EVALUATION OF MUSCLE FLAPS
IN THE TREATMENT OF
INFECTED ABDOMINAL AORTIC GRAFTS

by

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ABSTRACT

Standard therapy for abdominal aortic graft infection involving resection of the graft and extranatomic bypass, carries significant morbidity and mortality due to aortic stump rupture and limb loss. This thesis evaluates the feasibility of combining in situ graft replacement with a highly vascular tissue flap as an alternative approach to this problem.

In sixty nine pigs, a segment of a polytetrafluoroethylene (PTFE) graft was interposed in the infrarenal abdominal aorta. Staphylococcus aureus was then used to infect the graft. One week later the animals were divided into six treatment groups where two different types of pedicled muscle flaps were used to treat the infection. The flaps were designed from the rectus abdominis and the seromuscularis of the jejunum.

The results showed that graft infection could be controlled if either of the muscle flaps were used. Graft thrombosis did not occurred if the infected graft was changed with a new PTFE graft.

Vascularized tissue flaps avoid thus potentially fatal aortic stump rupture and may improve limb salvage in this difficult clinical problem.
RESUME

Le traitement actuel des infections des prothèses de l'aorte abdominale nécessite l'exérèse de la greffe infectée. Ceci est associé à une morbidité et mortalité trop élevées, principalement liées à la rupture du moignon aortique et à l'ischémie des membres inférieurs. Cette thèse évalue la possibilité de maintenir le flot sanguin à travers l'aorte en traitant l'infection à l'aide d'un lambeau musculaire pediculé. Ceci permettrait d'éviter les complications ci-dessus.

Soixante neuf cochons ont subit l'insertion d'un segment de greffe en polytétrafluoroéthylène, au niveau de l'aorte abdominale. La greffe fut par la suite infectée avec du Staphylocoque doré. Une semaine après, les animaux furent divisés en 6 groupes chez lesquels deux lambeaux musculaires différents furent essayés. Ces lambeaux provenaient du muscle droit de l'abdomen et de la séromusculaire du jéjunum.

Les résultats démontrèrent que les lambeaux musculaires étaient capables de contrôler l'infection. De plus, la thrombose de la greffe était évitée si la greffe infectée était remplacée par une nouvelle greffe.
PREFACE

The experimental work for this thesis was done while the candidate was training in General Surgery at McGill University, Montreal, Quebec, Canada. All work contained in the thesis is that of the candidate. The design of the flaps used in the experiments and the surgical interventions were performed by the candidate, based on personal original ideas. Under direct supervision, the pre and postoperative handling of the animals were performed by Mr N. Giannou and Mr S. Passemore, from the Royal Victoria Hospital Research Institute, and the animal technicians of the McIntyre animal centre. The bacteria used for inoculation and graft cultures were prepared by Mrs B. Giannias after instruction by the candidate. Computer desktop publishing and statistics were done by the candidate within the framework of commercial software. The drawings are photographs of originals painted by the candidate.
I would like to acknowledge the influence and contribution of the following individuals: Dr J.F. Symes, as my principal supervisor, whose availability, guidance and generosity were omnipresent; Dr A.M. Graham, for his continuous interest; Dr M. Ricci, for having established in the Cardio-Vascular Research Laboratories the appropriate environment to perform the experiments and for his suggestions in the redaction of many manuscripts; Dr N.V. Christou, for his help, through his laboratories, in the bacteriologic manipulations; Dr C.L. Kerrigan, for her suggestions in the design of the rectus abdominis flap; Dr K. Carter, for her help as a veterinarian in the different part of the experiments, Dr A.R. Mehran, my father, for his vigilance and perspicacity which allowed me to move away on time from the horror of a revolution in the middle east, and finally but not least, Ms L. Villeneuve for her patience and continuous moral support.
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INTRODUCTION

HISTORICAL PERSPECTIVE

Although descriptions of the diseases of the vascular system can be traced back to antiquity, and the need for cures has without doubt existed since human history began, vascular surgery is probably the youngest free standing surgical speciality. We will briefly review the milestones in the history of vascular surgery from the development of the anatomy of the vascular apparatus to the treatment of vascular diseases, relevant to the present work.

Anatomy of the vascular system

The anatomy of the vascular system did not attract the attention that the anatomy of the skeletal or visceral system received. The earliest anatomical manuscripts representing the arterio-venous system are probably part of the famous "five pictures series", the oldest of which were found by Karl Sudhoff (1853-1930) in a german manuscript from the cloister of Prufening (A.D. 1154)(1). The five pictures represented the osseous, nervous, muscular, venous and arterial systems to which the pregnant woman or a view of the generative organs were sometimes added.

These frog like human representations, depicting inaccuracies such as direct communications between the trachea and the intestine to the heart and the great vessels were reproduced for centuries and were distributed as far as China. It was not until the European Renaissance period that these manuscripts were abandoned.

All the great works on human proportions and human anatomy came from Florence, Italy. Leonardo da Vinci (1452-1519) was the founder of iconographic anatomy. Based on his works, Flemish born, Andreas Vesalius (1514-1564), the most commanding figure in european medicine after Galen and before Harvey, made anatomy what it is today, a living working science (front-cover). Precise descriptions of the heart and great vessels can be found in his main work De fabrica humani
corporis (1543)(2). The perfection of anatomic studies through his works and the dissections of many others to follow resulted in improved semiology and treatment.

**Early reports on vascular pathologies and their treatments**

The earliest reported descriptions of vascular pathologies were related to either acute traumatic injuries or aneurysm formation. The only therapy was ligation of the involved vessels. Anthyllus (antic greece)(1) a contemporary of Galen (131-201 A.D.) became famous for his well known method of treating aneurysms by applying two ligatures and cutting down between them which held the field until John Hunter (1728-1793). Galen himself used as reported by Osler (3) a "viscid, coaglaturable and obstructive medicine" as a heamostatic agent for the accidental arterial injury, caused by an inexperienced surgeon at the time of a venisection.

Aetius of Amida (1) who lived in the 6th century A.D. was the Royal physician at the court of Byzantium. He was the first to recognize 2 different types of aneurysms, those caused by dilation or those following arterial trauma (3). To him is due the first description of ligation of the brachial artery above the sac for the treatment of aneurysms.

Curiously, for one thousand years, vascular surgery as it was described by the early greek surgeons did not change until the era of William Hunter (1728-1793) (4). With his works vascular surgery ceased to be regarded as a mere technical mode of treatment, and began to take its place as a branch of scientific medicine, firmly grounded in physiology and pathology. His greatest innovation in surgery was the establishment of the principle that aneurysms due to arterial disease should be tied high up in the healthy tissues by a single ligature. The novel feature was not the single ligature but the sound pathologic reasoning upon which its used was based (i). Antonio Scarpa (1747(1752?)1832), a brilliant Venetian, well known for the triangle in the thigh which bears his name, was the first to regard arteriosclerosis as a lesion of the inner layers of the arteries (5). Sir Astley Cooper (1768-1841) of Norfolk, a pupil of John Hunter, was the most popular surgeon in London during
the first quarter of the 19th century. In 1817, he successfully ligated the first abdominal aortic aneurysm to be operated on. He performed the surgery without anaesthesia (6). Other methods of treatment of arterial aneurysms using intravascular wicks, acupuncture, and metal bands were introduced by Guido Bacelli of Rome (1832-1916)(1), Sir William MacEwen (1848-1924)(7) and William Halstead of New-York (1852-1922)(1) respectively.

The new era

A firmly structured system of therapeutic principles for the treatment of the abnormalities of the vascular system saw the light of day only in the fifth decade of our own century. The ground work of this late blooming was made possible through 50 years of research. The contributions of Jassinowsky, Jorfrer, Briau and Jaboulay and Watts (8) were important steps in the development of successful methods of vascular anastomoses. Carrel and Guthrie between 1905 and 1912 developed the suture technic on vessels we use now (9). During the same period an increased interest was shown in the use of prosthetic material to replace diseased segments of arteries. Tuffier (10) during World-War I attempted to use a paraffined silver tube to bridge arterial gaps but the results were hampered by thrombosis. In 1908, Carrel used tubes of glass, aluminium and gold in dogs (11) but again, significant thrombosis rates decreased the enthusiasm of replacing segments of arteries with artificial material for 40 years. In 1947, Hufnagel (8) reported the first long term (6 months) results using a highly polished methylmetacrylate tube interposed in the thoracic aorta of dogs. However around the same time interest focused on allograft materials. Arterial allografts became standard therapy in late forties and allograft banks were organised in the USA and Europe. By the late fifties it became evident that allogenic grafts were a poor choice as the patency rates rapidly declined, stimulating research with artificial grafts again.

