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By

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Glossary of Abbreviations

dB	Decibel
EAC	External auditory canal
HRP	Horseradish Peroxidase
LDF	Laser Doppler Flowmetry
LDPM	Laser Doppler Perfusion monitor
LS	Left side
NOE	Necrotizing Otitis externa
OE	Otitis externa
PU	Perfusion units
PPG	Photoplethysmography
RBCs	Red blood cells
RS	Right side
SPL	Sound pressure level
TM	Tympanic membrane

Abstract

The application of laser Doppler flowmetry in non-invasive measurement of blood perfusion in the human external auditory canal and tympanic membrane

A.Hamid El-Sawy

Laser Doppler flowmetry (LDF) has been used extensively over the last twenty years for measuring perfusion of the microcirculation in many clinical fields. One area that had not been studied extensively is in otology. In this study LDF was used to measure perfusion of the microcirculation in four sites of the external auditory meatus. Site 1: skin of the tragus. Site 2: skin of the cartilaginous part of the EAC. Site 3: skin of the deep bony part of the EAC. Site 4: the outer external surface of the tympanic membrane.

This study was carried out in three groups: Control subjects (n=43); otitis externa patients (n=20); Myringoplasty patients (n=18). Measurements were made in a controlled environment with standard technique using a Perimed 5000 LDF instrument. Data were presented as arbitrary perfusion units (PU) set by the manufacturer. In the control group, the results showed that there was significant variation in the difference between the four sites with a descending site order of 3:2:1:4.

The statistical distribution of the perfusion data showed that there was considerable variation between sites in their distribution patterns. No differences were apparent due to sex, age or core body temperature. There was no difference between right and left ear. There was no correlation between PU at each site, and intrasubject variability was not significant over two measures in n=13 subjects.

In the otitis externa patients very large increases in the median PU values for all sites were seen with up to seven fold increases in perfusion being measured. The site order changed to 3:2:4:1.

In the myringoplasty group no significant changes were seen pre- and post-operatively although individual patients exhibited substantial variation in individual pre- and post-site measurements.

These results provide the first detailed measures of perfusion in the external auditory meatus and tympanic membrane. Whilst these results and the LDF technique are of principally experimental interest, they provide a systematic basis for the use of LDF as a clinical tool in otology.

Declaration

This work is based on the work conducted by the author at ENT and Hearing services department, Leicester University Hospitals, University of Leicester during the period August 1998 and August 2000.

The work in this thesis is original, unless otherwise acknowledged in the text or by references.

Acknowledgements

I would like to thank all people who have been helping in the making of this thesis. Firstly, my supervisor and friend Dr Mike Mulheran for his support, scientific knowledge, enthusiasm, close supervision and continuing advice for every part of this thesis, and mainly for the great time he offered me to finish this work.

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Also, the Medical Physics department staff for their role in designing the head band and their help in any technical difficulties.

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Thanks also to all my friend and colleagues who took part in this study as candidates in the control subjects.

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Chapter 1

Introduction and Aims of work

1.1 Background

The laser Doppler technique was first used for studies of blood flow by Riva (1972) who recorded a Doppler shift in the backscattered light of a laser beam focused on retinal vessels. Stern (1975) interpreted the frequency broadening of back scattered light as that produced by Doppler shifts occuring when individual photons interacted with moving red blood cells.

Holloway (1977) described the first fibre optic based portable laser Doppler perfusion monitor. Over the last two decades, different laser Doppler flowmeter (LDF) instruments have become commercially available and widely found in experimental applications, due to their ease of use and non-invasive nature. Whilst there have been many clinical studies using LDF, this technique has not yet found widespread clinical application in diagnosis and management (Leahy *et al.,* 1999). The study described in this thesis was therefore primarily an experimental study using LDF to provide a measure of perfusion in the microcirculation of the external auditory meatus. This information could then be used to experimentally assess changes in perfusion in different pathological conditions.

The importance of blood to healthy living tissue was recognized since the earliest times of life. It was stated that blood flow provided the tissues with heat and life. Harvey (1578-1657) stated that, if the heart is functioning normally, life and health could be restored in the body. He also said that, many serious diseases gain access to the body when it is suffering from malnourishment and lack of warmth. Today, blood circulation is fully recognised as being necessary for maintenance of tissue vitality, growth, repair of injuries and sustaining the functions of different tissues.

1.2 Blood flow in the ear

Visual inspection of the external auditory canal and tympanic membrane is usually sufficient to determine colour, shape, position and motility of the tympanic membrane. However, such examination fails to define the adequacy of blood flow and nutritive capillary supply present (Schops *et al.,* 1987). The microcirculation plays a central role in the haemostatic control of the tissues by regulating the metabolic, haemodynamic and thermal state.

It is essential to measure blood flow at the microcirculatory level, to find out the changes in tissue perfusion in health and in the presence of different diseases or injury. Tissue blood flow can be considered as comprising both nutritional and non-nutritional flow. Changes in the non-nutritional component in skin at the body surface acts as a thermoregulatory mechanism. The nutritional component of the blood flow is small when compared to the total blood flow in the cutaneous tissue circulation. This component is the most important clinically for maintaining the normal function and different metabolic processes in tissues and its decrease below certain critical levels affects the tissues viability and leads to a variety of pathological alterations (Swain and Grant, 1989).

It is apparent that there is a clinical need for a continuous and reliable method to study the microcirculation. Several methods have been known and tried for assessment of tissue blood flow. Most of these methods applied in the past had drawbacks of being invasive and caused some alterations in the microcirculation, which affected the results. These methods include plethysmography; dye injection; thermocouples; isotope clearance; isotopically labelled microspheres, hydrogen washout and scintigraphy (Schabauer and Rook, 1994).

Laser Doppler flowmetry has been used extensively in microvascular research and clinical assessment of tissue perfusion over the last 20 years and also used for measuring blood flow in the tympanic

membrane in normal controls (Schops *et al.,* 1987). However, no studies have systematically used LDF to measure blood flow in the external auditory canal.

1.3 Aims of this study

In the absence of systematic studies in the literature, the aims of this study were as follow:

1. To evaluate the laser Doppler as a reliable and reproducible method for measuring the microvascular blood flow in the external auditory canal and tympanic membrane.

2. To study the changes between blood flow in normal tympanic membrane and external auditory canal and that in different inflammatory conditions of otitis externa.

3. To quantify the changes in tympanic membrane blood flow before and after myringoplasty and to what extent the degree of blood flow was related with the success rate of the operation.

Chapter 2

Review of literature

2.1 Anatomy and blood supply of external auditory canal and tympanic membrane

2.1.1 External auditory meatus

The meatus extends from the concha to the tympanic membrane. Its length from the floor of the concha is approximately 2.5cm and from the tragus about 4cm. It has two structurally different parts, the lateral 1/3 cartilaginous and the medial 2/3 osseous.

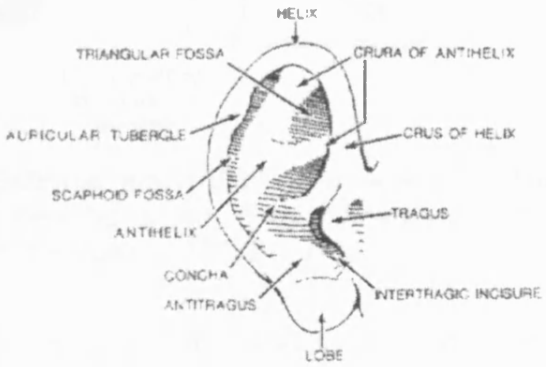

Figure 2.1. *Anatomical subdivisions of the pinna (Taken from: Alvord LS, Farmer BL. Anatomy and orientation of the human external ear. J Am Acad Audiol. 1997 Dec; 8(6):383-90).*

The ear canal has an S-shaped curve, at first directed anteromedially and up (outer part), then posteromedially and up and finally anteromedially and slightly down (inner part). The canal is oval in section with the oblique diameter directed posteroinferiorly, but nearly horizontal medially with two constrictions, one near the medial end of the cartilaginous part and the isthmus constriction in the osseous part about 2 cm from the bottom of the concha. The canal is closed medially with the tympanic membrane and its floor and anterior wall are longer than its roof and posterior wall.

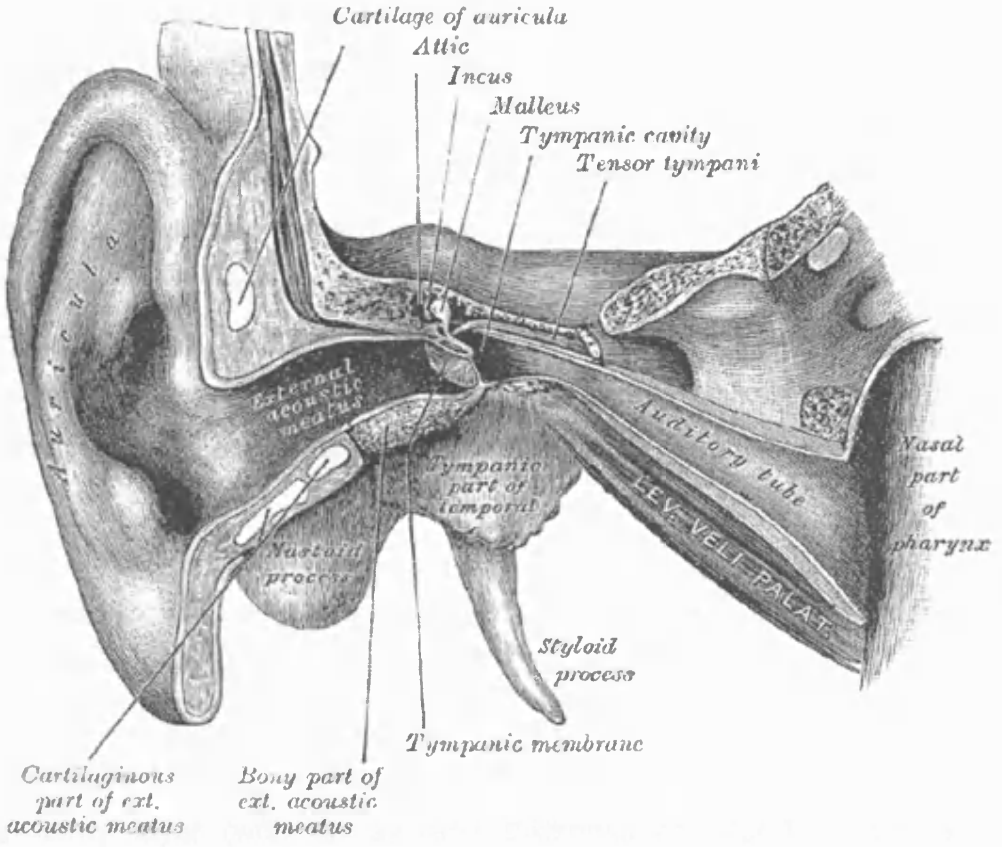

Figure 2.2. *External and middle ear anatomy (Taken from Peter L. Williams. The hearing organ, Gray's Anatomy : The Anatomical Basis of Medicine and Surgery, 38th Ed, (1995) Churchill Livingstone)*

The outer cartilaginous part is about 8mm long and is continuous with the auricular cartilage attached with the osseous circumference with fibrous tissue. The osseous part is about 16mm long and narrower than the cartilaginous part, with the anterior wall longer than the posterior wall by about 4mm and marked by the tympanic sulcus to which the tympanic membrane is attached. The osseous part is formed anteriorly, inferiorly and posteriorly by the tympanic part of the temporal bone, and posterosuperiorly by the temporal squamous bone.

The skin of the auricle is continuous into the ear canal and covers also the external surface of the tympanic membrane. The canal skin is thin, closely adherent to the cartilage and bone of the canal with absence of dermal papilla.

The subcutaneous tissue of the outer cartilaginous 1/3 is thick, with numerous ceruminous glands resembling the coiled tubular structures of the sweat glands. The glands' secretory cells are covered externally by myoepithelial cells and are either cuboidal or columnar when active. Their ducts open on the epithelial surface or into the nearby sebaceous glands of the hair follicles. There is a great variability of the skin anatomy due to regional differences, development, age, or effects of different diseases. However, there are general characteristics of all skin sites; divided into: epidermis; dermis; subcutaneous tissues.

2.1.2 The epidermis

The epidermis is only about 120 µm thick and contains many cells in different stages of differentiation (Forslind *et al.*, 1997).

The outermost layer of the epidermis is known as the stratum corneum or horny layer (with an average thickness of 30µm) (Leider and Tanenbaum, 1969). The epidermis consists of four layers, the basal, prickle, granular and horny layers. The basal layer is innermost consisting of a single row of columnar epithelial cells at the junction of the epidermis and the dermis. The epidermis is free from any capillaries and other blood vessels.

Cells in the basal layer of the epidermis undergo mitosis and, of the two daughter cells produced, one remains within the basal layer and the second migrates upward through the layers to the surface. This upward progression takes about 28 days and is accompanied by major changes in cellular content that lead to distinct layering in the appearance of the epidermis under the microscope (Tortora and Grabowski, 2000). Keratin production begins in the stratum spinosum. This continues through the stratum granulosum, where the cells lose their nuclei and die. The stratum lucidum and stratum corneum are composed of dead cells, packed with keratin and surrounded by lipid tissue.

In addition to the epidermal cells, or keratinocytes, the basal layer of the epidermis contains melanocytes. These cells produce melanin, which is inserted into neighbouring keratinocytes and provides protection against ultraviolet radiation. In Caucasian skin, the melanin tends to disintegrate as the epidermal cells move up through the layers of the epidermis (Burton, 1990).

2.1.3 Accessory structures of the epidermis

The epidermis also contains a number of accessory structures. Hair shafts are composed of a layer of epidermal cells that project down below the dermis. At the root of the growing hair is a knot of blood vessels. Also, associated with the hair shaft are sebaceous glands that produce sebum, a lipid secretion. These glands are most active in the hair follicles of the head, neck, back and chest (Burton, 1990). Eccrine sweat glands are found within the dermis, but their ducts travel through the epidermis to release sweat onto the skin surface.

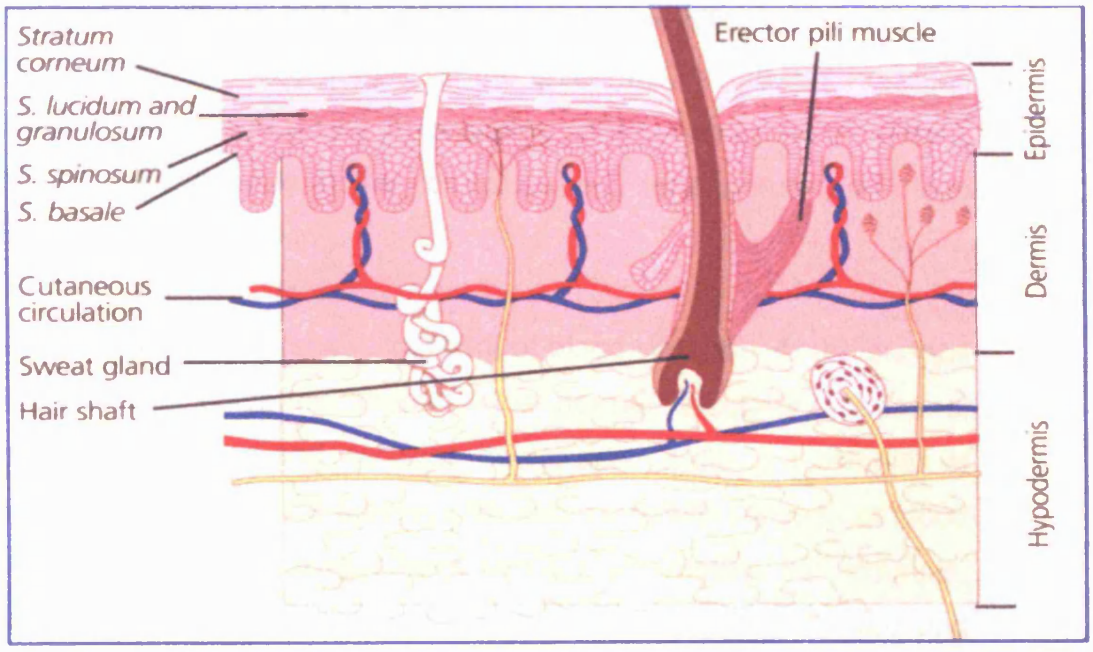

Figure 2.3 *The structure of the skin, epidermis, dermis and hypodermis.*

2.1.4 The dermis

While the cells of the epidermis are numerous and closely linked, those in the dermis are separated by a complex mesh of extracellular material. The main cells of the dermis are fibroblasts, but there are also immune and inflammatory cells, nervous tissue and blood vessels (Tortora and Grabowski, 2000). Collagen, elastin and other extracellular fibres are the major constituents of the dermis. These give the skin strength and flexibility (Clark, 1996).

The dermis lies below the epidermis with an average thickness of 3mm. The dermis is tough, elastic connecting type of tissue with a profuse network of glands, hair follicles, anastmosing capillaries, blood vessels, lymphatics and nerves. The dermal papilla, a nipple like projection rising into the epidermis contains the capillaries that are responsible for epidermal nutrition for the formation of new cells in the basal layer.

2.1.5 Functions of the dermis

The dermis provides structural strength and flexibility to the skin. It also contains the blood supply for the epidermis, which has no blood vessels of its own. The system of capillaries and venules in the dermis plays an essential role in the control of body temperature and blood pressure.

The dermis has an extensive network of blood vessels. At the surface closest to the epidermis there is the normal circulatory series of arterioles, capillaries and venules. Lower down there is a complex of deep veins that acts as a reservoir for approximately 1.5 litres of blood (Bray, 1999). Under sympathetic nervous stimulation, for example, during hypotension or haemorrhage, these veins are constricted and the blood they contain is pushed into the general circulation. At the same time, blood flow to the skin is restricted causing a pale, cool and mottled appearance (Porth, 1998). Skin is able to survive with

relatively little oxygen supply and, therefore, can tolerate reduced blood flow much better than other body tissues (Bray, 1999).

The skin of the hands, feet and face contains a large number of arteriovenous anastamosis. These blood vessels bypass the capillary bed. When the core body temperature increases, sympathetic nervous input to the skin is decreased, allowing these vessels and those of the deep vein complex to dilate (Bray, 1999). This leads to an increase in blood flow near to the body surface, allowing heat to be lost across the skin. If sympathetic stimulation is increased, more body heat will be retained, causing a rise in core body temperature.

