A STUDY OF THE EFFECT OF LOCAL AND DISTANT SEPSIS ON THE PATENCY OF MICROVASCULAR ANASTOMOSES

by

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For Lesley, Joanna, Katie and Stephanie

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SUMMARY

1. Introduction

Although Carrel laid the basic foundations of organ transplantation, vascular anastomosis and free tissue transfer in the early part of this century, it was not until almost 50 years later that the first successful free flap was reported by Seidenberg and colleagues. Now, using refined instrumentation, success rates of around 90% are reported in most large clinical studies involving the microvascular transfer of free tissues.

Many factors such as technique, irradiation, tension, diabetes and nicotine, are known to influence the patency of a microvascular anastomosis, but although the role of infection in vascular practice has been well documented, to date, little work has been published on the effect of sepsis on a microvascular repair.

The purpose of this study in the rat experimental model, was to investigate the role of distant septic inflammation, distant sterile inflammation, local wound sepsis and a transient sublethal bacteraemia on the patency of microvascular anastomoses.

2. Materials and Methods

One hundred female Sprague-Dawley rats were randomised into five groups:

(i) to receive an injection into the right axilla seven days prior to surgery, of a 2ml suspension of Pseudomonas aeruginosa containing 2×10^7 organisms, to produce a distant septic abscess,

(ii) to similarly receive 2ml of sterile turpentine oil for a remote sterile abscess,

(iii) to similarly receive 2ml of sterile saline as controls,

(iv) to receive a 2ml suspension of Pseudomonas aeruginosa, containing 2×10^5 organisms intravenously immediately prior to the release of microvascular clamps, to produce a transient sublethal bacteraemia,

(v) to have a 2ml suspension of Pseudomonas aeruginosa, containing 2×10^6 organisms, applied topically into the groin wounds at the time of surgery to produce a local wound infection.

The femoral arteries were divided and an end-to-end repair performed using interrupted 10/0 Prolene sutures. One week later the groins were re-explored and the anastomoses inspected for patency and specimens removed for light and electron microscopic study.

In a further study, blood was collected for haematological and coagulation studies to be performed.

3. Results

One week post-operatively, 39 of the 40 control anastomoses were patent. This was not significantly influenced by either a transient sublethal bacteraemia or a distant sterile abscess, where 35 anastomoses were patent in each ($p = 0.203$). Thrombosis was markedly increased to 13 of 40, in those animals with a local

wound infection ($p = 0.001$), but was greatest of all in the pyogenic abscess group (20 out of 40), even though it was remote from the microvascular repair ($p < 0.001$).

Those animals with a distant septic abscess had a significant increase in the total white cell count when compared to controls (11.95×10^9/L compared to 4.95×10^9/L) and a significantly higher percentage of polymorphonuclear leucocytes (63.6% compared to 14.2%) ($p < 0.001$). Those animals with distant sepsis, also demonstrated a significant increase in the platelet count ($1,475.20 \times 10^9$/L compared to 955.11×10^9/L) ($p < 0.05$).

There was also a profound decrease in this group's serum albumin (10.60mm compared to 15.89mm), α1.3 (2.30mm compared to 4.78mm), P2 (5.75mm compared to 11.0mm) and α1.6 (4.10mm compared to 6.78mm) protein peaks ($p<0.001$).

There were significant increases noted in the serum α1.7 (22.40mm compared to 4.56mm), α2.4 (10.90mm compared to 2.78mm) and β2 (10.90mm compared to 6.22mm) protein peaks ($p < 0.001$).

4. Discussion

Infection has a significant effect on wound healing and as long ago as 1907, Carrel reported on the failure of an experimental limb replantation due to sepsis. This present study, in the rat experimental model, has shown that sepsis, both distant and local, can adversely influence the patency of a microvascular anastomosis. This effect was associated, in the distant abscess

group, with a significant increase in the numbers of circulating platelets, changes in the white cell count and marked alterations in the serum protein levels.

5. Conclusions

Although the exact mechanism of anastomotic failure remains uncertain, these results demonstrate, that septic foci anywhere in the body, may promote occlusion of a microvascular anastomosis and should therefore be eliminated prior to reconstructive surgery involving the microvascular transfer of free tissue.

6. Possibilities For Future Research

Further work is currently underway to investigate more fully the roles of both cellular and humoral factors in the enhanced coagulation in the infected animals.

It is also proposed, using Gentamicin sensitive Pseudomonas aeruginosa, to determine the optimal time to begin antimicrobial therapy prior to microvascular surgery in the infected situation.

CHAPTER 1

INTRODUCTION

1.1 Historical background

The origins of reconstructive vascular surgery can be traced back to 1759, when Hallowell successfully repaired a wound of the brachial artery following a crude attempt at antecubital venesection (1), although Susratas had used an umbilical ligature around 1500 B.C. to arrest bleeding and Ambroise Paré controlled haemorrhage by mass ligation in 1552 (2). Toward the end of the last century, Eck created an experimental portacaval shunt using a continuous silk suture, Jaboulay performed end-to-end anastomosis of arteries (2) and Murphy successfully repaired traumatised vessels (3). In his paper of 1897 (3), Murphy described the successful repair of a bullet wound of the common femoral artery, using an invagination technique, held in place by interrupted silk sutures. Although Jassinowsky had recommended that vascular sutures should only be placed through the adventitia and media, it was Dorfler who advised an all-layer suture in vascular anastomosis for a successful repair (2).

The basic foundations of vascular anastomosis, organ transplantation and free tissue transfer were laid in 1908 by Carrel (4) and Guthrie (5). They adopted an all-layer method of repair and described the three-stay suture technique to stabilise the vessel ends and allow for small vessel anastomosis, a technique which is still sometimes used in current practice. Carrel, who received the Nobel Prize in 1912 (6, 7), probably performed the first experimental free composite tissue transfer, when the auricle, scalp and neck

glands, based entirely on the common carotid artery and jugular vein, were replanted from one animal into another (4). Around this time also, Guthrie successfully transplanted a canine head onto another dog (5).

1.2 Development of microvascular surgery

Nylén, in 1921, introduced the operating microscope into clinical practice (8). Using initially a monocular instrument, he operated on a case of chronic otitis media. Although the stereoscopic microscope was used shortly thereafter in otomicrosurgery, it took almost 40 years for it to be used successfully in the repair of small vessels, due to the limitations of surgical technique at that time (9). Jacobson and Suarez, using 25 times magnification, achieved 100% long term patency rates following the anastomosis of rabbit carotid arteries using 7/0 atraumatic silk sutures (9). Using miniaturised surgical instruments, they were able to operate on vessels measuring 1.4mm in diameter. Chase and Schwartz (10), using 4 times binocular loupes, were able to suture canine brachial arteries with an average diameter of 1.5mm, although their patency rate at 14 days was only 75%.

Seidenberg et al in 1958 (11), had previously reported on the repair of vessels of 1.5 to 4.0mm in diameter, although magnification was not used in their experimental study of over 300 canine vessels, and are credited with one of the first clinically successful free tissue transfers. Their paper described free jejunal transfer in mongrel dogs, but included a clinical case report of a one stage oesophageal reconstruction

using vascularised small bowel, following a laryngo-oesophagectomy performed in 1957 (12). The mesenteric artery was anastomosed to the inferior thyroid artery using a continuous 7/0 silk suture, and the vein was anastomosed to the common facial vein using a tantalum ring prosthesis. The reconstruction was successful, but the patient, a diabetic with severe hypertension, died of a cerebro-vascular accident on the seventh post-operative day.

Prior to these publications, Androsov, had in 1956 (13, 14), described a method of suturing vessels from 1.3mm in diameter using a vessel suturing apparatus which was first developed in 1945. He also reported on free intestinal transfer using this method in oesophageal reconstruction and discussed the successful replantation of a traumatically amputated forearm, which had been performed in the Soviet Union in 1951.

Shortly after these reports of successful small vessel anastomosis, a variety of skin and composite flaps were successfully transferred in the canine and rat experimental models (15, 16, 17). This laboratory work, confirmed that free tissue transfer and digital replantation were within the surgical capability of the early 1960's.

In 1906, Carrel and Guthrie (18) had successfully replanted an amputated canine limb which was viable for almost 36 hours, but was eventually lost due to a tightly constrictive bandage. Androsov successfully replanted a forearm in 1951 (13) and further upper limb reattachments were reported by

Horn (19) and Malt and McKhann (20) in 1964. Kleinert et al (21, 22) had managed to salvage severely injured upper extremities using vascular reconstruction and reported several unsuccessful attempts to restore completely severed digits. The late 1960's, saw hand (23) and digital replantation become a clinical reality (24). Komatsu and Tamai achieved the first successful clinical replantation of a completely amputated digit, when in 1968, they reported reattachment of a thumb, completely severed at the metacarpo-phalangeal joint (25), using microvascular techniques.

Despite all this work, it took until the 1970's, for the movement of tissues from one part of the body to another, with the re-anastomosis of their supply vessels at the recipient site using the operating microscope, to become a routine and reliable reconstructive technique (26-35). Antia and Buch (26), successfully transferred a dermis-fat graft by direct vascular anastomosis in 1971, but unfortunately there was considerable reabsorption due to infection. McLean and Buncke (27), transplanted omentum to a scalp defect in 1972 and the first distant transfer of a free skin flap, was reported by Daniel and Taylor in 1973 (28).

The 1980's have seen a rapid expansion in the field of reconstructive microsurgery (36, 37), particularly in the head and neck region (38-41), following severe trauma to the extremities (42, 43, 44) and even in the salvage of lower limbs with extensive soft tissue loss, when combined

with arterial bypass surgery (45).

1.3 Microsurgical instrumentation and techniques

The early attempts at microvascular surgery met with very limited success, because suture material and needles were imperfect, instruments were poorly designed and the vessel wall handling techniques were often crude and traumatic (46). Also, it was not until the introduction of the sulphonamides and penicillin, that any major progress was possible in preventing sepsis in severely traumatised tissues.

Since their introduction into clinical practice in the early 1960's, both the techniques and the instrumentation for microsurgery have become more refined (46, 47, 48). The use of vastly improved microsutures, made of monofilament nylon of high tensile strength (48, 49) and advanced needle designs (46, 48, 50), have allowed for improved anastomotic patency rates. The numerous microsurgical instruments now commercially available, originally designed from jeweller's forceps, associated with an atraumatic technique, have resulted in patency rates approaching 100% in experimental studies (51, 52, 53) and about 90% in most of the large published series of clinical cases (39, 41, 47, 54, 55, 56). The use of an operating microscope with 25 to 40 magnification, now makes it possible to repair vessels of about 1mm in diameter.

In the practice of microvascular surgery, both arterial and venous repairs are carried out, one of the most commonly

performed being an end-to-end anastomosis using eccentric biangulation of the stay sutures to allow the posterior edges to fall away from the needle point (47, 51). The posterior-wall-first technique has significant advantages over the eccentric biangulation method, as it is less complicated, more rapid and easier to perform (57). When there is marked vessel discrepancy, or in severe trauma to the lower limbs, an end-to-side repair may be used (58, 59). Interrupted placement of the sutures is the most widely practiced method (48), as a continuous repair tends to produce a degree of narrowing of the vascular lumen (51), resulting in poorer patency rates (60).

Other methods, such as the sleeve technique (61), use of CO_2 laser assistance (62), application of tissue adhesive (63) or fibrin glue (64) and the use of metal (65, 66) or polyethylene (67) rings, have been tried, but are generally not popular.

1.4 Factors influencing the patency of microvascular anastomoses

Despite the refinement of microvascular techniques over the last 25 years, histological studies have shown that there can be extensive damage at the site of anastomosis (53). Many different factors may have an influence on the patency of a small vessel repair (68, 69).

1.4.1 Surgical aspects

Pre-requisites for the surgeon, are freedom from fatigue, sound microsurgical experience and careful pre-operative planning (70). A meticulous surgical technique that produces

a tidy suture line with correct apposition of the vessel edges, will reduce the risk of intraluminal turbulence. In larger vessels, the surgery may be infinitely easier and the wider diameter permits a faster blood flow across the repair (69). Meticulous haemostasis is critical, as extravascular blood may cause vessel spasm, obscure the operative site and post-operatively, may cause extrinsic pressure on the vessel wall.

It has also been shown that vessels sutured under tension become stretched and their diameters may be reduced by more than 50%. If the tension is excessive, then there is more leakage of blood from the suture holes and the patency rate is reduced to around 67% (71), compared with nearly 100% in other experimental studies on normal vessels (51, 52, 53). In clinical practice, tension can be reduced or eliminated by bone shortening in replantation surgery or by the use of reversed vein grafts from the volar aspect of the forearm. Vein grafts inserted under tension in rats, show an increased rate of microaneurysm formation and a slight, but not statistically significant, tendency towards thrombosis (72).

After major surgical procedures there may be more post-operative complications when the haemoglobin level is high (73). Although anaemia can reduce blood viscosity, observations in the rat experimental model have demonstrated that it does not promote patency in microvascular surgical procedures (74).

In microsurgery the role of anticoagulants is controversial and there is no uniformity of their use in clinical practice (75, 76). About 70% of microvascular surgeons use aspirin and dipyridamole in combination, to prevent thrombotic occlusion in reconstructive microsurgery. Prostacyclins have been shown experimentally to be effective as anti-thrombotic agents (77, 78) and although dextran 70 seems to be of little value (79), a recent report has demonstrated that dextran 40 has a significant effect on the patency of rabbit arteries following microvascular trauma and vessel repair (80). Acland et al (81), have shown that even the solutions used to irrigate the vessel ends may have a harmful effect, and have recommended the use of lactated Ringer's solution for this purpose.

1.4.2 Anaesthetic factors

Maintenance of the blood flow across the microvascular repair is crucial and can be enhanced by reducing heat loss, correcting hypovolaemia and augmenting cardiac output (38). Small vessel spasm can be relieved by the use of nitroprusside (82), and the topical application of plain 1% lignocaine or papaverine.

Although sympathectomy may increase skin blood flow in patients suffering from vascular diseases or vasospastic disorders, it has no significant effect on microsurgical anastomoses in rats (83). In the clinical situation it does not prevent the vasospasm caused by the surgical manipulation

of small vessels (82), and there may even be a fall in free flap perfusion in the lower limb, when bupivacaine is used to produce an epidural sympathetic block (84).

1.4.3 Pathology of vessel and associated disease states

Vascular abnormalities such as atheroma, vasospastic disorders and local trauma may compromise an anastomosed vessel (85, 86). Calcification of the media, atheromatous plaques and thromboangitis may make vessel suturing technically more difficult. In disease states such as polycythaemia rubra vera, there may be abnormalities of blood flow due to an elevation of platelet numbers and this can be associated with intravascular thrombosis (85).

Hypertension, may be associated with thickening of the media and an increased risk of vasospasm. Although often seen in neurosurgical practice, hypertension, in the experimental situation, has no effect on thrombus formation or anastomotic stenosis (87). Nicotine, even in small doses, may lead to acute vascular insufficiency and there is evidence that it may even compromise a previously viable replanted digit (88). Observations in animals have demonstrated that diabetes seems to delay intimal healing and may reduce microvascular patency rates (89), but further studies are needed to confirm this.

1.4.4 Irradiation

In the irradiated patient a microvascular free flap can, theoretically at least, bring well vascularised tissues into the irradiated field. However, experimental studies have

shown up to a 50% loss of free flaps when the pedicle is anastomosed to irradiated vessels (90). This result does not seem to be affected by the timing of the pre-operative dose of irradiation. Watson (91) found that, although arterial anastomoses in irradiated vessels remained patent, there was an almost 50% occlusion rate in the venous anastomoses, which was often associated with extensive perivascular fibrosis. Similar findings have also been reported in other experimental studies (92). So whenever free tissue transfer is being used to provide cover for an irradiated area, either a flap with a long pedicle, or vein grafts, should be used to allow the anastomoses to be completed well outside the original field of treatment.

1.4.5 Infection

The role of infection in vascular practice has been well documented particularly when prosthetic conduits have been used in reconstructive vascular surgery (93-96). Such post-operative infections can be very serious and may result in the loss of a limb or even death of the patient, and accordingly an aggressive surgical approach has been widely recommended. More recently though, it has been suggested that adequate surgical drainage of infected tissues and high dose antibiotic therapy, may eradicate graft sepsis (97). In this study by Conn et al in 1986 (97), five of seven patients with infected aortic bifurcation grafts were treated conservatively. Three of the five however, eventually required graft replacement, and the study did not completely resolve the question of whether a conservative approach to

major vascular sepsis was feasible. Other reports have documented that about 70% of aortocoronary saphenous vein bypass grafts will remain patent in the presence of severe mediastinal sepsis, provided aggressive treatment including the surgical drainage of pus, suction irrigation and high dose antibiotics are utilised (98).

To date, little work has been published on the role of infection in microvascular surgery. Local sepsis can cause free flap loss due to vascular thrombosis (43, 82, 99) and marginal necrosis (100), and it is a known predisposing factor in the formation of pseudoaneurysms, which can lead to severe haemorrhage (101, 102). Rath et al in 1986 (103), reported the almost complete survival of a free latissimus dorsi musculocutaneous flap, despite the development of an infected pseudoaneurysm that required ligation of the supplying vessel during the third post-operative week. In this remarkable case, it seems that a great deal of the revascularisation must have come from the cutaneous inset of the flap.

Hayhurst et al (52) found that 7 out of 50 experimental animals had local wound infections following microvascular repair of the femoral vein, but only one of these had an occluded vessel. Luk and Chow in 1985 (104), showed that there was a 30% rate of microvascular anastomotic thrombosis in animals with a local wound infection, although this was not statistically significant from their control group. They also demonstrated that when a microsurgical anastomosis was performed in infected tissues, there was an occlusion rate of 75% (105) and that 20% of normal vessels crossing the infected area underwent

spontaneous thrombosis. They suggested that all anastomoses be performed outwith the zone of infection, and that flaps with a long vascular pedicle, or vein grafts, should be used to traverse the infected site.

In spite of the technical advances, some free flaps still fail due to an anastomotic occlusion in the early post-operative period. Many such cases may have local or distant sources of sepsis, where a transient bacteraemia could promote hypercoagulability (106, 107) and thus occlusion of the microvascular anastomosis.

Maxwell et al in 1979 (108), in an experimental study to evaluate the role of silicone cuffs in preventing false aneurysm formation around rat femoral artery anastomoses, found that 8 out of 20 microarterial anastomoses which were surrounded by a silicone cuff, had evidence of local infection with associated vascular occlusion. Ten of the remaining, non infected anastomoses, were patent.

Carrel in 1924 (109), showed that distant aseptic inflammation could adversely influence the healing of skin, and this effect has been more recently demonstrated in this laboratory in experimental abdominal, gastric and peritoneal wounds (110).

Howe (111, 112) observed that a clean surgical wound could become colonised by organisms in the circulation and it is well known that prosthetic vascular grafts can become secondarily infected by a transient bacteraemia induced at the

time of surgery (113). A distant bacterial focus of infection can also markedly inhibit the healing of stomach, muscle and peritoneum (110). In this latter study with Pseudomonas aeruginosa, over 50% of the animals with a distant septic abscess had colonisation of the abdominal wound by the organism, and it was postulated that the infection had been blood-borne.

1.5 Aims and design of the study

The purpose of this experimental study was to investigate the role of distant septic inflammation, distant sterile inflammation, local wound sepsis and a transient sublethal bacteraemia (induced at the time of surgery), on the patency of microvascular anastomoses.

In the initial part of this work patency was assessed by visually inspecting the microvascular repair seven days after surgery, looking for expansile pulsation distal to the anastomotic site and by using the milking and flicker tests (52, 114, 115). In the milking (or double forceps patency) test, blood is emptied from the artery distal to the anastomosis using two pairs of jeweller's forceps. Following release of the forceps nearest to the anastomosis, blood rushes to fill the empty segment, if the repair is patent. Specimens from the anastomotic site were then examined by light and electron microscopy and samples from a variety of sites were collected for microbiological study.

Following this, further investigations were performed, in which not only were the anastomoses inspected, but also blood samples were collected for a detailed haematological

study of cell types and numbers to be carried out. Further samples were assayed for evidence of hypercoagulability and the presence of acute phase inflammatory proteins, to assess the effects of local and distant sepsis on a variety of haematological parameters.