In 1952, Voorhees (12) proposed the first synthetic textile graft made of Vinyon. The material was shown to be unstable invivo and was replaced shortly after by Dacron (13). In 1957, Szilagyi used Dacron successfully for the first time in
human (14). Dacron is now used extensively in cardiovascular surgery. In high flow arterial reconstructions such as aortofemoral bypasses, Dacron grafts have yielded excellent long term patency rates. However, these grafts have been disappointing when utilised in lower flow states such as axillofemoral and femoropopliteal bypasses. Substitutes have been proposed to improve patency rates. Expanded polytetrafluoroethylene (PTFE) vascular grafts were first used as small arterial substitutes by Matsumoto (15). This graft has gained in popularity and is used now in both peripheral and central vascular surgery. Nevertheless long term patency rates have also been disappointing in the former position and the search for the best arterial substitute is still on. At the present time autogenous veins are the graft of choice for infrainguinal arterial reconstructions, while Dacron is used preferentially for the aortic position. Negatively charged gluteraldehyde-tanned grafts synthetized from collagen have shown interesting results in animal models and the information suggest that these grafts represent an improved method of conducting blood (16). Human trials with this type of graft are pending.

In conclusion, from this historical overview, one can appreciate how rapidly vascular surgery evolved from minimal scattered knowlege to what it is now. However many questions remain to be answered. This is particularly true in the field of bypass surgery, where graft thrombosis and infection are still possible troublesome complications.
EPIDEMIOLOGY OF VASCULAR DISEASES

In North America, the life expectancy is increasing. Consequently, a 34% increase in the population older than 65 years of age is predicted by the year 2010 (17). This suggest an increase need to manage diseases of the elderly in the first part of the 21st century.

The decrease in the death rate from circulatory diseases and the increase in death from malignant neoplasms since 1950 are widely recognised. There has also been a decrease in the number of deaths from abdominal aortic aneurysms. This is a matter of concern since deaths caused by peripheral vascular diseases are likely to increase as the elderly population increases.

Another important trend is the 29% decrease in the rate of death from diabetes mellitus in the 45 to 74 years old age groups from 1969 to 1971 compared to those from 1979 to 1981. This increase longevity may explain the increasing number of diabetics with vascular insufficiency problems (18).

On the basis of an approximate 5% sample, data available from the National Centre for Health statistics (USA) gives some insight into trends in the prevalence of diseases. Of patients discharged from non federal hospitals, the listed diagnosis of abdominal aortic aneurysms, myocardial infarction, and occlusion/stenosis of the carotid artery have been among those to increase the most in the past 6 years. In contrast the diagnosis of acute appendicitis, haemorrhoids, duodenal ulcers, inguinal hernias and diseases usually associated with the younger population have decreased (17).

From the same data base, the performance of abdominal aortic aneurysm repair, Arterio-venous fistula for haemodialysis, and coronary artery bypass are among those procedures that have increased the most in the united states in the past 6 years. When these procedures are presented in the broad category established by
the WHO ICD-9 codes, the number of operations on the cardiovascular system as reported by the National centre for health statistics is second only to operations on the eye (17).

This epidemiological review shows that despite a relatively stable mortality rate related to cardiovascular diseases in North America, there is an increasing need for procedures on the heart and vessels. The most important factors seem to be the aging of the population and improved survival of vasculopathy prone systemic diseases such as diabetes.
By far, the most common form of aortic disease is the result of atherosclerosis, while trauma, arteritis and dysplasia are less frequent. Because trauma represents an acute form of vascular disease and because arteritis and dysplasia are uncommon in North America, this discussion will be limited to atherosclerotic diseases only.

Atherosclerosis is a degenerative process of the major human elastic and muscular arteries. It is characterised by the formation of intimal plaques consisting of lipid accumulation, smooth muscle and inflammatory cells, connective tissue fibers and calcium deposits (18,19).

The lesions of atherosclerosis take different forms depending upon their anatomic location and the risk factors to which each individual may have been exposed. Atherosclerosis has been recognised in human for thousands of years. Lesions of atherosclerosis were identified in Egyptian mummies as early as the 45th century B.C. In the mid 19th century, Virchow (21) proposed that some form of injury to the arterial wall is associated with an inflammatory response which results in what was known at that time to be the degenerative lesion of atherosclerosis. This idea was subsequently modified by Anitschkow(22) and further included the role of platelets and thrombogenesis in atherosclerosis. Many of the modern views of atherogenesis stem from the work of John French (23), who suggested that the structural integrity of the endothelial lining of the artery represents a key element in the maintenance of normal arterial function and that alteration in endothelial integrity may precede a sequence of events that lead to the various lesions of atherosclerosis.
**Pathogenesis**

A number of risk factors of atherosclerosis have become reasonably well established by epidemiologic studies, to the incidence of clinically manifest disease. Among those factors that contribute to the formation of atherosclerosis are hyperlipidaemia, hypertension, cigarette smoking, male sex and diabetes mellitus (18).

One of the important factors is the role of circulating lipids specially cholesterol in the form of low density lipoprotein (LDL). LDL accumulates in the vessel wall and this results in metabolic changes that may play a key role in the initiation of atherosclerotic lesions. This increased cholesterol can be secondary to metabolic abnormalities, increased ingestion or increased in the intraarterial pressure (24). Increased in wall pressures and shear stress may also have a deleterious effect on the endothelial cells resulting in changes in their metabolism. It is well known that focal desquamation of the endothelium exposes the underlying subendothelial connective tissue to platelets and formation of atheromatous plaque. Other theories claim that the atherosclerotic lesions are hyperplastic or neoplastic responses to some local biochemical factors (25). It is also postulated that arterial segments at different locations disclose differences in their enzymatic makeup that may have an important role in the variability of lesions depending on the location of the vessels (26).

The examination of atherosclerotic plaques with modern techniques of cell and molecular biology has revealed that each lesion contains significant elements of each of three cellular phenomena. These are smooth muscle proliferation, the formation of large amount of connective tissue matrix including collagen, elastic fibers and proteoglycans, by proliferated cells, and the accumulation of intracellular and extracellular lipids (27). In each instance, the relative degree to which each of the cells responds to different atherogenic stimuli determines the unique combination of these three elements that defines the type and extent of the resulting lesion.
The lesions of atherosclerosis occur principally within the innermost layer of the arterial wall, the intima. They include the fatty streaks, the fibrous plaques and the so-called complicated lesions (28), which is a combination of the first two mixed with areas of wall hemorrhage and calcifications. Secondary changes have been noted in the media of the artery underlying the lesion, principally in association with the more advanced lesions of atherosclerosis.

**The role of the vasa vasorum**

Vasa vasorum are the capillary network responsible for the vascular supply of the arterial wall. Vasa vasorum provide also blood flow to the atherosclerotic plaques (29). The role of this vascularisation has various interpretations. According to Willems (30), the vascularity of atheroma influences the fate of the lesions. If the blood supply is adequate and there are no vascular accidents, the plaques undergo fibrosis; otherwise, hyalinization occurs. Vascularization of plaques is regarded as a less favourable event by other investigators (31). The poorly supported capillaries in the plaques may rupture, resulting in intramural hemorrhage and subsequent occlusion of the lumen of the vessel.

**Prognosis**

Morbidity associated with atherosclerosis arises from plaque enlargement and degeneration. Plaque enlargement may obstruct the lumen, resulting in stenosis and impairment of blood flow. Sudden obstruction of the lumen may result from the dissection of blood into or under the plaque or from hemorrhage within the plaque from the vasovasorum. Plaque ulceration may result in embolisation of atherosclerotic debris or thrombosis formation on the disrupted intima. Thrombosis may also occlude atherosclerotic vessels without obvious plaque disruption due to local modification of flow, and the creation of turbulence. Plaque degeneration and weakening of the vessel wall on the other hand, can result in the formation of aneurysmal dilation of the artery. Morphologic features underlying morbidity and mortality vary somewhat depending on the location. Extensive disease, often with multiple focal stenoses is characteristic of peripheral vascular diseases of the lower
extremities, while aneurysm formation is a major feature of abdominal aortic disease.