2.1.6 The subcutaneous tissue

The subcutaneous tissue lies below the dermis and consists mainly of fat with variable thickness from site to site.

2.1.7 Blood supply

The blood supply to both epidermis and dermis is through a very rich anastamosing superficial and deep plexus of small vessels. The complex capillary beds are supplied and drained of blood via underlying metarterioles, arterioles and venules (average diameter 50μm) that originate from longer arteries and veins deeper in the fatty subcutaneous tissue (Conard, 1971).

Anastamosis directly connecting pathways between arterial and venous vessels and further branching of vessels gives rise to a network of horizontal capillaries lying parallel to the surface of the skin in the subcapillary plexus. Pure capillaries devoid of muscle arise from subcapillary plexus to supply the papillae in the upper dermis.

2.2 Capillary blood flow

The average capillary loop is 0.2-0.4 mm in length and supplies a skin surface area of about 0.04-0.27 mm^2. The average distance between loops is 50-100 um (Curri, 1990). The diameter of the lumen of a

single capillary loop is just larger than that of RBCs, which can only move in a single file through the capillary. The average diameter of the capillary lumen between 8-10 um (Curri, 1990), and movement of RBCs through the capillary is characterized by strings of RBCs separated by thin columns of plasma. RBC movement is unidirectional and occurs over the entire length of the capillary. RBCs in the capillary network are dilute and the maximum blood volume in the capillary plexus has been found to be less than 0.5% (Fagrell *et al.,* 1980).

Blood flow to the skin is highly variable, especially in areas rich in arteriovenous anastamosis. Values for total skin blood flow measurements ranging from 1.3 to 13 ml/m²/s have been reported which is a very large range. Blood flow in the capillary loops is susceptible to spontaneous periodic fluctuation (of the order 2-6 cycles/min), which are not related to respiration or heart rate (Fagrell *et al.,* 1977). This is a characteristic of the vasomotor oscillation involving temperature control (Finley and Nugent, 1983) and appears to be a powerful, vital function of the autonomic nervous system.

2.3 Sub-capillary blood flow

Blood flow in the arterioles and venules lower down in the sub-capillary plexus is very different from that described for the capillary network. Although arterioles and venules have straight sections over much longer distances than capillaries, the RBCs contained within these vessels do not always follow straight patches (Gush *et al.,* 1984). This is a direct consequence of the asymmetric structure of the RBCs. Cells can not simply be considered as rigid spheroids, but rather as flexible distorting discs which can flip and turn.

2.4 The microcirculation

The microcirculation consists of an extensive capillary network and its associated structures (arterioles and venules) Figure 2.4.

The structure of the vascular bed varies considerably from one area to another in the human body. In most areas, blood enters the skin through small arteries penetrating the subcutaneous tissues obliquely to the skin surface. One small artery most often branches into several precapillary arterioles (30-80 um), which pass through one to three layers of venous plexuses that are parallel to the skin surface. The arterioles may divide into as many as ten terminal capillary loops, one to three of which are located in every skin papilla (Fagrell, 1984).

Blood enters the microcirculation through arterioles, which are surrounded by a thick continuous layer of smooth muscle. Contraction of smooth muscle reduces the internal diameter of this microvessel, and consequently increases the resistance to blood flow in the entire vascular bed. This feature makes the arteriole the major resistance element in the circulation and the principle determinant of the total peripheral resistance. Arteriolar smooth muscle tone also governs the amount of pressure transmitted from arteries to veins; hence capillary pressure falls when arterioles constrict and rises when arterioles dilate.

Blood flows from arterioles into a narrower vessel, the metarteriole, which is surrounded by a discontinuous smooth muscle layer and capillaries branch off from the metarteriole. The density of capillaries, which is an important determinant of the total area available for exchange between blood and the tissue, varies significantly between organs, with the lung exhibiting the largest capillary area ($3500cm^2/g$) compared with muscle ($100cm^2/g$). Importantly, the junction between the metarteriole and some capillaries is encircled by a simple band of smooth muscle called the precapillary sphincter. These sphincters determine the percentage of capillaries open to blood perfusion (see figure 2.4). Although all capillaries are normally open to perfusion and exchange in tissues like the heart, only 29% to 30% of capillaries are normally open in skeletal muscle and skin. In the latter tissues, relaxation of the precapillary sphincter allows for the recruitment of

more open capillaries and hence greater transcapillary exchange (Granger, 1998).

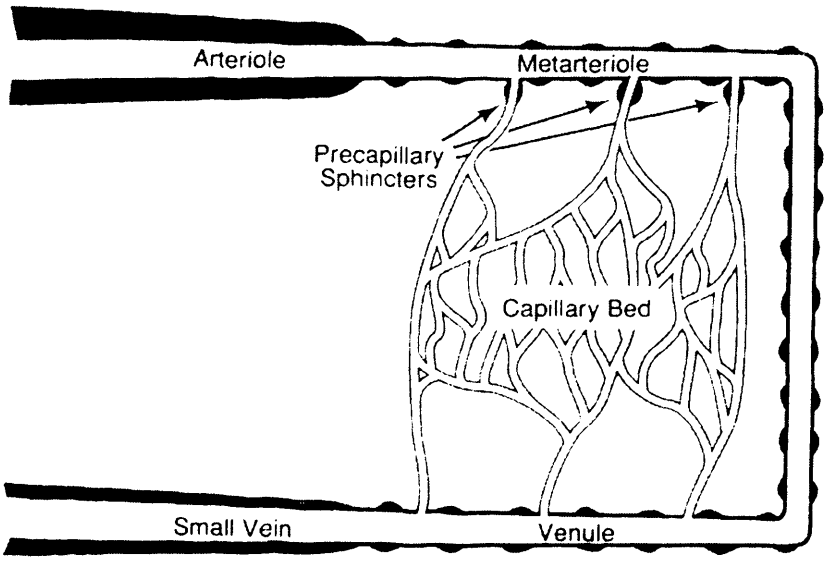

Figure 2.4. *Diagram of microcirculation showing the position of the precapillary sphincters. (Taken from Granger DN. Capillary Exchange (1998). In Essential Medical Physiology. 2nd Ed., Chapter 16, 218, Leonard R Johnson, by Lippincott-Raven Puplishers.*

Capillaries coalesce into a venule, which possesses a discontinuous, thin coat of smooth muscle that drains into small veins. Changes in venular smooth muscle tone can exert a significant influence on capillary exchange in as much as constriction of venules leads to an increased capillary pressure, whereas dilation of venules exerts the opposite effect.

The main purpose of cutaneous microcirculation in humans is to regulate the body temperature, and marked variation in the skin flow is necessary to accomplish that function. Only a very small portion of blood entering the skin is required to meet its metabolic need. The relative distribution of blood between the nonnutritional, thermoregulatory vascular bed and the nutritional papillary capillaries

also differs markedly from one area to another. For example, in fingers and toes, more than 90% of the blood flows only through the subcapillary vascular bed, and 10% or less flows through the nutritional capillaries (Figure 2.5).

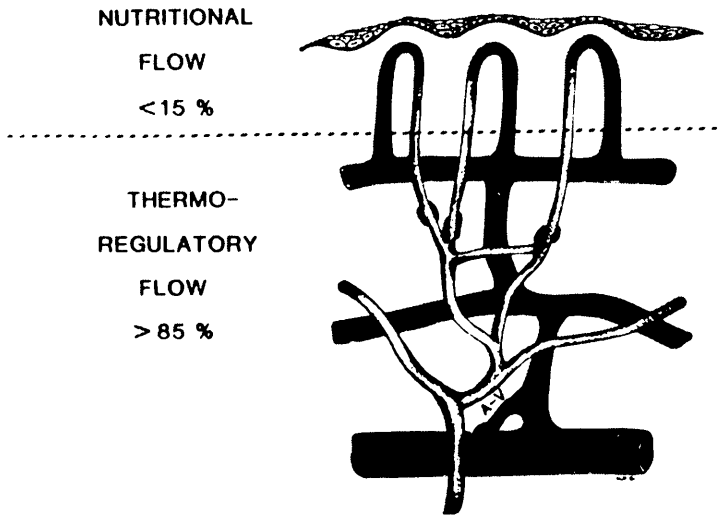

Figure 2.5. *Difference between nutritional and thermoregulatory blood distribution in human skin microcirculation (taken from Fagrell B, Peripheral vascular disease (1990). In Laser Doppler blood flowmetry, 1st ed., Chapter 11, 210. Kluwer Academic Puplishers.*

During cold exposure, microcirculatory flow in the skin may be extremely low, and it increases successively with skin temperature. A sudden and rather abrupt increase is seen at temperature of around 31-32°C (Fagrell, 1984). The flow in some areas can vary by a factor of 100-200. These marked variations in skin blood flow are possible because of the numerous arterio-venous anastamosis, or arterio-venous shunts, that allow the blood to be shunted directly from the small arteries and arterioles into the numerous sub-papillary veins and venules. With this unique arrangement, the temperature regulation of the body may be fulfilled with only a very limited change of blood flow in the nutritional skin capillaries (Fagrell, 1984). This role of control of thermoregulation is very likely to be reflected in ear canal and tympanic membrane blood flow.

2.5 The optical properties of the skin

The interaction between light and skin tissue involves two different processes, namely absorption and scattering. The detailed knowledge required for modelling and quantitatively assessing the transfer of optical radiation within the skin and microvascular structure is, however, extremely difficult to obtain because of the huge range of different cutaneous structures present among individuals. Nevertheless, there are some general qualitative bases about the optical properties of skin, which can be applied to most types of skin tissue.

2.5.1 Optical properties of the stratum corneum

Optical radiation impinging on the surface of the skin must pass through the stratum corneum before reaching the microvascular beds in the tissue below. The thickness, composition and morphology of the stratum corneum determines how much light enters the epidermis and beyond. At near normal incidence, a small fraction of light is reflected due to the change in the refractive index from air (na = 1) to the stratum corneum (ns = 1.55). This is often referred to as "Regular reflectance" and has been estimated to be between 4-7% of the total incident light (over the spectral range 250-3000 nm) for both black and white skin (Anderson and Parish, 1981a).

The optical interface between air and tissue also accounts for the presence of some diffuse backscattered light at the skin surface due to internal reflection. Because the stratum corneum has a relatively rough morphology, irregular reflectance from this surface cannot be considered specular. Thus, an incident beams from collimated radiation (such as that from the Laser) passing through this layer is reflected and made more diffuse. The bulk of the incident light, which is not reflected at the surface, passes through the epidermis and dermis and where it is absorbed and scattered. These two (Wavelength dependent) processes will determine the penetration depth of optical radiation into the skin.

Physical inhomogenecities in the cutaneous structures (such as RBCs, macrophages and blood vessels) provides inhomogenecities in the medium's refractive index, which account for varying degrees of light scattering. The extent to which light is absorbed in the skin is principally dependent on type and concentration of chromophores present and the spectral properties of the incident light.

2.5.2 Optical properties of the epidermis

In the avascular epidermis, scattering is relatively weak, compared to the effects of optical absorption by chromospheres. Transmission of visible light through the stratum corneum and epidermis is almost completely determined by the content and spatial distribution of the pigment melanin (Hardy *et al.,* 1965). At He-Ne laser wavelength (633nm) the transmittance of full thickness epidermis can vary by a factor of 5, from fair skinned Caucasians to dark skinned Negroes, due to the larger quantities of melanin present in the latter (Wan *et al.,* 1981a&b).

2.5.3 Optical properties of the dermis

The dermis, in which the intricate networks of microvascular beds are located, has distinctly different optical properties than the epidermis. Studies have shown (Anderson and Parrish, 1981b), that the dominant source of scattering is from cells (such as RBCs and macrophages), blood vessels (such as capillaries, arterioles and venules) and fibrous tissue (such as collagen fibres). Since the dimensions of RBCs are an order of a magnitude larger than the wavelength of optical radiation, most of the radiation incident on a single RBC will be strongly scattered in the forward direction as a function of inverse wavelength (Bonner and Nossal, 1980). If the scattering is strong, then most photons will undergo multiple scattering from the skin. This means that the spectral distribution of the light through the dermis will soon become isotropic in nature, regardless of the orientation of the microvascular bed.

The dermis can therefore be considered as a complex tissue matrix, where the degree of scattering is a function of inverse wavelength. It is the relationship that mostly defines the optical penetration depth. Depending on the wavelength of light passing through the dermis there may be also significant absorption effects. Major absorbers in the dermis include blood related pigments such as haemoglobin, oxyhaemoglobin, bilirubin and beta-carotenes, which are strongly absorbed in the short wavelength part of the spectrum.

Conversely, an "optical window" exists in the dermis in the spectral region 600-1000 nm. Anderson and Parish (1981b) studied in details the combined effect of absorption and scattering on the penetration depth of light through the skin at various wavelengths. Their results show that the penetration depth of red light (700 nm) in Caucasian skin was approximately 750 μm. This contrasted sharply with the value they obtained for blue light (400 nm), which is approximately 90um.

2.6 Vascular anatomy of external auditory canal

2.6.1 Arteries

These consist of the posterior auricular branch of the external carotid artery, the deep auricular branch of the maxillary artery and the auricular branches of the superficial temporal artery.

2.6.2 Veins

The veins drain into the external jugular and maxillary veins and the pterygoid plexus.

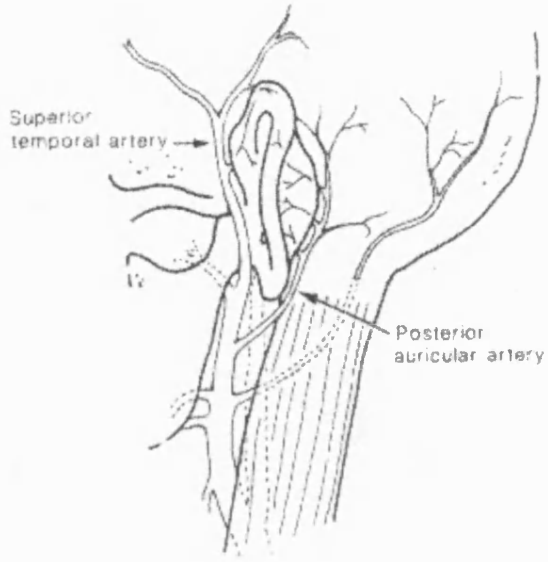

Figure 2.6. *Major arterial blood supply to the pinna (Taken from MiyamotoRT, Miyamoto RC. (1995). Pathology of the ear canal. Ballachanda BB, ed. The human ear canal. San Diego: Singular Puplishing Group, 64.)*

2.6.3 Lymphatics

They drain with the lymphatics of the auricle into the parotid lymph nodes especially the node in front of the tragus, the upper deep cervical lymph nodes and the mastoid lymph nodes.

2.6.4 Nerves

The nerve supply derived from the auriculotemporal branch of the mandibular, which supplies the anterior and superior walls of the meatus, the auricular branch of the vagus, innervating the posterior and inferior walls.

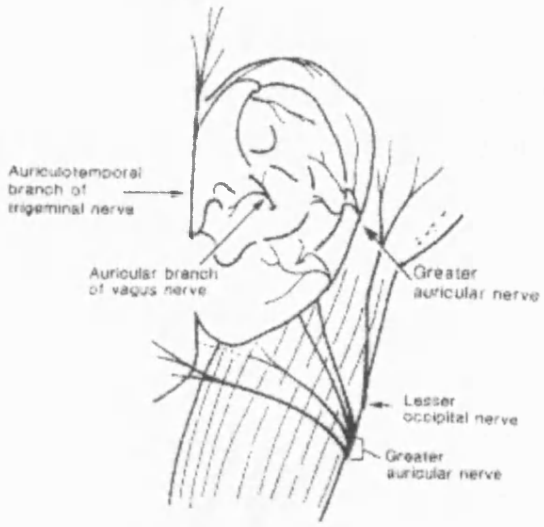

Figure 2.7. *Sensory nerves to the lateral surface of the ear (Taken from Goycoolea MV, Paparella MM, Nissen RL (1989). Atlas of Otologic Surgery. Philadelphia: WB Saunders, 9.)*

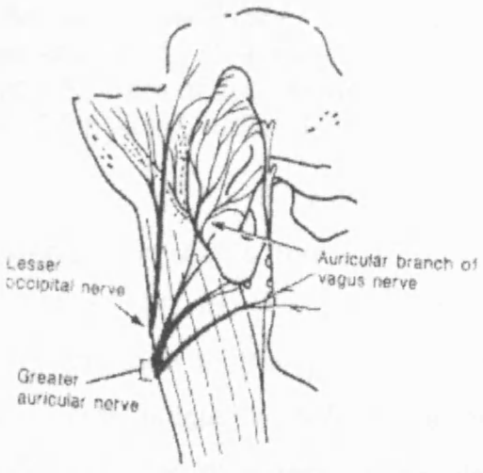

Figure 2.8. *Sensory nerves to the medial surface of the ear (Taken from Glasscock ME, Shambaugh GE. (1990). Surgery of the ear. 4th ed. Philadelphia: WB Saunders, 37)*

2.7 The tympanic membrane

The tympanic membrane separates the tympanic cavity from the external meatus. It is thin and semi-transparent, almost oval, though somewhat broader above than below. It is placed at an angle of about 55° with the meatal floor.

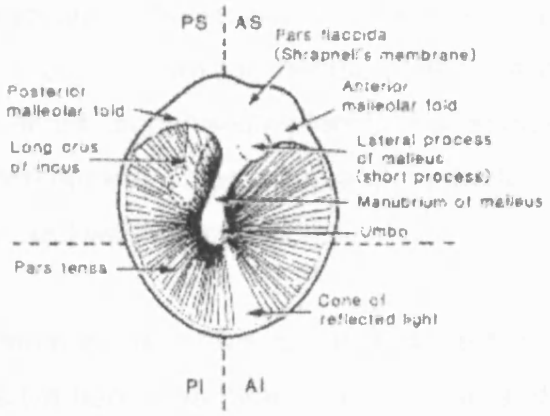

Figure 2.9. *Right tympanic membrane. Four quadrants: PS=postero-superior, AS=antero-superior, PI=posteo-inferior, AI=antero-inferior. (Taken from: Alvord LS, Farmer BL. Anatomy and orientation of the human external ear. J Am Acad Audiol. 1997 Dec; 8(6):383-90).*

Its longest antero-inferior diameter is from 9-10 mm, and its shortest is from 8-9 mm. Most of its circumference is a thickened fibrocartilaginous ring attached to the tympanic sulcus at the medial end of the meatus. This sulcus is deficient superiorly, where the anterior and posterior malleolar folds pass to the lateral process of the malleus, leaving between them the triangular pars flaccida, a thin lax part of the membrane. The membrane is elsewhere taut, the pars tensa.

