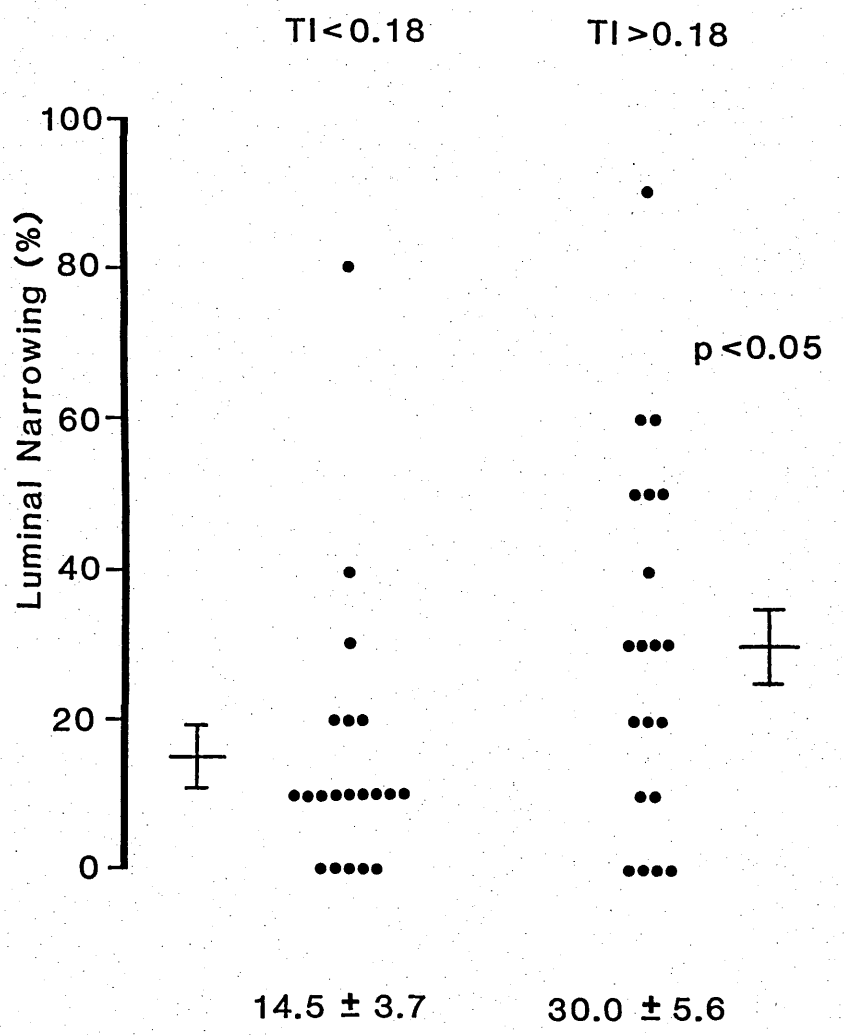


Figure 10.2 Patients with Thrombogenicity Index above the median of 0.18 were found to have a greater degree of luminal narrowing compared to those in the lower half of the range.