CHAPTER 2

MATERIALS AND METHODS

2.1 Preliminary investigations to develop the model

Previous observations in rats by De Haan et al in 1974 (110), had given guidelines as to the effective dose of infective agent to be delivered, sufficient to produce an abscess, but insufficient to kill the animal. Unfortunately, it was found that our laboratory strain of Pseudomonas aeruginosa had increased in virulence since 1974 (personal communication with microbiologist), and therefore a preliminary study was undertaken, using 30 ex-breeder female Sprague-Dawley rats, weighing between 300 and 400 grams, to assess the dose of Pseudomonas aeruginosa required to produce a distant pyogenic abscess by one week, a local wound infection and a transient sublethal bacteraemia. Pseudomonas aeruginosa was chosen as the infective agent, as it had previously been demonstrated that this organism was not normally present in our laboratory strain of the Sprague-Dawley rat (110), and because of the constraints of laboratory time, it was decided to allow a period of 7 days for the abscess to develop. The animals were therefore injected with varying concentrations of Pseudomonas aeruginosa into the subcutaneous tissues of the right axilla to produce a distant pyogenic abscess. It was found that a 2 ml suspension of 2×10^7 organisms, gave a large acute inflammatory mass measuring 2.0 x 2.5cms at 7 days. These animals were systemically unwell and lost around 15% of their body weight during the study. Further investigations on the remaining animals, showed that a local wound infection and a transient sublethal bacteraemia

could be produced by 2×10^6 and 2×10^5 organisms respectively.

Having successfully established the correct dose of the organism to produce the infection, the work was repeated using a further 15 rats, to make certain that these infected animals would survive the surgical trauma. The femoral arteries in the groin were chosen, as previous surgical exercises had shown that these vessels were ideally suited for a satisfactory micro-vascular anastomosis.

In a separate study, using a further 20 animals, it was found that the effective dose of commercially available turpentine oil, sterilised through a 0.22 μm millipore filter unit (Millipore Products, Bedford, MA01730), to produce an acute aseptic inflammatory mass measuring 2.0 x 2.0cms at one week, was 2 ml.

Having demonstrated the effective dose of sterile turpentine oil, further investigations were performed on 10 more animals to ascertain that they would survive the surgical manipulations.

During the course of these experiments, it was found that a 25 gauge needle allowed injection of the various agents into the subcutaneous tissue, without any leakage from the skin puncture site.

2.2 Rat groin dissections

Observations made during these preliminary studies, suggested that there was considerable variability in the anatomy of the rat femoral vessels, which had previously been inadequately described in the laboratory manuals of microsurgical practice (115, 116, 117). Therefore, prior to adventitial stripping of the femoral vessels, the following measurements were made in 100 consecutive groin dissections, using the 1mm square grid pattern supplied in the microvascular suture packs (71). Firstly, the transverse diameters of the undissected rat femoral artery and vein were recorded. Secondly, the distance between the inguinal ligament and the origin of the superficial epigastric vessels was measured and the site where the posterior (muscular) branch of the femoral artery arose, was documented. The presence of any duplication of these branches was also noted.

2.3 Design of the main study

One hundred female Sprague-Dawley rats weighing between 250 and 350 grams, were randomised to give 5 groups of 20 animals, prior to end-to-end anastomosis of both femoral arteries.

Group 1: A 2 ml suspension of Pseudomonas aeruginosa (containing 2×10^5 organisms) was injected into the external iliac vein immediately prior to the release of microvascular clamps, to produce a transient sublethal bacteraemia.

Group 2: A 2 ml suspension of Pseudomonas aeruginosa (containing 2×10^6 organisms) was applied topically into both the groin wounds at the time of surgery, to produce a local wound infection.

Group 3: A 2 ml suspension of Pseudomonas aeruginosa (containing 2×10^7 organisms) was injected into the subcutaneous tissues of the right axilla one week prior to anastomosis, to produce a distant pyogenic abscess.

Group 4: 2 ml of sterile turpentine oil was injected into the subcutaneous tissues of the right axilla one week prior to anastomosis, to produce a remote sterile abscess.

Group 5: 2 ml of sterile saline was injected into the subcutaneous tissues of the right axilla one week prior to anastomosis, as controls.

The animals were not restrained and were caged separately. Throughout the experiment they had free access to food and water. As previously stated there was random allocation and all groups were operated on concurrently by one microsurgeon (NRM). To prevent transmission of the organism from one animal to another, all instruments were autoclave sterilised between cases.

2.3.1 Operative technique

One week later the rats were anaesthetised with intraperitoneal sodium pentobarbitone (30mgs/kg body weight), the groins shaved and cleansed with chlorhexidine

gluconate 2.5% v/v in 70% w/w alcohol spray, and the femoral vessels exposed via an oblique groin incision. The artery was mobilised from the inguinal ligament to the superficial epigastric vessels and the posterior branches ligated with 10/0 prolene. The femoral artery was divided in Acland clamps, stripped of adventitia and flushed with heparinised saline. An end-to-end repair was performed using the posterior-wall-first technique (57). On average, 8 interrupted 10/0 prolene sutures were required. After clamp release, haemostasis was achieved by gentle pressure. Patency was assessed at 30 minutes by observing for expansile pulsation distal to the anastomotic site (114) and by use of the double forceps patency and flicker tests (52, 115, 116). Wound closure was with 3/0 silk and no local or systemic antibiotics were used in any of the groups.

One week later the groins were re-explored and the anastomoses inspected for patency (figures 1 and 2). Thrombosed specimens were immersed in either formol saline or Karnovsky's fixative. Patent vessels were cannulated proximally and gently irrigated with a fixative prior to removal of a 10mm segment centred on the suture line.

2.3.2 Light and Electron Microscopy

Specimens for study by light microscopy were processed routinely for paraffin wax embedding. Six μm thick paraffin embedded sections were taken through the

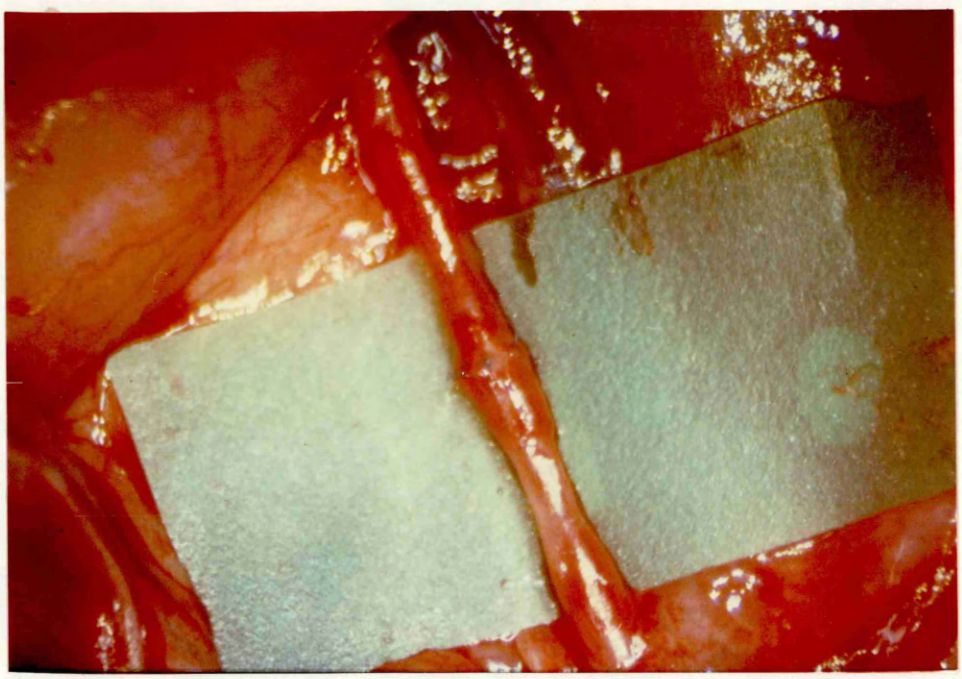

FIGURE (1): Patent microsurgical anastomosis of the femoral artery of a rat.

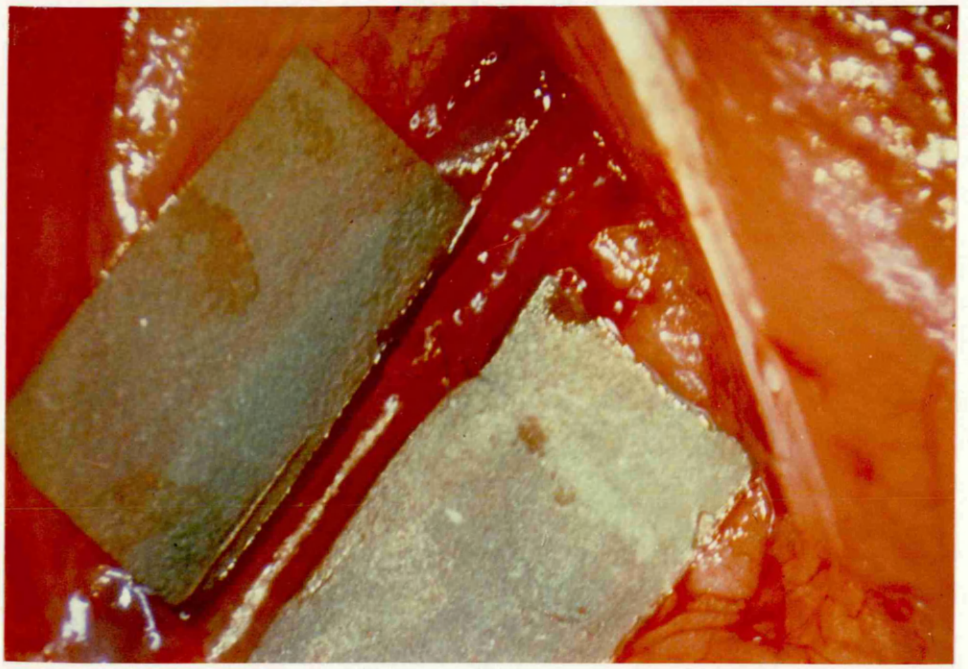

FIGURE (2): Thrombosed microsurgical anastomosis of the femoral artery of a rat.

anastomotic site either in a transverse or longitudinal direction. These serial sections across the suture line were then divided into groups of three; the first being stained with haematoxylin and eosin, the second with Miller's elastic stain and the third with the Brown-Bren stain. This pattern of staining was repeated for all of the serial sections examined. The Miller's elastic stain was used to study the morphology of the vessel wall and further sections were stained by the Brown-Bren technique, to demonstrate the presence of Pseudomonas aeruginosa.

The samples undergoing electron microscopic study were fixed in 3% gluteraldehyde in 0.1 M phosphate buffer. After fixation they were washed in 0.1 M phosphate buffer, dehydrated via graded alcohols to absolute ethanol and critically point dried using liquid CO_2. The dried samples were mounted on specimen stubs, sputter-coated with gold and examined with a Cambridge S200 series scanning electron microscope.

2.3.3 Microbiological study

Bacteriological specimens were taken from the axillae and groin wounds at the anastomotic site. Blood cultures were also collected. In four of the thrombosed anastomoses in the pyogenic abscess group, the thrombus was sent for ultrasonic disintegration prior to culture.

Seven animals in group 1 (transient bacteraemia), eight in group 2 (local sepsis), six in group 3 (distant

septic abscess), nine in group 4 (distant aseptic abscess) and one in group 5 (control) died before the anastomoses could be examined at 7 days. They were removed from the study and replaced with new animals. There were thus 20 animals (with 40 femoral artery anastomoses) in each of the 5 groups for study.

Results were analysed for statistical significance using the chi-square test.

2.3.4 Study of haematological parameters

In a separate study, 50 further animals were randomised into the same five groups, giving 10 animals in each for investigation. In this part of the work, normal saline was used to irrigate the vessel ends. Thirty minutes after microsurgical repair of the right femoral artery (24 hours for those animals in group 2 with local sepsis), a 5ml blood sample was carefully taken from the inferior vena cava using a 21 gauge needle. The specimen was sent for blood culture and a detailed haematological investigation was also performed. A full blood count was carried out on a calibrated Coulter S-Plus Mark IV Analyser and a whole blood film made to look for micro-platelet clumping. The two remaining samples were spun down in a refrigerated centrifuge, snap frozen and stored at -70° centigrade. Assays of fibrin degradation products, fibrinopeptides A and B and factors 10A and 2A were then performed. The blood film was also assessed for red cell morphology and

a differential leucocyte count was carried out.

Crossed Immunoelectrophoresis was performed on the samples (Appendix A), to identify changes in the serum protein levels.

In this part of the study, the results were analysed using one way analysis of variance.

CHAPTER 3

RESULTS

3.1 Anatomy and histology of the normal rat femoral vessels

In 100 consecutive groin explorations, the median external diameter of the undissected femoral artery was found to be 0.9mm, and that of the femoral vein to be 1.5mm. The median distance from the inguinal ligament to the origin of the superficial epigastric pedicle, was 1.3cm (table 1).

It was also demonstrated that there was duplication of the posterior (muscular) branch of the femoral artery in 13 instances, and of the posterior branch of the vein in 23 animals. It was shown that in around three-quarters of the dissections that the posterior (muscular) branch arose from the proximal one-third of the femoral artery and not as had been previously reported at its midpoint (116, 117), (table 2). Although, in two of the animals no posterior branch of the femoral artery was present, in all cases the corresponding branch of the femoral vein was always identified.

The normal rat femoral artery is composed of three layers. The innermost intimal layer comprises a single layer of flat, non-wettable endothelium, which lies on a basement membrane. This is separated from the media by the internal elastic lamina (figure 3). This layer has elastic fibres, smooth muscle and collagen and is separated from the adventitia by the external elastic lamina. The adventitial layer is loose connective tissue through which run small blood vessels and nerves.

Table 1. Dimensions of the femoral vessels.

Parameter	Median value	Range
diameter of femoral artery	0.9mm	0.6 - 1.8mm
diameter of femoral vein	1.5mm	1.0 - 2.0mm
length of femoral artery	1.3cm	1.1 - 2.0cm

Table 2. Site of origin of the posterior (muscular) branch of the femoral vessels.

Vessel	Upper 1/3	Middle 1/3	Lower 1/3	Absent Vessel
Artery	82	26	3	2
Vein	99	24	0	0

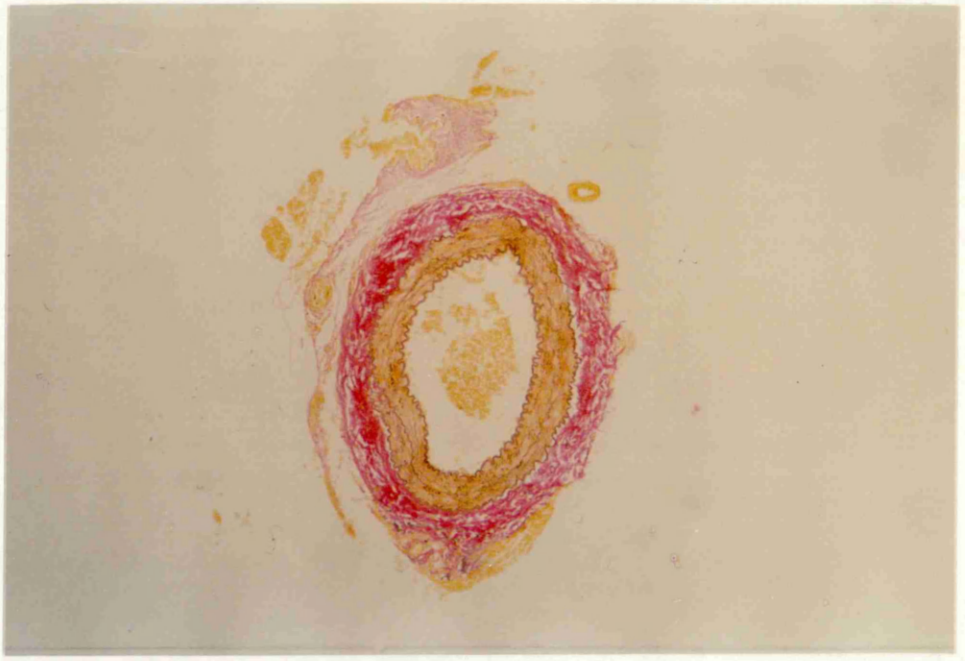

FIGURE (3): Transverse section of the normal rat femoral artery stained with Miller's elastic stain to show the elastic laminae coloured black. (Magnification x 40).

3.2 Surgical assessment of patency of the anastomoses

One week post-operatively, 39 of the 40 control anastomoses were patent. This was not significantly influenced by either a transient sublethal bacteraemia or a distant sterile abscess, where 35 anastomoses were patent in each (chi^2 = 1.62, p = 0.203).

Thrombosis was markedly increased to 13 of 40, in those animals with a local wound infection (chi^2 = 10.48, p = 0.001), but was greatest of all in the pyogenic abscess group (20 out of 40), even though it was remote from the site of the microvascular repair (chi^2 = 20.92, p < 0.001), (table 3).

In 5 of the animals in the study, the anastomoses required to be revised on the table at the time of the initial operation. All these revised anastomoses were patent at 7 days.

3.3 Histology of the anastomosed vessels

In the patent vessels of groups 1, 3, 4 and 5 (transient bacteraemia, distant pyogenic abscess, distant sterile abscess and controls), the histological appearances showed necrosis of the tunica media with denucleation of the smooth muscle cells. In some cases the sutures had caused a scalloped effect and there was evidence of subintimal hyperplasia both proximal and distal to the anastomotic site (figure 4). An infiltrate of acute inflammatory cells was often seen in the vessel wall, and around the sutures foreign body giant cells were found. In many cases there was marked thickening of the adventitial layer (figures 5 and 6). Those with local wound sepsis had an intense

Table 3. Post-Operative patency rates.

Group	Number Patent
Control	39/40
Distant sterile abscess	35/40 ($p = 0.203$)
Transient bacteraemia	35/40 ($p = 0.203$)
Local wound sepsis	27/40 ($p = 0.001$)
Distant septic abscess	20/40 ($p < 0.001$)

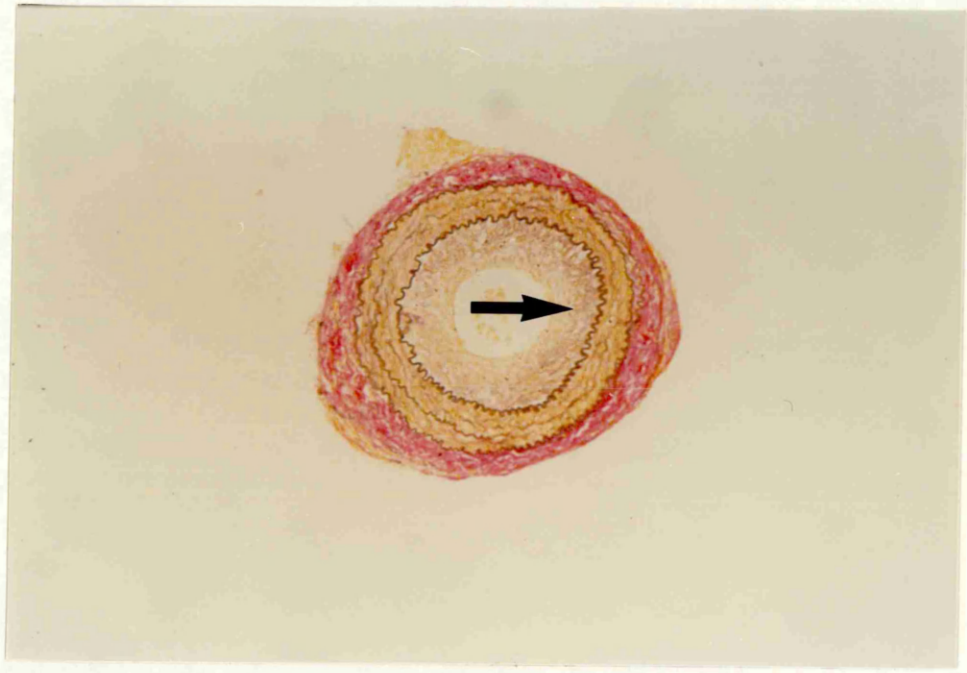

FIGURE (4): Narrowing of the lumen in a control animal due to subintimal hyperplasia (Arrow). (Magnification x 40).