The handle of the malleus is firmly attached to the membrane's internal surface as far as its centre, the umbo, which projects towards the tympanic cavity. Though this membrane as a whole is convex medially, its radiating fibres are curved with their concavities directed upwards.

2.7.1 Microstructure

Histologically, the tympanic membrane has three strata, an outer cuticular, an intermediate fibrous and an inner mucous. The cuticular stratum is continuous with the thin skin of the meatus and is keratinized, stratified squamous in type, devoid of the dermal papilla and hairless. Its subepithelial tissue is vascularized and may develop a few peripheral papillae. The fibrous stratum has an external layer of radiating fibres diverging from the handle of the malleus and a deep layer of circular fibres, peripherally plentiful but sparse and scattered centrally in the membrane. Marginally and centrally a fine network of elastic fibres is mixed with the collagen.

The mucous stratum is part of the mucosa of the tympanic cavity; it is thickest near the tympanic membrane's upper part and is covered by a layer of ciliated columnar epithelial cells. However, cilia occur only in patches or are entirely absent and are then replaced by a low cuboidal or simple squamous epithelium.

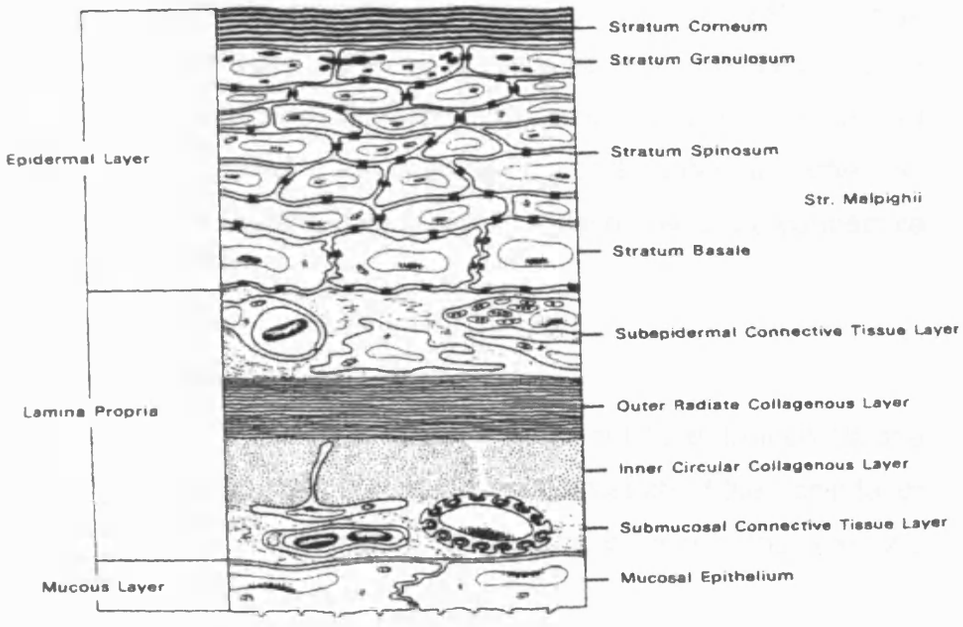

Figure 2.10. *anatomy of the layers of the tympanic membrane (Taken from: Lim DJ: tympanic membrane: EM Microscopic observation. Acta Otolaryngologica, 66:182, 1968).*

Electron microscopy has revealed that, the external epithelium is approximately 10 cells thick and has two zones, a superficial of non-nucleated squames and a deep zone like the epidermal stratum spinosum, with numerous desmosomes between cells, the deepest of which lie on a continuous basal lamina, but lack epithelial pegs and hemidesmosomes (Lim, 1995).

The internal layer is a single layer of very flat cells, with overlapping interdigitating boundaries carrying desmosomes and tight junctions between cells. Their cytoplasm contains only a few organelles; the luminal surfaces of these apparently metabolically inert cells have a few irregular microvilli and are covered by an amorphous electron dense material. Ciliated columnar cells are absent (Lim, 1995).

Most interestingly, the intermediate stratum contains filaments about 10μm in diameter, with links between filaments at 25nm intervals. The filaments are dispersed in outer radial and inner non-radial zones, the former more numerous; neither resembles collagen or elastin. Large fibroblasts occur between the external radial fibres and basal lamina of the external epithelium, while blood capillaries and their basement membranes lie just deep to the basal lamina of the internal epithelium. In the pars flaccida the fibrous stratum is replaced by loose connective tissue (Lim, 1995).

2.7.2 Arteries of the tympanic membrane

They arise from: the maxillary artery's deep auricular branch (to the outer, cuticular stratum) and the stylomastoid branch of the occipital or the posterior auricular artery and the tympanic branch of the maxillary to the tympanic mucosa.

2.7.3 Veins of the tympanic membrane

The superficial veins drain to the external jugular; those in the deep surface drain partly to the transverse sinus and dural veins and partly to the venous plexus of the pharyngotympanic tube.

2.7.4 Sensory nerve supply of the tympanic membrane

This is from the auriculotemporal branch of the mandibular nerve, the auricular branch of the vagus, the tympanic branch of the glossopharyngeal and possibly from the facial nerve.

2.7.5 Structure and function of the tympanic membrane

Detailed morphological descriptions of the tympanic membrane have been made by a number of investigators (Lim, 1968a&b, 1970; Johnson and Hawke, 1985; 1987; Hentzer, 1969; Youngs & Hawke, 1988; Chole and Kodama, 1989; Hartwein *et al.,* 1990; Ruah *et al.,* 1991; Von Unge *et al.,* 1991; Igarashi and Kawamata, 1993).

Although there are great variations in the size and thickness of the tympanic membrane among different species, the membrane of all laboratory animals and humans has two parts: pars flaccida (Shrapnell's membrane) and pars tensa. The latter is characterized by its unique fibre organization. The pars flaccida is continuous with the external canal skin and connective tissue layer. Regardless of the parts, it is important to understand that the tympanic membrane is composed of an epidermis, a connective layer (lamina propria) and a mucosal epithelial layer. Like all body tissue it undergoes changes during aging.

2.7.6 The epidermis of the tympanic membrane

The epidermis of the tympanic membrane is a typical keratinizing epithelium devoid of skin appendages, such as hair follicles and glands. The epidermis of the tympanic membrane is composed of a stratum corneum, stratum granulosum, a stratum spinosum, and a stratum basal. The cells of the stratum basal undergo cell division and

keratinization and migrate upward to become cells of the stratum granulosum and corneum, and ultimately are slough off as desquamated cornified cell debris.

The exact mechanism governing the cell cycle regulation (proliferation and quiescence), and the cell migration in the epidermis of the tympanic membrane is not yet fully elucidated (Lim, 1995).

It has been known that the epidermis of the human tympanic membrane migrates centripetally from the umbo and outwards to the external meatus. This pattern is suggested to be the self-cleaning mechanism of earwax and desquamating cell debris (Litton, 1968). The migration pattern of the epidermal layer of tympanic membrane has been investigated by a number of investigators (Litton, 1968; O'Donoghue, 1983 a&b; Michaels & Soucek, 1990). Using an ink dot technique, the pattern and rate of the epithelial migration in children with a normal tympanic membrane were examined and it was found that the epithelial migration occurred in a predominantly postero-superior direction at a mean daily rate of 131 microns per day. The umbo showed the greatest migratory rate (O'Donoghue, 1983a). The investigators observed the epithelial migration centre to be in the region of the umbo, manubrium, and short process of the malleus.

2.7.7 The lamina propria

Morphological studies indicate that the lamina propria of Shrapnell's membrane is largely made up with a loose connective tissue containing collagen and elastic fibres, and an intricate external and internal vascular plexus of blood capillaries, arteries, veins and nerve networks. The abundance of elastic fibres in the Shrapnell's membrane may account for the flaccid nature of that membrane.

The lamina propria of the pars tensa is made up of a subepidermal loose connective tissue layer which contains an external vascular plexus and a nerve network (both mylenated and unmylenated), a thin

submucosal loose connective tissue layer containing an internal vascular plexus and a nerve network, and a midfibrous layer which is composed of outer radial and inner circular fibres, in addition to parabolic fibres (Shimada & Lim, 1971).

2.7.8 Vascular supply of the Tympanic membrane

The vascular supply of the human tympanic membrane forms an external plexus, which derives from the tympanic branch of the deep auricular artery (branch of the maxillary artery), and an internal plexus, which derives from the stylomastoid branch of the posterior auricular artery. In the external plexus, the deep auricular branch sends one or two manubrial branches from above along the Shrapnell's membrane and along the manubrium in the pars tensa, the numerous small radial branches into the tympanic membrane from the circumference of the membrane.

The superficial veins open into the external jugular veins, and those on the inner surface drain partly into the transverse sinus and the veins of the dura mater, and partly into the plexus of veins of the Eustachian tube (Mawson, 1968). A number of other investigators studied the vascular supply and blood flow in the tympanic membrane in humans and animals (Albin *et al.,* 1985; Maher, 1988; Uno *et al.,* 1990; Masutani *et al.,* 1991; Yamagushi *et al.,* 1990; Triana *et al.,* 1990). The microvasculature of the neonatal mongrel dog tympanic membrane was investigated by Maher (1988); he observed close similarities between the dog and human tympanic membrane.

The pattern of the microvasculature of the guinea pig tympanic membrane, using scanning electron microscopy and resin casting method, was investigated and it was found that the vascular distribution is closely parallel with the radial fibres (Masutani *et al.,* 1991). Using light microscopy with India ink or coloured gelatine, and electron microscopy with horseradish peroxidase (HRP), Uno *et al.,* (1990) investigated the blood supply of guinea pig tympanic membrane and

described two arterial sources: the superior and inferior tympanic arteries, both of which arise from the posterior auricular artery. The superior tympanic artery gives off arterioles running centrifugally from the attachment of the manubrium, whereas the inferior tympanic artery gives off arterioles running centripetally from the tympanic annulus. Both sets of arterioles form a monolayered polygonal meshwork and drain into either the superior tympanic vein located at the manubrium or the inferior tympanic vein at the annulus (Uno *et al.,* 1990).

A number of investigators used several different techniques to study *in vivo* the blood circulation in humans and laboratory animals. Applebaum and Deutch (1985), using continuous xenon light source successfully measured endoscopic fluorescein angiography of normal human tympanic membranes. They showed that the mallear artery is apparently the major blood supply to the posterior half of the tympanic membrane, which is consistently better perfused than the anterior half; whereas branches of the blood vessels from the annular ring apparently supplied the anterior half of the tympanic membrane.

Schops *et al.,* (1987) successfully used the laser Doppler flowmetry to study the microcirculation of the human tympanic membrane and showed a steady value ranging from 70 to 120 (arbitrary perfusion units) as well as spontaneous oscillations due to rhythmical vasomotion.

Triana *et al.,* 1990, using intravital fluorescence microscopy with FITC labelled dextran, investigated the guinea pig tympanic membrane blood flow. The blood flow of the manubrial artery was measured to be 0.044 +/- 0.001 mm/min. The blood flow from the manubrial artery supplying the superior quadrants was 0.18+/- 0.01 mm/ min) and was statistically greater (p less than 0.001) than that of the inferior quadrants which was measured to be 0.08+/- 0.007 mm/ min (Triana *et al.,* 1990).

2.8 Methods of blood flow measurement in tissues

A variety of techniques have been used for a long time to study blood flow in blood vessels of the limbs and different organs. These techniques include methods based on measurement of volume and pressure changes, such as venous occlusion plethysmography and others based on X-ray to map bulk blood flow. Also, ultrasound and electromagnetic induction devices have been used to characterize flow in large single blood vessels. However, it is difficult to obtain information about state of microcirculation using any of these techniques. The principles of these techniques are based on different physical phenomena as changes in optical conductance, electrical impedances, or changes in temperature due to alterations in vascularity.

The main problem in tissue blood flow measurement is the effects of the technique on circulation. The ideal technique for microcirculation blood flow measurement should produce a continuous measurement without altering or disturbing the tissues. Also, our knowledge of microcirculation is based on animal studies and applications of these techniques to humans need further work. Clinical problems of inflammation, infections, allergies, trauma and healing involve changes in blood flow. Consequently, measurement of blood flow perfusion in different clinical conditions has a therapeutic and prognostic importance. Some of these techniques, which have been employed for measuring blood flow in microcirculation, are considered below.

2.8.1 Photoplethysmography

This technique is based on the principle that light is attenuated when passes through skin surface as a result of absorption, reflection and scattering processes (Weinman *et al.,* 1977). The amount of backscattered light is dependent on the volume fraction of red cells in tissue. Part of the light will be attenuated due to presence of tissues; light passing through blood vessels will be attenuated in proportion to

the amount of blood present. Therefore, the greater the volume of blood in skin under investigation, the greater the attenuation of incident light. The degree of attenuation with changes in blood volume can usually be measured with a simple photodetector.

Photoplethysmography is applicable for use all over the body surface and has been utilised extensively in microvascular and reconstruction surgery. PPG has been used to measure changes in tissue blood flow by measuring changes in amplitude of the signal from a photodetector due to scattering of the incident light by the blood. These optical properties of blood can be used to discriminate between oxygenated and deoxygenated blood. Total blood volume changes can be estimated by the transillumination method by measuring the amount of light passing through the skin in areas such as the ear lobule, where the light source is situated on one side and the detector on the other side.

There is also, a reflection mode, where the light source and detector can be placed adjacent to each other to enable the system to be used on any area of the body where light is directed down to on the skin and backscattered light is measured as it emerges back from skin. It should be noted that the orientation and reflective properties of red blood cells are an important contributing factor in determining the true nature of the signal (Robert, 1982).

In both methods two different components are recorded with the photodetector, the alternating (a.c.) or pulsatile component related to the cardiac cycle, which is a small signal and represents a small percent of total light reaching the detector. The other component is slowly varying (d.c.) signal, which closely related to blood flow changes in skin. By obtaining both components useful information can be obtained about total skin blood flow (Challoner, 1979).

Problems with this technique that it can not distinguish between superficial or "nutritional" skin blood flow and blood flow in the much larger vessels deep down in the dermis (Challoner, 1979). Another problem with this technique is the lack of base line stability and the difficulty in defining a "Zero" level. There are also difficulties in interpreting the photocurrent signals from the photodetectors if flow in deeper regions increases at the same time of decreased flow in superficial regions. This lack of fundamental knowledge about the nature of photocurrent signals, difficulties in calibration and studying relative changes lead to considerable debate as to what is really being measured (Challoner, 1979).

2.8.2 Thermal clearance methods

These methods are based on it is being possible to measure heat removal by blood flow through tissues. It is presumed to be related to both the volume and flow rate of regional skin blood flow. It has been developed specially for skin blood flow measurements and it is assumed that blood flow in the upper dermis will remove heat applied to the surface of the skin. Therefore, this system measures the nutritional blood supply to the epidermis.

Generally, the thermal clearance probe (sensing unit) comprises a central copper heating disc connected to an outer copper segmented annulus ring by an array of thermal couples (Challoner,1975). These two rings are thermally and electrically isolated from each other and thermocouples measure the temperature difference between the two rings to measure blood flow under the probe. These thermal clearance probes have limitations in their practical use. Slow response times (approximately 1 min.) due to poor conductivity of the skin tissues, and tissue conductivity is also greatly influenced by its water content.

Other problems using this technique, is that both sweating and oedema will affect tissue conductivity and result in overestimation of flow signal. In addition, the heating in tissues by the probe leads to a degree of

vasodilatation; making actual measurement of skin blood flow under normal conditions impossible. Due to all these difficulties in practical use and the non-linear characteristics thermal probes have not been used extensively (Challoner, 1975).

2.8.3 Radioisotopes

2.8.3.1 Radioactive isotope clearance

This technique is based on tissue clearance of rapidly diffusing inert isotopes as a means of measuring skin blood flow. This method was applied extensively to study skin and tissue blood flow both clinically and experimentally and relied on measurement of clearance or washout rates of intradermally injected isotopes as an indicator of local tissue perfusion (Sejrson, 1969). The most commonly used isotope is Xenon 133 which has affinity for fatty molecules, and makes interpretation of skin blood flow results difficult. Also, the trauma of intradermal injection of the radioactive isotope leads to vasodilatation, which makes the results inaccurate due to increase in tissue perfusion (Holloway, 1980).

The advantage of this method is that it can be applied to study all kinds of tissue blood flow problems. But, the disadvantage and problems in its use is that it does not give a continuous flow measurement, clearance curves are difficult to interpret and the trauma caused by intradermal injection seriously disturbs the flow. The last problem can be avoided by using Xenon isotope in a gaseous form and allowing it to diffuse directly through the skin (Sejrson, 1968a&b).

2.8.3.2 Radioactive microspheres technique

This technique is useful in measuring skin blood flow in animals by injecting very small radioactive labelled plastic spheres (typically 15um in diameter) into large blood vessels, which become trapped in the complex microcirculatory network. Then, the radioactivity of skin samples is measured after killing the animal to evaluate the level of

microspheres trapping in the microvascular network, which related to the regional blood flow in the tissue sample under examination. This technique has very limited application and its invasive nature means that it cannot be used in humans (Lundberg and Smedegard, 1981).

2.8.4 Erythrocyte velocity measurement

By continuous measurement of the velocity of red blood cells in the microcirculation, an important parameter for assessment of conditions in the microcirculation is provided (Wayland and Johnson, 1967).

2.8.5 Capillary microscopy

This is a useful method for blood flow measurement in a small number of blood vessels in the skin (Ryan, 1973). This technique gives information on a small number of vessels in the tissue, rather than overall assessment of microcirculatory blood flow. Accurate observation of red blood cells motion, blood vessels diameter and flow distribution can be made on vessels, which are transparent enough to visualize directly. Visibility is dependent on the thickness of epidermis, level of pigmentation and the density of capillaries which number varies from 10-60/mm^2 on different skin sites. This measurement is non-invasive, but blood flow changes in the microvasculature can occur from both the heating effect of observation lamps and the preparation process of fixing the tissue under examination.

2.8.6 Video microscopy

An advance of the above technique is to obtain in vivo measurement of red blood cells velocities in human nail fold capillaries (Butti *et al.,* 1975; Fagrell *et al.,* 1977). This method uses a light microscope and video camera to obtain recording of the single capillary under observation. The absolute RBCs velocity can then be determined by measuring the rate at which the plasma gaps between the RBCs advance in the televised scan during a frame-by-frame play back.