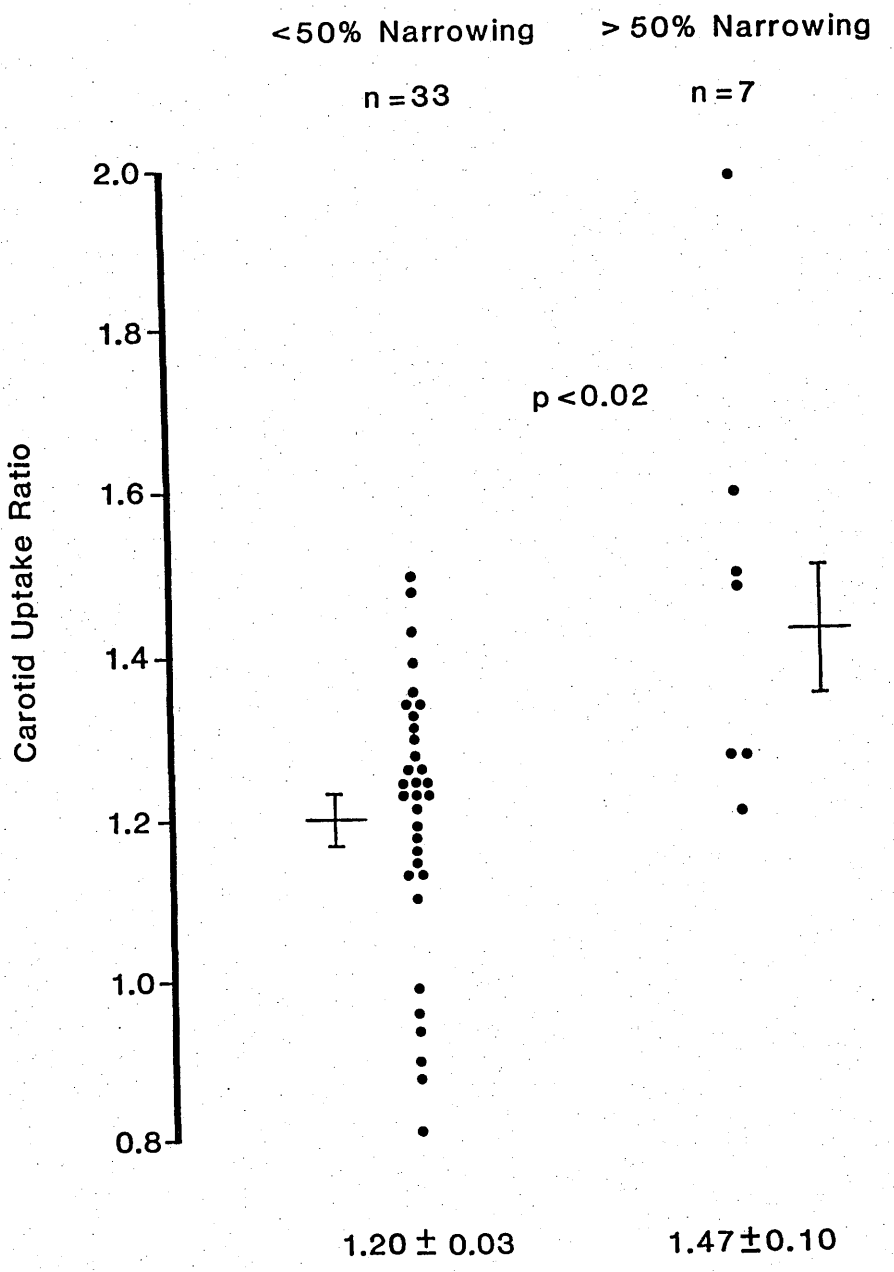


Figure 10.3 Postoperative CUR was statistically higher in those patients found to have developed restenosis of greater than 50 percent.