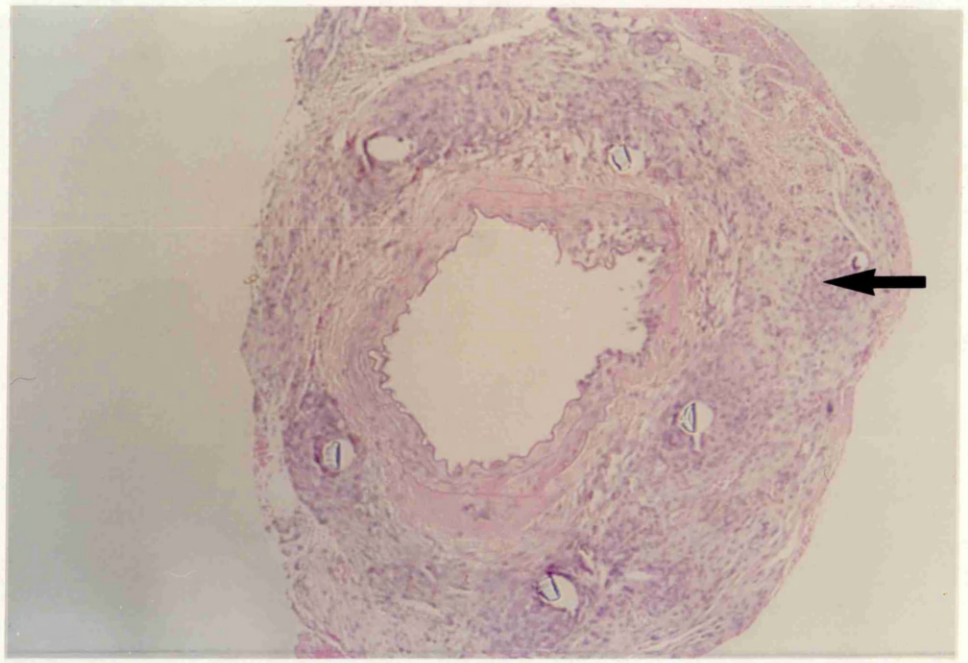

FIGURE (5): Patent anastomosis in animal with a distant pyogenic abscess stained with haematoxylin and eosin (Magnification x 40). There is medial necrosis, scalloping of the vessel wall related to the sutures and marked adventitial thickening (Arrow).

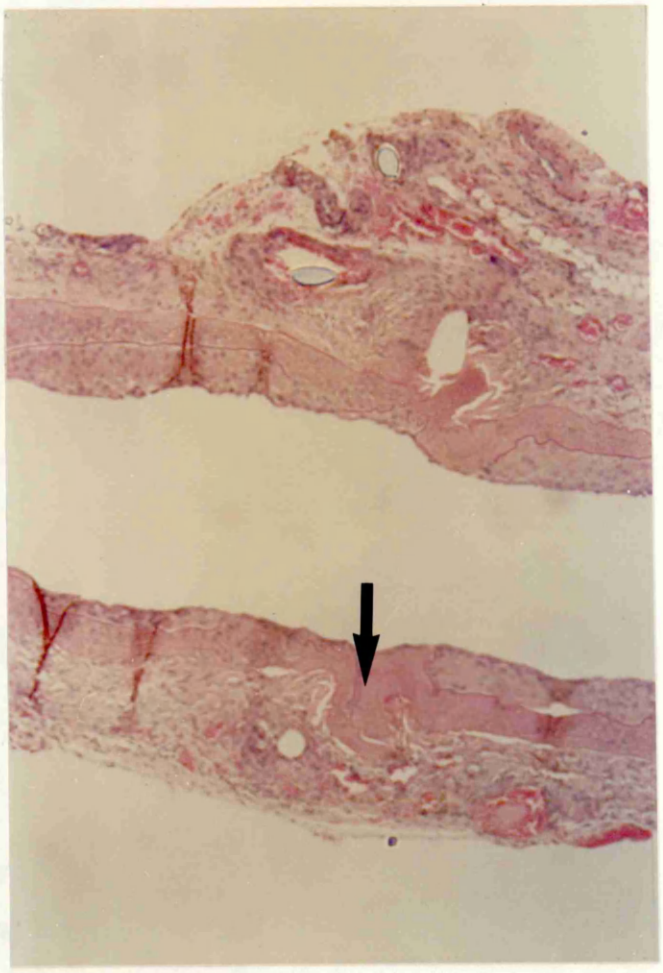

FIGURE (6): Longitudinal section of patent anastomosis in animal with distant abscess stained with haematoxylin and eosin (Magnification x 40). There is adventitial thickening, subintimal hyperplasia and outward buckling of the tunica media at the anastomosis site (Arrow).

vasculitis with clumping of polymorphonuclear leucocytes on the luminal surface of the intima (figure 7).

In the thrombosed vessels in groups 1, 3, 4 and 5 (transient bacteraemia, distant pyogenic abscess, distant sterile abscess and controls), there was disruption of the vessel wall with complete occlusion of the lumen, which was filled with organising thrombus (figure 8). Although many of the thrombi were recanalising by 7 days, there was insufficient flow in these vessels to give a positive patency test. It was not possible to demonstrate the presence of Pseudomonas aeruginosa in the thrombus using the Brown-Bren stain. Of those vessels assessed surgically to be patent, the correlation on histological assessment of patency was good. Those vessels not patent when assessed visually, were completely occluded by thrombus on the light microscopy sections.

Those with local wound sepsis had an intense vasculitis with a heavy polymorphonuclear leucocyte infiltration, both in the vessel wall and extending into the occluding thrombus (figure 9). The inflammatory response to the locally induced infection was of two types. In one, there was a thick fibrinous exudate, sometimes associated with enlargement of the inguinal lymph nodes, and in the other there was a copious watery inflammatory exudate, sometimes associated with lymphadenopathy, and accompanied by intense hyperaemia in the soft tissues around the femoral neurovascular bundle. The occlusion rate was similar in each. In 5 of these locally infected groins, the femoral vein had undergone spontaneous thrombosis.

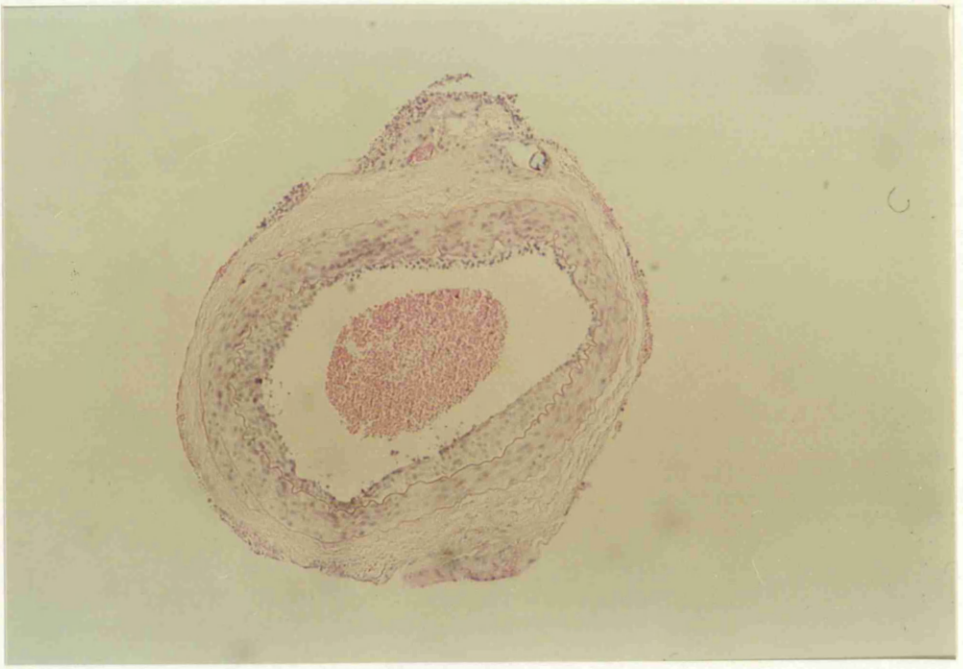

FIGURE (7): Patent anastomosis in animal with local wound sepsis stained with haematoxylin and eosin (Magnification x 40). The lumen is patent and there is some polymorphonuclear leucocyte clumping on the intimal surface.

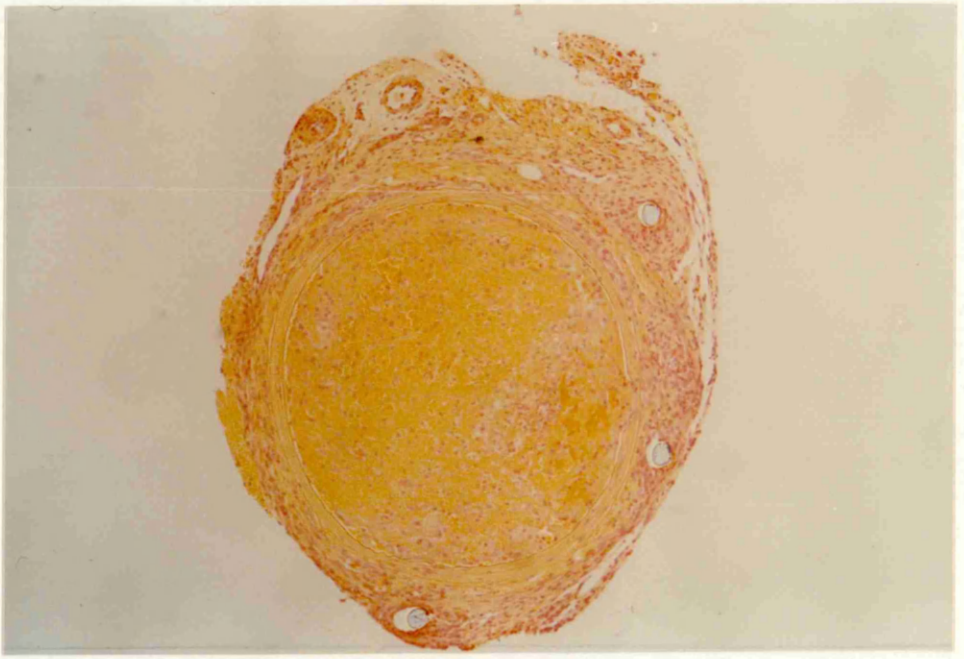

FIGURE (8): Thrombosed anastomosis in animal with distant pyogenic abscess stained with Brown-Bren stain (Magnification x 40). Lumen completely occluded by organising thrombus. No evidence of Pseudomonas aeruginosa in the specimen.

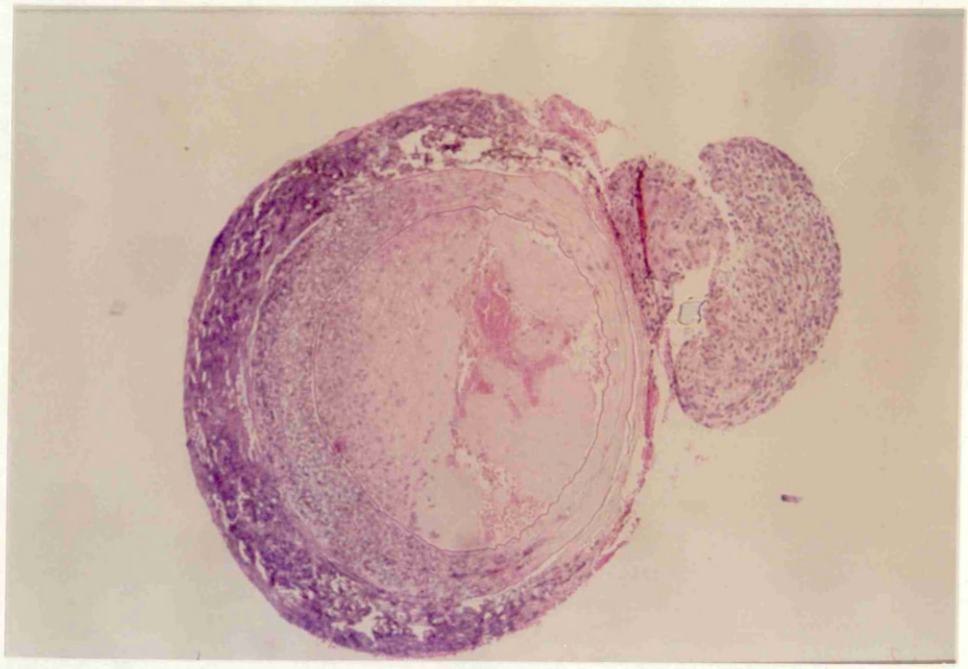

FIGURE (9): Thrombosed anastomosis in animal with local wound sepsis stained with haematoxylin and eosin (Magnification x 40). Note the vasculitis with presence of polymorphonuclear leucocytes both in the vessel wall and the occluding thrombus.

3.4 Scanning Electron Microscopy of the microvascular repairs

Scanning electron microscopy of the non-occluded vessels close to the anastomotic site, demonstrated a patent lumen and evidence of marked subintimal hyperplasia (figure 10). High power scanning electron photomicrographs of the vessel wall in the proximity of the anastomosis, showed the endothelial cells covered in a fine fibrin mesh on which were attached platelets and red blood corpuscles (figure 11). In several of the specimens examined, neo-intima was completely covering the microvascular sutures at the anastomotic site (figure 12).

In the thrombosed vessels in all groups, scanning electron microscopy showed complete occlusion of the lumen (figure 13). High power photomicrographs demonstrated a dense fibrin mesh in which were trapped both normal and crenated red blood cells (figure 14). In none of the thrombosed specimens from the distant pyogenic abscess group, was there any evidence of bacteria seen on scanning electron microscopy.

3.5 Microbiological results

In all the animals with local sepsis, Pseudomonas aeruginosa was recovered from the groin wounds, and in 11 of these animals there was an associated growth of either proteus, coliform, staphylococcal or streptococcal species. In group 3, with a distant pyogenic abscess, Pseudomonas aeruginosa was always cultured from the axillary focus of sepsis.

Although on two occasions in the preliminary study (to assess the dose of pseudomonas required to produce a distant pyogenic

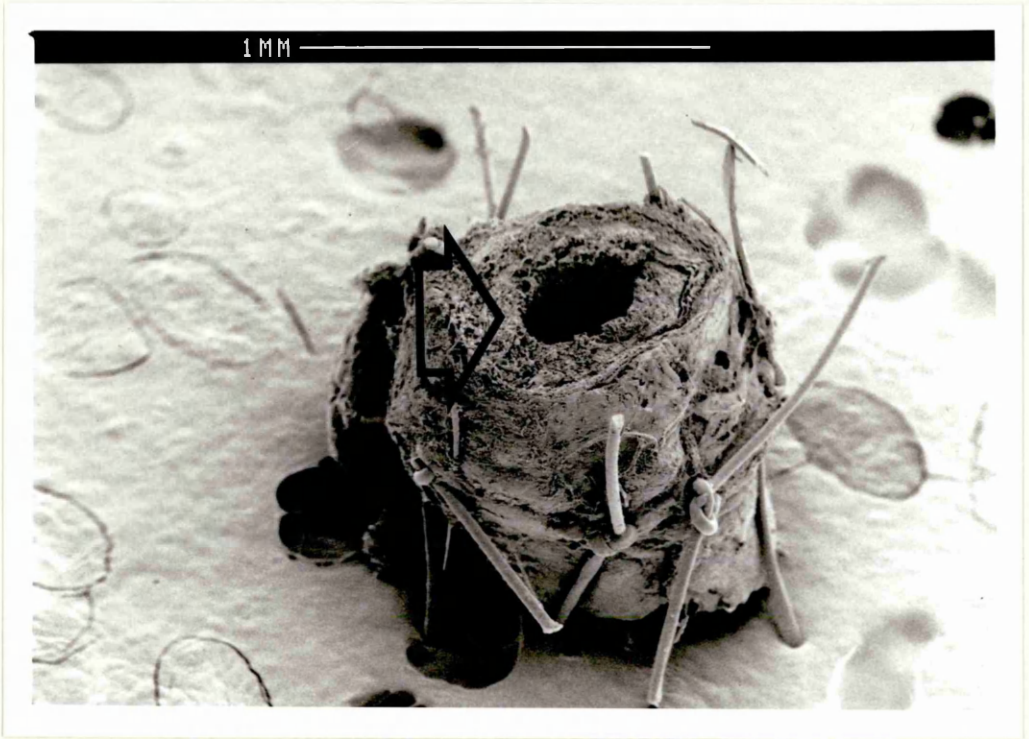

FIGURE (10): Scanning electron photomicrograph of patent anastomosis in control animal showing marked subintimal hyperplasia (Arrow).

FIGURE (11): High power scanning electron photomicrograph of vessel wall in control animal close to the anastomotic site, showing the endothelial cells covered with a fine fibrin mesh on which are attached red cells (Large Arrow) and many platelets (Small Arrow).

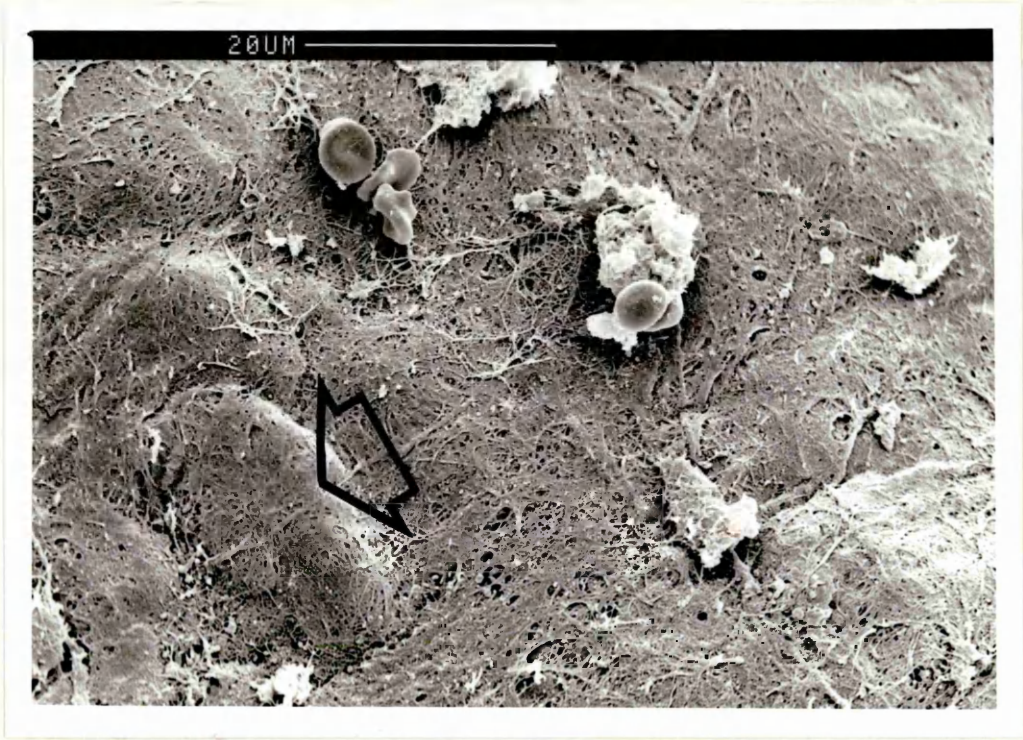

FIGURE (12): High power scanning electron photomicrograph at 7 days in control animal, showing complete coverage of a microvascular suture (Arrow) by neo-intima.