**TREATMENT OF DISEASES OF THE ABDOMINAL AORTA**

Whatever is the cause of abdominal aortic insufficiency, the treatment consists of restoration of flow by either primary repair (endarterectomy) if feasible or by bypassing the obstruction. The role of endarterectomy has decreased since the 1960s. The long-term results seem to be comparable to those of bypass grafting and the procedure is associated with more technical problems, blood loss and sexual dysfunction. Endarterectomy is now used mainly in the treatment of localized disorders where balloon angioplasty fails to relieve the stenosis. Percutaneous transluminal angioplasty is preferentially used in the treatment of short localized diseased areas. However, this type of lesion involves preferentially smaller vessels such as the iliac arteries (33). Thus, the use of artificial graft material represents the mainstay of treatment of aorto-iliac disease.

**Prosthetic materials used in the treatment of aorto-iliac disease**

The most common artificial graft material used in North America are obtained from Dacron or from expanded polytetrafluoroethylene (PTFE).

**The Dacron graft**

The architecture of the Dacron graft is formed of cross-linked fibers (fig.1). The porosity allows tissue ingrowth and good tissue incorporation during the healing phase. After implantation and deposition of the pseudointima, the inner lining becomes a highly sophisticated autogenous protein.

![Figure 1: Life tables for patency rates for Dacron grafts in different locations (37)](image-url)
Figure 1: Scanning electron microscopy of A: Dacron and B: PTFE grafts. Note the difference of graft interstices in each graft. (With permission, Dr Guidoin, Laboratoire de Chirurgie Experimentale, Université Laval, Quebec).
conduit which is hypotrombogenic (34). The thickness of the layer of proteinaceous deposits of the inner wall is increased when a filamentous surface (velour) in contact with the blood is used. This may reduce the lumen of a small calibre graft to a critical degree (35). On the other hand the presence of velour on the outer surface of the graft improves the tissue incorporation of the graft. Dacron grafts are fabricated with crimping to increase its resistance to kinking and to maintain its round shape. This crimping however produces irregularities of the inner wall which is acceptable in high flow, large calibers vessels but may be one of the reasons this type of graft does not maintain satisfactory patency rates in smaller vessels, such as in infrainguinal bypasses (fig.1).

The PTFE graft

Introduced in Japan in 1973 (15), the PTFE graft, is the only currently popular prosthesis graft that is not a textile. The graft is white, smooth and flexible and was introduced as a alternative to Dacron in small diameter vessels. The inner surface is made of a node-fibril configuration (fig.2). The wall spaces rapidly fills after insertion with serum protein and collagen. The healing phase is categorised as one of incorporation with fibroplastic penetration rather than a simple encapsulation. In the human the proteinaceous lining of the graft remain non cellular and stays thin, 25 to 50 m to the most. Like any other artificial graft material in human the endothelialization does not seem to extend more than 1.5-3.5cm from the anastomosis line (36).
Presently most PTFE grafts are inserted in a femoropopliteal position but aortic-iliac models are commercially available. The long term patency rates of aortofemoral bypasses using PTFE are excellent. In the infrainguinal position the PTFE graft maintain a similar patency rate compared to Dacron (fig.2)(37).
VASCULAR GRAFT INFECTION

The incidence of graft infection reported in the literature varies between 0.6 and 6% (table I)(38-44). On the other hand, The reported mortality rate of graft sepsis varies between 20 and 75% (38-44), making graft infection uncommon but the worst complication of vascular surgery.

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<tr>
<th>Author</th>
<th>Number of patients</th>
<th>percent infection</th>
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<tr>
<td>Hoffert(38)</td>
<td>201</td>
<td>6</td>
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<tr>
<td>Moore (39)</td>
<td>50</td>
<td>5</td>
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<tr>
<td>Nevelsteen (41)</td>
<td>352</td>
<td>2.3</td>
</tr>
<tr>
<td>Szilagyi (42)</td>
<td>2145</td>
<td>1.5</td>
</tr>
<tr>
<td>Brodwater (43)</td>
<td>241</td>
<td>0.8</td>
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<tr>
<td>Lind (44)</td>
<td>175</td>
<td>0.6</td>
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Pathogenesis of prosthetic graft infection

The primary mechanisms of graft sepsis are 1) operative contamination, 2) bacteraemia, and 3) mechanical erosion (graft enteric fistula) and bacterial exudation.

The main source of operative contamination is the skin flora, especially when incision in the groin is necessary (48). Iatrogenic trauma to the lymphatics in the groin is another reported factor in the higher infection rates in this area (49).

Concurrent infections elsewhere in the body can be sources of bacteraemia. Although bacteraemia is a rare cause of graft infection in human (50) graft colonization have been repeatedly reproduced in experimental models (51-53). Following implantation of a Dacron or PTFE graft, the susceptibility to haematogenous bacterial contamination has been reported to last from 6 weeks to 6 month (45,54,55). It is likely that the entrapment of bacteria by the graft decreases as the formation of the proteinaceous pseudointima is completed (45). However in
a recent study, Formichi and Guidoin (56) observed the presence of bacteria in 46% of long term PTFE implants in humans, even though only 29% were considered clinically infected. The incomplete healing of the inner wall of arterial graft in humans allows this bacterial trapping, and eventually their growth can be reactivated under particular circumstances (57).

Another disastrous source of graft contamination is erosion of prosthetic grafts into adjacent portions of bowel, creating prosthetic-enteric fistulas (45,59). Graft contamination, on the other hand, can also occur without enteric contamination by translocation of bacteria from the bowel lumen into the peritoneal cavity during aortic procedure. Although this source of contamination has been studied (60,61), its role in graft infection is not clear. Bacterial translocation may be one of the reason for the positive cultures obtained from clean intrabdominal procedures and for the 15 to 30% positive arterial cultures obtained from the wall of abdominal aortic aneurysms (62,63).

The search for the most infection resistant prosthetic material is still very active. Autogenous tissue grafts (artery and vein) are more likely to resist infection than artificial prostheses which do not act as a foreign body in the wound. But when they do become infected, degeneration followed by haemorrhage is likely. In addition these materials are not suitable in the aortic position.

Surprisingly enough both Dacron and PTFE have been found to be superior to each other in terms of resistance to bacteremic infection in experimental setting (58,64). The general tendency however, is to use preferentially the PTFE graft in an infected field. A large clinical experience with PTFE in dialysis access, suggests that, despite repeated violations of the graft, it remains resistant to infection (65). Additionally, it has been utilized successfully in contaminated fields following traumatic arterial injuries (66).
Bacteriology of prosthetic graft infection

There has been a slow but definite alteration over time in the organisms producing deep graft infection (67). In 1962, Javid et al (68) found that all infections in his study were caused by staphylococcal species. In 1970, Conn (69) discovered that 68% of infections were caused by gram negative organisms. *Staphylococcus aureus* involves predominantly superficially situated grafts such as those crossing the inguinal area, while *Escherichia Coli* and other enterobacteriaceae tend to involve the abdominal segments of infected grafts.

Early vascular graft infection is usually secondary to perioperative contamination by skin organisms. Late vascular graft infection on the other hand is usually secondary to haematogenous spread and originates from the bowel.

Prophylaxis

The best measure to avoid vascular graft infection is prevention. Adherence to strict surgical asepsis, treatment of bacteraemia prior to surgery, coverage of the graft with healthy tissue as a barrier between the gastrointestinal tract and the graft, and appropriate antibiotic prophylaxis, all constitute proper surgical technique. Prophylactic antibiotic-therapy should be routinely used. In 1978, (70) a controlled double blind clinical study revealed a decrease in vascular graft infection in patients receiving prophylactic antibiotic. Another study by Pitt et al (71) revealed similar findings with both local and intravenous antibiotics decreasing graft infection significantly. The antibiotic of choice should have antistaphylococcal and at least some gram negative activity. For these purposes first generation cephalosporins are the best choice. However another approach to prophylaxis has been the attempt to bond antibiotics directly to the graft. This has been shown to be effective in some experimental studies (72,73).

Management of aorto-iliac prosthetic graft infection

The main clinical presentation of vascular graft sepsis is the development of unexplained fever. There may be also back pain or renal failure from ureteral
obstruction from the septic process. Increasingly, it is recognized that development of a false aneurysm at either the proximal or distal suture lines may represent the first manifestation of a graft infection. Retroperitoneal haemorrhage from one of the suture lines or aorto-duodenal erosion or fistula are other less frequent manifestations.