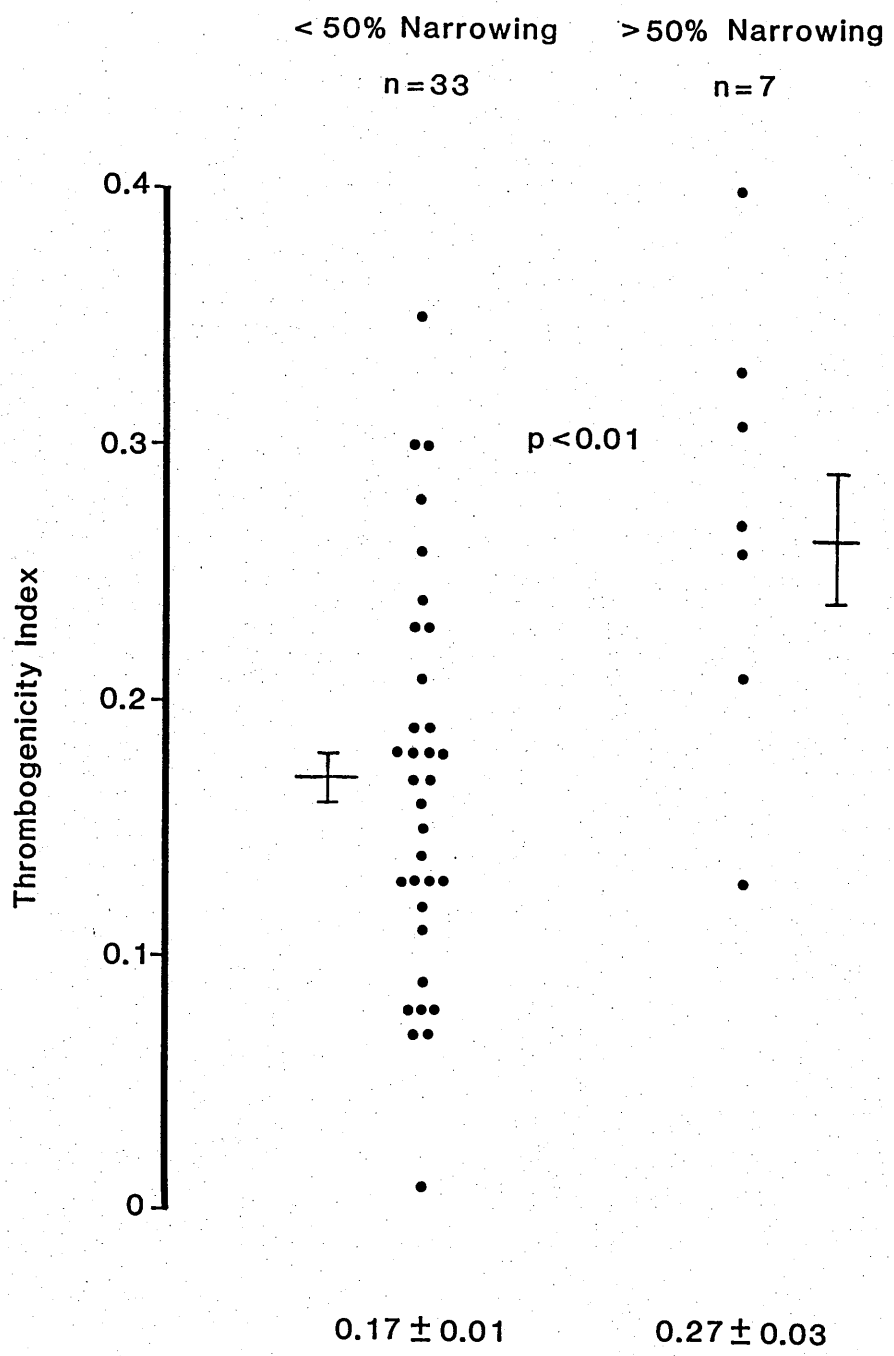


Figure 10.4 Retrospective analysis of patients with significant restenosis showed these to have had increased postoperative platelet uptake measured by Thrombogenicity Index.