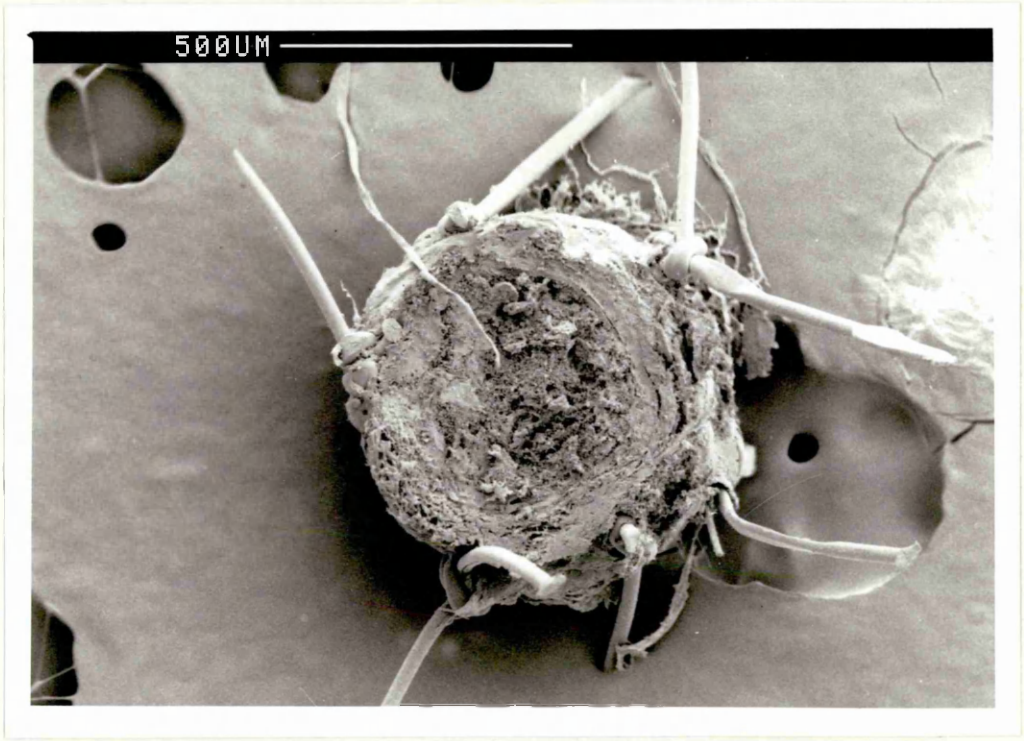

FIGURE (13): Scanning electron photomicrograph of occluded anastomosis in animal with a distant pyogenic abscess.

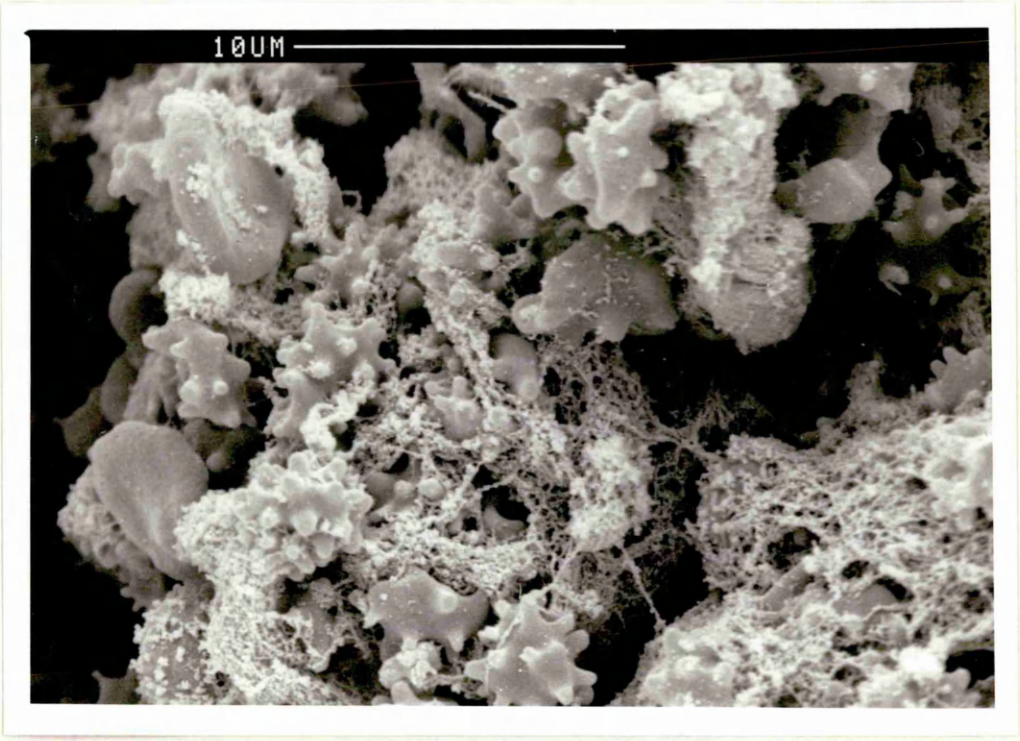

FIGURE (14): High power scanning electron photomicrograph of thrombus occluding anastomosis in animal with a distant abscess. Note red cells both normal and crenated trapped in the fibrin mesh.

abscess), Pseudomonas aeruginosa had been cultured from the groin wounds, in none of the 20 animals in the subsequent work was the organism found at that site. This finding, in the preliminary study, could possibly have been due to contamination, since the wounds themselves looked healthy.

However, in 4 of the 20 animals with a distant septic abscess in the main study, pseudomonas was grown from the blood culture (table 4). Because of these findings it was decided to perform post-mortem studies on the remaining animals. In 5 of the 10 animals in this distant pyogenic abscess group which underwent post-mortem examination, multiple abscesses (from which pseudomonas was grown) were found in the liver, spleen, kidneys and lungs (figures 15 and 16). Histological sections from the spleens of these animals showed the presence of extra-medullary haemopoiesis. Also in this group, 4 anastomotic thrombi (from 2 animals) were sent for ultrasonic disintegration prior to culture. In one animal, faecal streptococci and lactose-fermenting coliforms were grown and in the other proteus species and faecal streptococci were found. Pseudomonas aeruginosa was not grown from any of the four anastomotic thrombi.

Overall, 35 animals in groups 1, 3, 4 and 5 (transient bacteraemia, distant pyogenic abscess, distant sterile abscess and controls) had a superficial wound infection, and in each only a scanty growth of organisms was cultured from the wound swab (table 5). None of the group with a distant septic focus of infection grew pseudomonas from the groin wound, despite the

Table 4. Blood culture results in distant pyogenic abscess group.

Animal (Number)	Organisms grown from blood culture
Distant abscess (2)	Pseudomonas species
Distant abscess (4)	Pseudomonas species
Distant abscess (5)	Pseudomonas species
Distant abscess (7)	Pseudomonas species
Distant abscess (12)	Staphylococcus species
Distant abscess (13)	Staphylococcus species
Distant abscess (14)	Coliform species
Distant abscess (16)	Coliform species
Distant abscess (17)	Staphylococcus species
Distant abscess (19)	Staphylococcus species

FIGURE (15): Spleen of animal with distant pyogenic infection showing multiple abscesses from which Pseudomonas aeruginosa was grown.

FIGURE (16): Kidney of a different animal with distant sepsis showing multiple abscesses due to Pseudomonas. aeruginosa

Table 5. Animals with a local wound infection.

Group (Number)	Wound Swab	Anastomosis
Control (5)	Skin flora on (R)	Patent
Control (17)	β haemolytic streptococcus (R)	Patent
Sterile abscess (1)	Corynebacterium on (R)	Patent
Sterile abscess (9)	Corynebacterium on (R)	Patent
Septic abscess (1)	Staph. epidermidis on (R)	Thrombosed
Septic abscess (3)	Staph. aureus on (L)	Thrombosed
Septic abscess (3)	Staph. aureus and coliforms (R)	Patent
Septic Abscess (6)	Staph. epidermidis on (L)	Thrombosed
Septic abscess (10)	Proteus and Staph. epidermidis on (L) and (R)	Thrombosed
Septic abscess (11)	Proteus on (L) and (R)	Thrombosed
Septic abscess (13)	Staph. species on (R)	Patent
Septic abscess (14)	Staph. species on (L)	Patent
Septic abscess (15)	Coliforms on (L) and (R)	Thrombosed
Septic abscess (16)	Coliforms on (L)	Patent
Septic abscess (16)	Coliforms on (R)	Thrombosed
Septic abscess (17)	Staph. species on (L)	Thrombosed
Septic abscess (17)	Staph. species on (R)	Patent
Septic abscess (18)	Staph. species on (L)	Thrombosed
Septic abscess (19)	Staph. species on (L) and (R)	Patent

Table 5. (Continued) Animals with a local wound infection.

Group (Number)	Wound Swab	Anastomosis
Transient bacteraemia (1)	Staph. species on (R)	Patent
Transient bacteraemia (2)	Coliforms and pseudomonas species on (L) with also Staph. species on (R)	Patent
Transient bacteraemia (3)	Staph. species on (L)	Patent
Transient bacteraemia (4)	Pseudomonas species on (R) and (L)	Patent
Transient bacteraemia (5)	Coliform species on (L)	Patent
Transient bacteraemia (6)	Pseudomonas species on (L) and Staph. species on (R)	Thrombosed
Transient bacteraemia (7)	Pseudomonas species on (R) and (L)	Patent
Transient bacteraemia (8)	Pseudomonas and Staph species on (R) and (L)	Patent
Transient bacteraemia (10)	Escherichia coli on (L)	Patent
Transient bacteraemia (11)	Pseudomonas and proteus species on (L) with also β haemolytic streptococci Group G on (R)	Patent
Transient bacteraemia (13)	Proteus on (R)	Patent
Transient bacteraemia (14)	Staph. aureus, and β. haemolytic streptococci Group G on (L) and (R)	Thrombosed
Transient bacteraemia (15)	Staph. aureus coliforms and proteus species on (L)	Patent
Transient bacteraemia (16)	Proteus and pseudomonas species on (L)	Patent
Transient bacteraemia (17)	Pseudomonas species on (L)	Patent
Transient bacteraemia (18)	Coliform species on (L)	Patent

fact that in 4 animals the organism was cultured from the blood sample. It is interesting to note that in 8 of the animals with a transient bacteraemia at the time of clamp release, pseudomonas was found locally in the groin wounds. This was presumably due to local contamination by blood leaking from the anastomotic sites on clamp release. Three-quarters of the distant pyogenic abscess group developed a local wound infection in the groin. As all these animals were systemically unwell, it may be that their defences against bacterial infection were depressed by the chronic septic abscess. In none of the control animals with a local wound infection was there occlusion of the underlying anastomosis. The presence of Pseudomonas aeruginosa could not be demonstrated in either the thrombi or the vessel walls in any of the above groups using the Brown-Bren stain.

On one occasion the turpentine abscess became infected due to the animal biting itself and a scanty growth of staphylococcus was obtained. Both anastomoses in this animal were occluded by thrombus.

3.6 Haematological results

A full analysis of the blood films taken in the second part of the main study was performed, and the mean haematological values were documented.

3.6.1 Red cell morphology

The control group displayed the normal features of red cell morphology for the age and sex of the animals (tables 6 and 7). There was however a significant increase in the haematocrit and haemoglobin levels in the control group, when compared to the normal unoperated animals ($p < 0.05$), which was possibly due to post-surgical haemoconcentration (table 18 and Appendix B1).

The assessment of the films of those animals with a distant sterile abscess and a transient sublethal bacteraemia was essentially normal (tables 8 and 9), although there was some individual variation in the groups. Those animals with a distant sterile abscess had a significant reduction in their haemoglobin level as compared to the controls ($p < 0.05$), (table 18 and Appendix B2). A transient sublethal bacteraemia had a marked effect on both the haematocrit and the haemoglobin level ($p < 0.001$), (table 18 and Appendix B3).

Those with local sepsis showed a mild anaemia (tables 10, 18 and Appendix B4), with evidence of red cell membrane fragility and some regenerative activity (demonstrated respectively, by the presence of crenated red cells and normoblasts). There was again in this group, a significant reduction in the haematocrit value ($p < 0.05$), (table 18 and Appendix B4).

The distant septic abscess group had markedly increased changes in red cell morphology, and a significant lowering of the

Table 6. Red cell morphology : Normal animals

Animal Number	PCV (%)	Aniso-Cytosis	Poikilo-Cytosis	Poly-Chromasia	Rouleaux Formation	Howell Jolly Bodies	Normo-Blasts	Haemo-globin-isation	Crenation
N1	37.4	0	0	0	0	0	0	✓	++
N2	45.5	0	0	slight	0	0	0	✓	0
N3	41.3	0	occas-ional	±	±	0	0	✓	0
N4	36.5	±	±	±	0	0	0	✓	±
N5	41.7	±	±	±	0	0	0	✓	+
N6	37.2	±	0	±	0	0	0	✓	±
N7	36.5	0	±	±	+	0	0	✓	+
N8	40.8	+	++	±	+	0	0	✓	+
N9	39.9	±	±	+	+	0	0	✓	0
N10	35.9	±	+	±	++	0	0	✓	++

COMMENT : The normal series displays the normal features for the age and sex of the rats.

Table 7. Red cell morphology : Control animals.

Animal Number	PCV (%)	Aniso-Cytosis	Poikilo-Cytosis	Poly-Chromasia	Rouleaux Formation	Howell Jolly Bodies	Normo-Blasts	Haemo-globin-isation	Crenation
C1	40.8	- - - - - - - - - - - -			Unreadable		- - - - - - - - - - - - - - - - - -		+++
C2	45.3	±	±	±	0	0	0	✓	0
C3	44.7	+	+	+	0	0	0	✓	0
C4	40.6	+	0	+	0	0	0	✓	0
C5	45.0	±	±	+	0	0	0	✓	0
C6	43.7	0	0	±	0	0	1	✓	0
C7	38.1	++	+	+	+	0	0	✓	0
C8	48.4	±	±	+	occas-ional	0	0	✓	0
C9	49.6	±	0	±	0	0	0	✓	0
C10	43.0	0	0	±	0	0	0	✓	0

Comment: The control group displays the normal features for the age and sex of the rats.

Table 8. Red cell morphology : Distant sterile abscess

Animal Number	PCV (%)	Aniso-Cytosis	Poikilo-Cytosis	Poly-Chromasia	Rouleaux Formation	Howell Jolly Bodies	Normo-Blasts	Haemo-globin-isation	Crenation
TA1	26.9	+++	+++	+++	0	occas-ional	3	Target cells ++	+
TA2	38.9	+	0	+	0	0	1	✓	+++
TA3	45.7	- - - - - - - - - - - - - - -			Unreadable		- - - - - - - - - - - - - - - - -		
TA4	46.5	- - - - - - - - - - - - - - -			Unreadable		- - - - - - - - - - - - - - - - -		
TA5	40.5	++	++	++	0	occas-ional	0	✓	0
TA6	40.0	+	+	+	0	0	0	✓	0
TA7	38.1	++	+++	++	0	0	0	✓	++
TA8	38.6	+	occas-ional	+	0	0	0	✓	0
TA9	38.4	0	0	+	++++	0	0	✓	0
TA10	45.0	occas-ional	0	+	occas-ional	0	2	✓	0

COMMENT : The sterile abscess group displays largely normal results, but individual animals (TA1, TA7 and TA9) show aberration.

Table 9. Red cell morphology : Transient bacteraemia.

Animal Number	PCV (%)	Aniso-Cytosis	Polkilo-Cytosis	Poly-Chrom-asia	Rouleaux Formation	Howell Jolly Bodies	Normo-Blasts	Haemo globin-isation	Crenation
TRB1	40.5	±	±	0	0	0	0	✓	0
TRB2	35.2	±	+	++	++++	0	0	✓	0
TRB3	34.1	+	+	+	0	0	0	✓	0
TRB4	40.3	+	+	+	0	0	0	✓	0
TRB5	37.3	+	+	+	0	0	0	✓	0
TRB6	38.4	0	0	+	0	0	0	✓	0
TRB7	38.8	+	+	+	0	0	0	✓	0
TRB8	40.6	+	+	++	0	0	0	✓	0
TRB9	39.4	+	+	+	0	0	0	✓	0
TRB10	37.9	0	0	+	0	0	0	✓	0

Comment : The group with transient bacteraemia display the normal features for the age and sex of the animals.

Table 10. Red cell morphology : Local sepsis.

Animal Number	PCV (%)	Aniso-Cytosis	Poikilo-Cytosis	Poly-Chromasia	Rouleaux Formation	Howell Jolly Bodies	Normo-Blasts	Haemo-globin-isation	Crenation
LS1	43.7	+	+	++	+++	occas-ional	3	✓	0
LS2	39.3	±	+	+	+	0	3	✓	+
LS3	40.6	+	++	+	occas-ional	0	0	occas-ional target cells	++
LS4	42.5	- - - - - - - - - - - - - - - - - Unreadable - - - - - - - - - - - - - - - - -							
LS5	42.3	±	+	+	slight	0	1	✓	++
LS6	43.0	+	+	±	++++	0	0	✓	+
LS7	37.9	+	++	+	+	0	0	✓	++
LS8	38.6	±	+	+	++	0	0	✓	++
LS9	36.1	+	+	+	+	0	0	✓	++
LS10	38.5	- - - - - - - - - - - - - - - - - Unreadable - - - - - - - - - - - - - - - - -							

COMMENT : The local sepsis group show a mild anaemia with some evidence of regenerative activity and membrane fragility.

haemoglobin and haematocrit levels ($p < 0.05$), (tables 11, 18 and Appendix B5). There was increased red cell dyscrasia with enhanced crenation due to abnormal red cell membranes There was also evidence of marked regenerative activity in this group.

3.6.2 Differential leucocyte count

Assessment of the differential leucocyte counts showed that the control group displayed the normal numbers and distribution of leucocytes for the age and sex of the animals (tables 12, 13, 18 and Appendix B1).

Those animals with a distant sterile abscess showed an occasional reversal of the lymphocyte/neutrophil ratio with a shift to the left, as demonstrated by the presence of more immature cells and monocytes (table 14). There was also some activation of lymphocytes in this group. Overall, there was a significant increase in the percentage of polymorphonuclear leucocytes ($p < 0.05$), and reduction in the percentage of lymphocytes (table 18 and Appendices B2 and B6).

In the group with a transient sublethal bacteraemia, there was a significant leucocytosis ($p < 0.05$), mainly due to an increase in the numbers of small reactive lymphocytes (tables 15, 18 and Appendix B3).

In those animals with local sepsis at the site of anastomosis, although the total number of leucocytes was normal (tables 16, 18 and Appendix B4), there was a significant change in the

Table 11. Red cell morphology : Distant septic abscess.

Animal Number	PCV (%)	Aniso-Cytosis	Poikilo-Cytosis	Poly-Chromasia	Rouleaux Formation	Howell Jolly Bodies	Normo-Blasts	Haemo-globin-isation	Crenation
DA1	47.7	+	+	+	0	0	0	✓	+++
DA2	36.1	++	+	++	0	0	2	✓	++
DA3	33.6	+	+	++	0	0	1	✓	++
DA4	36.4	+	+	+	0	0	0	✓	+++
DA5	40.6	+	+	+	0	0	1	✓	+++
DA6	35.4	+++	+	+++	0	0	1	✓	+
DA7	34.7	++	+	++	++	0	1	✓	+
DA8	35.7	+	+	++	++++	0	0	✓	+
DA9	38.7	+	+	++	slight	0	0	✓	++++
DA10	37.3	+	+	++	+	0	0	✓	++

COMMENT : The distant abscess group shows an overall pattern of increased red cell dyscrasia and a more profound anaemia with evidence of regenerative activity and membrane fragility.

Table 12. Differential leucocyte Count : Normal animals.

ANIMAL No.	TOTAL WBC	SMALL LYMPHO	LARGE LYMPHO	NEUTRO-PHIL	MONO-CYTE	EOSINO-PHIL	BAND CELL	UNCLASS-IFIABLE MONO	PLASMA CELL	MYELOID CELL
N1	5400	4320	324	540	216	0	0	0	0	0
N2	6600	5610	0	924	0	66	0	0	0	0
N3	6900	4968	69	1725	0	69	0	69	0	0
N4	5000	4250	100	450	50	100	50	0	0	0
N5	4600	3174	0	1380	0	46	0	0	0	0
N6	5800	3596	290	1856	0	0	0	58	0	0
N7	5000	4000	200	750	0	50	0	0	0	0
N8	3200	2016	160	992	0	0	0	0	0	32
N9	5800	4930	398	348	0	116	0	58	0	0
N10	2500	1875	25	525	25	25	25	0	0	0

COMMENT : The normal series displays the normal numbers and distribution of leucocytesfor the age and sex of the rats.