This is an accurate technique, but is still time consuming and limited in application. The nail fold site is most commonly used due to the ease in which it can be immobilised from the effects of cardiac and respiratory movements. Video-microscopy studies are not readily used in routine clinical investigations or the study of capillary blood flow over long periods of time.

2.8.7 Fluorescein angiography

This technique has been used to determine skin blood flow by a number of workers (Lund, 1976). By intravenous injection of Fluorescein, areas with impaired microcirculation will show a delayed clearance of the injected dye. Sodium Fluorescein is used and the skin is illuminated under ultraviolet radiation resulting in a yellow green fluorescence. By repeatedly photographing the site under examination, the initial onset of fluorescence and the time delay interval required to reach maximum fluorescence are estimated. This method is limited to providing some general information on overall distribution of blood supply. It does not help in direct quantitative or continuous blood flow measurement.

2.9 Laser Doppler flowmetry

2.9.1 Historical background

The Laser was originally conceived by Schawlow and Towns (1958) and demonstrated experimentally by Mainman (1960). The advances in Laser in early 1960's helped to a great extent in microcirculatory blood flow measurements. Yeh and Cummins (1964) proposed that macromolecule velocity in solution could be determined from Doppler frequency shifting of laser light backscattered from moving particles. Helium-Neon (He-Ne) laser was used to study the flow profile in transparent tubes. In the following years, the technique was also used to measure airflow in tunnels and tubes, and was known as laser Doppler anemometry (Masbernat *et al;* 1975, Durst *et al;* 1981).

Riva *et al.*, (1972) were the first to use this technique to measure blood flow through narrow capillary (glass) tubes and in rabbit retinal vessels. Blood flow in these arteries was measured using a 10mW He-Ne laser. This high output power was not suitable for obtaining acceptable signal to noise ratios for human retinal experiments. Tanaka *et al.*, (1974) used a reduced laser power (18uW) and a more sophisticated laser system for blood flow measurement in human retinal vessels. Advancement in blood flow measurement using laser Doppler flowmetry was the development of laser Doppler microscope to evaluate flow velocities and profiles in transparent tissues such as small vessels in the mesentery of mouse (Einav *et al.*, 1975).

Tanaka and Bendek (1975) studied flow velocities of red blood cells in the femoral vein of a rabbit by an optical fibre of small diameter based on laser Doppler system. Stern (1975) was the first to study blood flow in the microcirculation using laser Doppler technique. He used 15mW He-Ne laser to prove the effectiveness of the system by recording photocurrent changes in human skin and fingertips before and after occlusion of the brachial artery with a pressure cuff around the upper arm and during intake of alcohol to monitor its effects on microvasculature as a well-known vasodilator.

Stern *et al.*, (1977) also used a Xenon clearance technique to measure flow in the skin of volunteer subjects who had been exposed to ultraviolet induced erythema.

Portable and practical laser Doppler instruments for use in clinical settings were developed by Holloway and Watkins in 1977. They employed both delivery and collection of light from the tissue via optical fibres incorporated with a photodiode detector to compare measurements of forearm skin blood flow obtained using laser Doppler flowmetry with results obtained using Xenon clearance method. Their study demonstrated close correlation between the two methods with supporting laser Doppler flowmetry as a useful technique in clinical

applications. Watkins and Holloway (1978) reported that this system responded linearly to blood flow.

Holloway (1980) reported clinical use of this system to measure blood flow responses to intradermal injection trauma which was supported by the criticism of the other methods of monitoring blood flow which depend on administration of an intravenous injection e.g. radioisotope clearance method. He concluded that the increased blood flow due to injection trauma could be up to ten times larger than resting values.

Nilsson *et al.,* (1980), developed a new laser Doppler instrument incorporating a novel photodiode detection system to overcome the problem of laser's noise reported by Watkins and Holloway (1978), which allows measurements to be made only approximately during 50% of the observation time. He introduced a double channel detector system, which coupled the signal to two identical photodetectors and signal processing units. They showed that the flowmeter gave a linear response to blood flow over a moderate range of red blood cells velocities and concentrations. At high RBCs concentrations, the output signal from the processor become non linear with blood flow due to increased multiple photon scattering in moving RBCs and predominance of mixing process at the surface of photodetector.

Nillson *et al.,* (1982) developed an improved signal processor to overcome this problem, by electronic correction of the output signal to establish a linear response between the instrument output and skin blood flow concentrations. Due to commercialisation of this instrument it has been used extensively in clinical work to assess the degree of arterial insufficiency. Bonner and Nossal, (1980) suggested that laser Doppler flowmetry could measure changes in blood flow in vessels at different depths by changing the distance between the delivering and collecting fibres.

Hirata *et al.*, (1988) used probes with optic fibres separations of 0.3 mm and 0.7 mm to measure finger blood flow during local hand warming and whole body warming. They found that values obtained with 0.7 mm probe measure total blood flow through the skin as correlated with using and comparing venous occlusion plethysmography. The probe with 0.3 mm fibre separation is an indicator of the level of capillary blood flow. Duteil *et al.*, (1985) developed a double wavelength laser Doppler system to discriminate between superficial and total skin blood flow. An Argon-ion laser (548 nm) and He-Ne laser (633 nm) which had different penetration depths in the dermis was used to achieve depth sensitivity. They found that a constant ratio could be obtained between the Doppler shift frequencies at both wavelengths.

Boggett *et al.*, (1985) measured skin blood flow by laser Doppler to distinguish between photocurrent signals of the Doppler and non-Doppler origin. By introducing a gross frequency shift into one of the 2 laser beams illuminating the skin, discrimination was obtained between signals contributing to the photocurrent spectra which are Doppler shifted in origin, and those (such as number fluctuations, fibre line movements and laser amplitude noise) which were not. This clearly indicated that most of the processed signal was due to Doppler frequency shifting. Obeid *et al.*, (1988) investigated the possibility of using different wavelength laser sources to provide depth discrimination in LDF skin blood flow measurements.

In order to discriminate between total and superficial skin blood flow, three different wavelength laser sources were employed (a He-Ne 'red' laser, a He-Ne 'green' laser and a NIR laser diode) which had different penetration depths in the skin. Flow parameters were measured for both in vitro fluid flows in a polythene tube and *in vivo* skin blood flow in the forefinger by comparing the photocurrent spectral response of the three laser systems for both in vivo and in vitro blood flow situations.

A significantly reduced response of the He-Ne green laser light to blood flow was observed.

The characteristics of the photocurrent spectra with the green laser appeared to be consistent with a response solely limited to blood flow in the superficial dermis, where the concentration and mean velocity of the moving RBCs were small. In addition, little or no difference could be observed between the relative response of the He-Ne and infra red laser to blood flow both in the model and the in vitro situation.

2.9.2 Theory of laser Doppler flowmetry

Laser Doppler flowmetry has developed over the past 20 years to be become a monitoring technique widely used for clinical assessment of tissue viability. Laser Doppler flowmetry has been used for many years to measure the movement of macromolecules in fluids.

Stern (1975) was the first to suggest using the technique to measure microvascular blood flow. All commercially available clinical LDF systems are designed to measure microvascular blood perfusion and the important advantage over other methods is the direct measurement of blood delivered to tissues. The technique has been used extensively to measure blood flow in different tissues but its principle use is in measurement of the skin blood flow.

The Austrian Physicist Christian Doppler described the Doppler principle in 1842. This phenomenon is experienced by everyone when a railway train comes to a crossing and the pitch of the whistle decreases as it passes (Holloway, 1980).

The scattering of light as it traverses a medium has been used for many years to observe and study the bulk properties of matter. The Doppler principle depends on the frequency change of the original wave when reflected by moving objects. The wave energy emitted by moving objects will shift in frequency in proportion to the *velocity* of

object. If the frequencies of emitted and reflected wave are known, the *difference* between the two is the *Doppler shifted frequency.*

When the initial wave is emitted from a stationary source at an object moving towards the source, the relative frequency shift occurs both on its way to and from the moving object. The relative frequency shift is then seen twice, as the source appears to have a relative velocity itself (Oberg, 1990).

The magnitude of Doppler frequency shift is given by the general Doppler equation:

$$\Delta f = 2\,\acute{\eta} v/\lambda \cos\theta$$

- θ = angle between colliding photon and red blood cells.
- λ = wavelength.
- v = red blood cells velocity.
- ή = tissues refraction index (approximately).

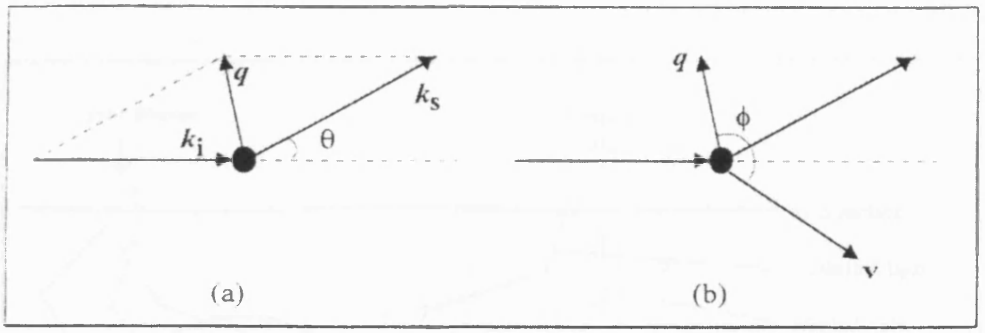

Figure 2.11. *Scattering of photon by a moving RBC.*

The interaction between light and tissues is a process of multiple scattering and absorption, in which the tissue has a higher refractive index than air. About 4-5 % of incident laser beam on skin surface will be reflected back and the remaining light beam penetrates the tissues and will undergo scattering and absorption processes. The Periflux system 5000 employed in this study, utilised a solid state diode laser with a far-red light of 780nm and a maximum power output of 1mW at the probe tip.

The laser light was conducted to tissue surfaces by an optical fibre and penetrated the tissue surface to a depth of 1-1.5 mm. With this degree of penetration only micro vessels contribute to the backscattered signal. The single frequency laser light is scattered by both stationary connective tissue and moving RBCs. Only the part of light scattered by moving red blood cells undergoes a frequency shift, which is relates to the RBCs velocity. The Doppler shifted light is however, not of a single frequency, but a spectrum of frequencies as the RBCs move at different velocities and light is scattered at many different angles (Stern *et al.*, 1977).

The backscattered different wavelengths light consists of a mixture of *shifted* and *unshifted* light. This results in a *frequency beating* which perceived as the *Doppler shift*. The presence of more than one spectral line in the laser output may cause beat frequencies to occur on the photodiode, generating enough noise to obscure the faint Doppler signal.

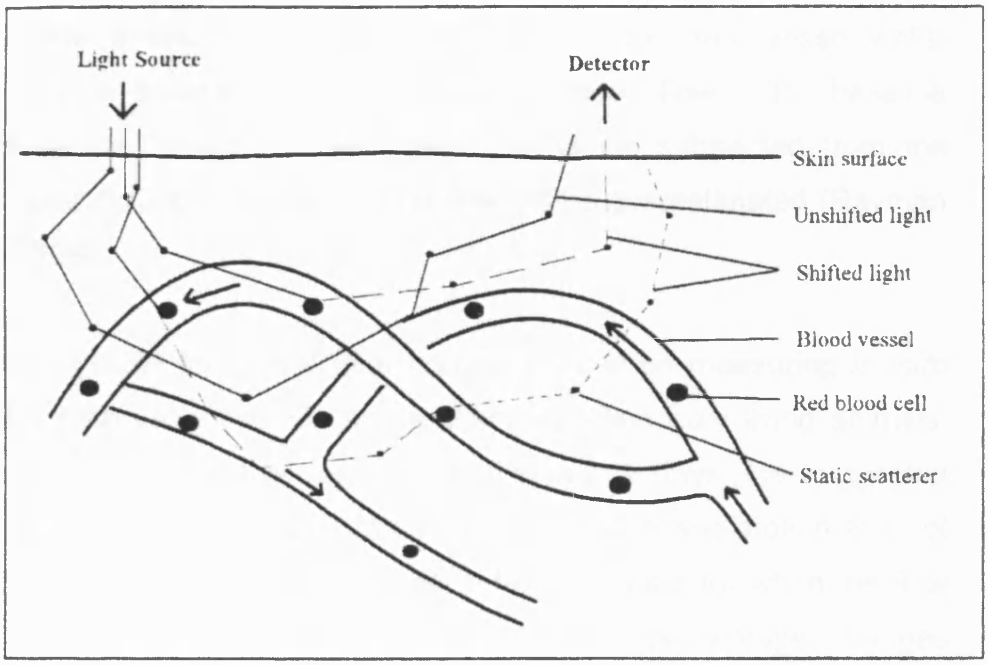

Figure 2.12. *Laser light scattering and shifting by RBCs.*

Nilsson *et al.,* 1980, proposed a dual photodetection system, by which laser noise and noise from external sources can be suppressed while the blood flow signal is enhanced, so the signal to noise ratio is increased. Laser Doppler instruments measure the Doppler shifted and backscattered signals from all moving structures within the measuring volume of tissues which ranges between 1-1.5 mm^3, directly under the endpoint of the optical fibre (Bonner and Nossal, 1980).

The backscattered collected signals are mainly from red blood cells. Also, other blood components as leucocytes and platelets add to the signal with a small fraction not disturbing the reading due to low relative volume fractions of these cells (Tenland, 1982). There is also a non-blood flow contribution from movement of other tissues in the region under study. On complete occlusion of blood flow when blood flow signal drops to zero, these movements due to these other sources become apparent and the output signal does not reach zero.

This continued signal is explained due to small to-and-fro movements of red cells as fluid exchanged across the vessels, and also, to a non blood flow contribution from movement of muscle cells, vessel walls, and various tissues which is unrelated to blood flow. This baseline signal is the "biological zero", must always be subtracted from the flowmeter output, otherwise, blood flow will be overestimated (Rayman *et al.,* 1986).

Tenland (1982) found a similar non-zero signal on measuring *in vitro* tissues preparations a few hours after dissection from living animals. This signal disappeared completely after a few days. He suggested that this portion of the signal was due to internal tissue motion and not due to blood flow, and this needed to be accounted for when the flow signal is expressed in absolute values or in percentage changes (Tenland, 1982).

Noise signal, however does not originate from large vessels passing through the measurement volume. They have no influence on the signal, as their walls are too thick and dense to permit more than a limited amount of light to pass (Tenland, 1982). In large blood vessels RBCs are transported in rouleaux formations. These rouleaux formations act as larger particles producing a higher degree of forward scattering of light when examined by laser Doppler flowmetry.

Compared to a homogeneous distribution of moving red cells in tissues, the flux of cells in large vessels is underestimated (Tenland, 1982). The output signal from the transducer is expressed in volts and is proportional to blood flow. It is independent of direction, as RBC movement in capillary bed is multidirectional. Therefore, RBCs flux is a *relative* rather than *absolute* measure of blood flow (Schlehr *et al.,* 1987).

2.9.3 Calibration

Calibration of the instrument to the two measuring points 0PU and 250 PU using the motility standard allows restoration of the original calibration of the instrument. This provides stable and consistent readings over a long period of time. The feasibility of LDF in experimental medicine and clinical applications is dependent on how well the output signal from the flowmeter is correlated with the microcirculatory blood flow under study (Nilsson *et al.,* 1980).

Laser Doppler flowmetry is portable, completely non-invasive with no effect on the local circulation compared to other techniques, which are clinically unsuitable or difficult to apply. The laser Doppler flowmetry has a negligible influence on the microvascular bed under study (Tenland, 1982). The laser light produce no measurable heating effect on the tissues under study and blood low rate can be measured continuously without contact between the probe and measuring area (Damber *et al.,* 1982). They found, however, no effect from

wavelengths exceeding 450 nm. A diode laser of wavelength 780nm, therefore, does not interact with blood flow.

The main limitations of laser Doppler flowmetry, in certain applications, are the sensitivity to movement artefacts, the non-linear relation to flow at high volume fractions of red cells in tissues and the limited depth of penetration of laser light which may change in different tissues depending on their light scattering properties (Edwall *et al.,* 1987).

Also, the unknown relationship of the output signal to tissue pigmentation, tissue thickness, vascular bed geometry and blood haemoglobin content make true quantitative measurements difficult to obtain and may influence the recorded flow values to some extent (Tenland, 1982; Oberg *et al.,* 1984).

All LDF instruments using flexible optical fibres for signal transmission are affected by movement artefacts to varying degrees, where false non perfusion signals appear if the fibre line is suddenly moved and are superimposed upon the flux signal as steep upward spikes on the regular record which confused the assessment of blood flow. In this study, we overcame this movement artefact in part by, fixing the fibre to a headband applied around the patient's head, minimizing patient movements and analysing the results from time intervals where the measurements are continuous with no spikes.

No compensation for the degree of oxygen content needs to be made as changes in oxygen pressure within the tissues has a minor effect on the flowmeter response at uniform red cells concentrations and velocity (Oberg *et al.,* 1984). The haemoglobin content and tissue pigmentation also influence the flowmeter response to some extent. The use of infrared lasers overcame the influence of skin pigmentation on flowmeter response, as the absorption of infrared light by melanin is comparatively low (Hardy et al, 1965).

Attempts to calibrate LDF instruments to produce absolute values of volumetric flow are impossible, although, it correlates well with other methods for measuring blood flow such as capillary microscopy, occlusion plethysmography, radioactive microspheres (Stern *et al.,* 1977; Shepherd and Reidle, 1982). No other method measures blood flow in exactly the same volume of tissue.

2.9.4 Applications of laser Doppler flowmetry

2.9.4.1 Skin blood flow

Stern in 1975 was the first to use LDF in measuring skin blood flow in the fingertips. Holloway and Watkins (1977) compared laser Doppler flowmetry and Xenon-clearance techniques to measure forearm skin blood flow and demonstrated a linear relationship. Salerud *et al.,* (1983) found the rhythmical activity variation in blood flow in healthy human skin to differ widely between subjects, although, the frequency of oscillation was uniform from one site to another. They also found that raised skin temperature and local anaesthetic paste strongly affected the variations in blood flow frequencies (Salerud *et al.,* 1983). Waeber *et al.,* (1984) using laser Doppler flowmetry found a reduction in skin blood flow after cigarette smoking.

Most of the laser Doppler measurements from the skin surface measure blood flow from capillary loops and most parts of the subcapillary plexuses. The role of skin microcirculation is twofold, 90% to regulate the body temperature and the remaining 10% is for skin metabolic needs (Nitzan *et al.*, 1988). Skin microcirculation has been studied using laser Doppler flowmetry utilizing the fact that laser Doppler gives continuous recordings of blood flow and is non-invasive, so not changing or disturbing the flow.