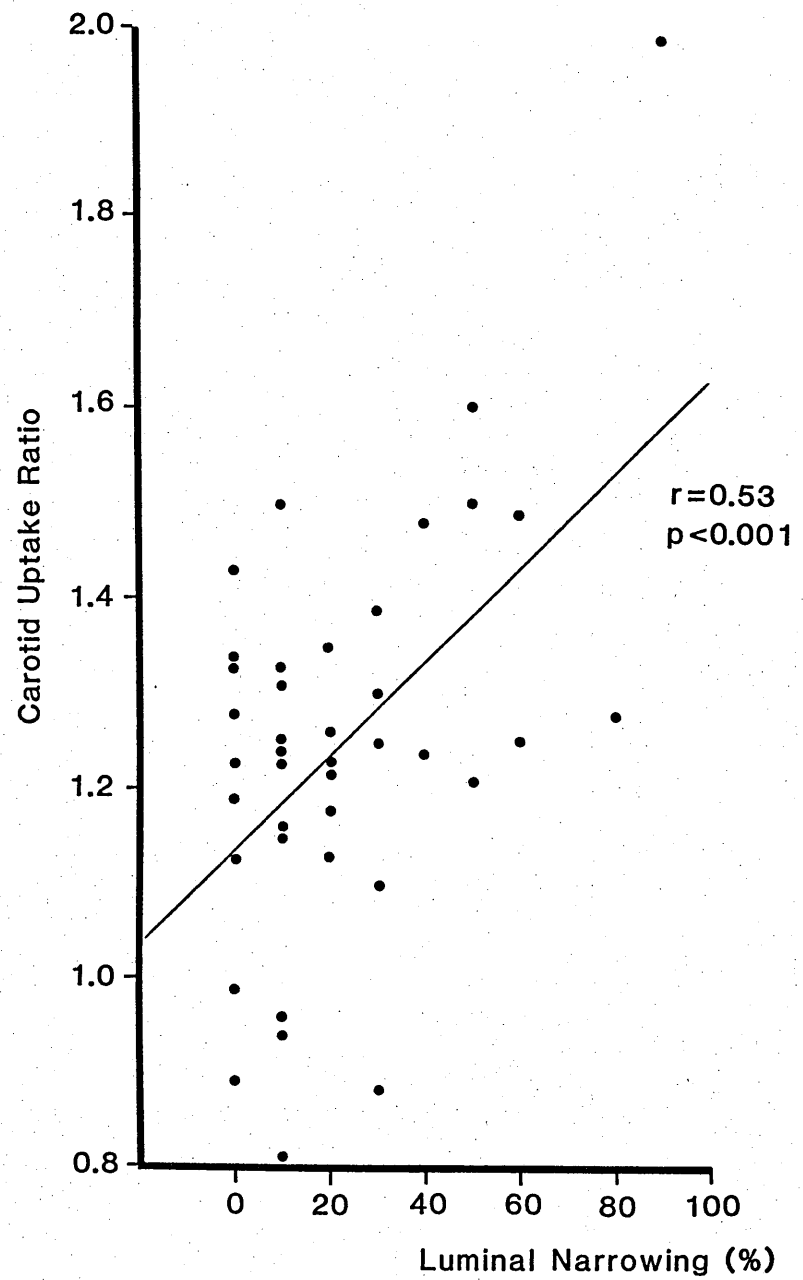


Figure 10.5 There was a direct correlation between CUR measured 72 hours after surgery and the development of luminal narrowing in all 40 patients.

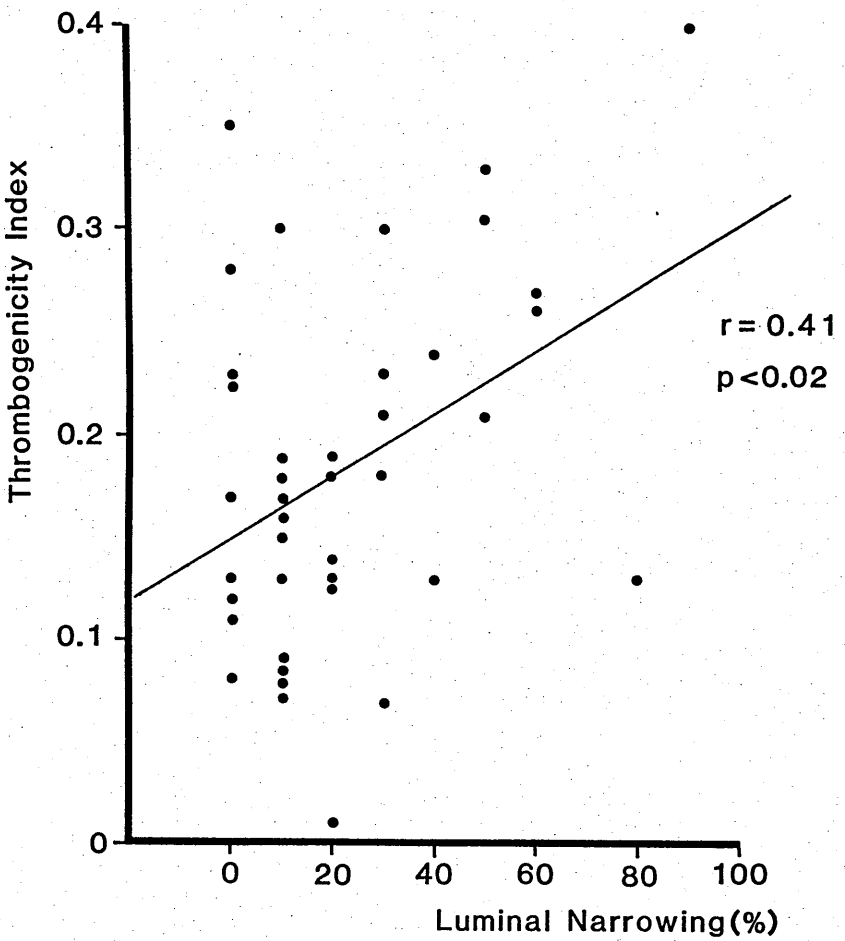


Figure 10.6 Thrombogenicity Index was related to the development of subsequent luminal narrowing in all 40 patients studied.