Table 13. : Differential leucocyte Count : Control Animals

ANIMAL No.	TOTAL WBC	SMALL LYMPHO	LARGE LYMPHO	NEUTRO-PHIL	MONO-CYTE	EOSINO-PHIL	BAND CELL	UNCLASS-IFIABLE MONO	PLASMA CELL	MYELOID CELL
C1	4700	3337	141	940	94	94	94	0	0	0
C2	4700	2914	470	1269	0	47	0	0	0	0
C3	5700	4788	228	570	0	114	0	0	0	0
C4	6600	5016	132	990	198	264	0	0	0	0
C5	5600	4892	280	448	0	0	0	0	0	0
C6	4800	4032	144	576	0	48	0	0	0	0
C7	3500	2345	210	805	35	35	0	70	0	0
C8	5400	4536	540	270	0	54	0	0	0	0
C9	5100	4029	153	918	0	0	0	0	0	0
C10	3400	2720	136	544	0	0	0	0	0	0

COMMENT : The control group displays the normal numbers and distribution of leucocytes for the age and sex of the rats.

Table 14. : Differential leucocyte Count : Distant sterile abscess.

ANIMAL No.	TOTAL WBC	SMALL LYMPHO	LARGE LYMPHO	NEUTRO-PHIL	MONO-CYTE	EOSINO-PHIL	BAND CELL	UNCLASS-IFIABLE MONO	PLASMA CELL	MYELOID BA CELL
TA1	4500	2700	450	765	260	45	45	0	180	0	0
TA2	4700	3337	376	846	0	0	0	0	141	0	0
TA3	1800	---------- UNREADABLE ----------									
TA4	2500	---------- UNREADABLE ----------									
TA5	4900	2646	441	1519	245	0	0	0	49	0	0
TA6	9400	1880	0	6352	0	564	376	0	0	0	188
TA7	7300	4307	657	2190	146	0	0	0	0	0	0
TA8	4600	2300	184	1564	368	92	92	0	0	0	0
TA9	4500	2880	45	1440	0	45	90	0	0	0	0
TA10	5000	3050	50	1500	150	150	0	0	100	0	0

COMMENT : There is occasional reversal or reduction of lymphocyte/neutrophil ratio with left shift and monocytosis but without significant leucocytosis except for number 6, where there was some infection in the axilla. There is some activation of lymphocytes.

Table 15. : Differential leucocyte Count : Transient bacteraemia.

ANIMAL No.	TOTAL WBC	SMALL LYMPHO	LARGE LYMPHO	NEUTRO-PHIL	MONO-CYTE	EOSINO-PHIL	BAND CELL	UNCLASS-IFIABLE MONO	PLASMA CELL	MYELOI CELL
TRB1	9900	7920	0	1089	198	99	0	693	0	0
TRB2	7200	6336	0	360	288	72	0	144	0	0
TRB3	6400	5824	0	448	128	0	0	0	0	0
TRB4	7100	6319	0	781	0	0	0	0	0	0
TRB5	9200	8648	0	460	92	0	0	0	0	0
TRB6	6800	5576	0	1156	68	0	0	0	0	0
TRB7	4800	4080	0	720	0	0	0	0	0	0
TRB8	9600	8064	0	1152	384	0	0	0	0	0
TRB9	4700	4465	0	235	47	0	0	0	0	
TRB10	5900	5310	0	590	0	0	0	0	0	

Comment: In the group with a transient bacteraemia there is a marked leucocytosis mainly due to an increase in the numbers of small reactive lymphocytes.

Table 16 : Differential leucocyte Count : Local sepsis.

ANIMAL No.	TOTAL WBC	SMALL LYMPHO	LARGE LYMPHO	NEUTRO-PHIL	MONO-CYTE	EOSINO-PHIL	BAND CELL	UNCLASS-IFIABLE MONO	PLASMA CELL	MYELOID CELL
LS1	4500	1630	90	1980	135	90	270	45	180	90
LS2	3300	1056	0	1716	132	33	264	0	0	99
LS3	6100	2684	366	2623	122	0	122	61	122	0
LS4	4500	---------- UNREADABLE ----------								
LS5	3100	930	186	1271	155	93	186	0	31	186
LS6	3600	1584	0	1332	72	36	360	36	36	144
LS7	5500	2430	110	2970	0	0	0	0	0	0
LS8	4600	3128	92	1288	0	0	92	0	0	0
LS9	5200	2912	104	1976	104	52	52	0	0	0
LS10	2700	---------- UNREADABLE ----------								

COMMENT : There is a reduced lymphocyte/neutrophil ratio with shift to left in neutrophils. There is monocytosis and some activation of lymphocytes. There is a normal number of leucocytes.

lymphocyte/neutrophil ratio ($p < 0.001$), with a shift to the left in the polymorphonuclear leucocytes. Many more immature band cells were noted, and there was a monocytosis and some activation of lymphocytes (Appendix B6).

The group with a distant septic abscess had a significant leucocytosis ($p < 0.001$), with neutrophilia ($p < 0.001$), a shift to the left with many more juvenile cells and a reversal of the lymphocyte/neutrophil ratio (tables 17, 18 and Appendix B5). There was marked nuclear hypersegmentation of the polymorphonuclear leucocytes, and in some an increase in the number of monocytes was found.

When comparing the control group to those with distant sepsis (table 18), there was a statistically highly significant increase in the total white cell count and percentage of polymorphonuclear leucocytes in the infected group ($p < 0.001$), (Appendices B5 and B6).

3.6.3 Platelet estimation

Overall, the platelet count demonstrated a large range of normal values (Appendices B1-5), although there was an upward trend in the presence of inflammation (table 18), both in the aseptic and septic abscess groups.

A distant septic abscess was shown to cause a significant increase in the platelet count, when compared to the control animals ($p = 0.008$), (table 18 and Appendix B5).

Table 17 : Differential leucocyte Count : Distant septic abscess.

ANIMAL No.	TOTAL WBC	SMALL LYMPHO	LARGE LYMPHO	NEUTRO-PHIL	MONO-CYTE	EOSINO-PHIL	BAND CELL	UNCLASS-IFIABLE MONO	PLASMA CELL	MYELOID CELL
DA1	6500	1950	260	3835*	130	0	195	130	0	0
DA2	8800	2200	264	5456*	352	0	88	88	352	0
DA3	9400	1974	282	6204	94	188	94	94	470	0
DA4	16900	4235	676	11323*	338	0	169	169	0	0
DA5	12800	2816	640	8960*	384	0	0	0	0	0
DA6	12300	3198	369	7872*	738	0	123	0	0	0
DA7	7700	2514	156	4581	77	77	0	0	0	0
DA8	14700	3822	0	10731*	0	0	0	0	0	0
DA9	21200	4240	0	16748*	0	0	0	0	0	147
DA10	9200	2852	0	6072	276	0	0	0	0	0

* Hypersegmentation of neutrophil nucleus.

COMMENT : There is leucocytosis with a polymorphonuclear neutrophilia and left shift with reversal of the lymphocyte/neutrophil ratio with nuclear hypersegmentation. There are many reactive lymphocytes and occasional monocytosis.

Table 18 : Haematological Results (Mean ± Standard Deviation)

Parameter	Normal	Control	Distant Sterile Abscess	Transient Bacteraemia	Local Sepsis	Distant Septic Abscess
Haemoglobin	13.92 ± 0.89	15.85 ± 1.34 ≠	14.09 ± 1.88 *	13.34 ± 0.83 **	15.01 ± 0.91	13.59 ± 1.41 *
Haematocrit (%)	39.27 ± 3.09	43.92 ± 3.52 ≠	39.86 ± 5.59	37.98 ± 2.73 **	40.25 ± 2.54 *	39.15 ± 5.74 *
White Cell Count ($x10^9$/L)	5.08 ± 1.38	4.95 ± 0.98	4.92 ± 2.16	7.16 ± 1.87 *	4.31 ± 1.11	11.95 ± 4.60 **
% Polymorpho-nuclear leucocytes	19.60 ± 11.64	14.20 ± 7.19	28.80 ± 20.67 *	11.50 ± 6.40	44.40 ± 9.54 **	63.60 ± 7.24 **
% Lympho-cytes	75.60 ± 11.92	81.80 ± 6.48	62.30 ± 18.94 *	87.10 ± 5.43	46.40 ± 10.78 **	28.20 ± 6.39 **
Platelet Count ($x10^9$/L)	1074.30 ± 75.91	955.11 ± 223.58	1257.70 ± 292.35 *	987.10 ± 101.46	803.56 ± 150.29	1475.20 ± 474.96 *

≠ control V's normal $p < 0.05$

* distant sterile abscess, transient bacteraemia, local sepsis and distant septic abscess V's control $p < 0.05$

** distant sterile abscess, transient bacteraemia, local sepsis and distant septic abscess V's control $p < 0.001$

3.6.4 Measurement of activated clotting factors

Specimens were assayed for measurement of activated clotting factors, but unfortunately due to limitations in the method, caused by inadequate facilities in the laboratory available at that time, these factors had clearly denatured and were not detectable on any of the assays.

3.7 Counter-current Crossed Immunoelectrophoresis results

The results of the counter-current crossed immunoelectrophoresis of the serum samples are shown in Appendices C1-5 and D1-6, and are summarised in table 19.

The normal and control groups showed very little difference in peak heights. There was a slight reduction in the albumin and α1.5 peaks in the control animals, possibly related to post-operative fluid loss (table 19, Appendices C1, D1 and D2). The reduction in the α1.5 peak was statistically significant ($p < 0.05$), (table 19 and Appendix C1), (figures 17 and 18).

Those animals with a transient sublethal bacteraemia showed close resemblance to the control group with a small reduction in the peak heights of albumin and α1.5 proteins (table 19, Appendices C3 and D4). This group also recorded a significant decrease in the levels of α1.6 and β3 proteins ($p < 0.05$), (table 19 and Appendices C3 and D4), (figure 19).

Animals with local wound sepsis showed a reduction in albumin ($p < 0.05$) and P2 (pre-albumin 2) peak heights ($p < 0.001$), (table 19, Appendices C4 and D5). It was also observed that

Table 19. Summary of Mean Peak Heights (m.m.) ± Standard Deviation

Peak	Normal	Control	Distant Sterile Abscess	Transient Bacteraemia	Local Sepsis	Distant Septic Abscess
Albumin	17.40 ±2.32	15.89 ±1.27	13.30 ±0.82 **	15.00 ±1.34	13.50 ±1.78 *	10.60 ±2.32 **
α1.2	3.00 ±0.47	3.11 ±0.78	3.20 ±0.42	3.36 ±0.81	3.10 ±0.57	3.50 ±0.53
α 1.7	4.60 ±1.07	4.56 ±0.53	18.60 ±3.57 **	4.27 ±0.90	14.60 ±1.17 **	22.40 ±2.41 **
α 1.4	6.40 ±0.52	6.33 ±0.50	7.00 ±0.94	5.82 ±0.75	7.20 ±1.40	6.89 ±1.05
α 1.3	5.00 ±0.67	4.78 ±0.44	4.40 ±0.70	5.36 ±1.03	4.30 ±0.95	2.30 ±0.95 **
P2	11.70 ±1.49	11.00 ±1.87	8.70 ±1.49 *	10.91 ±1.76	6.80 ±1.48 **	5.75 ±1.75 **
α 2.4	3.33 ±0.71	2.78 ±0.67	10.10 ±4.98 **	3.27 ±0.65	13.20 ±2.20 **	10.90 ±2.96 **
β2	6.70 ±0.67	6.22 ±1.09	10.70 ±1.57 **	6.45 ±0.82	10.60 ±1.17 **	10.90 ±1.20 **

≠ control V's normal $p < 0.05$

* distant sterile abscess, transient bacteraemia, local sepsisand distant septic abscess V's control $p < 0.05$

** distant sterile abscess, transient bacteraemia, local sepsis and distant septic abscess V's control $p < 0.001$

Table 19. : Summary of Mean Peak Heights (m.m.) ± Standard Deviation (Continued)

Peak	Normal	Control	Distant Sterile Abscess	Transient Bacteraemia	Local Sepsis	Distant Septic Abscess
α1.5	8.00 ± 1.25	6.56 ± 1.13 ≠	9.50 ± 2.88 *	6.73 ± 1.01	9.80 ± 1.03 **	9.40 ± 1.35 *
α1.6	6.60 ± 0.97	6.78 ± 1.20	4.90 ± 0.74 **	5.82 ± 0.60 *	4.90 ± 0.88 *	4.10 ± 1.37 **
α1.8	3.60 ± 0.70	3.56 ± 0.53	3.90 ± 0.88	3.18 ± 0.75	4.60 ± 0.52 **	3.60 ± 0.97
α2.7	5.40 ± 0.52	5.11 ± 0.33	6.40 ± 1.07 *	4.64 ± 0.67	6.10 ± 0.57 **	5.60 ± 0.70
β3	4.30 ± 0.95	4.78 ± 1.20	5.40 ± 0.52	3.73 ± 0.90 *	4.80 ± 1.14	5.30 ± 0.82
α1.1	5.50 ± 0.53	5.11 ± 0.93	6.20 ± 0.79 *	4.82 ± 0.60	4.80 ± 0.42	6.20 ± 1.03 *
β7	6.50 ± 0.71	6.33 ± 0.71	6.40 ± 0.84	6.27 ± 0.79	6.10 ± 1.45	6.50 ± 0.71
α2.5	-	-	9.67 ± 1.15	-	-	-

≠ control V's normal $p < 0.05$

* distant sterile abscess, transient bacteraemia, local sepsis and distant septic abscess V's control $p < 0.05$

** distant sterile abscess, transient bacteraemia, local sepsis and distant septic abscess V's control $p < 0.001$

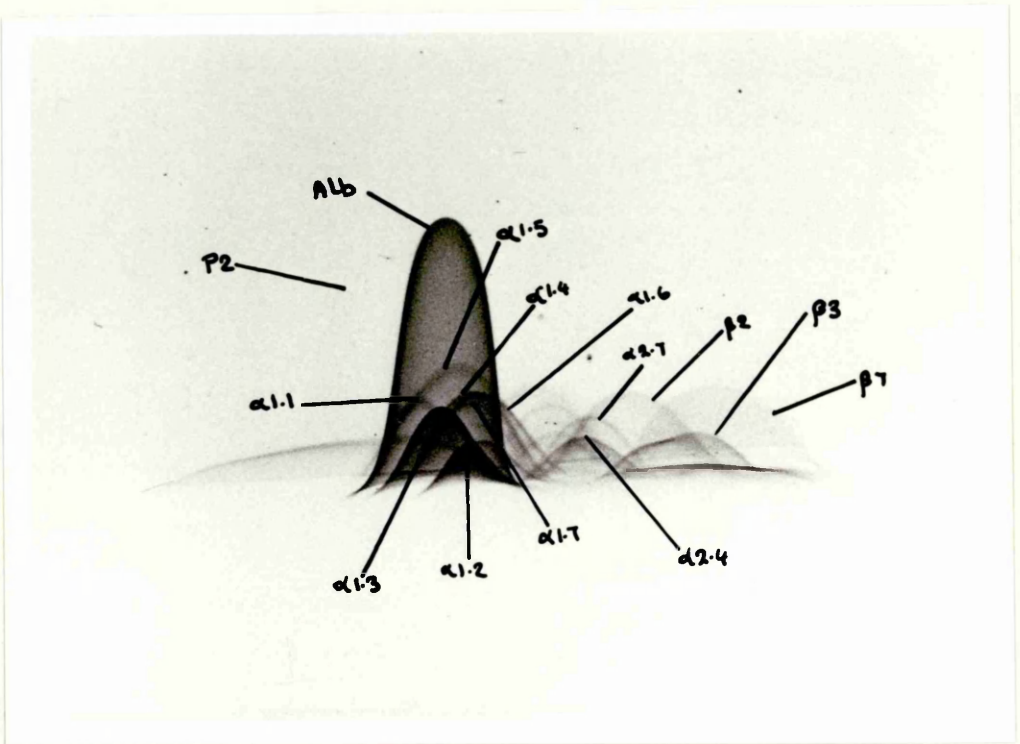

FIGURE (17): Countercurrent crossed immunoelectrophoresis of serum from a normal (unoperated) animal.

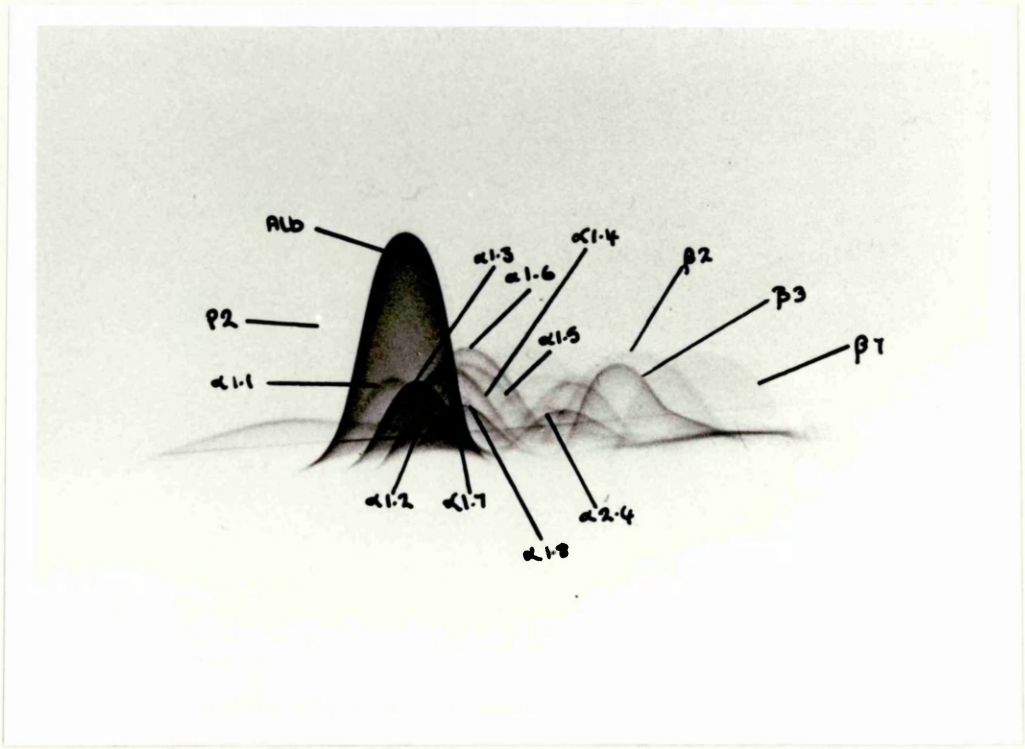

FIGURE (18): Countercurrent crossed immunoelectrophoresis of serum from a control animal.

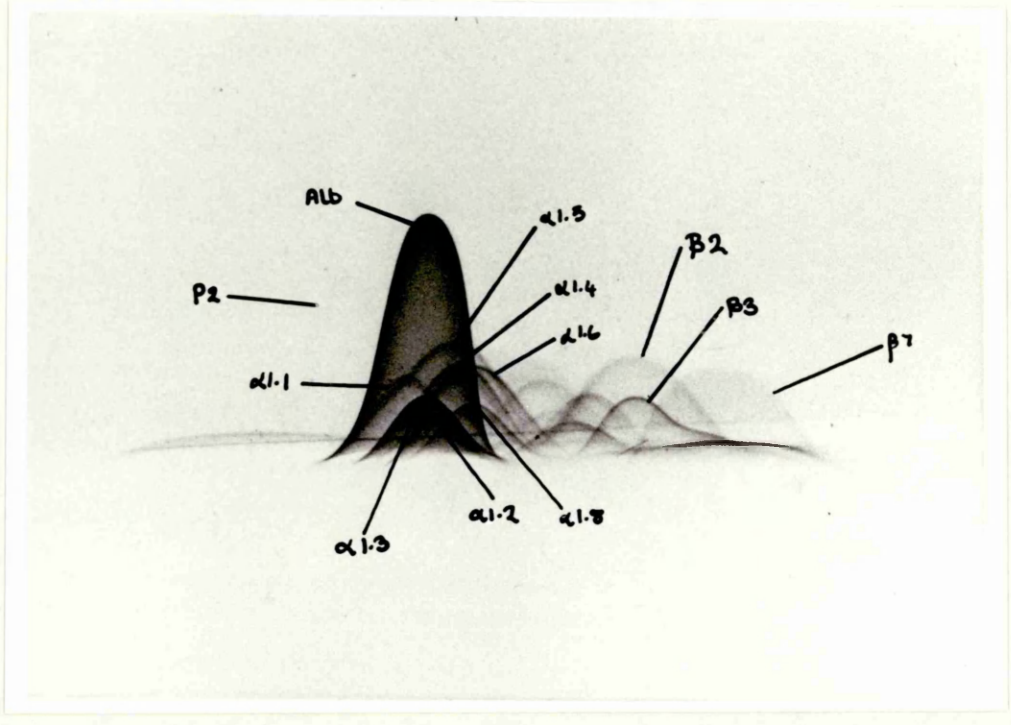

FIGURE (19): Countercurrent crossed immunoelectrophoresis of serum from animal with a transient bacteraemia.

there was a large increase in α1.7, α2.4 and β2 proteins ($p < 0.001$), (table 19), probably due to stimulation of the immune mechanisms and complement activation (table 19, Appendices C4, D5 and E1). In this group there were also increases in α1.4, α1.5 and α2.7 proteins noted, the latter two being statistically highly significant ($p < 0.001$), (table 19, Appendices C4 and D5). A small, but significant decrease in α1.6 was recorded ($p < 0.05$) and also in α1.1, and little or no change was demonstrated in α1.2, α1.3 and β7 proteins. The increases recorded in α1.8 and β2 proteins were statistically significant ($p < 0.001$). There was no clear α2.5 peak (haptoglobin) observed in this group, showing that there was no evidence of gross haemolysis, (figure 20).