Holloway and Watkins (1977) and Holloway (1980) compared laser Doppler and Xenon 133 clearance to measure skin blood flow and they found a general correlation between the two methods. Holloway

(1980) found a sevenfold increase in blood flow for 20 minutes following insertion of an injection needle.

Variability in skin blood flow is under control by different environmental conditions. Rotation of the fibreoptic probe on the skin by 90 degrees caused significant changes in perceived blood flow due to heterogeneity of the microvascular bed (Tenland *et al.,* 1983 and Salerud *et al.,* 1983). Marszalek (1996) stated that changes in the skin blood flow due to vasoconstriction or vasodilatation of the cutaneous vessels are thermoregulative responses during cold or heat stress, respectively.

The application of laser light based on Doppler shift is a relatively new method for skin blood flow measurement. At present several companies operating throughout the world produce laser Doppler flowmeters. The laser Doppler method is designed particularly for cutaneous tissue.

Depending on the wavelength, the laser light penetrates into the tissue at different depths (from 0.6 to 1.5 mm). The laser light with the wavelength of 633 nm enables the measurement of thermo-regulative and nutritional blood flow in the skin. The range of the measurement and application of the device provide an opportunity for developing a universal standard.

Eun (1995) stated that LDF was an excellent non-invasive technique for the measurement of cutaneous microcirculation. The list of applications of LDF is long; it can be applied to monitor inflammation caused by various drugs, chemicals, and allergens related to blood flow. Through blood flow measurement, the pathophysiology of various skin diseases can be verified and certain treatments can be partially monitored.

2.9.4.2 Otological blood flow measurements

Miller *et al.,* (1984) introduced laser Doppler flowmetry for dynamic measurements of cochlear blood flow. Using several methods for manipulation of cochlear blood flow, i.e. systematic phenylephrine administration, 10% CO2 in air, direct electrical stimulation of the cochlea and haemodilution they concluded that there were several advantages with the laser Doppler system as a non-invasive and non-destructive method with easy set up and continuous measurements of cochlear blood flow.

Yokoyama *et al.,* (1988) found a linear relationship between laser Doppler flowmetry and hydrogen gas clearance in studying the effect of norepinephrine and phentolamine on cochlear blood flow. Norepinephrine induced elevation of blood pressure with an increase in cochlear blood flow about 1 minute after administration. Phentolamine a vasodilator caused a drop in blood pressure, but only a slight decrease in cochlear blood flow.

Gan *et al.,* (1997) used a single-point laser Doppler interferometer to evaluate implantable hearing devices' mass, shape and orientation, attachment, electromagnetic coupling and acoustic properties. Gan *et al.,* (1997) considered laser Doppler interferometer as an international standard for accurate, consistent comparison of performance of all implantable hearing devices.

Filipo *et al.,* (1997) measured cochlear blood flow with a laser Doppler flowmeter at the level of the basal turn of the cochlear lateral wall, both in normal and hydropic cochleae, before and after osmotic infusion. This study considered that basal values in the normal cochlea were much higher than in hydropic one and both mannitol and glycerol markedly influenced the local blood flow in the normal cochlea, giving few or no changes in the hydropic ones.

Degout *et al.,* (1997) showed that regulation of the blood flow to the cochlea by the sympathetic nervous system occurs in humans at the level of the cochlear microcirculation during increases in blood pressure and that involvement depends on the pressure level. The cochlear blood flow in this study was measured by LDF.

Goode *et al.,* (1996) described a laser Doppler system that can be used clinically for the measurement of the tympanic membrane, malleus and prosthesis head displacement in response to sound inputs of 80 to 100 dB sound pressure level. It also has the potential for use in the operating room to perform measurement of prosthesis and stapes displacement. The information provided by such testing gives the otologist knowledge of tympanic membrane and ossicular function that is unique in evaluating middle ear function; it should help select the best type of reconstruction in a given case and direct us toward new and better methods of tympanic membrane and ossicular reconstruction. The results of umbo displacement measurement in 95 human ears are reported.

Nakashima and Yanagita (1995) measured blood flow in nine patients suspected of having perilymphatic fistulas using laser Doppler flowmetry. During exploratory tympanotomy, the tip of a laser Doppler probe was attached to the promontory near the anterior superior portion of the round window niche.

Sillman *et al.,* (1988) reported that changes in blood flow to the inner ear have been thought to influence or underlie a number of cochlear diseases, including some forms of noise induced hearing loss, sudden hearing loss, and Meniere's disease.

Important devices have been made in two technologies for the study of cochlear blood flow. The first is in the area of vital microscopic studies of cochlear microcirculation, and the second is based on the introduction of laser technology in the form of laser Doppler flowmetry.

In the report by Sillman *et al.,* (1988), measurements are given of changes in cochlear circulation caused by carbon dioxide breathing, systemic haemodilution and direct electrical stimulation of the cochlea. From these changes, it was observed that cochlear blood circulation responds to systemic blood pressure alterations and is subject to local flow control mechanisms. Linearity and speed of response of the laser Doppler instrumentation were also shown.

Alberta *et al.,* (1992) stated that laser Doppler flowmetry is one of the methods of choice in measuring cochlear blood flow and the studies carried out in humans demonstrated the reliability of laser Doppler flowmetry and its usefulness in understanding inner ear physiology. They concluded that laser Doppler flowmetry is a safe and reliable technique for microcirculation assessment.

Schops *et al.,* (1987) were the first group to study the microcirculation in human tympanic membrane using laser Doppler flowmetry in 7 normal volunteers. The fibre optic probe was inserted through the external ear canal and positioned as close as possible to the tympanic membrane for recording the spontaneous rhythmical variations in the blood flow of tympanic membrane. They concluded that laser Doppler flowmetry is a suitable non-invasive method for the study of tympanic membrane blood flow in health and disease.

Apart from experimental studies in cochlear blood flow there have been only few clinical applications by Schops *et al.,* (1987). This current study was an attempt to provide some insight into the basic properties of blood flow in the external auditory meatus and tympanic membrane. Two groups of clinical Otological interest are patients with otitis externa and tympanic membrane perforations. Small groups of those patients were investigated in this study. A brief consideration of the underlying pathology is given in sections 2.10 and 2.11.

2.10 Otitis externa (OE)

Otitis externa (OE), defined as inflammation of the auricle, external ear, or tympanic membrane. The severity can range from mild inflammation to life-threatening infection (Agius *et al.,* 1992). It is commonly seen by family physicians and affects 4 out of each 1000 every year. In most cases the significant pain of OE compels the patient to seek care urgently.

OE can be categorized as localized or diffuse. When it persists for more than 6 months, it is considered chronic and is more commonly bilateral. It is thought to be caused by local trauma to the external canal, diabetes, high humidity, and loss of the canal's protective coating of cerumen, eczema, use of a hearing aid or stethoscope, or glandular obstruction. It is commonly seen in swimmers, particularly in the summer months (Agius *et al.,* 1992). The most frequent symptoms are discharge, pain, hearing loss, itching, and tinnitus.

Acute otitis externa is a common, painful infection of the outer ear canal. The most common clinical manifestation of otitis externa is pain, followed by erythema, oedema, itching, discharge, and hearing loss. Assessment of severity of otitis externa, rated by physicians on a scale from 0 to 3 based on physical findings and symptoms, indicates 44 percent of patients have mild disease, 43 percent have moderate disease, and 13 percent have severe disease (Cassisi, 1986). Pseudomonas is responsible for approximately 60% of infections, Staphylococcus for 15%, fungi for 10%, and other organisms for the remaining 15% of infections (Russell *et al.,* 1993).

Complications of otitis externa include ear canal stenosis, myringitis and tympanic membrane perforation, regional dissemination of infection (auricular cellulitis, chondritis, and parotitis), and progression to malignant otitis externa, which can be fatal (Bojrab *et al.,* 1996).

Treatment for otitis externa can include cleaning the ear as well as topical creams, ointments, or drops containing antiseptics, antibiotics, and steroids (Hirsch, 1992). Necrotizing (malignant) otitis externa (NOE) is the most severe form of OE and is most often seen in elderly patients with diabetes. One case series in a referral population found a mortality rate of 53% (Zaky *et al.,* 1976). Pain, purulent discharge, bilateral involvement, and external canal granulation tissue are common symptoms.

The ear canal is a blind sac with an anterior recess. Trauma to the canal, accumulation of keratin, or a change in pH can trigger inflammation and infection. Clark *et al.,* (1997) found that aerobic bacteria account for 91% of bacterial causes; anaerobes, 4%; and mixed infections, 4%. The most common offending organisms are Pseudomonas aeruginosa (50%), Staphylococcus aureus (23%), anaerobes and gram negative organisms (12.5%), and yeast, such as Aspergillus and Candida (12.5%). The increased pH of pool water is believed to make infection more likely, since bacteriologic studies fail to show a direct link between swimming pool contamination and the organisms of OE.

There are no published studies of the accuracy of the medical history, physical examination, or office laboratory tests for the diagnosis of OE. Diagnosis is usually made based on physical examination findings: pain on movement of the auricle, oedema, redness, and foul-smelling discharge (Leung *et al.,* 2000). Swelling often obscures the tympanic membrane.

The main principles of treatment are local cleansing of debris, drainage of the infection, re-establishment of the normal acidic environment, use of topical and systemic antimicrobials, and prevention of recurrent infections. The best evidence (grade of evidence: A) demonstrates equivalent results with ear cleaning, an ear wick, and any of the choices of topical agents including acidifying agents, antibiotics,

antibiotic and steroid combinations, or antifungal agents. Frequent dosing (3 to 4 times daily) for at least 4 days is supported by the studies. Two studies demonstrated equivalent efficacy with topical ciprofloxacin or ofloxacin dosed twice daily compared with antibiotic and steroid combinations dosed 4 times daily (Jones *et al.,* 1997).

However, these agents are also more expensive than older topical antibiotics. The evidence for single topical treatments and oral antibiotics is weaker (grade of evidence: B). Physicians should treat patients with one of the following regimens for at least 4 days

2.11 Myringoplasty

Myringoplasty is defined as an operation in which the reconstructive procedure is limited to the repair of tympanic membrane perforation without middle ear exploration or ossicular chain evaluation whereas tympanoplasty is performed to eradicate disease in the middle ear and to reconstruct the ossicular integrity (Sheey, 1984).

Tympanic membrane (TM) perforations arise from chronic middle ear infections, trauma, or placement of ventilation tubes. Most acute TM perforations begin healing after 12 hours when squamous cells at the edges of the perforation begin to proliferate (Dunlap and Schuknecht, 1958). When the healing process is complete, a neomembrane or replacement membrane is formed (Yamashita, 1985). This neomembrane is thinner than the normal TM as a result of lack of a middle fibrous layer. A chronic perforation results from failure of epithelial growth across the perforation.

When the TM fails to heal spontaneously, surgery is often necessary to close the perforation. Attempts have been made to repair perforations of the tympanic membrane dating as early as 1640 by Banszer when he used a small tube of elk horn surrounded by pig's bladder. Since then, a host of materials have been used for the repair of TM perforations.

Berthold, who introduced the term "myringoplasty," successfully repaired a TM perforation using a full thickness skin graft in 1878. Other materials used included split thickness skin grafts, vein grafts, sclera, perichondrium, temporalis fascia, cartilage inlay, and fat (Glasscock and Kanok 1977, Eavey 1998).

Chronic tympanic membrane (TM) perforations are a common problem in otolaryngology. Perforation of the TM most commonly arises as a result of either trauma or otitis media, usually presenting clinically with conductive hearing loss and chronic infection (Gladstone *et al.,* 1995). It has been estimated that the incidence of TM rupture resulting from trauma is 8.6 per 1000 persons (Griffen, 1979). Although the exact incidence of TM perforations attributable to infection is unknown, it is highly prevalent in developing countries and among indigenous populations (Gladstone *et al.,* 1995).

Although the TM has demonstrated a remarkable ability for regeneration and spontaneous healing, chronic perforations do commonly occur and may require grafting as a means of repair. The development of chronic TM perforations after trauma or infection depends on a variety of factors. The continued presence of infection with possible membrane necrosis may give rise to a chronic perforation without healing. In addition, the mechanism of trauma and the size of the perforation also influence the TM healing ability (Griffen, 1979; Kristenson, 1992).

There are several major reasons why the complete closure of a chronic TM perforation is desirable. Patients experience a dramatic improvement in hearing, prevention of otitis media, and the tolerance of water in the ear canal. In addition, with complete closure of the defect, recurrent otorrhea is unlikely to occur with respiratory tract infections and otitis media.

The normal TM consists of three layers, including a surface epithelium, a middle fibrous lamina propria, and an internal mucosa. In most cases of acute TM perforation, the membrane heals spontaneously in a three-stage process (Gladstone *et al.,* 1995). The initial stage, which is similar to other cutaneous lesions, involves haemostasis and inflammation. The next stage differs in that the epidermal layer first closes the perforation, followed by the inner mucosal layer. The fibrous lamina propria is the last layer to regenerate across the perforation, and evidence even suggests that it often fails to do so, leaving a dimeric layer with only loose, disorganized fibres between layers (Goeverts *et al.,* 1988).

Persistent infections and large TM perforations resulting from trauma may impair the normal healing process and lead to chronic perforations. In these cases, it is generally thought that TM grafting is required to provide the needed mechanical support for membrane healing. The primary goal of repairing a TM perforation is to prevent otitis media and to restore hearing. In this regard, several techniques and graft materials have been developed, each with its advantages and disadvantages.

In the 1970s and early 1980s, homograft material including lyophilized dura and cadaveric TM was popular; however, these materials are seldom used today, because of concerns about the transmission of infectious diseases such as Creutzfeldt-Jakob disease.

Today, the most commonly used autograft is the temporalis fascia (Storrs, 1961) which provides a thin, pliable, and sturdy graft material. In addition, the temporalis fascia has the advantage of being harvested at the same surgical site as the affected ear. In this technique the temporalis fascia is removed from a postauricular site and laid under the perforation, eventually becoming incorporated into the middle fibrous layer of the TM.

Although myringoplasty has proven to be effective, with a success rate of 88% to 95% (Sheehy and Anderson 1980, Vartiainen and Nuutinen 1993) it is limited by 1) the need for expensive equipment in an operating room setting, 2) the microsurgical skills of the surgeon, and 3) the donor site morbidity that results. These disadvantages are especially important in developing countries, where the incidence of TM perforations is high but medical resources are often limited.

Again, from this literature review it is evident that new information about changes in the tympanic membrane blood flow before and after myringoplasty is of importance to the otologist.

Chapter 3

Materials and Methods

3.1 Instrumentation:

- The instruments used during this study were:
- Laser Doppler flowmeter (Periflux system 5000).
- Laser Doppler probe (Probe 403-Stainless Steel Probe).
- Personal computer.
- Outpatient diagnostic microscope.

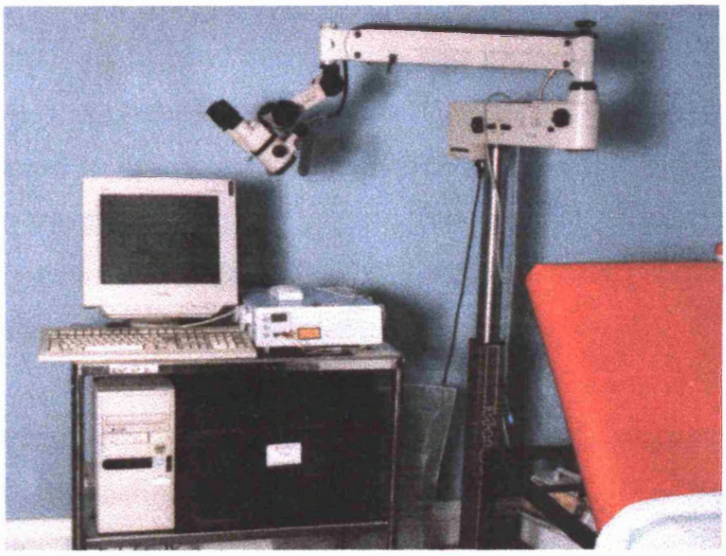

Figure 3.1. *The LDF connected to the PC*

3.1.1 Laser Doppler system (Periflux 5000)

The laser Doppler system (Periflux 5000) consisted of a multi-channel, multi-functional system that allowed different types of blood flow measurements to be made simultaneously in up to four different sites. The unit came with its own custom written Perisoft software to analyse data. The Periflux system 5000 (Figure 3.2 ***)*** consisted of one Periflux PF 5001 main unit and up to four function modules that could be inserted into the PF 5001 housing unit.

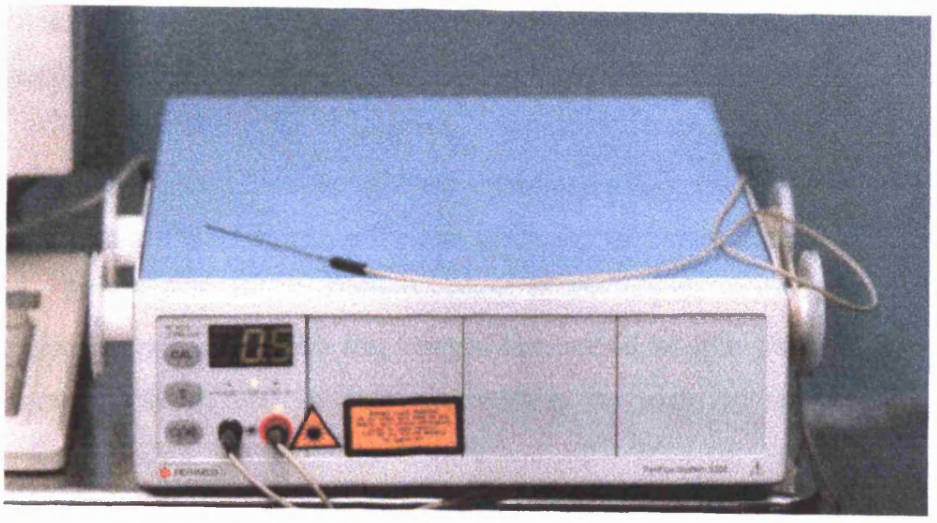

Figure 3.2. *The LDF connected to the study probe*

The function module used in this study was PF 5010 laser Doppler unit. This was a one channel laser Doppler unit which generates the laser light delivered to the probe. The collecting channel for backscattered light within the probe also fed back to this module. This backscattered signal was collected back to the photodetector and processed in the instrument prior to being delivered to the PC.