When vascular graft infection is suspected the acute septic process can frequently be controlled with aggressive antibiotherapy but this therapy cannot always eliminate the bacteria in and around the graft. Because persistent infection will endanger life and because serious infection frequently shows no external manifestation, removal of the prosthetic graft is a necessity.

Graft excision alone is seldom sufficient since blood flow must be restored through an extraanatomic route, avoiding the infected region. In the case of the abdominal aorta this is done by axillofemoral bypasses as originally proposed by Blaisdell and Hall (74), and now modified to axillobifemoral bypasses. The overall patency rate of axillofemoral bypasses at five years, varies between a disappointing low 30% to 81% (75,76,77), slightly higher for the bilateral procedure.

It has recently been suggested that removal of the infected graft after the extraanatomic bypass (sequential) or few days after the later (staged operation) is associated with a better overall result that if the graft is removed first followed by the axillofemoral bypass (traditional procedure) (78).

Following the excision of the graft the proximal aorta is closed. This is associated with a significant number of complications. Disruption of the stump from residual infection and subsequent massive haemorrhage is the most feared one (79). Many techniques increasing the mechanical strength of the aortic stump closure have been described (80,81,82) but they do not aim at eradicating the infection. Shah and coworkers (83) used a seromuscular patch of the jejunum to cover the stump. In a small group of patients they obtained very good clinical results probably by the
propensity of the good vascularity of the patch, to eradicate the infection. Another complication of stump closure of the aorta is renal artery occlusion secondary to proximal propagation of intraluminal thrombus (84,85).

Dissatisfied with the need to excise and bypass the graft, Walker and Cooley (86) proposed a more conservative approach by in situ replacing the infected abdominal aortic graft with a new graft. They found that using the omentum as a protective barrier between the graft and the bowel was helpful. Eighty three percent of their 18 patients, were still alive on average 5.2 years after the surgery.

In conclusion, aortic graft infection is a rare but disastrous complication. At the present time management consists of appropriate antibiotic therapy, graft removal and restoration of blood to the lower extremities via axillofemoral bypasses. This management is far from being satisfactory because of the high morbidity and mortality associated with blow-out of the aortic stump and failure of the bypass.
MUSCLE FLAPS

The use of muscle flaps as a reconstructive tool has evolved from an operation for the lower extremity to one applicable to all body regions. The rapid growth of this reconstructive technique has been directly related to increased awareness of the precise anatomy of the blood supply of muscle. In fact every muscle is a potential flap if the surgeon has an accurate knowledge of its functional and vascular anatomy.

One of the theories proposed for chronic persistent infections such as those seen in osteomyelitis is the presence of a relatively avascular structure in the wound where the infecting organisms escape natural host defense mechanisms. Providing a new source of blood flow to the wound would theoretically help the treatment of infection. In 1946, Stark (87) in order to increase the vascular supply to infected bone, used muscle flaps to cover areas of osteomyelitis. He obtained results superior to simple incision and drainage, commonly used at that time to treat osteomyelitis.

The idea of using muscle flaps to cover chronic wounds was used and perfected by Ger (88,89). Ger eventually formulated the principles, established the operative procedures and demonstrated a wide range of applications for muscle flaps. Muscle flaps are now aside from their reconstructive usefulness, are valuable adjuncts in the treatment of chronic wound infections (90,91).

The use of muscle flaps in vascular surgery

A prosthetic vascular graft is a foreign body. Healing of a graft in humans involves the development of a thick, relatively acellular and avascular outer capsule which is poorly attached to the prosthesis by prongs of tissue extending into the interstices of the graft (92). In the case of the abdominal aorta, the capsule is surrounded by loose retroperitoneal tissue which is also relatively avascular. When a graft infection occurs, there is thus a favourable milieu for the development of a chronic infection. This milieu contains a foreign body surrounded by a relatively avascular barrier to host immune functions. One can thus appreciate the importance
of excising the graft in the treatment of the infection. However, because of the serious morbidity involved with the removal of the graft, one may consider the potential benefits of applying a vascularized tissue flap against the infected graft. Encouraging results using muscle flaps, in the treatment of vascular graft infections have been recently reported by Horneffer (93) and Cruz (94).

Horneffer et al demonstrated that muscle flaps could be used to patch small aortic defects left by the removal of an infected graft. Cruz and coworkers showed that a rotational flap of sartorius can protect vascular prostheses with bacterial inoculums up to 1x10^4 organisms, but at a greater bacterial contamination its efficacy was no longer significant.

Another source of vascularized tissue flap is the omentum. The efficacy of the omentum to patch gastrointestinal perforations has been known for many years. The omentum has also been used for the coverage of infected grafts in experimental and clinical settings with some success (95,96). The presence of different angiogenic factors in the omentum (97), can be one of the potential benefits of using the omentum in the treatment of a relatively avascular wound.

The mechanism of function of muscle flaps in the treatment of graft infections

Muscle flaps are equipped with a rich capillary network and their efficacy in treating wound infection is directly related to this anatomic characteristic. Hypoxia is known to increase the susceptibility of tissues to infection, possibly as a consequence of impaired leucocyte function (98,99). Thus increasing the oxygen delivery and wound oxygen tension in the infected tissues results in improved leucocytic action and bacterial elimination.

When comparing musculocutaneous flaps with random skin flaps, Mathes (90) was able to demonstrate that 1) in response to a challenge of *S. aureus* the musculocutaneous flap was able to decrease the bacterial counts and survive, and 2)
tissue oxygen tension were significantly higher in the musculocutaneous than in the random skin flaps. Muscle flaps are thus excellent biological dressings which not only offer coverage for exposed tissues but also have excellent indirect bacteriocidal activities through their rich vascular supply.

The design of a muscle flap for the abdominal aorta

In the selection of a muscle flap, certain technical criteria must be respected: 1) when designing an island flap there must be enough leverage at the rotation point to cover the wound totally, 2) the vascular supply of the muscle must be preserved at all cost and 3) there must be only limited functional loss from the donor site.

The use of flaps in vascular surgery is very recent. The latissimus dorsi has been used experimentally to cover defects of the thoracic aorta (93), and the sartorius can easily be rotated over a graft in the groin (94). On the other hand, the abdominal aorta because of its retroperitoneal position is not easily accessible by surrounding muscles. The psoas muscle which is situated on both sides of the aorta has a segmental blood supply which makes the rotation of a viable flap over and around the aorta very difficult. Moreover the psoas muscle maintains close contact with important nervous elements of the lumbar plexus which would be at high risk of injury if the muscle is mobilized. Although the omentum can reach the abdominal aorta, few factors limit its usefulness for the treatment of a graft infection: 1) the considerable variation between the length, mobility, and availability from patient to patient, 2) lower degree of vascularity compared to muscle flaps, particularly in obese patients where the omentum is invaded by thick adipose tissue, and 3) the suturing of the omentum around the abdominal aorta produces a band in the abdomen predisposing the patient to internal herniation of the bowel and volvulus of the stomach. An original source of muscle flap was proposed by Shah (83). He used a pedicled seromuscular patch of the jejunum to cover infected aortic stumps. Although the flap was originally designed to offer increased mechanical strength to the stump, it proved to be efficacious probably by eliminating the infection.
In conclusion one can appreciate the theoretical usefulness of using a muscle flap in the treatment of aortic graft infections. The aim of this thesis is the design and evaluation of different type of muscle flaps for this purpose.
THE CHOICE OF THE ANIMAL MODEL
IN EXPERIMENTAL VASCULAR SURGERY

Although vascular graft infection is uncommon, the association of high morbidity and mortality demands a search for better means of prevention and therapy. Because of this low prevalence, the design of clinical studies is difficult, requiring large number of patients. For this reason animal models of vascular graft infection provide a valuable tool to explore better therapeutic modalities.

Different animal species have been used as models in experimental vascular surgery. However results can only be extrapolated to clinical practice only if the animal faithfully reproduces the human situation. In order to obtain this, a few criteria must be respected: 1) the model should be of sufficient size to allow similar surgical conditions as in humans, 2) the healing physiology of the animal should be close to human's, 3) the resistance to infection in the model should be homogenous and close to man, 4) the cost of purchase and humane maintenance should be reasonable and practical allowing the use of large number of models and long term follow-ups.