Animals with a distant septic abscess showed a significant decrease in the albumin, α1.3, P2 and α1.6 peaks ($p < 0.001$), (table 19, Appendices C5 and D6). There were large increases recorded in the α1.7, α2.4 and β2 peaks ($p < 0.001$), (table 19, Appendices C5 and D6), and a smaller increase in α1.2, α1.4 and α1.5 peaks. There was little or no change in α1.8, α2.7, β3 and β7 peaks, and again this group demonstrated no clear α2.5 activity (haptoglobin), (figure 21).

Those animals with a distant sterile abscess showed a significant reduction in the albumin, P2 and α1.6 peak heights (table 19, Appendices C2 and D3). There was a very large increase in α1.7, α2.4 and β2 peaks ($p < 0.001$) and a smaller increase in

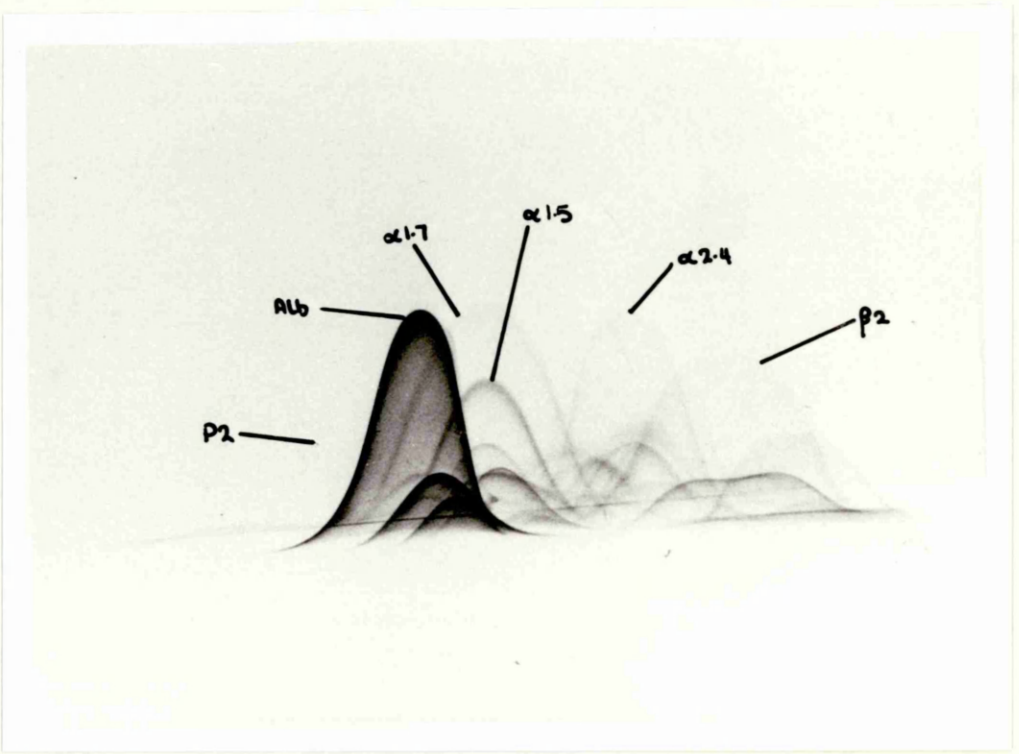

FIGURE (20): Pattern obtained after countercurrent crossed immunoelectrophoresis in animal with local sepsis. Note the reduction in albumin and P2 peaks and the large increase in α1.7, α2.4 and β2 proteins.

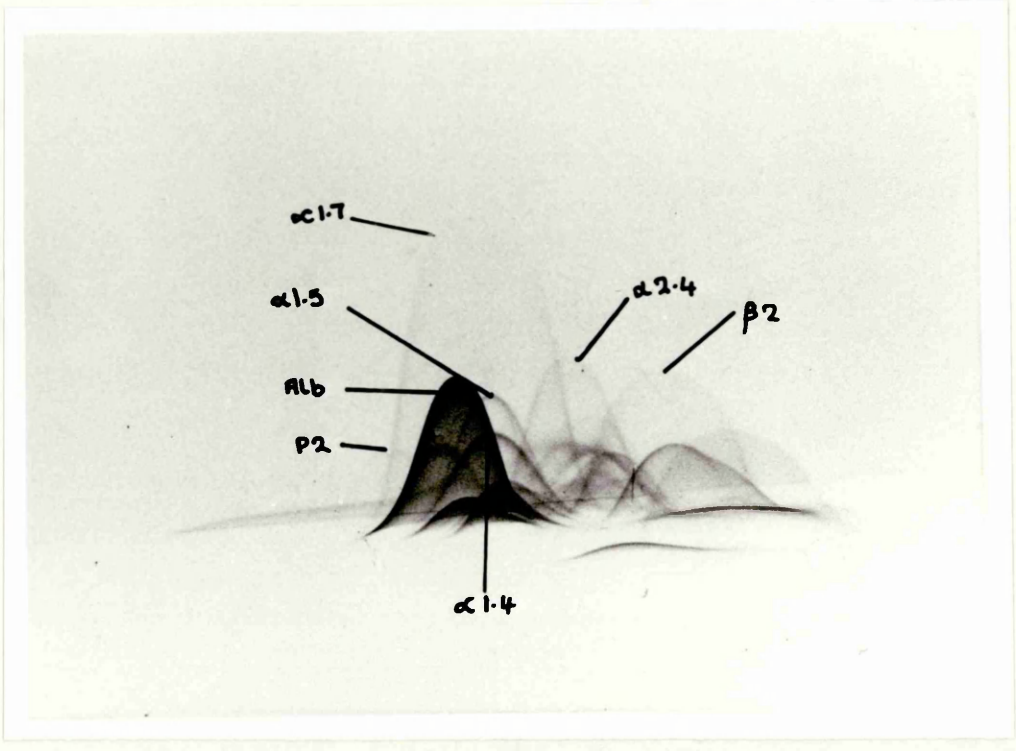

FIGURE (21): Pattern obtained after countercurrent crossed immunoelectrophoresis in animal with distant sepsis. Note the reduction in albumin and P2 peaks and the large increase in α1.7, α2.4 and β2 proteins.

α1.4, α1.5 and α2.7 levels. There was little or no change in α 1.2, α1.3 α1.8, β3 and β7 peaks. There was however in this group a very clear α2.5 peak (haptoglobin) observed in 3 of the samples and this seemed to be associated with large changes in the α2.4, β2 and α1.5 peaks. In this particular group there seemed to be a wide variation of peak heights in the individual animals (Appendix D3), (figure 22).

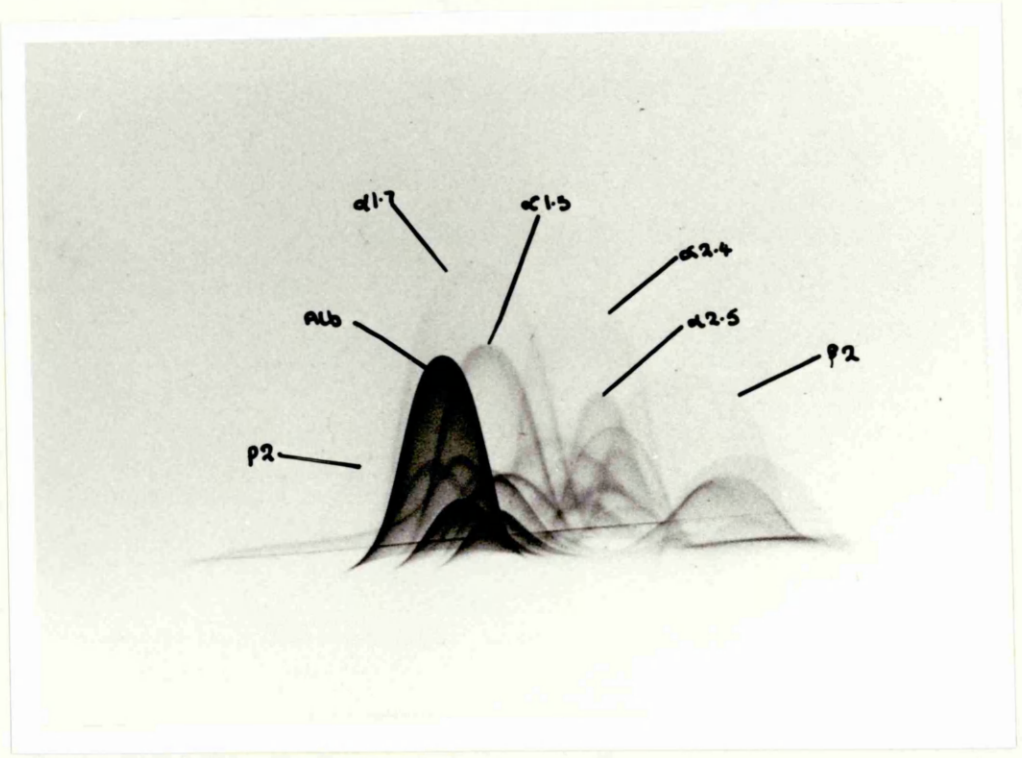

FIGURE (22): Pattern obtained after countercurrent crossed immunoelectrophoresis in animal with a distant sterile abscess. There is a reduced albumin and P2 peaks with a large increase in α1.7, α2.4 and β2 proteins. Also there is a clear α 2.5 peak (haptoglobin).

C H A P T E R 4

DISCUSSION

Microvascular free tissue transfer is defined as the movement, in a single operative procedure, of tissues from one part of the body to a distant site, with their immediate revascularisation using microsurgical techniques. The transfer is made possible by careful microvascular anastomosis of the supplying artery and draining vein of the flap, which depends for its early survival on the patency of the sutured vessels.

Although the concept of "transplantation in mass" of tissues was first postulated by Carrel over eighty years ago (4), it is only in the last 10 to 15 years that composite free tissue transfer has become well established in plastic and reconstructive surgical practice. Carrel stated in 1908 that "by using the method of transplantation in mass it becomes possible to perform the transplantation of a whole anatomic region, with its main artery and vein. The limits of the anatomic specimen must be approximately those of the field of distribution of the main artery". He then went on to describe the first successful experimental free composite tissue transfer (4) and along with his colleague Guthrie (5, 118) he performed, in the experimental situation, the successful transplantation of heart, kidney, thyroid gland and ovary and the replantation of limbs. In a further presentation on vascular surgical techniques in 1907 (119), Carrel stated that "a rigid asepsis is absolutely essential for success" and found that when the vessel anastomosis became occluded by thrombus that this was either produced by infection or by a failure of surgical technique. He also recorded the failure of a canine lower limb replantation due to local wound sepsis at the site of the vascular anastomosis.

Infection is well known to have a significant effect on wound healing (110, 120) by altering collagen metabolism leading to delayed epithelial growth with associated necrosis of tissues and local microvascular thrombosis (121). The factors influencing the healing of wounds are innumerable, and can be classified into those having a local effect and those acting systemically. Infection, has the capacity to delay wound healing by both methods. Ellis et al (110, 120) using the rat experimental model found that distant inflammation (both sterile and septic) could adversely influence the early healing of peritoneum, muscle, skin and stomach wall. This correlated with the clinical impression of delayed wound healing in patients with a collection of pus at a distance from the operative site. In their study of distant sepsis using Pseudomonas aeruginosa, it was also found that a transient sublethal bacteraemia had a similar effect, and since over half the animals with a distant focus of sepsis had growth of pseudomonas in the laparotomy wound, they postulated that the infection had been blood-borne.

Single stage free composite tissue transfer is now a reliable technique in reconstructive plastic surgery with consistently good results in experienced hands (25 - 35). Even so, some flaps still may fail post-operatively despite a technically sound microvascular repair of the anastomosed vessels. Although there is good clinical evidence that free flaps can be lost due to vascular occlusion associated with local sepsis (55, 99, 100, 122), surprisingly, despite many publications on the role of infection in vascular surgery, little experimental (104, 105) or clinical work has been done on its role in microsurgical practice.

In fact one of the most recent standard text-books on microvascular surgery (37) devotes only 7 lines to the effect of infection on microvascular anastomoses. O'Brien et al (123) despite their claim that infection was rare in their series of almost 300 free tissue transfers, reported a 15% incidence of non-thrombotic local complications (which included local infection). However the exact number of cases with local sepsis was not given.

Recently, Godina in his paper on the microsurgical repair of severely traumatised limbs (43), conclusively demonstrated that infection in free flaps greatly increased the failure rate. In his extensive review of over 500 free tissue transfers, it was found that a post-operative wound infection significantly increased the flap failure rate, led to delayed bone healing and markedly prolonged the total hospital stay. This correlation between local sepsis and occlusion of the vascular pedicle to the free tissue transfer was found to be greatest in the group undergoing reconstructive microvascular surgery between 72 hours and 3 months following the initial trauma. The occurrence of the local sepsis was related to the inadequacy of the initial surgical toilet of bone and soft tissues, which allowed for infection deep to the flap and thrombosis of its vascular supply.

It was concluded that the early radical debridement and coverage of complex limb trauma by microvascular free tissue

transfer, in association with high dose, antimicrobial therapy, allowed the optimal conditions for flap success and early wound healing free from the complications of local wound sepsis.

The aim of the present study, using the rat experimental model, was to investigate the role of distant inflammation (both pyogenic and aseptic), local wound infection and a transient sublethal bacteraemia on the patency rates of microarterial anastomoses.

Seven days following microvascular repair of the femoral artery, 39 of the 40 control anastomoses were patent. This patency rate of over 97% for the control group was in good agreement with those of other published experimental studies (51-53, 124, 125). Patency was assessed intra-operatively by observing expansile pulsation distal to the microvascular anastomosis and by the use of the milking and flicker tests (52, 114-116, 126). Although recent studies would seem to suggest that the milking (or double forceps patency) test may have limited value in assessing vessel patency (127, 128), and may even be injurious to the intimal lining (129), in this study it correlated closely with the histological findings at the anastomotic site. The occlusion rate was not significantly influenced by either a distant sterile abscess or a transient sublethal bacteraemia, where 35 anastomoses remained patent in each ($p = 0.203$). However

thrombosis was markedly increased in animals with local wound sepsis, 13/40 ($p = 0.001$), but was greatest of all in those animals with a pyogenic abscess, even though it was remote from the site of microvascular repair, 20/40 ($p < 0.001$). These results are partly in keeping with those of De Haan et al in 1974 (110) who found that distant sepsis delayed the healing of experimental laparotomy wounds. They also demonstrated that distant aseptic inflammation and a transient bacteraemia had a marked inhibitory effect on wound healing, but these findings were not confirmed with regard to microarterial wound repair in the present study. Although these current results conflict with those previously reported (110), there were differences in the animal model and dose of bacterial inoculum used. In contrast to De Haan et al (110), who were measuring the bursting strength of abdominal laparatomy wounds, this present work was investigating thrombosis following the microsurgical repair of small arteries.

The normal healing processes and the histopathological features of small vessels following microvascular surgery have been well documented, both in the experimental (53, 130-134) and in the clinical situation (135, 136). It is a commonly held misconception that by using a gentle surgical technique and by having accurate apposition of the vessel ends that there is minimal pathological change in the vessel wall. Despite an accurate anatomical repair, the microscopic architecture of the vessel wall can be significantly damaged by surgery (53, 130, 133). Acland and Trachtenberg (53), in a very elegant study, showed that despite 100% patency rates at the time of examination of the microvascular repair, within 60 minutes of surgery, there was widespread

loss of the intimal lining and in 70% of cases there was extensive damage to the tunica media. This consisted of medial necrosis with denucleation of the smooth muscle cells. There was compression of the elastic laminae by the suture loop, platelet plugging of the needle holes and by 48 hours in 60% of the vessels there was some disruption of the apposed vessel edges. Baxter et al (130) in the rabbit model, found buckling of the cut vessel edges at the anastomotic site, pinching of the vessel wall at suture sites, medial necrosis particularly when too many sutures had been inserted too tightly and marked subintimal hyperplasia due to proliferation of the medial smooth muscle cells. This subintimal thickening had the potential to reduce greatly the vessel lumen. This work on small vessel repair complemented a previous study on larger vessels (137) which showed that, following vascular injury, there was migration of smooth muscle cells resulting in a subintimal plaque. In addition Baxter et al noted damage to the adventitia (130). Other studies, (131-134) have confirmed these findings and it has been suggested that factors released by platelet deposition may stimulate the medial myofibroblasts to produce the subintimal hyperplasia (132).

These reports provide good evidence that the microsurgical repair of small arteries and veins can severely alter the vessel wall morphology, and the results of the current study corroborate those findings. In all the groups in the present experimental work, there was widespread histological damage noted following the microvascular repair. Those animals with a transient bacteraemia, distant pyogenic abscess, distant sterile abscess and controls, had extensive necrosis of the tunica media with

loss of the smooth muscle cells. In the patent vessels at the site of arterial repair, the suture loops had caused a marked scalloped effect. In several of the animals with distant sepsis there was a marked thickening of the adventitial layer. Both at and adjacent to the site of the repair, there was extensive subintimal hyperplasia, which in some animals had produced a considerable reduction in the diameter of the vessel lumen. Despite this finding, those vessels demonstrated a good blood flow distal to the anastomotic site.

Local sepsis, as one would expect, produced a marked vasculitis with a heavy infiltrate of acute inflammatory cells in the vessel wall and occluding thrombus. An important question which still remains unresolved, is why there should have been two different responses to the induction of local sepsis. However, similar findings have been documented by other authors (105).

Lidman and Daniel (131) found that re-endothelialisation was complete by 48 hours in all areas between the microvascular clamps, apart from the anastomotic site, which took around 10 days to occur. There was also some dilatation of the vessel wall at the anastomosis and again there was marked subintimal hyperplasia and hyaline degeneration of the media. They found that the media did not regenerate and noted that significant thickening of the adventitia was uncommon. A long-term histological study was reported by Lidman et al in 1984 (134) who found that the subintimal hyperplasia reached a peak at about 12 weeks and then regressed a little, although it remained to compensate for the thinning of the media due to the hyaline necrosis. Their long term patency in 81 anastomoses was 100% (134), despite the obvious

histological trauma caused by the microvascular sutures. These experimental findings have also been noted in man when arterial anastomoses have been examined after digital replantation (135) and free flap transfer (136). In the latter report by Karl et al, there was extensive subintimal hyperplasia and medial necrosis in the presence of a tight suture loop.