The value from the photodetector was electronically processed and the signal was converted into the perfusion value. The main unit is connected to the PC for further analysis and saving of data. The 5010 module also displayed perfusion values on the front panel.

3.1.2 Time constant: (Signal processing)

The time constant is a filter that is used to smooth the signal and thus avoid irrelevant peaks. This is especially important since the Periflux LDPM probe is sensitive to movement, which may create peaks (artefacts). Using the short time constant (0.03 sec.) the artefacts are clearly seen and using a long time constant (3.0 sec.) the artefact is integrated into the curve, which thus will be smoothed and not suitable to record quick perfusion changes, so we used the (0.2 sec.) time constant to avoid both effects and to obtain a better curve. This was

chosen to filter out the majority of movement artefact signals, whilst allowing a majority of true signal arising from RBC movement.

The backscattered Doppler shifted signal was also narrowly filtered on both sides of the 780 nm infra red laser signal between 20 Hz and 12 kHz. Detailed technical consideration of the signal processing of the Doppler signal is outside the immediate scope of this clinical project. Extensive description of this processing is given in the thesis by Tenland (1982).

3.1.3 The Laser Doppler probe (Probe 403)

The probe (Figure 3.3) used a silica fibre with core diameter of 0.125 mm that gave flexibility and a small bending radius. The probe was re-enforced with Kevlar for increased strength. The cable connecting the probe was 2 m in length, which terminated in a Kevlar coated probe head. The metal probe itself (Figure 3.4) was 80mm in length and 1mm in diameter. The probe head was connected to a manipulator for fixation and positioning. The cable carries three optical fibres, which fed to the probe tip, one to transmit the light from the laser to the tissues and the two fibres to carry the backscattered light to the photodetector.

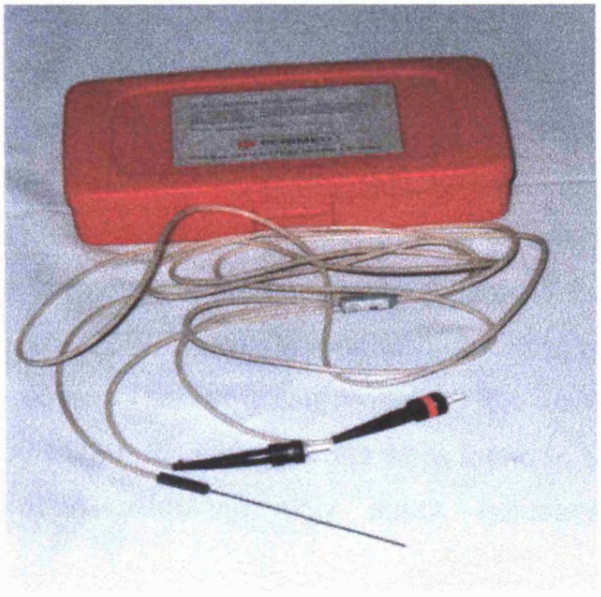

Figure 3.3. *The Laser Doppler probe (Probe 403)*

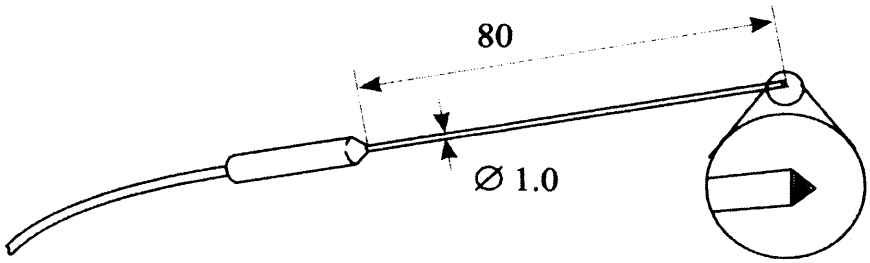

Figure 3.4 *The LDF probe 403 (stainless steel probe).*

3.1.4 Measurement Volume:

The use of 403 probes afforded a measurement volume of 1mm³ (Karanfilian *et al.,* 1984; Slaaf *et al.,* 1990; Nilsson *et al.,* 1980; and Bollinger *et al.,* 1991). This was dependent on the fibre separation distance (0.25mm). The probe tips were sterilized by soaking in glutaraldehyde (Cidex) for 12 hours following its use.

3.1.5 LDPM probe Calibration:

Probe calibration was based on measurement of Brownian motion in an aqueous colloidal suspension of latex particles at 22°C. This provided the upper limit of an arbitrary scale for motility. The set points for motility were 0 PU (Perfusion unit) and 250 PU. Zero PU was set by placing the probe against a white plastic zeroing disc; the perfusion value obtained was normally 0PU +/- 5%.

The upper value of 250 PU was set by, placing the probe into a white walled container filled with the calibration colloid. The probe tip was carefully immersed into the motility standard at a minimum distance of 5mm away from the container wall and bottom. Care was taken to avoid air bubble formation at the tip. While calibrated the unit gave a reading of 250 PU +/- 5%. These values were found to be stable over repeated calibrations. The Brownian motion of the particles at 22°C was taken as 250 PU +/- 5. These values were found to be stable over repeated calibration, requiring only minor adjustments to the equipment.

Prior to this procedure the machine was allowed to warm for 20 minutes. The instrument calibrated for the probe used to obtain the same flux sensitivity (the same reading on measuring same perfusion in the same tissue in different occasions).

Calibration was performed to the two measuring points 0 PU and 250 PU. The PF1001 motility standard (Figure 3.5) was used to provide a standardised perfusion value equivalent to a perfusion of 250 PU, while zero is automatic.

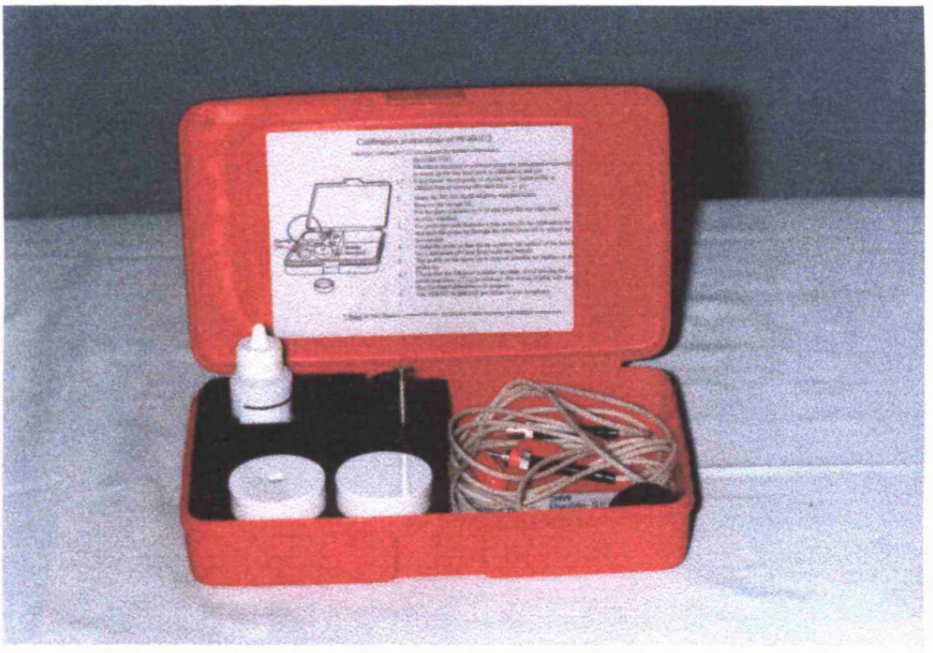

Figure 3.5. *The LDF calibration kit*

3.1.6 Modified head band and probe holder:

We used the head band of an ENT examination mirror supplied with 3 ball and socket metal joints for fixing and fitting the probe for measuring blood flow values from different points along the external ear canal and tympanic membrane (Figure 3.6).

The head band was placed on the patient's head, with the ball and socket joints and the probe positioned over the right ear first for studying the right side blood flow, then moved to the left side.

Figure 3.6. *The modified head band and probe holder.*

3.1.7 Positioning of the LDPM probes and cables:

In this study we used a specially designed headband with three ball and socket joints developed at the Medical Physics department, Leicester University Hospitals, to support the probe when applied to the area under assessment. The probe head was held in position by a small clamp as shown in (Figure 3.6). This arrangement largely reduced movement artefact as any head movement was transmitted to the probe assembly.

As we used the non-touch technique, this probe holder through the headband and slit in the joint, helped us to avoid any movements for

the probe or the tissue under study during the measurement. We moved the probe tip till it was close to the tissue under study and perfusion values was obtained and displayed on the instrument front panel.

In this study a non-touch technique was employed. Under direct visual inspection using the microscope, the probe was placed just above the skin surface by about 0.5-1mm. This has been established to be within the optimal distance to perform a laser Doppler flow measurement. Within 0.1-1mm no change in signal amplitude was observed.

If the probe was moved greater than about 2mm from the surface, the signal was dramatically reduced. If the probe was pushed into the skin surface, this typically resulted in a decrease of signal due to compression of the underlying vasculature.

Prior to placement of the probe, patients lay supine on the couch and they were asked to relax and to avoid movements of the body or the head. Generally, movements of the patient did not affect the probe signal, as long as the headband and probe did not move.

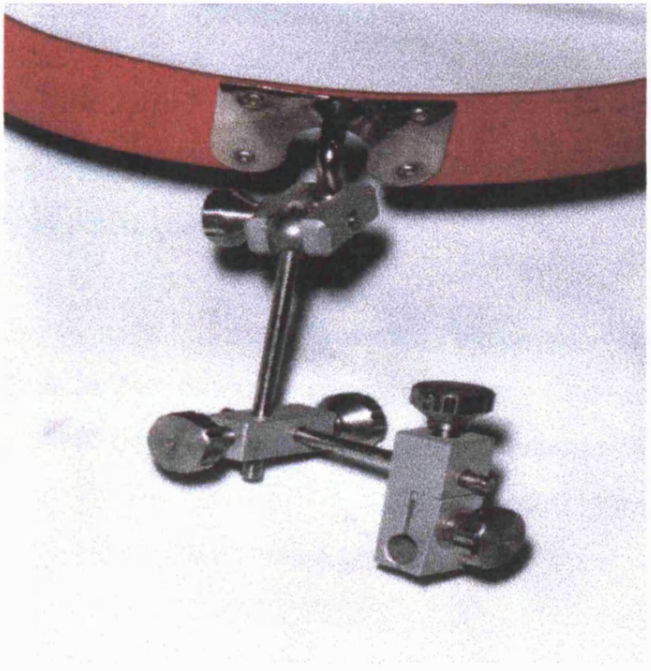

Figure 3.7. *The probe holder ball and socket joint*

3.1.8 Preparation and instrument setting:

The light source used in Periflux system 5000 is 780nm solid-state diode Laser with a maximum laser beam power output at the probe tip of 1mW, which classified the instrument as class 1 type BF for electrical safety standards. According to the instrument manufacturer instructions, we allowed the instrument to warm up for 20 minutes before starting any measurements.

3.2 Ethical Committee Approval, Information Sheets and Consent forms:

Prior to beginning the study, ethical approval was obtained from the Leicestershire Health authority clinical research ethical committee (Ref. no. 5096 - 20August 1998). All patients and volunteers involved in this study read the patient information sheets about the study, and explanation of the procedure as approved by Leicestershire health clinical research ethical Committee. Also, all patients groups signed the consent forms to share in this study with freedom to withdraw at any stage.

3.3 Study design: justification and aims.

The design of this study invited recruitment of three separate groups. These were:

-Control group.

-Otitis externa patients group.

-Myringoplasty patients group.

-Control group: Prior to this study, very little information was available about blood flow in the external auditory canal. The control group would provide novel results about this, and measurement in this group was justified for its own sake. It also, provided the results for comparison in the other experimental groups.

-Otitis externa patients group: otitis externa is a very common infection of the external auditory canal and contributes to a large amount of the clinical workload. It is therefore, highly relevant to study this patient group. The experimental aim of measuring from this patient group was to provide quantitative estimates of the increase in blood flow accompanying the infection. This has never previously been attempted.

-Myringoplasty group: myringoplasty is a common surgical procedure. Myringoplasty is defined as an operation in which the reconstructive procedure is limited to the repair of tympanic membrane perforation without middle ear exploration or ossicular chain evaluation whereas tympanoplasty is performed to eradicate disease in the middle ear and to reconstruct the ossicular integrity (Sheey, 1984).

3.3.1 Measurements from the control group of the study with evaluation and validation of the laser Doppler flowmetry:

There is not enough information about application of laser Doppler for studying ear blood flow in humans, and one of the first trials was to validate and evaluate the instrument for measurement from different points in patients' ears.

This group of volunteer controls included colleagues, medical students, and staff working at Leicester University Hospitals. It included 43 subjects (16 female and 27 males) with age range 18-70.

3.3.1.1Selection Criteria:

All volunteer controls selected to be:

-Healthy, non-smokers, not under medical care for any medical problems and not taking any medical treatments.

-No past or recent history of ear infections or problems.

-Clear, clean external ear canals and normal intact tympanic membranes.

-No recent ear problems, hearing changes, earache, otalgia, tinnitus or dizziness over the past 12 months.

-Not eating or drinking for one hour before the measurement.
-All accepted to take part in the study after reading the patient information sheet and signing the consent forms.

3.3.1.2 Procedure:

We performed this study on groups of controls and patients. Cases were recruited from the ENT department, Leicester University Hospitals. Information sheets given to the patients and consent obtained to take part in the study. The study was based at the Hearing Services Department, Leicester University Hospitals, where we used a sound proofed room to avoid the effect of the outside noise and temperature on the tympanic membrane during the study. The room temperature was maintained at 22° C during the study.

Prior to LDF measurement each patient was allowed to relax for 20 minutes and acclimatise in the room where the measurement took place. This allowed the heart to stabilise at the resting rate, also, to allow the patient to acclimatise to the room temperature if they came from an environment with a significantly different temperature, e.g. from the outside in cold weather.

3.3.1.3 Making the LDPM measurements (Procedure):

Measurement for each patient was performed bilaterally on the right and left ears. Four sites on each side measured in this order:
- skin overlying the tragus.
- skin overlying (of) the cartilagenous part of the EAC.
- skin overlying (of) the deep bony part of the EAC.
- Outer external surface of the tympanic membrane.

The reasons for choosing these four sites were as follows. Site 1 provided a convenient external site representing external skin blood flow. Sites were representative sites of the two differing parts of the ear canal. Site 2 is the cartilaginous outer third of the canal containing ceruminous and sweat glands along with hair follicles. By contrast at

the position of site 3, there were no ceruminous glands or hair follicles. The specific choice of the recording site on the TM was determined by the fact that it was easier to position the probe at this site without touching the canal or causing discomfort. Blood flow in the posterior half of the TM has also been reported to be higher than in the anterior half (Maher, 1988).

The patient lay comfortably on his back over a couch in the sound proofed room, in a relaxed physical and mental state. The patients wore the headband and the probe applied to the study area without any touch or compression (about 1mm from the surface under study).

Readings were taken for approximately 1-3 minutes from each site. The readings were saved to the Perisoft windows used in connection to the Periflux for further analysis. This procedure was performed first in the right ear then the left ear, with each subject remaining in the supine position on the couch for the whole duration of procedure (approximately 30 minutes for both ears). After the study the head band and probes were removed and probe inserted for sterilisation in Cidex.

All patients' data were entered in the Periflux software installed in the PC before starting the study. Analysis of the results was carried out by selecting a continuous period of 30 seconds with regular flow pattern and no artefacts.

Of the forty three control patients, 13 agreed to be measured on a second occasion to provide information about intrasubject variability. These measurements were conducted on the same day in the afternoon usually about four to six hours after the first set of measurements had been made. These second measurements were carried out in the same environmental and experimental conditions.

3.3.2 Measurements from patients with otitis externa:

This group includes 21 patients (14 males and 7 females), with age range between 19-70 years selected from the ENT outpatient department, Leicester University Hospitals.

3.3.2.1 Selection Criteria:

All patients in this group were selected to be:

-Healthy, non-smokers, not under medical care for any medical problems and not taking any medical treatments.

-No past history of otitis media or perforated tympanic membranes or active ear discharge.

-Not eating or drinking for one hour before the measurement.

-All accepted to take part in the study after reading the patient information sheet and signing the consent forms.

3.3.3 Group of patients undertaking myringoplasty operations:

This group included 19 patients having myringoplasty operations for dry central tympanic membrane perforations. Patients for this study group were selected from the ENT department pre-admission clinic. They were attending for pre-clerking for their planned myringoplasty operations.

3.3.3.1 Selection Criteria

All patients in this group were selected on the following basis:

-Having dry central tympanic membrane perforations not discharging for the past 3 months.

-Their operation is a simple myringoplasty with endaural incision and temporalis muscle facial graft.

-Healthy, non-smokers, not under medical care for any medical problems and not taking any medical treatments.

-Not eating or drinking for one hour before the measurement.

-All accepted to take part in the study after reading the patient information sheet and signing the consent forms.

This patient group underwent their measurements one week before their surgery and 3-6 months after surgery provided they had successful surgery, their grafts had taken and their ear canal had healed and was clean on examination. Blood flow measurements were obtained from both sides before and after surgery and recorded on the PC for further analysis.

3.3.3.2 Surgical procedure (Myringoplasty):

All patients underwent simple myringoplasty operation, by endaural incision under general anaesthetic, with temporalis muscle facial graft, overnight stay in the hospital and discharge the next morning. Their aural BIPP pack was removed at the outpatient clinic 2-3 weeks postoperatively with further review in 6 weeks, 3 months and 6 months. Their post-operative measurement was performed after 3-6 months.

3.3.3.3 Follow up and postoperative measurements:

During follow-up sessions the same procedures were repeated in the same order and the results stored in the Periflux software for further analysis. For the surgical group follow-up the surgical site was examined to identify any infection or abnormality, and those not fulfiling the selection criteria were excluded from the study.

3.3.4 Calculations and statistical analysis:

All data were entered in an Excel spread sheet. Data were organised and collected prior to detailed analysis in MINITAB. Data sets were first examined for the nature of their distribution using the Anderson-Darling normality test. This was done to determine what subsequent statistical analysis should be performed.

In the majority of cases, non-parametric testing was employed due to the non-normal appearance of the distribution. Kruskal Wallis ANOVA, followed by Mann Whitney was used for analysis of data sets. A significance level of $P<0.05$ was adopted throughout.