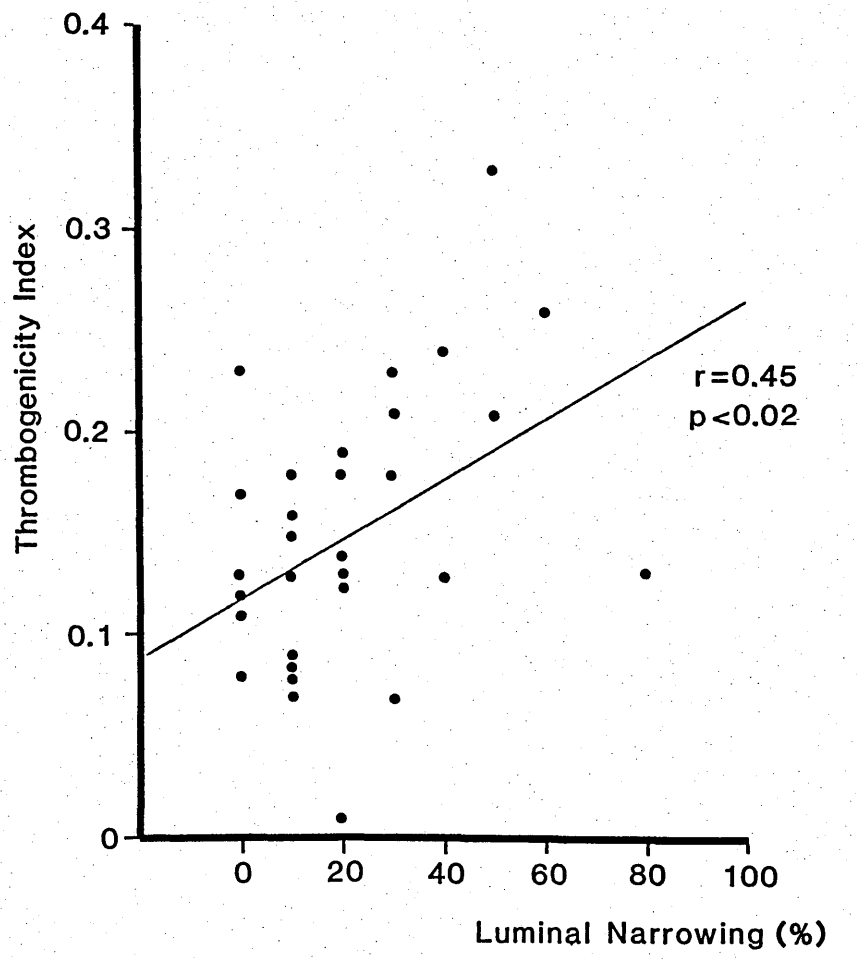


Figure 10.7 The ten patients who required patch angioplasty may represent a subgroup with high thrombogenicity and low rates of restenosis. In 30 patients postoperative TI after standard endarterectomy showed an improved correlation to subsequent luminal narrowing.

10.4 Discussion

These results indicate that there is an association between the postoperative accumulation of platelets on the arterial wall after endarterectomy and the subsequent developement of luminal narrowing. Although it has previously been suggested that this relationship exsists, there was little or no evidence in patients after carotid endarterectomy (Stoney & String 1976; Ross 1985; Das et al 1985). Postoperative thrombogenicity has been found to predict graft failure in femoro-popliteal bypasses but this may be explained by increased platelet uptake in prosthetic grafts compared to autogenous vein (Goldman et al 1983a). Graft failure is more common with synthetic materials and may be independent of platelet kinetics if other factors outweigh the effects of thrombogenicity. In addition the mechanism of graft failure, with progressive accumulation of platelets and formation of a pseudo-intima on prosthetic grafts, differs from the cellular proliferation which causes intimal hyperplasia.

The significance of raised levels of CUR and TI following carotid endarterectomy has not been established. As yet insufficient numbers have been studied for the range of these values to be calculated and therefore these data have been analysed in 2 groups, above and below the median values. This shows only a trend towards greater luminal narrowing with CUR, but a significant increase in patients with higher thrombogenicity.

It was also possible to analyse the results of postoperative platelet accumulation in patients who subsequently developed significant restenosis of greater than 50% diameter reduction. This group of 7 patients had significantly higher levels of CUR and TI in the early postoperative period compared to those patients in whom the arteries remained widely patent.

Not surprisingly the linear correlation between Carotid Uptake Ratio or Thrombogenicity Index and luminal narrowing was poor as many other components must contribute to restenosis (Figs 10.5-7). This may be skewed by factors which are known to alter either thrombogenicity or luminal narrowing. Patch angioplasty affects both of these measurements with increased thrombogenicity, compared to direct suture (Chapter 8), while preventing restenosis (Katz et al 1987). By excluding these and considering only the 30 patients in whom the arteriotomy was directly sutured, the correlation between TI and luminal narrowing improved.

The non invasive techniques, used to evaluate the degree of restenosis in this series, assess the internal diameter of the vessel lumen (Thomas et al 1984). Minor luminal narrowing was demonstrated anatomically by B-mode ultrasound and Doppler frequency analysis was used to confirm increased blood velocity in more severe restenosis (Hames et al 1984). These measurements of luminal narrowing reflect the degree of intimal hyperplasia in the arterial wall. In some cases it is possible to visualise the external wall of the artery and

measure the thickness of the intimal layer (Plate 2.7). This is not always satisfactory as the plane between the adventitia and the surrouding connective tissue may be obliterated (Plate 4.3).