The two most commonly used animal models in experimental vascular surgery are mongrel dog and swine (sus scrofa). The choice of the dog as an animal model of human surgery has been based on tradition in many instances (100). No single animal species is exactly analogous to man and the choice of an appropriate model should be based on physiologic and anatomic considerations.

Swine may be preferable to dogs in cardiovascular research for many reasons: 1) the cardiovascular system of the swine has been used extensively as a surgical model for humans. The distribution of the coronary blood supply and the wound healing characteristics of the myocardium are almost analogous to man (101,102). 2) The growth of the heart and the great blood vessels from 6 weeks to 6 months of age is similar to the growth of these vessels in human infants from birth to sexual
maturity (103). 3) The aorta of the pig has a true vasavasorum like humans and consequently exhibits similar wound healing characteristics (102,103). 4) The mature pigs possesses the three serum immunoglobulins, IgG, IgA, and IgM, Which on the basis of their physicochemical and antigenic relationships are analogous to their human counterparts (104) and overall pigs may demonstrate a pattern of bacteremic graft infection that is similar to humans (53,105). 5) Swine tend to be more healthy and yield more consistent results than pound dogs, and because pigs are always from bred sources, they are provided in uniform, predictable sizes and physiologic behaviour (106). 6) For long term studies dogs need to be conditioned, thus doubling their purchasing price which is already more elevated than pigs. 7) Housing of pigs in groups is easy and cannibalization of surgical wounds does not occur. 8) There has been increasing public concern in the use of pet animals as experimental models and consequently procurement of dogs is becoming more difficult. 9) Swine are available through both agricultural sources and commercial suppliers of laboratory animals.

**Swine anatomy**

**The infrarenal abdominal aorta**

Detailed anatomic descriptions of the infrarenal abdominal aorta of swine are scarce and incomplete. The study described in this thesis was performed on the infrarenal aorta and based on a thorough knowledge of its tributaries to surrounding structures, particularly the rectus abdominis muscle. As a preliminary work it was felt necessary to study the anatomy of the infrarenal aorta by the means of anatomic dissections. Ten pigs were studied and detailed dissections of the interested areas were performed with emphasis on the main tributaries and territories of vascularisation. The compilation of the results of this study are represented in plates 1 and 2. Note the one to two pairs of lumbar arteries located between the renal arteries and the trifurcation, the trifurcation of the aorta into common external iliac arteries and the common iliac trunk, and the inferior epigastric artery. The deep inferior epigastric artery was a tributary of the common external iliac artery in 7 cases, in the two others the artery was originating from the superficial division of the external iliac artery, and in one the artery was originating from the common external...
iliac artery. In comparison to humans, the division of the external iliac artery is proximal to the inguinal ligament. The diameter of the infrarenal aorta in its widest diameter in a 15 kg animal is 6 to 7mm.

The anterior abdominal wall

The anterior abdominal wall of the pig is nearly identical to man. The same muscle groups with similar insertion and blood supply can be found in the pig (plates 3 to 5). Like its human counter part, the rectus abdominis has a dual vascular supply provided by the superior and deep inferior epigastric pedicles. Numerous collaterals between the superficial inferior epigastric artery and the deep branch can be found on the lateral border of the muscle.

Porcine intestinal tract

The gross anatomy of the porcine gastrointestinal tract is different from humans but has analogous physiologic functions, probably because swine are true omnivores. Significant differences include: 1) the vascular arcades of the small intestine form in the muscularis mucosa rather than the mesentery. 2) Most of the colon is spiral in shape, located in the left upper quadrant of the abdomen, and consists of centrifugal and centripetal coils of caecum and transverse colon. The sigmoid colon is partially retroperitoneal and runs parallel and to the right of the aorta. The small bowel is located in the mid portion of the abdomen and its vascular supply is from the superior mesenteric artery which provides also blood supply to most of the colon as well. The blood supply of the sigmoid colon is from the inferior mesenteric artery which originates on the anterior surface of the aorta just proximal to the trifurcation.

Antibiotherapy in pigs

Antibiotherapy in swine, choice of drugs, pharmacokinetics of different agents and dosage on a weight unit is similar to humans. The choice of drug is based on the location and type of bacteria involved. In this experimental setting, the antibiotic
should be effective against the infecting organism and must attain high tissue levels without toxicity at high doses.

Cephalosporins are used more frequently in veterinary presurgical prophylaxis. We have used Cefazolin which is a first generation cephalosporin with good gram positive activity and some gram negative coverage. It is easily available, inexpensive and pharmakokinetic data has been generated in animals (107). The use of cefazolin in swine abdominal surgery should be associated with a low incidence of wound or pulmonary infections. Since it is used almost uniformly in clinical vascular surgery as a single prophylactic agent its use reproduces an important element of the clinical setting.

Penicillins are effective mainly against gram positive bacteria. This group of drugs, used in swine, find its greatest indication in the treatment of systemic infections caused by streptococci, staphylococci, and corynebacterium. One of the major advantages of penicillin are the long acting formulas such as procaine penicillin. This salt form of penicillin maintain therapeutic levels for periods up to 24 hours following a single intramuscular injection. This a real advantage when one compares with sodium or potassium salts which need to be administered every 2 to 4 hours (108). The use of procaine penicillin is attractive in the treatment of infection caused by susceptible organisms because it allows single daily treatments which is particularly handy when dealing with large numbers of animals on long term therapy.

In conclusion to this section we have found the swine a particularly suitable model for experimental vascular surgery. The knowledge of porcine physiology and anatomy are essential background components to the work presented in the thesis.
PURPOSE

The purpose of this study is to explore new therapeutic options in the treatment of abdominal aortic graft infections, using a swine model. Muscular flaps will be designed and applied around an infected graft. The efficacy of the flap to treat the infection without having to excise the graft will be assessed. Maintenance of aortic continuity should eliminate both the risks of aortic stump disruption as well as artery occlusion and the need for extraanatomic bypasses.

This study will base itself on two different sources of muscle flaps, one of striated muscle, obtained from the rectus abdominis and another of smooth muscle, dissected from the seromuscularis of the jejunum.
METHOD

EXPERIMENTAL PROTOCOL

Sixty nine white Landrace pigs were enrolled in the present study. The mean body weight at the beginning of the experiment was 15 to 20 kg. A three cm segment of a six mm PTFE graft was interposed in the infrarenal aorta between the renal arteries and the inferior mesenteric trunk. One cc of a suspension containing $10^8$ S. aureus was injected after the closure of the retroperitoneum around the graft and the abdomen was closed. One week later the animals were subjected to a second operation. At that time all grafts were still patent and were included in a retroperitoneal abscess containing 5 to 10 cc of yellowish pus growing in all cases S. aureus.

The animals were divided into 6 experimental groups (table II). Group 1 (control) had incision and debridement of the abscess only. Group 2 had incision and debridement, and change of the graft with a new PTFE graft. Group 3 had same treatment as group 2 and in addition an island rectus abdominis muscle flap was positioned around the graft. Group 4 was similar to group 3 except that the flap was obtained from the seromuscularis of the jejunum. Group 5 and 6 were similar to groups 3 and 4 respectively except that the PTFE graft was left unchanged.

The animals received 250mg of cefazolin IV preoperatively and procaine penicillin $G^2$, 40'000 U/Kg/day intramuscularly for 7 days post-op.

Three weeks after the second surgery the animals were sacrificed and the grafts harvested aseptically. The grafts patency were assessed and the grafts were submitted for bacterial cultures and light microscopic studies to assess the degree of

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1 Kezol®, Lilly, Scarborough, Ontario
2 Ayercilline®, Ayerst, Quebec
healing. Details of each step of the experiment will follow.

**SURGICAL PROCEDURES**

*Graft infection*

Under proper anaesthesia the animal was positioned supine and the skin was prepped with providine detergent and solution. The animal was then draped and a midline skin incision, from the pubic bone to the mid point between the umbilicus and the xiphoid process was carried down the linea alba using a #15 blade. The cautery was used for haemostasis and incision of the linea alba. Upon entering the abdomen, the large and small bowel were brought out of the wound and wrapped in sterile moist towel to expose the posterior abdominal wall structures.