Chapter 4

LDF measurements in the control group

4.1 LDF measurements from four sites in the external auditory canal and tympanic membrane.

The control group consisted of 43 patients, 27 males and 16 females. The age range was 18-70 years with a median age of 41 years. The 25th and 75th percentile covered 34-47 years (see figure 4.1).

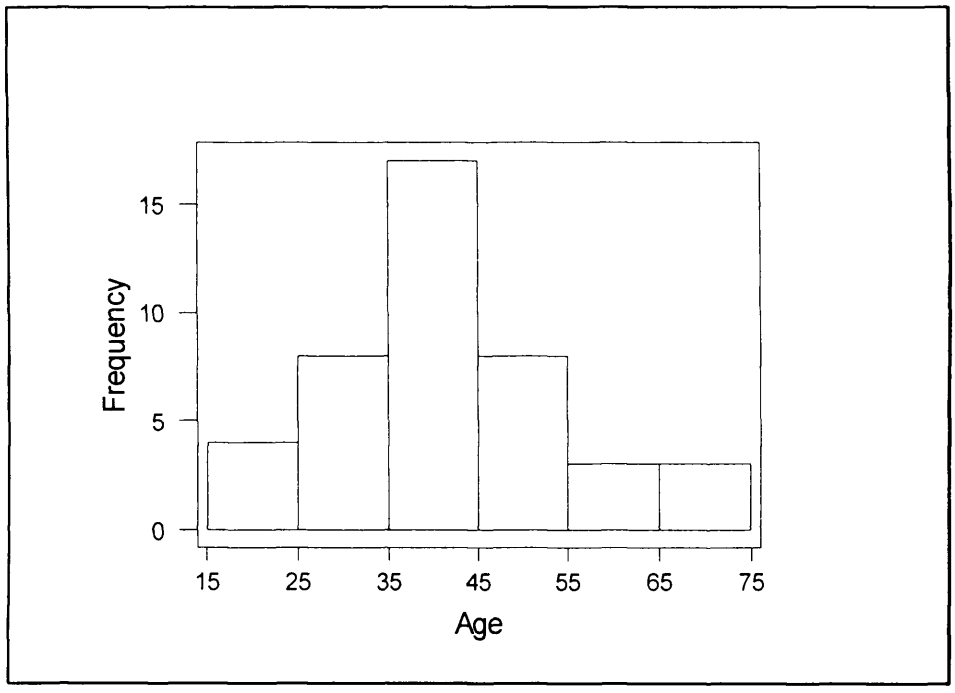

Figure 4.1. *The age distribution of control group.*

In this control group, measurements were made at four sites:

-Site 1. Skin over the tragus.

-Site 2. Outer cartilagenous part of EAC.

-Site 3. Deep bony part of EAC.

-Site 4. Tympanic membrane.

For each site the range of PU values, and the nature of the distribution were analysed.

4.1 .1 Control Group

4.1.1.1 Site 1

Fig 4.2 and 4.3 show the frequency histogram and Anderson-Darling normal probability plot for the right side site 1. The histogram plot (Figure 4.2) shows the large, seven-fold range of perfusion units from about 20-140. This substantial variation in range is also seen for site 1 in the left ear (figure 4.4).

Interestingly, the shapes of distribution of both plots do not appear statistically equivalent. Right site 1 apart from outliers has a relatively even spread of values at the lower end. In contrast left site 1 is more negatively kurtotic, i.e. has broad top and relatively steep sides.

In both histograms plots the majority of values clustered between 30-90 PU. The Anderson Darling plots also yield very different values for both sites (Right site1, $A^2 = 0.735$, $p=0.5$ and Left site1, $A^2 = 0.341$, $p=0.48$). These values are suggestive at rest; the blood flow is loosely controlled at this external site and is likely to reflect multifactorial influences of local blood flow.

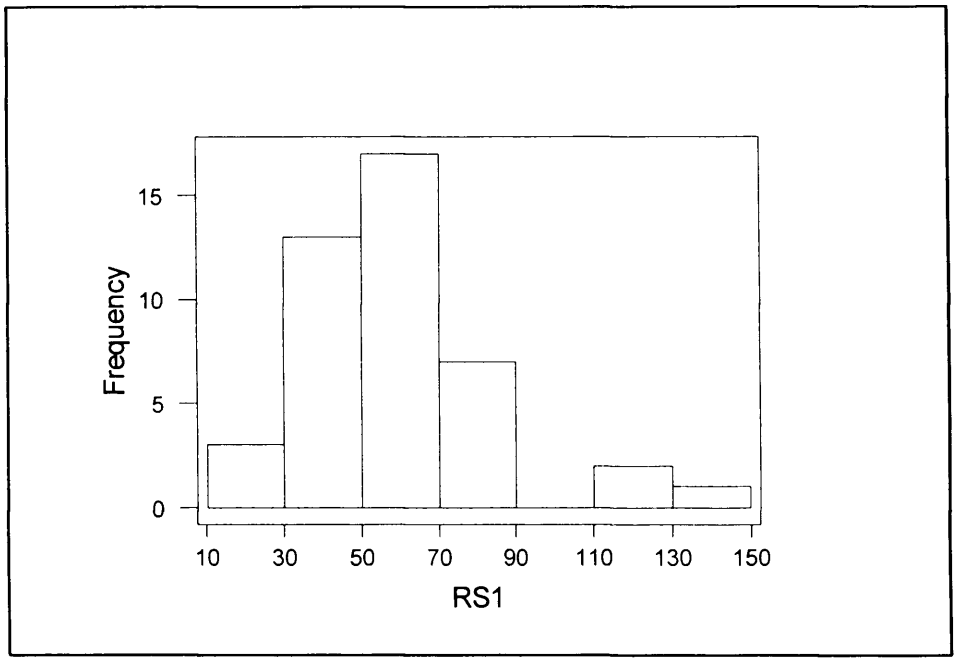

Figure 4.2. *Frequency histogram for right site 1 (RS1), note clustering between 30-90 PU, and wide PU range in normals.*

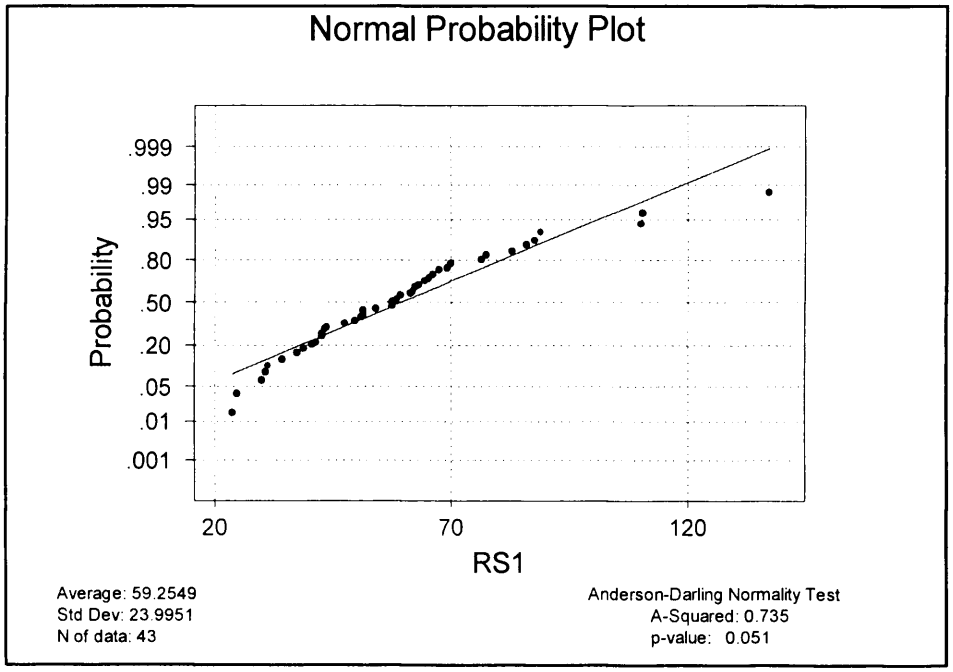

Figure 4.3. *Anderson-Darling probability plot for RS1. The A^2 value and marginal probability are not supportive of a normal distribution.*

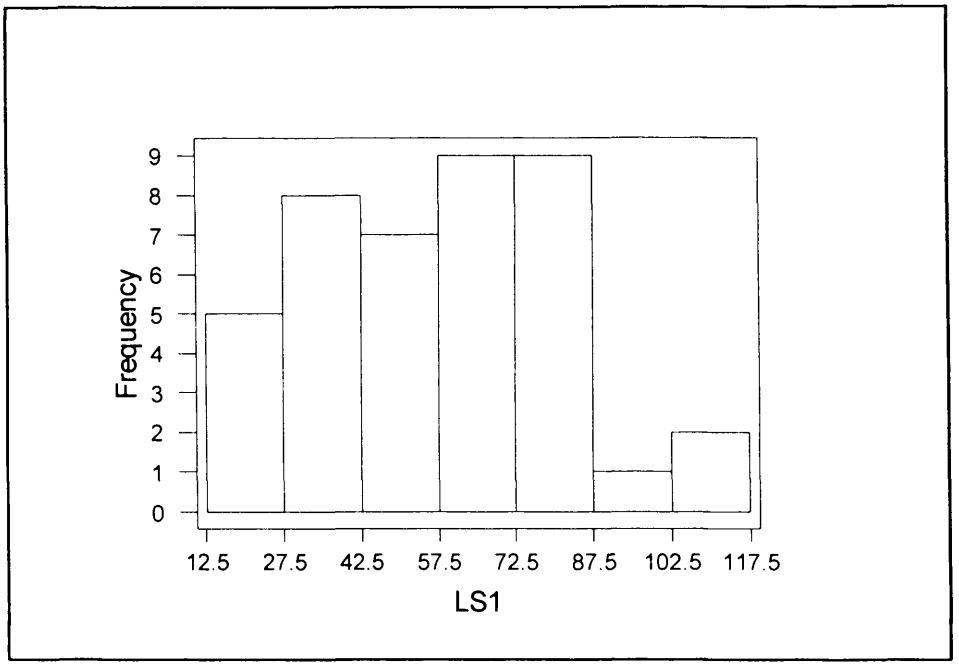

Figure 4.4. *Frequency histogram for left site 1 (LS1). Note clumping of values between 25-85 PU. There is also a wide PU range in normals.*

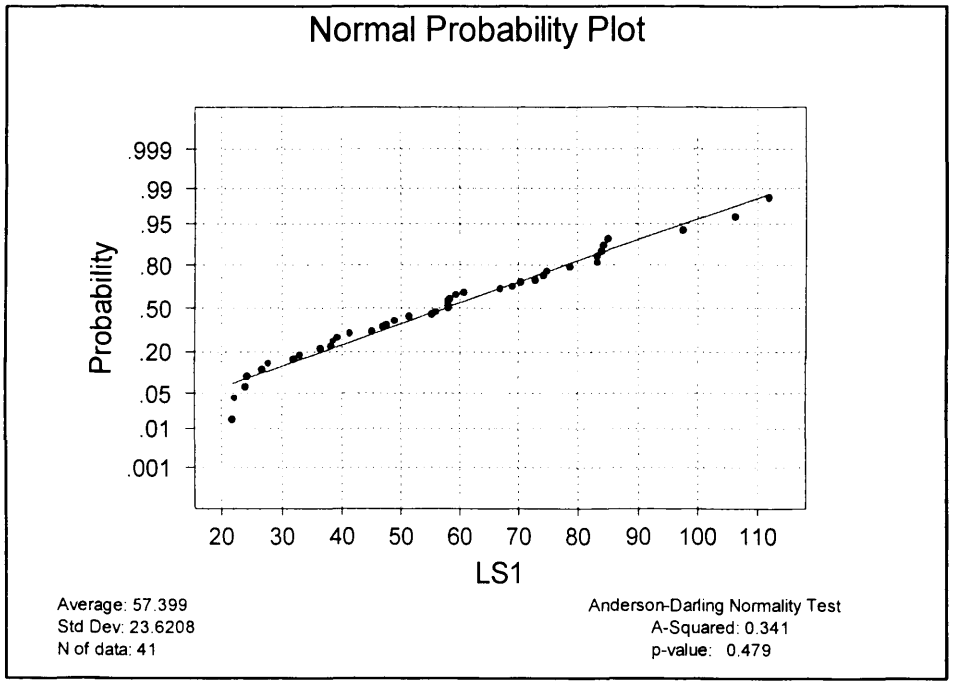

Figure 4.5. *Anderson-Darling probability plot for left site 1. In contrast with the right ear, the A^2 value and probability of 0.48 are supportive of a normal distribution.*

4.1.1.2 Site 2

The frequency histograms and Anderson-Darling plots for site 2 are shown in figures 4.6-9. Site 2 was typically located within 0.8mm within the external auditory canal. The range was similar to right site 1, varying from 22 –145 PU. On the left site 2, the upper range extended to 189 PU, but this was due to few outliers over 140 –190 PU.

Again, as with right side 1 and left side 1 there is large range, but there is a shift in median values of 72 and 75 PU for the right site 2 and left site 2 respectively. The pattern of clustering of values, appear to be somewhat different between ears.

At right site 2 the cluster is spread about 30- 100 PU, but in the left site 2 the clustering is more constrained between 40- 90 PU. Curiously, the Anderson-darling test for right site 2 returns as non-significant (P = 0.76) value, which supports the consideration of the distribution as normal.

Although, the frequency histogram in figure 4.6 has a fewer upper range values appearance, the appearance of frequency histogram in figure 4.8 for left site 2 does however look more normal, but the Anderson-Darling test returns a significant (A^2= 1.02 , P= 0.009) value, suggesting it should not be considered as a normal distribution. This is due to the outliers between 90- 190 PU.

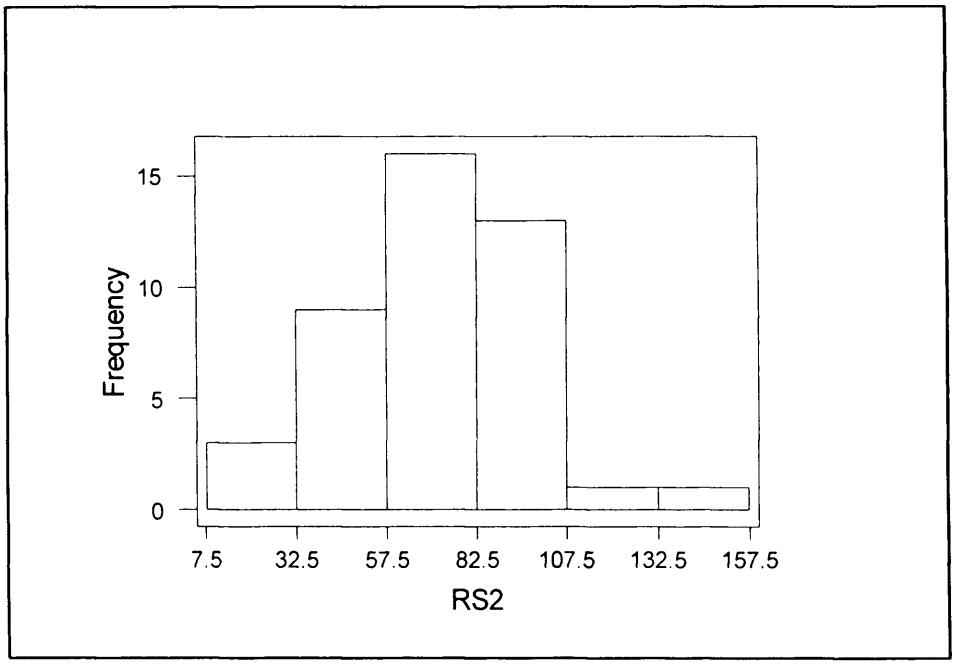

Figure 4.6. *Frequency histogram for right site 2 (RS2). This has a more normal appearance with majority of PU values between 30-100 PU. There is also a wide PU range in normals.*

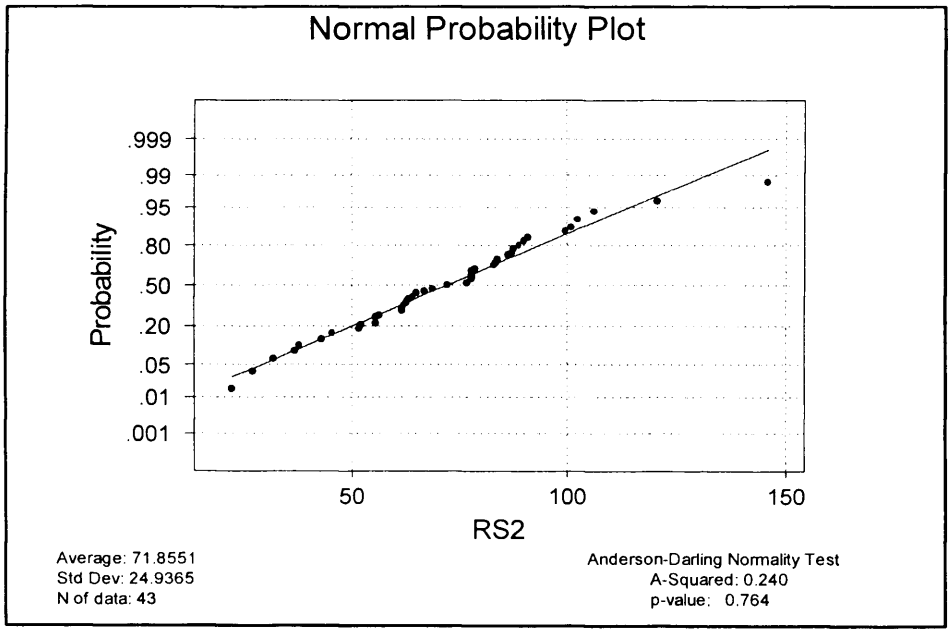

Figure 4.7. *Anderson-Darling probability plot for Right site 2. The A^2 value and probability of 0.48 are supportive of a normal distribution.*