Using biplanar imaging, patients have been classified into lesser degrees of luminal narrowing, between 10 and 40 percent as well as more severe restenosis from 50 to 90%. In addition, 9 patients were found to have no evidence of luminal narrowing and in 3 patients, following patch angioplasty, marked dilatation of the internal carotid artery was discovered (Plate 4.1). The comparative diameters of these arteries were far greater than those seen in a normal carotid bulb however normal blood flow was confirmed by Doppler spectral analysis in these cases. Patch angioplasty may prevent restenosis but excessive widening may adversely affect laminar flow and account for the greater platelet accumulation seen after this type of repair.

10.5 Conclusion

Duplex imaging has been used to identify minor degrees of luminal narrowing and more severe restenosis. Platelet accumulation measured in the early postoperative period following carotid endarterectomy seems to recognise those patients at risk of developing luminal narrowing. This would suggest that platelets are associated with the processes which produce intimal hyperplasia and restenosis.

SECTION IV DISCUSSION

Chapter 11

Discussion and Conclusions

11.1 Measurement of Platelet-Vessel Wall Interaction

The hypothesis investigated in this thesis was that platelet deposition in the carotid artery related to the development of intimal hyperplasia and restenosis following endarterectomy. Platelet deposition is thought to be the initial step in the process of tissue repair and these data confirm that platelets accumulate after endothelial trauma in rabbits and following carotid endarterectomy in patients. In the rabbit model platelet accumulation was detected on the arterial wall within 48 hours of endothelial trauma and confirm the results of similar experiments (Crowley & Pierce 1981). In patients, platelet uptake was measured from the second to the fourth postoperative days and expressed as Carotid Uptake Ratio and Thrombogenicity Index.

Carotid Uptake Ratio is synonymous with the Deposition Index described by Stratton and represents an isolated comparison between the operated artery and the contralateral reference side (Stratton et al 1987). In the present series, radiolabelled platelets were injected at 48 hours and CUR was calculated 72 hours after surgery. Stratton and colleagues infused radiolabelled platelets within 1 hour of arteriotomy closure and measured platelet deposition at 24 hours. They consistently demonstrated platelet accumulation at this stage but failed to detect any change in the intensity of platelet activity between this early measurement and subsequent recordings up to 96 hours following surgery. By introducing radiolabelled platelets at 48 hours and measuring CUR at 72 hours,

sustained platelet accumulation has been identified at the site of endarterectomy after the intense, early, collagen mediated aggregation. These delayed studies identify smaller changes in local platelet activity which may be obscured by the early intense deposition. This initial platelet adherence is in response to the exposure of collagen with secondary deposition resulting from the aggregation of circulating cells to the platelets already attached to the arterial wall. The collagen-platelet response is complete within minutes of establishing blood flow however the studies in these patients reflects the balance between the accumulation and dissaggregation of circulating platelets at the site of endarterectomy (Baumgarten 1973). The rate of this platelet accumulation has been measured over 3 days and expressed as Thrombogenicity Index which reflects the intensity of platelet deposition over this period (Goldman et al 1982a). Platelet-vessel wall interaction is thought to continue from the time of arteriotomy closure when flow is reestablished until healing has produced a neointimal lining (Reidy et al 1983). Although Thrombogenicity Index only assesses platelet activity over this short 3 day period it has been used to compare the rate of platelet accumulation in different groups of patients.

Visual and computer analysis by gamma imaging have been shown to be the most appropriate method for the identification of platelet uptake in the carotid arteries (Powers 1984) as probe counting proved dis-appointing in the detection of carotid thrombogenicity. Despite

careful probe geometry, background activity from the blood pool in the tissues of the neck may be too great to allow consistent detection of small changes in carotid radioactivity. Gamma imaging techniques were able to consistently measure platelet uptake over the endarterectomy segment however gamma camera definition failed to differentiate between platelet uptake in the common and internal carotid arteries. In all patients studied, endarterectomy was extended into the common carotid artery and in some cases for more than 2cms proximal to the bifurcation. This portion of the endarterectomy represents a larger surface area than the internal carotid artery and a greater part of the platelet aggregation will occur in this region. Current concepts suggest that aggregating platelets initiate smooth muscle hyperplasia by the release of growth factors directly into the subendothelium which then act locally to stimulate cellular proliferation (Goldberg et al 1980; Ross 1985). If this is true, platelet uptake detected in the common carotid artery would be unlikely to directly affect the development of intimal hyperplasia in the internal carotid artery. However, endarterectomy of the common carotid artery may activate circulating platelets such that they were more likely to aggregate downstream on the internal carotid artery (McCollum 1981) and could therefore increase internal carotid thrombogenicity and resulting intimal hyperplasia.