More recently the scanning electron microscope has given very detailed and accurate information on the ultra structure of the microvascular repair (138-142). It is well known that fibrin enmeshes platelet aggregates at the site of vascular injury (143) and a recent study of microvascular anastomoses in traumatised vessels, has suggested that the fibrin mesh may be a more important factor than platelet aggregation, in the pathogenesis of the occluding thrombus (144). Scanning electron photomicrographs taken at the time of clamp release, have demonstrated platelet adherence to a fibrin network at the anastomotic site and the microvascular sutures. This early platelet adherence peaks after a few hours following clamp removal (138-142). This phenomenon of the early peak at 30 minutes of platelets adherent to the anastomotic site has also been demonstrated using P32 labelled platelets (145). There is severe and widespread intimal damage with flattening of the longitudinal ridges usually seen in dissected vessels. Initially there is an increase in the number of acute inflammatory cells at the anastomotic site and by 3 to 7 days there is endothelial regeneration from the adjacent normal cells, which migrate over the thrombotic pannus to completely cover the sutures, which

become buried beneath the new intimal layer These very early changes were not demonstrated in the present study, because the specimens were harvested one week following surgery. However, it was possible in the patent vessels, to demonstrate at 7 days significant subintimal hyperplasia and neo-endothelium covering the site of arterial repair. High power photomicrographs of the occluded vessels, showed a very dense network of fibrin, in which were enmeshed both normal and crenated red cells. No platelets were noted in these thrombi, but they were observed attached to the luminal surface of the vessel wall close to the anastomotic site. Although pseudomonas was grown from the blood of several of the animals with a distant septic focus, it was never cultured from the anastomotic thrombus nor seen on histological or scanning electron microscopy specimens. Recent experimental work has suggested that there is a significant reduction in the median area of re-endothelialisation of a microarterial anastomosis in diabetic animals at 10 days (89) but this was not found in any of the groups in the present work using scanning electron microscopy, there being complete re-endothelialisation by 7 days in the patent specimens.

The detrimental role of infection on wound healing is well known (110, 120) and following De Haan's work (110), it was postulated that the significant increase in the anastomotic occlusion rate in animals with distant sepsis could be due to blood-borne spread of the organism to the area of active tissue regeneration (111, 112). Although in 20% of the distant abscess group, pseudomonas was successfully grown on blood culture, unlike the previous work on abdominal wounds (110), the organism

could not be cultured either in the groin wounds or anastomotic thrombi. Multiple abscesses due presumably to septic embolisation, were found in several of these animals at post-mortem examination, confirming the blood culture finding that the organism had been present in the circulation in this group with a remote pyogenic abscess. Although the results of the present work do conflict with those of the healing of abdominal wounds (110), they might just be reflecting the differences in the study model and in the mechanism of healing between skin and arterial wall.

Having failed to confirm that the thrombotic episodes were related to wound sepsis from the transient pseudomonas bacteraemia, the role of other potential mechanisms, such as enhanced activation of coagulation, were investigated. It has been shown experimentally in diabetic rats that endotoxin markedly enhances fibrin deposition in the microvascular circulation, leading to a generalised Shwartzman reaction (107). This increased susceptibility to intravascular coagulation can be significantly altered by prostacyclin infusion (106). Other studies, using the pig model, have demonstrated that the endotoxin induced changes in the coagulation mechanism, pulmonary microthrombosis and other haematological disturbances can be prevented by Cyproheptadine, a serotonin antagonist (146). Unfortunately, due to technical limitations, activated clotting factors were not detectable in any of the assays performed. It was however, shown that sepsis, both distant and local, had a marked effect on both red and white cell morphology. This finding was more pronounced in the group with distant sepsis, showing that because they had been inoculated with ten times the dose of pseudomonas aeruginosa, they were more systemically unwell than those animals with a local wound infection in the groin.

In the normal rat peripheral blood a slight polychromasia and anisocytosis is not uncommon. Normoblasts are normally not present in the adult animal and the lymphocyte/neutrophil ratio is around 3/1. Reticulo-endothelial studies have shown that in healthy adult rats it is rare to see signs of splenic haemopoiesis except under conditions of extreme stress (147). This present work has demonstrated that only local and distant sepsis had a significant effect on red cell morphology, the latter being more profound. In the remote abscess group there was an increase in the size and shape of the red blood corpuscles, with enhanced crenation due to abnormality of the red cell membranes. The marked polychromasia seen in the infected animals, demonstrated that there was more regenerative activity in this group in response to the toxic anaemia. Histological study of the spleens of the animals with distant sepsis demonstrated the presence of marked extra-medullary haemopoietic activity in response to the toxic insult. This finding was not observed in any of the other groups. The extra-medullary haemopoiesis could have been partly responsible for the significant leucocytosis and thrombocytosis observed in those animals with with a distant pyogenic abscess.

Analysis of the differential leucocyte counts demonstrated again that the infected groups showed significant change. Although in those animals with local sepsis the mean white cell count was not dissimilar to the normal group, there was a greatly reduced lymphocyte/neutrophil ratio, with an increase in polymorphs, as the immediate response to the locally-induced infection. The overall count seemed to be dropping, possibly due to the trapping of lymphocytes in the infective focus and the surrounding

inflammatory exudate around the infection site. Polymorphonuclear leucocytes "sticking" to the anastomotic site could also have had an influence on thrombosis. In the distant abscess group several animals showed the presence of plasma cells, suggestive of an antibody response. Also in this chronic infection group there were more monocytes present, which are young macrophages "in-transit". In this group there was a significant increase in white cells ($p < 0.001$) and a significant reversal of the lymphocyte/polymorphonuclear leucocyte ratio ($p < 0.001$) when compared to the control animals.

The normal platelet count in healthy adult rats varies enormously, ranging between 430 and 840 x 10^9/l (147). Animals with a distant septic focus had a significant thrombocytosis ($p = 0.008$) when compared with control animals, which was associated with a statistically highly significant increase in the rate of occlusion of the microarterial repairs ($p < 0.001$). There was also an increase in platelet numbers in animals with distant aseptic inflammation ($p = 0.02$). However, although more anastomoses were occluded in this group when compared with control animals, the results were not statistically significant. It would therefore appear that other factors, possibly humoral, may be associated with the increased tendency to thrombosis in animals with a distant pyogenic abscess.

While analysis of the specimens for the mediators of the coagulation cascade was unsuccessful, the results of the crossed immunoelectrophoresis studies are extremely interesting. Tiselius in 1937 demonstrated that the plasma proteins could be separated

in an electric field on the basis of their mobility, into four main classes: albumin, α, β and γglobulins. Each class was in fact heterogeneous and consisted of a group of closely related proteins. About twenty years later, the addition of a specific antiserum to the technique (immunoelectrophoresis) has allowed further identification of the various serum components based on their antigenic properties (148).

The results of this study showed that there were marked changes in the levels of serum proteins detected. A brief summary of the rat protein profile (which is analagous to the human) and their postulated functions, is shown in Appendix E.

Overall, four proteins showed a decrease in concentration (albumin, P2, α1.6 and α1.3) and six exhibited an increase (α2.4, β2, α1.5, α2.7, α1.1 and α1.7). There was very little difference noted in the peak heights in the normal, control or transient bacteraemia samples. The reason for this finding in the bacteraemic group, is that the blood sample was probably collected before any dramatic changes in serum protein levels could be registered.

There were large reductions seen in the levels of albumin and P2 in the local infection and distant septic and sterile abscess groups. This change was noted particularly in those animals with distant sepsis and less pronounced in the local sepsis animals. In all three groups there were dramatic increases in the peak heights of α1.7 (immune regulation), α2.4 and β2 proteins ($p < 0.001$). Less pronounced increases were noted in the levels of α1.4, α1.5 and α2.7. Why distant septic and distant sterile

inflammation should cause similar dramatic changes in the levels of the serum proteins remains unclear, when the inflammatory stimulus was different in each case. It has been demonstrated that complement is an important trigger mechanism in the early phase of the acute inflammatory response (149) and yet in most groups there was little or no change in the α1.8 peak apart from those with local wound sepsis ($p < 0.001$). These findings may be related to the timing of the samples as the peak level possibly had occurred at an earlier time in many of these groups. There was a different α2.5 (haptoglobin) response in several of the animals in the distant sterile abscess group, which seemed to be associated with large changes in the α2.4, β2 and α1.5 peaks. This protein (haptoglobin) is known to bind and conserve haemoglobin by producing complexes which are removed by the reticuloendothelial system. Estimation of its level can give an indication of the degree of haemolysis present. Why there was a different haptoglobin response to chemically induced inflammation (which may also be seen with heavy metals) is uncertain, as there was no evidence of gross haemolysis found on the blood film assessment in the sterile abscess group. One would perhaps have expected to see this change in the animals with haemolysis secondary to sepsis, but this was not the case.

Although this study was designed to investigate the effect of local and distant sepsis on the patency of microvascular anastomoses, as an incidental finding further information was gained on the vascular anatomy of the rat femoral vessels.

Some of the current laboratory dissection manuals give little guidance on the anatomy of this particular region (150). Most of the microsurgical dissection handbooks include a small sub-section on the relevant anatomy (115-117), although the details given can be conflicting. Brunelli (117) stated that the deep femoral (profunda) vessels came off half way between the inguinal ligament and superficial epigastric pedicle, which was in agreement with Serafin et al (116). The latter authors also mentioned the possibility of duplication of these deep (profunda) branches. However, Acland (115) described the common femoral artery as running from the inguinal ligament for a distance of 1.5cms where it divided into superficial and deep branches, and just proximal to this division the epigastric artery was given off.

In 1935, Greene described the circulatory system of the rat in some detail (151). The femoral artery extends from the inguinal ligament to where the vessel passes between the two muscles adductor brevis and caudo-femoralis, just distal to the origin of the superficial epigastric artery. The branches given off in order, as it traverses the femoral triangle are: the superficial circumflex iliac, muscular, superficial epigastric, highest genicular, saphenous and the "profunda femoris". The muscular branch is given off from the postero-medial side of the femoral artery, about one third of the distance down the thigh, near to the level of the insertion of pectineus. It crosses the adductor muscles and supplies both them and the two gracilis muscles. The superficial epigastric artery comes off distal to this muscular branch and supplies the subcutaneous fat of the

region, the skin of the medial thigh and groin, and via its anastomotic branch the skin of the abdominal wall. The "profunda femoris" artery is not present in the rat. The perforating branches are absent and both its medial and lateral circumflex femoral branches are given off by the hypogastric artery, proximal to the inguinal ligament. There is however a remnant, a muscular branch, given off just proximal to the caudo-femoralis muscle, although it does not perforate the adductor muscle group. The origin of this branch is situated distal to that of the superficial epigastric artery.

Given this conflicting evidence, it would appear that the deep ("profunda") vessel, described by various authors as coming off halfway between the inguinal ligament and the superficial epigastric artery, is in fact the muscular branch of the femoral artery. Acland (115), is in fact anatomically more accurate in his description of the common femoral artery and he does describe several muscular branches of this artery arising from its posterior surface.

During the course of the present dissections, it was noted that the posterior branch, frequently referred to in the microvascular practice manuals as the "profunda femoris" artery (116, 117), arose more proximally than the midpoint of the thigh.

In 100 consecutive groin dissections, the median length of the femoral artery from the inguinal ligament to the superficial epigastric branch was 1.30cms (range 1.1 - 2.0cms). This correlates well with Acland's measurement of the total length of

the common femoral vessel at 1.5cms (115). The median external diameter of the undissected femoral artery (0.9mm) and femoral vein (1.5mm) is in good accord with those reported by Serafin et al (116), where the range of arterial diameter was given as 0.6-1.0mm and venous diameter as 1.0-2.0mm

The major difference found was related to the site of origin of the posterior (muscular) branch of the femoral vessels. Duplication of this artery was found in 13% of the arterial dissections and in 23% of the venous examinations. In two, there was no posterior (muscular) artery present, and there were thus 111 muscular arteries and 123 muscular veins for assessment. It was found that in the majority of cases (approximately 75%), the origins of the posterior (muscular) artery and vein arose in the proximal one-third of the thigh and not as had been previously documented in microsurgical guides (115, 116) at its midpoint. These findings support the observations of Greene (151) and give further information regarding the vascular anatomy of this most important region for microsurgical practice.

C H A P T E R 5

CONCLUSIONS, IMPLICATIONS OF THE STUDY AND THE POSSIBILITIES FOR FURTHER RESEARCH

The purpose of this study, using the rat experimental model, was to investigate the effect of local and distant sepsis on the healing of microarterial anastomoses. The results presented in this thesis, have demonstrated that chronic distant sepsis increases the occlusion rate of microvascular anastomoses to a significantly greater extent than either aseptic inflammation, transient bacteraemia or even local infection. Although the exact mechanism of the anastomotic failure remains uncertain, it was related to a significant increase in the number of platelets in the group with remote sepsis and associated with changes in red cell morphology, white cell count and a marked elevation of α1.7, α2.4 and β2 serum protein levels.

Although it is difficult to extrapolate an experimental finding to the clinical situation, it would appear that a septic focus, both locally and at a distance, is an important risk factor to be taken into account when planning reconstructive microvascular free tissue transfer.[43] As sepsis has the potential to promote occlusion of a microvascular anastomosis, it would seem prudent to eliminate such infective foci prior to reconstructive surgery involving the microvascular transfer of free tissue. Of course, this is not always possible, as many free flaps are now being performed in the acute clinical situation (42-44). Essential prerequisites for such innovative surgery to be successful are, complete surgical excision of all contaminated, contused and avascular tissues and the use of high dose antibiotic therapy.

The future is extremely exciting for reconstructive microvascular surgeons, with new flap donor sites being regularly described, second attempts at free-tissue transfer being considered when the initial procedure has failed (152) and free tissue transfer being performed on patients with conditions which until recently were deemed a contraindication to such surgical techniques (45, 153).

Perhaps even, with the less toxic immunosuppressants of the future, Carrel's concept of stored tissue and limb banks (119) is not too far away. Systemic heparin has been shown experimentally to improve the patency rates of traumatised small vessel anastomoses (144, 154, 155), and recently May et al (156) were able to successfully salvage a failing free gracilis muscle transfer, by infusing heparin directly into the vena comitans of the flap. Although streptokinase has been used as a thrombolytic agent for many years, its use in the salvage of free tissue transfers (157) and a failing thumb replant (158), has only recently been described. The new, and even more potent agent, human recombinant tissue plasminogen activator (159, 160), although only in the experimental stages at present, may herald a new era in microvascular practice, with its capacity to re-open small vessels occluded by thrombus. This would permit the successful reperfusion of the tissues and thus allow flaps which would otherwise necrose, to survive by "buying time" for neovascularisation to occur from the surrounding tissues (103, 161-163).

Further experimental work is currently underway in this laboratory, not only to assess the role of humoral factors in the

enhancement of coagulation in the infected animals, but also to determine the optimal time to begin anti-microbial therapy prior to reconstructive microvascular free tissue transfer in the infected situation.

Using a similar animal model, it should also be possible to investigate more fully the role of other factors known to be detrimental to wound healing, and which are commonly encountered in clinical practice (such as malignant disease, jaundice, uraemia, diabetes and protein deficiency), on the healing and patency of microvascular anastomoses.

APPENDIX A

Crossed Immunoelectrophoresis Method

Solutions

(1) Barbitone acetate buffer pH 8.6 (Stock for tank and gel)

4.52g Barbitone (heat to dissolve)

35.5g Sodium barbitone

26g Sodium acetate (triacetate)

Cool and make up to 4 litres. Adjust pH to 8.6

(2) Tank Buffer

Take 3 litres Stock barbitone - acetate buffer (1)

and 2 litres Distilled water

(3) 1% Agarose in barbitone acetate buffer

1g Agarose (Pharmacia agarose A)

50mls Stock barbitone - acetate buffer

50mls Distilled water

Heat to boiling until agarose is dissolved. Transfer to 56^{o}C

Water bath (or store at < 4^{o}C and remelt gently)

(4) Bromophenol blue (marker)

Small spatula tip of dye in 100 mls Distilled water

(5) Coomassie brilliant blue stain (0.5%)

450mls 96% Ethanol

450mls Distilled water

100mls Glacial acetic acid

Mix and add 5g Coomassie brilliant blue Reagent

Shake until most of the stain has dissolved and leave overnight

Filter stain before use

(6) Destainer

450mls 96% Ethanol

450mls Distilled Water

100mls Glacial Acetic Acid

(7) 0.9% Sodium Chloride (Serum dilution and plate washing)

(8) Antisera

Dako anti (rat) serum 2178 5% in agarose

Apparatus

1. Immunoelectrophoresis tank with cooling platter
2. Power pack to provide at least 150mA at 250V
3. 56°C water bath
4. Boiling water bath
5. Well cutter 2.5mm diameter and suction pump
6. Glass plates (8.2 x 8.2cms)
7. Filter paper wicks 3mms thick (8.2 x 13cms)
8. Diamond pencil to mark plates
9. 10mls glass pipette
10. 5µl micropipette
11. Levelling table
12. Staining racks and dishes

Method

1. Place clean numbered plates (8.2cms x 8.2cms) on levelling table.

2. Fill warmed 10mls pipette with hot 1% Agarose and run contents onto plate.

3. Leave to set (approximately 10 mins).

4. Cut holes about 1.5cms from each of lower corners of the plate (negative pole end).

5. Place plates on apparatus.

6. Lay filter paper wicks onto edges of the plate gel (ensure even contact across plate).

7. Turn on cooling water.

8. Add one drop of Bromophenol blue to right-hand well.

9. Add 5µl of diluted sample to left-hand well.

10. Place lid on apparatus.

11. Connect leads so samples lie close to the negative electrode.

12. Turn on power pack and adjust to give 200V (approximately 20mA). (Voltage may be raised to 250V or lowered to 100V for convenience).

13. Continue electrophoresis until Bromophenol blue marker reaches far end of the plate (will travel faster than the proteins) approximately 1½ - 2½ hours.

14. Turn off power pack and disconnect leads.

15. Place plates on levelling table.

16. Cut gel parallel to sample track, 0.5cms from edge of sample well and scrape off excess gel.

17. Pipette antiserum into test tube containing sufficient agarose to make up volume of 10mls.

    eg 5% antiserum = 0.5mls antiserum + 9.5mls agarose

    Rapidly mix (avoiding bubbles) and pour onto plate. Allow to set.

18. Connect electrodes from power pack to tank the opposite way around from that used in the first dimension.

19. Place plates so that sample strip lies next to the negative electrode. Connect to buffer with new filter paper wicks.

20. Turn on power pack to give a constant voltage of 45V and continue electrophoresis for at least 16 hours.

Washing and Staining

1. Cover gel with a piece of filter paper, taking care to avoid trapping any air bubbles. (1 damp layer on first).

2. Place several layers of filter paper on top followed by several layers of Bow towels. Cover with several sheets of glass plate (about 2kgs weight) and press for 10-15 mins.