The peritoneum over the infrarenal aorta was incised with the Metz and Baum scissors. The aorta was then freed from the surrounding lymphatic cisternae and ducts, from the renal arteries to the takeoff of the inferior mesenteric artery. One to two pairs of lumbar arteries originate at this level from posterior wall of the aorta which need to be divided. Four thousand units of heparin\(^1\) was then used to anticoagulate the animal. The aorta was then cross clamped with atraumatic vascular

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1: Heparin Leo\(^6\), Leo Laboratories, Ontario.
clamps, distal to the renal arteries and just proximal to the inferior mesenteric artery. A two cm segment of the aorta was excised and between the retracted ends a three cm segment of six mm PTFE graft was sutured. For the end-to-end anastomoses 6-0 Novafil\(^1\)(TE-1, 12mm, curved needle) was used. The peritoneum was closed over the aorta using 3-0 Dexon\(^1\) (CE-4 19mm, curved needle). One cc of a bacterial suspension containing \(10^6\) *Staphylococcus Aureus* sensitive to penicillin (MIC 0.003 mg/l) was inoculated around the graft, retroperitoneally. The bowel was repositioned and the linea alba closed with a running 2-0 Maxon\(^1\) (T-12, 37mm curved needle). The skin was approximated subcutaneously with a running 3-0 Dermalon\(^2\) (straight needle) and the animal was sent to the recovery room.

**The second operation, the experimental groups**

1) **Group 1 (control) and group 2:**

Under appropriate anaesthesia and antibiotic prophylaxis, the animal was prepped and draped and the abdomen entered as described above. The abdominal content was removed to expose the retroperitoneum. In all cases, there was a 5-10ml abscess situated posterior to the sigmoid colon around the graft. The dome of the abscess was incised using the cautery with caution to not damage the inferior mesenteric artery or the ureters. The abscess content was suctioned and the cavity irrigated with a 0.2% solution of providine and the walls burned with the cautery. A swab of the pus was always taken for confirmatory cultures. The graft was then exposed along with 1 cm of proximal and distal native aorta. In group 1 the graft was not changed and the procedure terminated here. In group 2, the pig was anticoagulated and the aorta cross clamped. The graft was replaced with a new PTFE graft. The abdominal content was repositioned and the abdominal wall closed as described previously.

\(1\) Davis and Geck, Danbury, USA
\(2\) Ethicon, N J , USA
2) **Group 3 and 5, the rectus abdominis flap**: (figures 4 and 5)

In these groups prior to the opening of the peritoneum, a dissection of an island flap of the rectus abdominis based on the inferior epigastric pedicle was performed (109,110). The first step consisted in the elevation of the anterior rectus sheath from the muscle, from the distal insertion on the pubic tubercle to the mid portion of the muscle. The muscle was then divided 15 cm from the pubic bone using the knife to avoid burning the muscle. Haemostasis of the proximal remaining muscle was achieved with the electrocautery. The distal muscle was then elevated from the posterior rectus sheath and the parietal peritoneum. The posterior rectus sheath covers only the superior two-third of the rectus muscle. The dissection was carried out down to the distal insertion which was also divided. At this point the muscle was only attached to the inferior epigastric artery and vein. The vascular pedicle is carefully separated from surrounding tissues down to its origin from the external iliac arteries. Extreme care is taken to avoid injury to the pedicle by the instruments or by over stretching. Once the dissection was finished the muscle was wrapped in a moist gauze and placed on the thigh of the animal.

At this point the parietal peritoneum was elevated from the antero-lateral and posterior abdominal wall until the retroperitoneal abscess was exposed retroperitoneally. the abscess wall was then opened, the content aspirated and the
Figure 5: A: Dissected rectus flap based on the inferior epigastric pedicle; B: The flap around the graft.
wall cauterised. The graft was changed in group 3 and the patent graft left in place in group 5. Care was taken to avoid injury to the inferior vena cava which becomes very adherent to the abscess wall. In order to prevent catastrophic bleeding we used a 5x30mm rectangle of PTFE as a separation between the 2 vessels at the time of the first surgery which was removed when the graft was reexposed one week later.

Once the graft was in place and a 5mm proximal and distal segment of the aorta had been cleaned, the island flap was brought into the abdominal cavity through the retroperitoneal passage and wrapped 360° around the graft using interrupted 3-0 Dexon (CE-4, 19 mm suture). The proximal and distal end of the aorta were well covered by the flap. The abdomen was closed using running 0 Maxon (T-20, 48 mm, curved needle), approximating the anterior rectus sheath on one side and the linea alba on the other. The subcutaneous tissue was approximated with interrupted 3-0 Dexon (CE-4, 19 mm, curved needle) and the skin was closed with either nylon sutures or skin staplers.

3) Groups 4 and 6: the seromuscular jejunal flap: (figure 6 and 7)

In these groups, the abscess was debrided first in the usual fashion and then the graft either changed in group 4 or left in place in group 6. A 15 cm segment of the proximal jejunum which easily reached the aorta was clamped and then separated from the rest of the bowel. The mesentery of the segment of jejunum was divided down the lymph nodes at the base of the mesentery. This offered good leverage of the seromuscularis. The continuity of the bowel was restored with a running 4-0 silk' (19mm, curved needle) suture and a second reinforcing layer with an interrupted suture of the same material. The mesentery of the donor jejunum was also closed with a running 4-0 silk. The antimesenteric border of the segment of jejunum was then opened and the mucosa cleaned with providone. The mucosa was

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1: Ethicon, N.J., USA
Figure 7: Different steps in the preparation of the seromuscular jejunal flap. A: submucosal injection of saline; B: the separation of the seromuscularis; C: the flap in place.
Figure 6: The seromuscular jejunal flap in position around the graft

separated from the seromuscularis after saline infiltration in the submucosa. This allowed the mucosa to peel away from the seromuscularis. There was usually a fair amount of bleeding from the rough surface of the muscularis which subsided after few minutes of compression.

The seromuscularis was wrapped 360° around the graft. The space between the mesenteric pedicle and the posterior abdominal wall was reduced with a interrupted 3-0 Dexon to avoid potential internal hernias. The abdomen was closed in the usual fashion.

ANIMAL MANAGEMENT

All pigs were obtained from a specific pathogen-free breeding herd from the McDonald farms, Ste Anne de Bellevue, Montreal. The pigs were housed at 22 degree celsius at a 14:10 light cycle at the Royal Victoria and McIntyre animal
facilities. Pigs received commercial diet mixed with tap water until the evening prior to surgery. Normal feeding was resumed 24 hours after surgery. The animals were gaining about one kg per week during the length of the experiment.

Animal care in the project complied with the "Principles of Laboratory Animal Care" and "The Care and Use of Laboratory Animals" by the Canada Council on Animal Care, 1980.

ANAESTHESIA

Ketamine hydrochloride¹ (22 mg/Kg IM) given simultaneously with atropine² (0.05 mg/kg) were used for preanaesthesia. Halothane³(2%) was then given by mask. When the animal was well sedated an IV line with normal saline was started in a marginal ear vein.

Anaesthesia was induced by sodium pentobarbital⁴ (10 mg/kg IV) followed by endotracheal intubation with a 6.0 Fr soft cuff endotracheal tube. The anaesthesia was maintained with halothane (1%), O₂, and mechanical ventilation (TV 300cc, RR 16, pressure controlled respirator). Recovery from anaesthesia was obtained by stopping the halothane and maintaining the animal on the respirator until adequate ventilation was resumed.

Intraoperatively when bleeding was less than 250-300cc, 500cc normal saline was used as only fluid replacement. If the bleeding was in excess of 300 cc, 250cc of homologous, uncrossmatched blood along with 500 cc normal saline were used to restore the fluid volume.

1 Rogarsetic®, Rogar laboratories, Montreal, Quebec
2 Astra, Mississauga, Ontario
3 Fluothane®, Ayerst, Montreal, Quebec
4 Somnotol®, MTC Pharmaceuticals, Mississauga, Ontario
Post operatively the animal was placed under a heat lamp, maintaining an ambient temperature of 30 degrees celsius. The IV line was removed shortly after the operation. Food and water were resumed the day after surgery.

Post operative analgesia was obtained by acetaminophen\(^1\) (325 mg po every 4 hrs when necessary) for all animals and levorphenol\(^2\) (2 mg IM every 6 hours when necessary) for those felt to be in greater pain.

**GRAFT EXCISION AND ANALYSIS**

At 21 ± 2 days, the animals were taken back to the OR and under sterile technique as described previously, the abdomen was entered. The retroperitoneum was exposed, clamps were applied proximal and distal to the graft and the graft along with the muscle flaps if present excised. Graft patency was assessed by direct visualisation of a transverse cut-section of the graft. One half of the graft was saved for microscopic studies and the other half reduced in pieces and sent for bacterial cultures. Photographs of all graft were taken for records.