11.2 Factors Affecting Carotid Platelet Uptake

In comparison to vascular prostheses which continue to accumulate platelets throughout their life (Stratton et al 1982), the absence of platelet accumulation two months after operation suggests that the carotid artery has healed to a confluent endothelium by this time. This agrees with the small histological reports where the endarterectomy has been shown to heal by about 30 days following surgery (Dirrenberger & Smidt 1978). Platelet accumulation was not identified on the operated arteries 10 days after surgery in patients undergoing staged bilateral endarterectomy and this suggests that platelet deposition has resolved at this earlier stage (Lusby et al 1983). Although regeneration of true endothelium has not been reported in patients, evidence from animal models suggest that the neointimal lining of smooth muscle cells, which forms after endarterectomy, develop a secretory, anti-thrombotic function (Groves et al 1982).

Systemic platelet inhibition with a standard regimen of aspirin plus dipyridamole reduced carotid thrombogenicity in patients. This presumably affects the thromboxane mediated platelet aggregation by interuption of arachidonic acid metabolism (Packham & Mustard 1980). Local prostacyclin activity is thought to diminish the platelet response on the subendothelium following the passage of a balloon catheter compared to formal endarterectomy (Crowley & Pierce 1981) but the estimation of local prostacyclin production and the effects of cyclo-oxygenase inhibition following endarterectomy have yet to be

investigated. Although platelet inhibitory therapy has widespread applications in vascular surgery (Kohler et al 1984), its use in carotid surgery has always been on an empiricle basis (Edwards et al 1985). No studies to date have demonstrated a reduction in platelet activity or lower rates of restenosis with these drugs (Glover et al 1985). Whether or not platelet inhibition influences the incidence of restenosis following carotid endarterectomy requires to be investigated in a randomised, controlled trial.

Local factors which increase platelet aggregation are thought to be those which produce turbulence or low flow states (Zarins et al 1982). This may result from operative errors such as intimal flaps or areas of narrowing in the arterial repair which interfere with laminar flow (De Palma et al 1977). Widening of the arterial repair by patch angioplasty certainly enlarges the origin of the internal carotid artery but at the expense of increased platelet deposition compared to routine endarterectomy. This increased platelet accumulation may be either on the patch material, on the longer suture line or may be on the endarterectomised vessel wall where excessive widening produces a situation analogous to aneurysm formation. Thrombogenicity was at least as marked with vein as with Dacron when used for patch angioplasty and this suggests that excessive widening rather than the choice of patch material is the most significant factor. The high thrombogenicity of venous patches confirmed the findings of canine study in which post mortem thrombus was more marked on vein patch material compared to the endarter-

ectomised arterial wall (Lusby 1983). This indicates that even autogenous patch material is responsible for considerable platelet accumulation.

11.3 Relationship between Thrombogenicity and Restenosis

Significant restenosis of greater than 50% diameter reduction was found in 13% of patients following carotid endarterectomy in agreement with other published series (Ackroyd et al 1986). Fortunately these lesions seldom caused recurrent symptoms of cerebrovascular insufficiency as has been previously reported (Aldoori & Baird 1987).

Platelet deposition following endarterectomy, measured as Carotid Uptake Ratio and Thrombogenicity Index, has been found to relate to the subsequent development of luminal narrowing and restenosis. Patients with increased thrombogenicity in the early postoperative period were found to progress to greater degrees of narrowing measured one year later compared to less thrombotic arteries. A similar relationship between postoperative platelet accumulation and patency has been demonstrated in patients, with respect to pseudo-intimal hyperplasia seen after the implantation of vascular grafts, but has not been described following endarterectomy (Goldman et al 1983a). However the pathogenesis of graft failure differs from restenosis after endarterectomy in that pseudo-intima lining prosthetic grafts is caused by the progressive accumulation of platelets and fibrin on the wall of the arterial conduit. Intimal

hyperplasia is thought to develop in the wall of the endarterectomised artery as a result of the platelet response following surgery. This work provides more evidence to suggest that arterial restenosis caused by intimal hyperplasia is mediated by platelet deposition. The basis for this relationship is derived from on studies which suggest that platelet derived growth factors may influence vascular smooth muscle hypertrophy and result in intimal hyperplasia but at present is only substantiated by animal work and cell culture studies (Bowen-Pope et al 1985). These growth factors have been isolated from platelet extract and shown to be required for normal cell replication stimulating growth of vascular smooth muscle cells in vitro. Although these substances have been isolated and identified, measurement in vivo has not yet been achieved and this prevents the assessment of their effect on vascular smooth muscle in patients (Ross 1985).