3. Wash for at least 1 hour in 0.9% sodium chloride.

4. Press again for 10-15 mins.

5. Wash for 2 hours in 0.9% sodium chloride.

6. Wash for 30 minutes in distilled water.

7. Dry under current of warm air.

8. Stain by immersion in Coomassie solution for 10 minutes.

9. Destain in 3 changes until background slightly blue (about 6 mins).

10. Dry.

Sample dilution	- 1:20	(20µl + 380µl sodium chloride)
Antiserum dilution	- 5%	(0.5mls antiserum + 9.5mls agaros
First electrophoresis dimension	200 volts	(2 hours)
Second electrophoresis dimension	45 volts	(18 hours)

Run (1) Sample numbers assayed : N4, C1,LS2, DA3, TA1

Run (2) Sample numbers assayed : TRB3, N1, C5, LS3, DA4

Run (3) Sample numbers assayed : TA6, TRB8, N8, C7, LS1

Run (4) Sample numbers assayed : DA7, TA2, TRB6, N5, C2

APPENDIX B

Haematological Results (Mean ± Standard Deviation)

Parameter	Normal	Control	
Haemoglobin (gm/dL)	13.92 ± 0.89	15.85 ± 1.34	(p = 0.0013)
Haematocrit (%)	39.27 ± 3.09	43.92 ± 3.52	(p = 0.0057)
White Cell Count ($x10^9/L$)	5.08 ± 1.38	4.95 ± 0.98	(p = 0.8093)
% Polymorpho-nuclear leucocytes	19.60 ± 11.64	14.20 ± 7.19	(p = 0.2277)
% lymphocytes	75.60 ± 11.92	81.80 ± 6.48	(p = 0.1655)
Platelet count ($x\ 10^9/L$)	1074.30 ± 75.91	955.11 ± 223.58	(p = 0.1301)

Haematological Results (Mean $\pm$ Standard Deviation)

Parameter	Control	Distant Sterile Abscess	
Haemoglobin (gm/dL)	15.85 $\pm$ 1.34	14.09 $\pm$ 1.88	(p = 0.0270)
Haematocrit (%)	43.92 $\pm$ 3.52	39.86 $\pm$ 5.59	(p = 0.0677)
White Cell Count ($x10^9/L$)	4.95 $\pm$ 0.98	4.92 $\pm$ 2.16	(p = 1.0000)
% Polymorpho-nuclear leucocytes	14.20 $\pm$ 7.19	28.80 $\pm$ 20.67	(p = 0.0492)
% Lymphocytes	81.80 $\pm$ 6.48	62.30 $\pm$ 18.94	(p = 0.0064)
Platelet Count ($x10^9/L$)	955.11 $\pm$ 223.58	1257.70 $\pm$ 292.35	(p = 0.0224)

Haematological Results (Mean ± Standard Deviation)

Parameter	Control	Transient Bacteraemia	
Haemoglobin (gm/dL)	15.85 ± 1.34	13.34 ± 0.83	(p = 0.0001)
Haematocrit (%)	43.92 ± 3.52	37.98 ± 2.73	(p = 0.0005)
White Cell Count ($x10^9/L$)	4.95 ± 0.98	7.16 ± 1.87	(p = 0.0039)
% Polymorpho-nuclear leucocytes	14.20 ± 7.19	11.50 ± 6.40	(p = 0.3858)
% Lymphocytes	81.80 ± 6.48	87.10 ± 5.43	(p = 0.0629)
Platelet Count ($x10^9/L$)	955.11 ± 223.58	987.10 ± 101.46	(p = 0.6853)

Haematological Results (Mean ± Standard Deviation)

Parameter	Control	Local Sepsis	
Haemoglobin (gm/dL)	15.85 ± 1.34	15.01 ± 0.91	(p = 0.1190)
Haematocrit (%)	43.92 ± 3.52	40.25 ± 2.54	(p = 0.0155)
White Cell Count ($x10^9/L$)	4.95 ± 0.98	4.31 ± 1.11	(p = 0.1883)
% Polymorpho-nuclear leucocytes	14.20 ± 7.19	44.40 ± 9.54	(p = 0.0000)
% Lymphocytes	81.80 ± 6.48	46.40 ± 10.78	(p = 0.0000)
Platelet Count ($x10^9/L$)	955.11 ± 223.58	803.56 ± 150.29	(p = 0.1108)

Haematological Results (Mean ± Standard Deviation)

Parameter	Control	Distant Septic Abscess	
Haemoglobin (gm/dL)	15.85 ± 1.34	13.59 ± 1.41	(p = 0.0018)
Haematocrit (%)	43.92 ± 3.52	39.15 ± 5.74	(p = 0.0379)
White Cell Count ($x10^9$/L)	4.95 ± 0.98	11.95 ± 4.60	(p = 0.0002)
% Polymorpho-nuclear leucocytes	14.20 ± 7.19	63.60 ± 7.24	(p = 0.0000)
% Lymphocytes	81.80 ± 6.48	28.20 ± 6.39	(p = 0.0000)
Platelet Count ($x10^9$/L)	955.11 ± 223.58	1475.20 ±474.96	(p = 0.0082)

Lymphocyte/Neutrophil Ratio

Group	L/N Ratio	
Normal (N)	3.8/1	
Control (C)	5.8/1	
Distant Sterile Abscess (DSA)	2.2/1	*
Transient Bacteraemia (TRB)	7.8/1	
Local Wound Sepsis (LS)	1.1/1	**
Distant Septic Abscess (DA)	0.4/1	**

* distant sterile abscess V's control ($p < 0.05$)

** local wound sepsis and distant septic abscess V's control ($p < 0.001$)

APPENDIX C

SUMMARY OF MEAN PEAK HEIGHTS (mm) ± STANDARD DEVIATION

PEAK	NORMAL	CONTROL	
ALBUMIN	17.40 ± 2.32	15.89 ± 1.27	(p = 0.1014)
α 1.2	3.00 ± 0.47	3.11 ± 0.78	(p = 0.7129)
α 1.7	4.60 ± 1.07	4.56 ± 0.53	(p = 0.9215)
α 1.4	6.40 ± 0.52	6.33 ± 0.50	(p = 0.7807)
α 1.3	5.00 ± 0.67	4.78 ± 0.44	(p = 0.4079)
P 2	11.70 ± 1.49	11.00 ± 1.87	(p = 0.3778)
α 2.4	3.33 ± 0.71	2.78 ± 0.67	(p = 0.1057)
β 2	6.70 ± 0.67	6.22 ± 1.09	(p = 0.2613)
α 1.5	8.00 ± 1.25	6.56 ± 1.13	(p = 0.0174)
α 1.6	6.60 ± 0.97	6.78 ± 1.20	(p = 0.7229)
α 1.8	3.60 ± 0.70	3.56 ± 0.53	(p = 0.8892)
α 2.7	5.40 ± 0.52	5.11 ± 0.33	(p = 0.1713)
β 3	4.30 ± 0.95	4.78 ± 1.20	(p = 0.3459)
α 1.1	5.50 ± 0.53	5.11 ± 0.93	(p = 0.2700)
β 7	6.50 ± 0.71	6.33 ± 0.71	(p = 0.6167)
α 2.5	-	-	

SUMMARY OF MEAN PEAK HEIGHTS (mm) ± STANDARD DEVIATION

PEAK	CONTROL	DISTANT STERILE ABSCESS	
ALBUMIN	15.89 ± 1.27	13.30 ± 0.82	(p = 0.0001)
α 1.2	3.11 ± 0.78	3.20 ± 0.42	(p = 0.7557)
α 1.7	4.56 ± 0.53	18.60 ± 3.57	(p = 0.0000)
α 1.4	6.33 ± 0.50	7.00 ± 0.94	(p = 0.0756)
α 1.3	4.78 ± 0.44	4.40 ± 0.70	(p = 0.1827)
P 2	11.00 ± 1.87	8.70 ± 1.49	(p = 0.0085)
α 2.4	2.78 ± 0.67	10.10 ± 4.98	(p = 0.0004)
β 2	6.22 ± 1.09	10.70 ± 1.57	(p = 0.0000)
α 1.5	6.56 ± 1.13	9.50 ± 2.88	(p = 0.0106)
α 1.6	6.78 ± 1.20	4.90 ± 0.74	(p = 0.0007)
α 1.8	3.56 ± 0.53	3.90 ± 0.88	(p = 0.3199)
α 2.7	5.11 ± 0.33	6.40 ± 1.07	(p = 0.0031)
β 3	4.78 ± 1.20	5.40 ± 0.52	(p = 0.1537)
α 1.1	5.11 ± 0.93	6.20 ± 0.79	(p = 0.0132)
β 7	6.33 ± 0.71	6.40 ± 0.84	(p = 0.8645)
α 2.5	-	9.67 ± 1.15	-

SUMMARY OF MEAN PEAK HEIGHTS (mm) ± STANDARD DEVIATION

PEAK	CONTROL	TRANSIENT BACTERAEMIA	
ALBUMIN	15.89 ± 1.27	15.00 ± 1.34	(p = 0.1484)
α 1.2	3.11 ± 0.78	3.36 ± 0.81	(p = 0.4886)
α 1.7	4.56 ± 0.53	4.27 ± 0.90	(p = 0.4170)
α 1.4	6.33 ± 0.50	5.82 ± 0.75	(p = 0.0953)
α 1.3	4.78 ± 0.44	5.36 ± 1.03	(p = 0.1291)
P 2	11.00 ± 1.87	10.91 ± 1.76	(p = 0.9214)
α 2.4	2.78 ± 0.67	3.27 ± 0.65	(p = 0.1104)
β 2	6.22 ± 1.09	6.45 ± 0.82	(p = 0.5906)
α 1.5	6.56 ± 1.13	6.73 ± 1.01	(p = 0.7226)
α 1.6	6.78 ± 1.20	5.82 ± 0.60	(p = 0.0320)
α 1.8	3.56 ± 0.53	3.18 ± 0.75	(p = 0.2248)
α 2.7	5.11 ± 0.33	4.64 ± 0.67	(p = 0.0704)
β 3	4.78 ± 1.20	3.73 ± 0.90	(p = 0.0386)
α 1.1	5.11 ± 0.93	4.82 ± 0.60	(p = 0.4041)
β 7	6.33 ± 0.71	6.27 ± 0.79	(p = 0.8644)
α 2.5	-	-	

SUMMARY OF MEAN PEAK HEIGHTS (mm) ± STANDARD DEVIATION

PEAK	CONTROL	LOCAL SEPSIS	
ALBUMIN	15.89 ± 1.27	13.50 ± 1.78	(p = 0.0040)
α 1.2	3.11 ± 0.78	3.10 ± 0.57	(p = 1.0000)
α 1.7	4.56 ± 0.53	14.60 ± 1.17	(p = 0.0000)
α 1.4	6.33 ± 0.50	7.20 ± 1.40	(p = 0.0968)
α 1.3	4.78 ± 0.44	4.30 ± 0.95	(p = 0,1859)
P 2	11.00 ± 1.87	6.80 ± 1.48	(p = 0.0000)
α 2.4	2.78 ± 0.67	13.20 ± 2.20	(p = 0.0000)
β 2	6.22 ± 1.09	10.60 ± 1.17	(p = 0.0000)
α 1.5	6.56 ± 1.13	9.80 ± 1.03	(p = 0.0000)
α 1.6	6.78 ± 1.20	4.90 ± 0.88	(p = 0.0011)
α 1.8	3.56 ± 0.53	4.60 ± 0.52	(p = 0.0004)
α 2.7	5.11 ± 0.33	6.10 ± 0.57	(p = 0.0003)
β 3	4.78 ± 1.20	4.80 ± 1.14	(p = 1.0000)
α 1.1	5.11 ± 0.93	4.80 ± 0.42	(p = 0.3509)
β 7	6.33 ± 0.71	6.10 ± 1.45	(p = 0.6684)
α 2.5	-	-	

SUMMARY OF MEAN PEAK HEIGHTS (mm) ± STANDARD DEVIATION

PEAK	CONTROL	DISTANT SEPTIC ABSCESS	
ALBUMIN	15.89 ± 1.27	10.60 ± 2.32	(p = 0.0000)
α 1.2	3.11 ± 0.78	3.50 ± 0.53	(p = 0.2162)
α 1.7	4.56 ± 0.53	22.40 ± 2.41	(p = 0.0000)
α 1.4	6.33 ± 0.50	6.89 ± 1.05	(p = 0.1724)
α 1.3	4.78 ± 0.44	2.30 ± 0.95	(p = 0.0000)
P 2	11.00 ± 1.87	5.75 ± 1.75	(p = 0.0000)
α 2.4	2.78 ± 0.67	10.90 ± 2.96	(p = 0.0000)
β 2	6.22 ± 1.09	10.90 ± 1.20	(p = 0.0000)
α 1.5	6.56 ± 1.13	9.40 ± 1.35	(p = 0.0010)
α 1.6	6.78 ± 1.20	4.10 ± 1.37	(p = 0.0003)
α 1.8	3.56 ± 0.53	3.60 ± 0.97	(p = 0.9215)
α 2.7	5.11 ± 0.33	5.60 ± 0.70	(p = 0.0734)
β 3	4.78 ± 1.20	5.30 ± 0.82	(p = 0.2810)
α 1.1	5.11 ± 0.93	6.20 ± 1.03	(p = 0.0278)
β 7	6.33 ± 0.71	6.50 ± 0.71	(p = 0.6167)
α 2.5	-		

APPENDIX D

Countercurrent Crossed Immunoelectrophoresis : Normal Animals

SAMPLE NUMBER	ALBUMIN	α1.2	α1.7	α1.4	α1.3	P2	α2.4	β2	α1.5	α1.6	α1.8	α2.7	β3	α1.1	β7	α2.5
N1	18	3	6	7	5	12	3	6	9	6	4	6	4	6	7	-
N2	19	3	3	7	5	12	4	8	9	8	4	6	6	6	7	-
N3	18	3	6	6	5	14	4	7	8	6	3	5	3	6	7	-
N4	21	3	5	7	5	14	4	7	8	7	3	5	3	6	6	-
N5	16	3	5	6	4	10	3	6	7	5	3	6	5	5	6	-
N6	20	3	3	6	5	10	2	6	10	8	5	6	5	5	7	-
N7	13	2	5	6	4	10	3	7	6	7	3	5	4	5	7	-
N8	16	3	4	6	6	12	-	6	9	6	4	5	4	6	5	-
N9	17	4	4	7	6	12	3	7	7	6	4	5	4	5	6	-
N10	16	3	5	6	5	11	4	7	7	7	3	5	5	5	7	-
Mean Values	17.4	3.0	4.6	6.4	5.0	11.7	3.3	6.7	8.0	6.6	3.6	5.4	4.3	5.5	6.5	-

Countercurrent Crossed Immunoelectrophoresis : Control Anima.

SAMPLE NUMBER	ALBUMIN	α1.2	α1.7	α1.4	α1.3	P2	α2.4	β2	γ1.5	α1.6	α1.8	α2.7	β3	α1.1	β7	α2.5
C1	16	3	4	6	5	12	2	7	7	5	3	5	4	4	7	-
C2	15	2	4	6	4	9	3	4	6	6	3	5	7	4	6	-
C3	16	4	5	6	5	11	2	6	7	9	4	5	5	5	6	-
C4	15	3	4	6	5	10	3	7	6	7	3	5	6	6	7	-
C5	18	4	5	7	5	11	3	6	5	6	4	5	5	6	7	-
C6	15	3	5	7	5	10	3	6	7	7	4	5	4	5	6	-
C7	17	4	5	7	5	12	3	8	9	8	4	6	5	6	7	-
C9	14	3	4	6	4	9	2	6	6	6	4	5	3	4	5	-
C10	17	2	5	6	5	15	4	6	6	7	3	5	4	6	6	-
Mean Values	15.8	3.1	4.5	6.3	4.7	11.0	2.8	6.2	6.5	6.7	3.5	5.1	4.7	5.1	6.3	-

Countercurrent Crossed Immunoelectrophoresis : Distant Steri
Abscess

SAMPLE NUMBER	ALBUMIN	α1.2	α1.7	α1.4	α1.3	P2	α2.4	β2	α1.5	α1.6	α1.8	α2.7	β3	α1.1	β7	α2.5
TA1	14	3	25	9	3	8	16	13	12	4	5	7	5	7	8	9
TA2	14	3	12	6	5	10	5	9	6	5	3	5	5	6	6	-
TA3	12	3	20	6	4	6	16	12	13	5	5	8	5	6	6	9
TA4	14	3	20	7	4	7	19	13	15	6	5	8	6	6	7	11
TA5	12	3	18	7	4	10	9	10	9	4	4	6	6	6	6	-
TA6	14	3	20	8	4	10	7	11	9	5	4	6	5	6	6	-
TA7	13	3	18	7	5	8	7	9	8	4	3	6	5	6	5	-
TA8	13	4	20	7	5	10	9	11	8	6	4	7	6	8	6	-
TA9	14	4	14	6	5	10	6	9	7	5	3	6	6	5	7	-
TA10	13	3	19	7	5	8	7	10	8	5	3	5	5	6	7	-
Mean Values	13.3	3.2	18.6	7.0	4.4	8.7	10.1	10.7	9.5	4.9	3.9	6.4	5.4	6.2	6.4	9.7

Countercurrent Crossed Immunoelectrophoresis : Transient Bacteraemia

SAMPLE NUMBER	ALBUMIN	α1.2	α1.7	α1.4	α1.3	P2	α2.4	β2	α1.5	α1.6	α1.8	α2.7	β3	α1.1	β7	α2.5
TRB1	18	4	5	7	6	14	4	8	7	7	4	6	6	6	8	-
TRB2	14	3	4	6	5	10	3	6	7	6	4	4	4	4	6	-
TRB3	14	5	3	5	7	12	3	6	6	5	3	5	4	5	6	-
TRB4	15	4	5	7	6	12	3	6	8	5	4	4	3	5	6	-
TRB5	15	3	5	6	4	12	4	7	8	6	3	5	3	5	5	-
TRB6	13	3	4	6	4	7	3	5	6	6	3	4	3	5	6	-
TRB7	16	3	6	6	4	11	3	7	8	6	4	4	4	5	6	-
TRB8	15	2	3	5	6	11	4	6	6	5	3	4	4	4	6	-
TRB9	16	4	4	6	6	10	4	7	6	6	3	5	3	5	7	-
TRB10	15	3	4	5	6	11	3	6	5	6	2	5	3	5	6	-
TRB11	14	3	4	5	5	10	2	7	7	6	2	5	4	4	7	-
MEAN VALUES	15.0	3.3	4.3	5.8	5.3	10.9	3.2	6.4	6.7	5.8	3.1	4.6	3.7	4.8	6.2	-

Countercurrent Crossed Immunoelectrophoresis : Local Sepsis

SAMPLE NUMBER	ALBUMIN	α1.2	α1.7	α1.4	α1.3	P2	α2.4	β2	α1.5	α1.6	α1.8	α2.7	β3	α1.1	β7	α2.5
LS1	13	3	13	6	4	7	11	9	9	4	4	6	4	5	5	-
LS2	11	3	16	7	3	6	13	9	8	4	4	6	3	5	3	-
LS3	15	3	14	8	5	8	16	11	11	6	5	7	6	6	8	-
LS4	16	4	16	8	5	9	17	12	11	6	5	6	5	5	7	-
LS5	15	4	14	6	6	9	12	10	9	5	5	6	5	5	6	-
LS6	11	3	13	5	3	5	12	10	9	4	4	5	4	4	5	-
LS7	14	3	15	8	5	7	15	11	10	5	5	6	5	5	7	-
LS8	12	3	14	7	4	6	13	12	10	5	4	7	7	5	7	-
LS9	13	3	15	7	4	5	13	10	10	6	5	6	5	5	6	-
LS10	15	2	16	10	4	6	10	12	11	4	5	6	4	4	6	-
Mean Values	13.5	3.1	14.6	7.2	4.3	6.8	13.2	10.6	9.8	4.9	4.6	6.1	4.8	4.8	6.1	-

Countercurrent Crossed Immunoelectrophoresis : Distant Septic Abscess

SAMPLE NUMBER	ALBUMIN	α1.2	α1.7	α1.4	α1.3	P2	α2.4	β2	α1.5	α1.6	α1.8	α2.7	β3	α1.1	β7	α2.5
DA1	10	4	25	-	1	-	10	12	8	2	4	5	6	7	7	-
DA2	6	3	23	7	1	-	10	11	8	3	2	5	4	5	7	-
DA3	10	4	22	7	2	5	12	11	9	4	4	6	6	6	6	-
DA4	13	4	27	7	3	5	9	13	11	4	3	6	5	7	7	-
DA5	9	3	19	6	2	4	7	9	8	3	3	5	4	5	5	-
DA6	11	3	20	6	3	7	8	10	9	5	3	5	5	5	7	-
DA7	10	3	22	6	2	5	10	10	9	5	4	6	5	6	7	-
DA8	14	4	24	9	3	7	17	12	12	6	5	7	6	7	6	-
DA9	10	3	21	6	2	4	12	10	10	3	3	5	6	6	7	-
DA10	13	4	21	8	4	9	14	11	10	6	5	6	6	8	6	-
Mean Values	10.6	3.5	22.4	6.9	2.3	5.7	10.9	10.9	9.4	4.1	3.6	5.6	5.3	6.2	6.5	-

APPENDIX E

Summary of rat protein profile

PEAK	PROTEIN NAME	FUNCTION
P2	Pre-Albumin 2	-
Alb	Albumin	General Transport
α1.1	-	-
α1.2	α1 Macroglobulin	-
α1.3	-	-
α1.4	Caeruloplasmin	Oxygen Scavenger, Cu transport, Ferroxidase activity
α1.5	-	-
α1.6	-	-
α1.7	α1 Acid Glycoprotein	Immune Regulation : repair and resolution
α1.8	-	Clotting protein
α2.4	-	-
α2.5	Haptoglobin	Binds and conserves Haemoglobin
α2.7	Haemopexin	Binds and conserves Haem
β2	-	-
β3	C3 Complement Component	Opsonisation and Chemotaxis
β7	Transferrin	Iron Transport

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