**Bacteriologic preparations**

1) **Preparation of *S. aureus* for inoculations:**

The preparation of the bacterial inoculum involved the following steps; a) a swab of a pure culture of penicillin sensitive *S. aureus* obtained from a wound was incubated in 10cc of BHI (brain heart infusion broth) at 37\(^\circ\) for 4 hours, this produces a bacterial concentration of 4.5x10\(^3\) organism/ml. b) 50 l of the initial solution was then diluted in 5ml BHI. c) the solution was then serially diluted to obtain a final concentration of 10\(^3\) organisms/ml. d) The process described was repeated for each operating day.

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1. Tylenol\(^R\), McNeillab, Fort Washington, USA
2. Levodromoran\(^R\), Roche, Ontario
2) Graft cultures:

In order to increase the bacterial recovery, the grafts were first cultured in 10 cc of BHI for 5 days at 35° Celsius and then plated on blood agar plates. The growth of *S. Aureus* on the plates was interpreted as a positive graft culture. The growth of other organisms were interpreted as contaminants. No quantitative cultures were taken. The different steps involved in the identification of the contaminants are reported in figure 8.

![Figure 8: The different steps in the identification of the contaminants.](image)

**Microscopic preparations**

Four specimen in each group were analyzed by light microscopy. The purpose of this part was to assess at an microstructural level the difference in healing between the flapped vs non flapped grafts. Cut sections of the graft were placed in neutral formaldehyde 10% for 24 hrs then embedded in paraffin, cut in 5-8um thick sections and finally stained with haematoxylin and eosin.

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1 Sigma pharmaceuticals, USA
Statistical analysis

Thrombosis or infection are discrete independent variables. When compared to the controls, the values can be plotted in a 2 by 2 table. If the expected value for each cell is less than five, a Fisher's exact test with one degree of freedom and an alpha value of 0.05 is calculated. If the expected value in each cell is equal or more than five a Chi square value with the same degree of freedom and alpha value is calculated (111). Since in this study, multiple comparisons to one control is performed, Bonferroni's test (112) for homogeneity has been used. Bonferroni's test is very specific but decreases the power of the analysis. The P value to reach statistical significance is then 0.05/5 tests = 0.01.
RESULTS

Fifty two animals completed the study, 10 in groups 1 to 4 and 6 in groups 5 and 6.

**GRAFT THROMBOSIS** (fig.9, table III)

There was a significant discrepancy ($p < 0.01$) between the groups treated with a muscle flap and the graft change (groups 3 and 4) compared to those treated with a flap without change of the graft (groups 5 and 6). In group two where the graft was changed but no flaps were used the thrombosis rate was between the rates of groups 3-4 and groups 5-6.

![Histogram of graft thrombosis and infection results.](image)

**GRAFT INFECTION** (fig.9, table III)

The groups where a muscle flap was used showed lower infection rate compared to groups 1 and 2 where no flap was used. The infection rate was significantly lower in groups 3 and 4 where the infected graft was also changed. In 4 cases gross sepsis with a perigraft abscess was present, but in the others infection
Figure 9: Results, cross-sections through the graft at 3 weeks, A: control; B: group 3.
Figure 9 (cont'd): C: cross-section of a graft in group 5 at 3 weeks.
Table III: Frequencies of graft thrombosis and infection in different treatment groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Infection</th>
<th>$P_{ct}$</th>
<th>Thromb</th>
<th>$P_{thromb}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7/10</td>
<td></td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4/10</td>
<td>0.37</td>
<td>4/10</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>0/10</td>
<td>&lt;0.01</td>
<td>1/10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>1/10</td>
<td>0.02</td>
<td>1/10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5</td>
<td>1/6</td>
<td>0.12</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2/6</td>
<td></td>
<td>6/6</td>
<td></td>
</tr>
</tbody>
</table>

*: Fisher's exact test, compared to control.

was demonstrated only after cultures.

The list of the graft contaminants and their frequencies are reported in table IV. There was no relation between the number of contaminants and the different groups.

LIGHT MICROSCOPY

The healing reaction around the grafts in groups 1 and 2 was characterized by a dense desmoplastic reaction with infiltrates of fibroblasts, multinuclear giant cells and occasional lymphocytes. In groups 3 and 4, the graft was well incorporated in the muscle, this was more evident in the rectus abdominis group, where the muscle was in direct contact with the graft. There was an eosinophilic noncellular infiltrate in the interstices of the grafts. The orientation of the fibers closest to the graft was longitudinal. The same orientation was found in the seromuscular fibers. In group 4 the leaves of the flap situated in the posterior surface of the graft were almost completely replaced by a fibrous acellular tissue. These parts of the flap were the most distant from the vascular pedicle. In groups 4 and 5, there was a thin fibrous layer between the grafts and the flaps and thus the degree of healing based on the thickness of the perigraft fibrous layer, was somewhere between the groups 1-2 and groups 3-4.
Table IV: List of organisms grown on the grafts other than S. aureus.

<table>
<thead>
<tr>
<th>contaminants</th>
<th>group1</th>
<th>group2</th>
<th>group3</th>
<th>group4</th>
<th>group5</th>
<th>group6</th>
</tr>
</thead>
<tbody>
<tr>
<td>enterobacteriaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>proteus sp</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>others</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>(E. coli, klebsiella)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>streptococci sp</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>pseudomonas sp</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>total</td>
<td>8</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

**COMPLICATIONS**

Seventeen animals died from immediate complications following the first or the second surgery. The complications are listed in table V. Two animals died from uncontrollable intraoperative bleeding from inadvertent trauma to the IVC. From a total of 12 rectus flap, two failed and were associated with the death of the animal. The post mortem examination of one animal showed a thrombosed graft in an abscess cavity containing fresh fecal material. It could not be determined whether the flap necrosis and persistent sepsis, caused the bowel perforation or the iatrogenic bowel ischemia and stool contamination caused the flap to necrose. In the second animal, diffuse intraabdominal sepsis was found. The flap was necrotic and the graft thrombosed.

Two other rectus flaps showed partial survival (group 3 and group 5) but none were infected with *S. aureus*. In one animal a rectus flap was elevated but while dissecting the retroperitoneum the colon was accidentally entered. In this case the rectus flap was used to cover the perforation which healed well without the need to
Table V: List of complications associated with death in the postoperative periods.

<table>
<thead>
<tr>
<th>Complications</th>
<th>OR#1</th>
<th>OR#2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaesthesia:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>overdose</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>traumatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intubation</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Bleeding</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Sepsis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wound dehiscence</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>purulent peritonitis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aortoenteric fistula</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sigmoid gangrene</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acute graft thrombosis</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Small bowel obstruction:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intussusception</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>suture loop</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

divert the colonic flow. In this case the animal was treated in the study as a group 2. All seromuscular flaps were viable at the end of the experiment. Five animals were sacrificed because of acute massive ischemia of the lower extremities secondary to graft thrombosis which occurred in the first few hours after the surgery. These animals were felt to be too sick to undergo reoperation. Three other grafts thrombosed immediately after their insertion at the time of the second surgery despite appropriate anticoagulation. Thrombectomy with a 3 Fr Fogarty catheter was followed by good flow. Two of the grafts were found to be eventually thrombosed at 3 weeks. Fifteen animals required transfusion and this was associated in one with anaphylactoid cardiac arrest intraoperatively, the diagnosis of which was delayed and the animal could not be resuscitated.

1 American Edwards laboratories, Puerto-Ricco, USA.
Table VI: Morbidity factors associated with the surgical procedures.

<table>
<thead>
<tr>
<th>Complications</th>
<th>OR #1</th>
<th>OR #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound infection</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Abdominal wall dehiscence</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Lower limb paresis</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>transient</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>permanent</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Small bowel volvulus</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Colon perforation (iatrogenic)</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Cardio-resp.-arrest</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Thrombectomy</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

In those who survived (table VI), the most common complication was staphylococcal infection around the suture materials used to close the abdomen. This was usually treated by simple incision and drainage and daily packing of the wound until healing was completed. In the perioperative period, seven animals developed transient paresis of one or two lower extremities. Special care was given to these animals to avoid pressure wounds.
DISCUSSION

The most feared complication of vascular surgery is aortic graft infection. Aortic prosthetic infection is a relatively uncommon occurrence, which is associated with death if not treated aggressively with large doses of antibiotics and removal of the graft. Complications after such treatment are associated with the persistence of the inflammatory and septic process around the aortic stump or the failure of the extraanatomic bypasses that are used to restore blood to the lower extremities. The objective of this study was to evaluate an alternative therapeutic option that would allow the maintenance of the anatomic flow through the graft while treating the infection.