The precise role of platelets in the development of intimal hyperplasia remains unclear but can be regarded in one of the following three catagories. In the first instance, platelet deposition, measured as thrombogenicity, may simply be a marker which identifies patients likely to develop restenosis but is indepentent of the process which causes intimal hyperplasia. This could identify patients where the endarterectomy was less than satisfactory, reflect severe arterial disease or demonstrate an individual predisposition to arterial restenosis (De Palma et al 1977).

Secondly, platelets may be one of many factors in the development of luminal narrowing. In the same way that the aetiology of atheroma seems to be multifactorial, platelet activity is unlikely to be the only precursor to the development of arterial restenosis. In common with atheroma, other factors such as hyperlipidaemia, hypertension, diabetes and smoking may influence the aetiology of restenosis but their contributions to this process, individually or in combination, have not been evaluated. How platelets interact in the presence of these and other factors to produce the cellular proliferation of intimal hyperplasia requires further investigation.

And finally platelet deposition and the effect of growth factors may be the single most important activator in this process. Despite the isolation of PDGF and studies which demonstrate its effects on protien sythesis and RNA production, the significance of its contribution to cellular replication and hypertrophy in patients is unknown. Verstraete suggested that PDGF was unable to promote cell growth and only prepares cells for division by inducing 'competence' (Ross 1985). Further studies in cell culture will determine if this competence can be endowed by other factors or if this represents the rate determining step by which PDGF is capable of initiating cell division in isolation. The translation of this experimental work to animal and human models will answer these questions.

11.4 Implications for Platelet Inhibition and Surgical Technique

Despite the widespread use of anti-platelet agents in patients undergoing carotid endarterectomy, no randomised clinical trial has been performed showing benefit with this treatment, either in freedom from neurological complications or restenosis. The platelet accumulation which has been shown to occur following carotid endarterectomy is reduced by platelet inhibition but has not been demonstrated to lower the incidence of subsequent restenosis. Similarly the efficacy of platelet inhibitory therapy in preventing clinically imporant events has yet to be established. As with other trials investigating the treatment of cerebrovascular disease, to prove the benefit of platelet inhibitory therapy would require a large randomised controlled study examining postoperative platelet accumulation, restenosis and the recurrence of cerebrovascular symptoms or stroke (Taylor, Sackett & Haynes 1984). The logistics of such a trial would be hampered by the numbers involved, the prolonged follow up and the attrition rate of these elderly patients from coronary artery disease.

The method of closure of the arteriotomy following carotid surgery adds another variable to such prospective evaluation. Patch closure is usually determined by the surgeon's impression of the diameter of the artery and his choice of patch material is one of personal bias. In order to avoid excessive widening, the optimal diameter of the vessel after vascular reconstruction should reflect the original size and configuration of the artery and a policy of

selective patching might be adopted. Following endarterectomy large vessels are not significantly narrowed by arteriotomy repair but stenosis of small arteries might be avoided by patch angioplasty with the width of such patches restricted to reproduce an internal carotid artery of preoperative external dimensions. Thus the configuration of the artery and the method of repair should be investigated in a suitable controlled trial when platelet deposition can be related to patency, luminal narrowing and clinical outcome.

It seems likely that further research into the cellular response to the trauma of endarterectomy will prove that platelet mediated cell growth is associated with intimal hyperplasia. The abolition of this response either by drugs, induced thrombocytopenia or the use of antibodies to platelet derived growth factor should be possible in the future. This could have widespread implications if platelet interaction with the arterial wall continues to be linked with atherogenesis.

11.5 Conclusions

Platelet deposition on the arterial wall has been demonstrated both in a rabbit model of intimal trauma and in patients following endarterectomy. This deposition of radiolabelled platelets could be measured externally by gamma camera immediately following surgery but was no longer detectable at 2 months. Patch repair of the arteriotomy increased postoperative platelet uptake but inhibitory therapy may be used to reduce thrombogenicity. Platelet accumulation

in the early postoperative period was related to the subsequent development of luminal narrowing following endarterectomy.

These techniques represent a reliable model for measuring platelet-vessel wall interaction in humans and may be used to investigate the effects of therapeutic modalities designed to prevent intimal hyperplasia and postoperative restenosis. Measurement of platelet interactions with the arterial wall may further our understanding of the cellular response to trauma, growth control and tissue repair.

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