Although the efficacy of muscle flaps in the treatment of osteomyelitis has been known for many years, their use in vascular graft infections has been limited. The effect of the muscle flaps is mainly related to their rich vascular supply allowing an increase delivery of oxygen and antibiotics around the relatively avascular infected wound. We have explored in this study these principles.

Muscle flaps that would reach the retroperitoneum around the infrarenal aorta were designed and their efficacy in treating a grossly infected graft were evaluated. The muscle grafts were obtained from the rectus abdominis or the seromuscularis of the jejunum.

The experimental protocol was oriented initially to the development of a reproducible, clinically relevant aortic graft infection model. The most commonly cultured organism in graft infections was used to infect a retroperitoneal graft. The closure of the parietal peritoneum over the graft was found to be essential in order to localize the infection around the graft. The delay between graft infection and
treatment was set at one week (53). This was suitable because all the grafts were infected and the grafts were patent at that time. The main complication associated with this step was a large incidence of wound infections.

Good mobilization of the rectus abdominis was obtained by dissecting the vascular pedicle down to its origin either from the common external iliac artery, the profunda femoris or the superficial femoral artery. The inferior epigastric pedicle can support most of the muscle and thus the flap can reach almost anywhere in the lower abdomen. For the upper abdomen, flaps can be designed from the latissimus dorsi or the rectus abdominis based on the superior epigastric pedicle. The preservation of the anterior rectus sheath allows closure without tension and good support of the abdominal wall.

The seromuscular patch was derived from the proximal jejunum. This type of flap is very friable and thin but is extremely vascular. the seromuscular flap is particularly mobile in the upper abdomen but the infrarenal aorta is the lowest point to which it can be mobilized. In order to avoid internal herniation, once the flap has been sutured around the graft, the mesenteric pedicle should be attached to the retroperitoneum. This was not possible in every case but we did not see symptomatic herniation. Blood loss is more important in this type of flap, most of the animals that needed transfusion were in this group. The procedure has the potential disadvantages of requiring more operating time and further contamination from deliberately opening the bowel. However in practice this has been shown to add little risk of further infection (83). We feel that this type of flap may be particularly useful in cases of graft enteric erosion or fistula where the bowel lumen is usually already exposed. In the seromuscular group, complications were similar to the rectus group, except for more intraoperative death from anaesthetic complications or haemorrhage due to the longer procedure and more blood loss from the flap.
Graft thrombosis was present in all animals in groups 5 and 6, where a flap was used and the graft was not changed. The reason for this large discrepancy, compared to the groups where the graft was changed, is not clear. There was no association with the presence of infection, indeed only 3 out of 12 cases in the last two groups, were infected. The cause of thrombosis is thus probably not related to the presence of infection but probably to the deposition by the infectious process within the interstices of the PTFE graft of some thrombogenic substances. These substances cannot be cleared by the flap. They must be acellular because the PTFE is impermeable to cells. Judged by human standards two other reasons can explain these high thrombosis rates: 1) Some animal species including swine maintain elevated levels of coagulation factors and parameters that would be interpreted as indicating hypercoagulability if they were found in human patients (113). 2) Compared to man, swine have a lower aortic diameter/weight ratio and maintain a large amount of collaterals assuring sufficient vascular supply to the lower extremities. In other terms this would suggest that the flow in the pig’s aorta is comparatively lower. This may facilitate platelet deposition and thrombosis over a predisposed surface. Another factor of possible significance is the duration of graft implant in the animals where the grafts were not changed. In these animals the grafts were left in place for 28 days instead of 21. The longer a graft is in place, the likelihood of thrombosis is higher (see PTFE lifetables).

This study has reproduced the expected data in terms of infection. We have found similar results with the rectus abdominis or the seromuscular flap. Graft infection rates, although statistically not significant, were more reduced if the graft was changed. This trend suggests that the use of the muscle flap to clear the infection is limited to the load of bacteria to be cleared. This has been confirmed by Cruz (94) in the study of bacterial clearance from inguinal grafts using a sartorius flap. Changing the graft, draining the abscess and appropriate antibiotherapy should reduce the bacterial load to a minimum for maximal graft effect.
A large amount of other bacteria were cultured from the grafts. They were found in seromuscular cases but also in the other groups where the bowel was not entered. All the contaminants were gut organisms except *S. epidermidis* which was probably skin born. Translocation of bacteria from the bowel to intraabdominal abscesses has been proven experimentally (114). These bacteria are similar to the species shown to translocate into mesenteric lymph nodes and other intestinal organs (115) and in response to immunosuppression (116). Phagocytic cells could be of primary importance in facilitating this migration. Phagocytic cells may engulf intestinal bacteria, transport them to another site, fail to accomplish intracellular killing and then liberate the phagocytosed organisms (114). The route of translocation is probably through the lymphatic system (115). Aortic graft surgery entails significant lymphatic damage which could be a source of leakage of translocated bacteria from the surrounding gut.

Other mechanisms of translocation of enteral organisms from the gut have been demonstrated. Endotoxin promotes the translocation of indigenous bacteria from the gastrointestinal tract of the mesenteric lymph node (117), probably secondary to an increase intestinal permeability (118). The presence of a sterile foreign material in the abdominal cavity can also attract intestinal bacteria (119). Fry (120) in a study of culture material recovered from bowel bags used during reconstructive abdominal vascular surgery found almost routinely typical enteric gram negative organisms. In a similar study, Russel (121) found no enteric organisms. However, for prophylaxis, Fry used oxacillin while Russel used cephalotin which covered the enteric organisms. Despite these controversial reports, it seems that even the mild transient congestion that the bowel is invariably subjected to in the bowel bags, is a factor involved in translocation. This is confirmed by Fiddian-Green (122) who concludes that the development of infection of an aortic graft is predictable from the presence and duration of sigmoid ischemia during the surgery.
Based on these results, conditions such as concurrent sepsis elsewhere, intraabdominal graft infection or transient bowel ischemia predispose the patient to bacterial translocation. It seems wise to administer to these patients long term antibiotherapy with not only staphylococcal but also enteric coverage. In the study reported here, enteric organisms were recovered at 21 days after surgery. Further studies looking at the length of translocation in relation to the healing of aortic graft infection is necessary to optimize the antibiotherapy. The need for long term antibiotherapy is supported by observations of Malone et al (123) who demonstrated that the risk of arterial/aortic disruption in patients treated for a graft infection with positive arterial wall cultures, is decreased by the long-term use of culture specific antibiotics (6 weeks).

The results of this study did not reproduce the data obtained by the "conservative" approach of Walker and Cooley (86). These authors proposed replacement of the infected aortic graft and antibiotherapy as the only treatment. They also used the greater omentum as a protective barrier between the repaired small bowel and the graft without any formal wrapping of the graft, in about half their cases. The authors reported good results in 83%. This approach is reproduced in group 2. In this group 4 out of 10 grafts were infected and the same number thrombosed. Although this type of treatment seems to represent an improvement compared to the classical method, it is not perfect and additional method of treating the graft infection are necessary. Muscle flaps can be as shown here a useful adjunct to preservation of the graft and antibiotherapy.

We have used in this study PTFE grafts. This type of graft is recommended in infected wounds, but the graft does not allow tissue penetration and complete incorporation into the healing process. Indeed we have found the adherence of the graft to the muscle especially to the seromuscular flap to be minimal. The use of a
porous braided graft such as Dacron in this setting, using muscle flaps, might promote a better healing process. The cellular penetration of the graft could then improve the endothelialization of the intimal surface, and perhaps lead to a reduce thrombosis rate.
CONCLUSION

The study reported in this thesis proposes a new option in the treatment of infected aortic grafts, using muscle flaps. Very good results are obtained if the flap is apposed against a freshly changed graft. Both grafts showed similar results in terms of outcome and complications, the rectus flap was however technically easier to use. In this work, using small diameter grafts, the graft change is essential to avoid graft thrombosis. Long term studies with larger diameter porous grafts, with appropriate enteric antibiotic coverage are still necessary. Once this is done, further evaluation of the usefulness of the technics reported here, in a human setting is recommended.
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