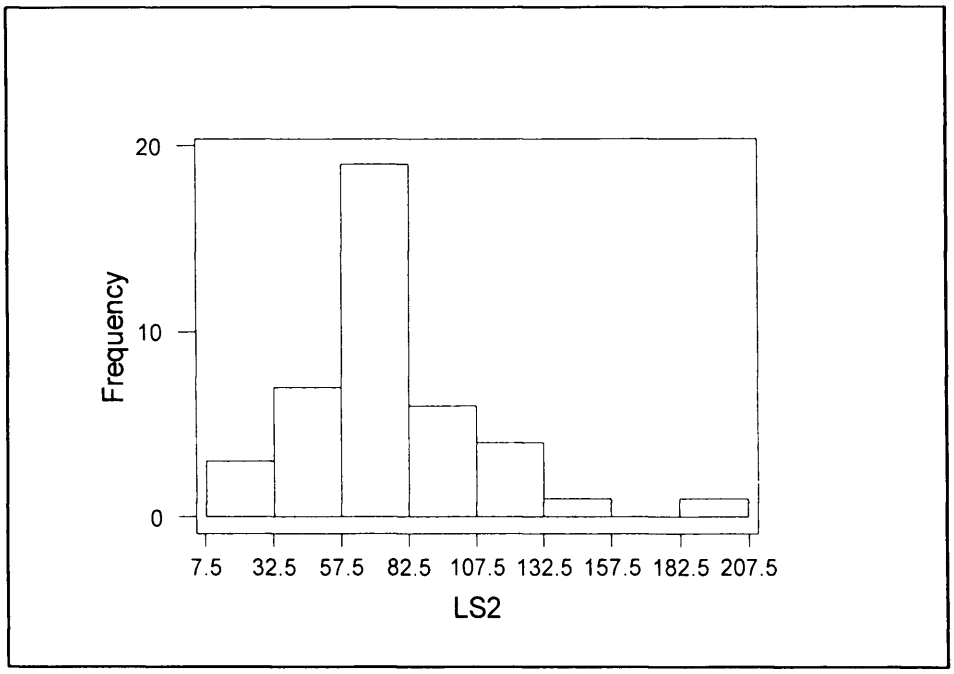

Figure 4.8. *Frequency histogram for Left site 2 (LS2). This has a normal but very peaked appearance. The majority of PU values cluster between 30-100 PU. There are a few outliers as seen below.*

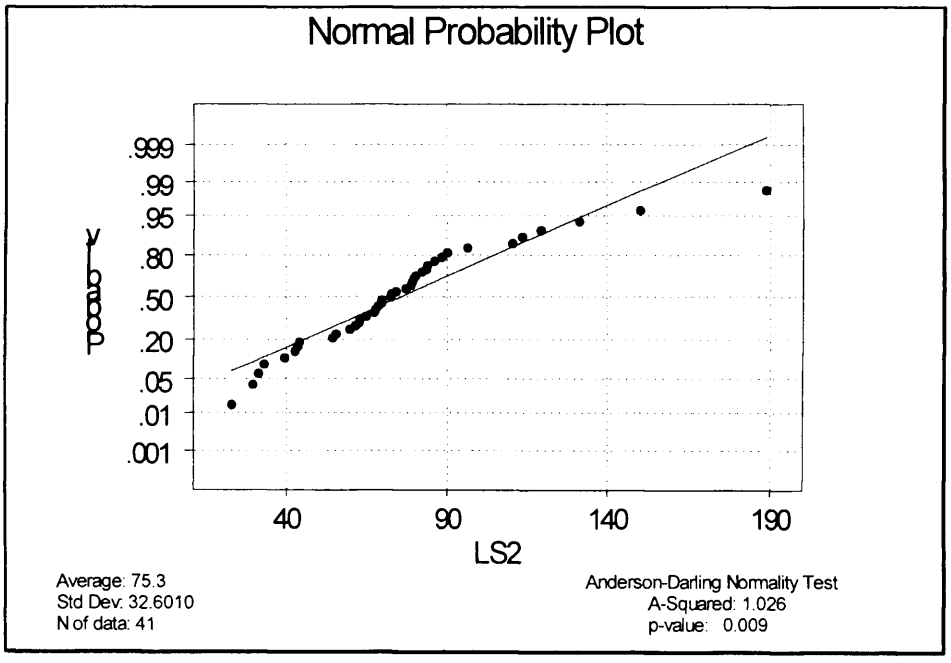

Figure 4.9. *Anderson-Darling probability plot for left site 2. Consideration of individual points especially round the middle of the distribution leads to an unexpectedly high A^2 value of 1.03 with a probability of 0.01. Statistically, this is not a normal distribution but the histogram would support the idea of it being normal.*

4.1.1.3 Site 3

Site 3 was located 16mm within the external auditory canal. The frequency histogram and Anderson-Darling plots for site 3 are shown in figures 4.10-13.

The *proportionate* range decreased somewhat. For the right site 3 this is 44-207 PU and 34-146 PU; i.e. the proportionate range was about 4-5 fold in comparison with 7 fold for sites 1 and 2.

However, the median values are higher than site 2 being 112 PU and 111 PU for right and left sites 3 respectively. Moreover, there appears to be a tighter clustering from 80-140 in the right side and 95-130 in the left side respectively. Both frequency histograms differ somewhat in appearance, with evidence of skewing to higher PU values in the left ear.

In both cases the Anderson-Darling test returns values that do not support treating distributions as normal. The right site 3 is borderline (A^2= 0.73, p= 0.052) value.

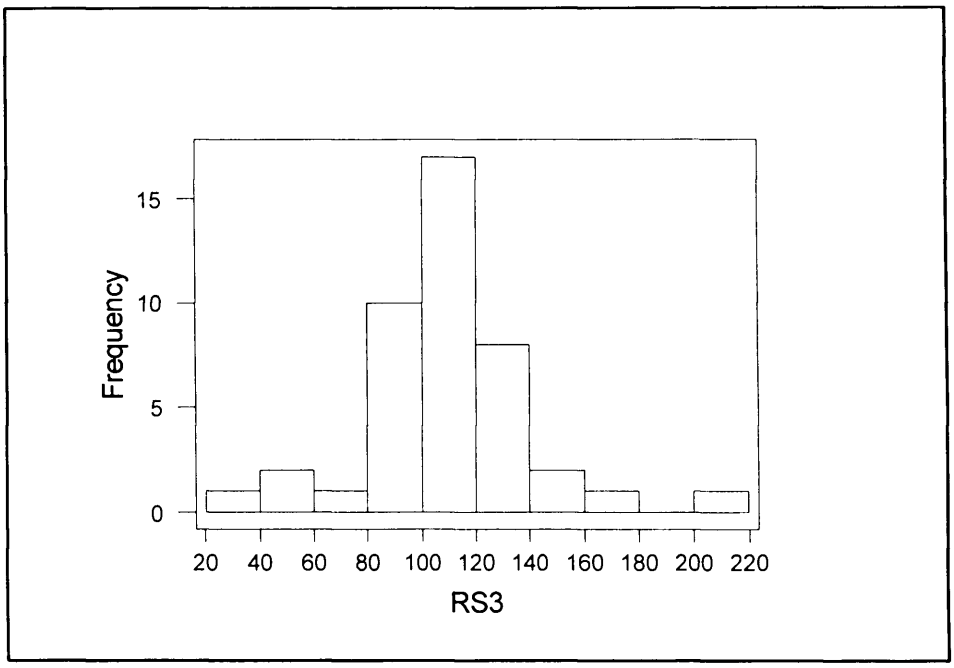

Figure 4.10. *Frequency histogram for Right site 3 (RS3). This has a normal, but again a very peaked appearance with a spread of outliers. The majority of values cluster between 80-140PU.*

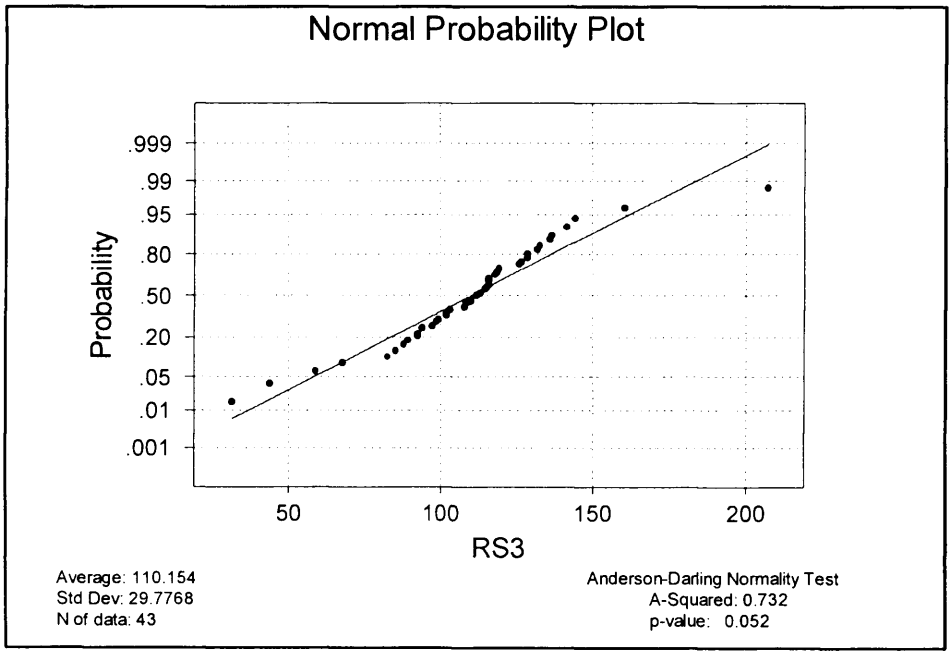

Figure 4.11. *Anderson-Darling probability plot for Right site 3. Consideration of individual points at either side of the distribution mean yields a marginal lead to an unexpected high A^2 value of 1.03 with a probability of 0.01. Typically this is not a normal distribution but the histogram would support the idea of it being normal.*

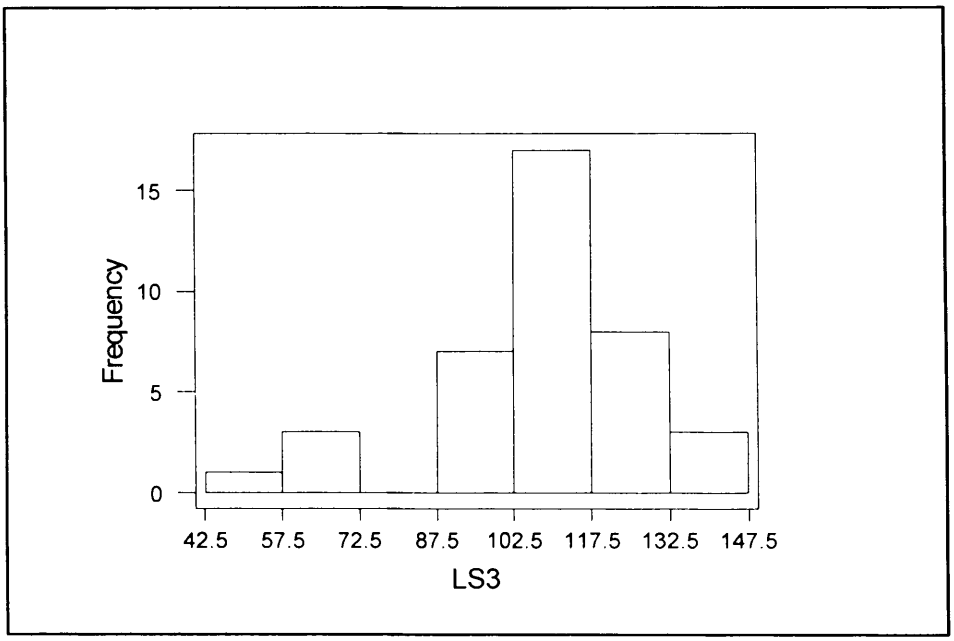

Figure 4.12. *Frequency histogram for Left site 3 (LS3). This has a normal appearance but is right skewed with fewer outliers, and a very peaked appearance with a spread of outliers. The majority of values cluster between 80-140PU.*

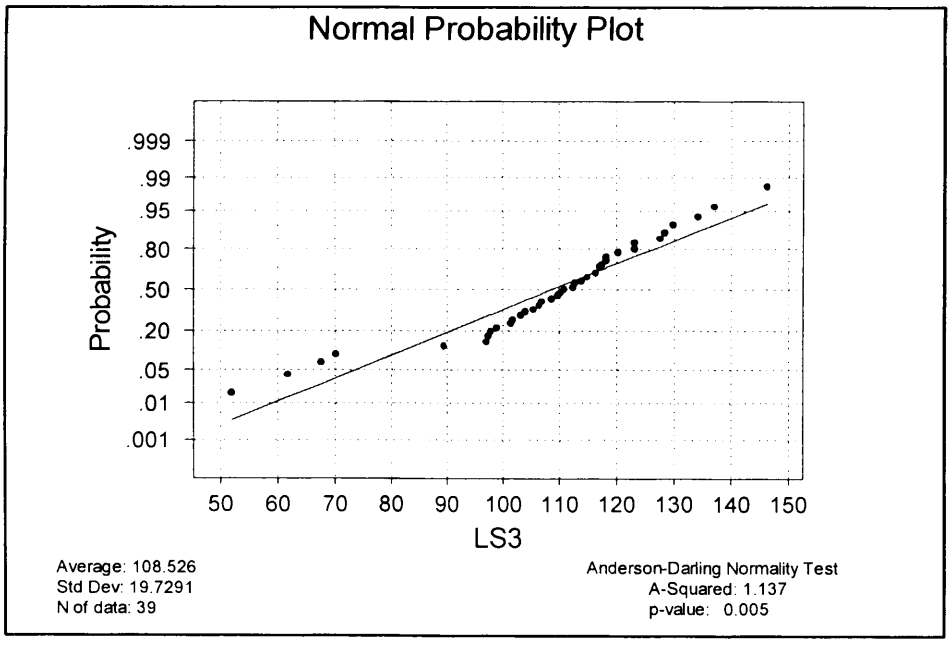

Figure 4.13. *Anderson-Darling probability plot for Left site 3. The skewing to the high PU values with a few outliers leads to a high A^2 value of 1.14 with a probability of 0.005. This means the distribution should be best analysed using non-parametric testing.*

4.1.1.4 Site 4

Site 4 is the tympanic membrane and figures 4.14-17 show the relevant frequency histogram and Anderson-Darling plots for this site. These plots showed marked contrast with the previous plots in both range and distribution. The ranges for right site 4 and left site 4 are 14-78 PU and 18-81 PU respectively; i.e. this represents about a 4-6 fold range.

However, what is most clear is the clustering towards lower PU values of 15-35 in both ears, with the scattering of outliers between 40-80 PU. The median values were 26 and 28 PU for right and left site 4 respectively. The Anderson-darling test values were highly significant (P=0.0001) for both sides, and the frequency histogram bearing this clearly has a non-normal distribution.

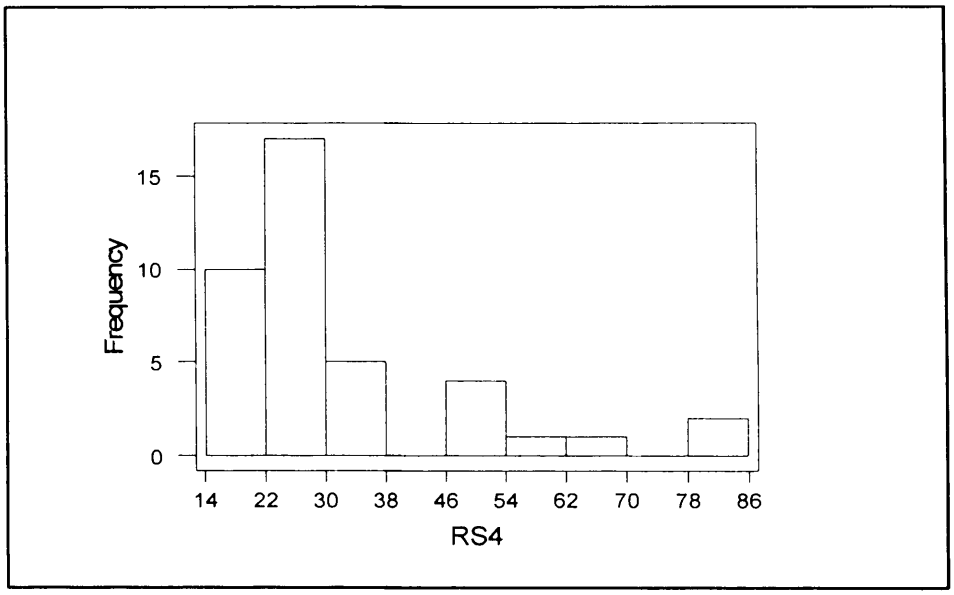

Figure 4.14. *Frequency histogram for Right site 4 (RS4). This has a much skewed distribution with outliers towards higher values. The majority of values cluster are between 15-35 PU.*

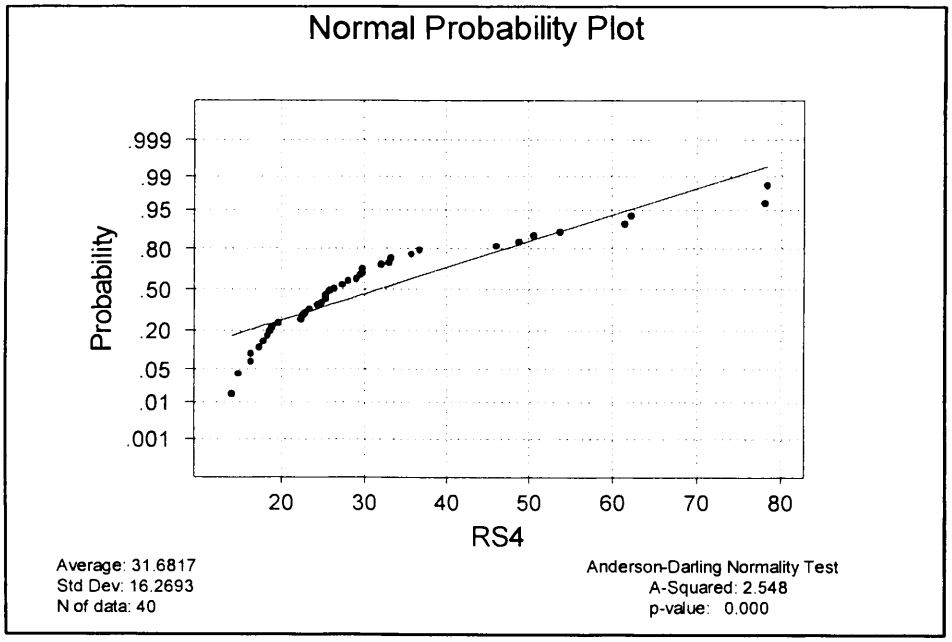

Figure 4.15. *Anderson-Darling probability plot for Right site 4. The skewing to the low PU values with great deviation from the normal, leads to a very high A^2 value of 2.55 with a probability of <0.0001. This means the distribution should definitely be analysed using non-parametric testing.*

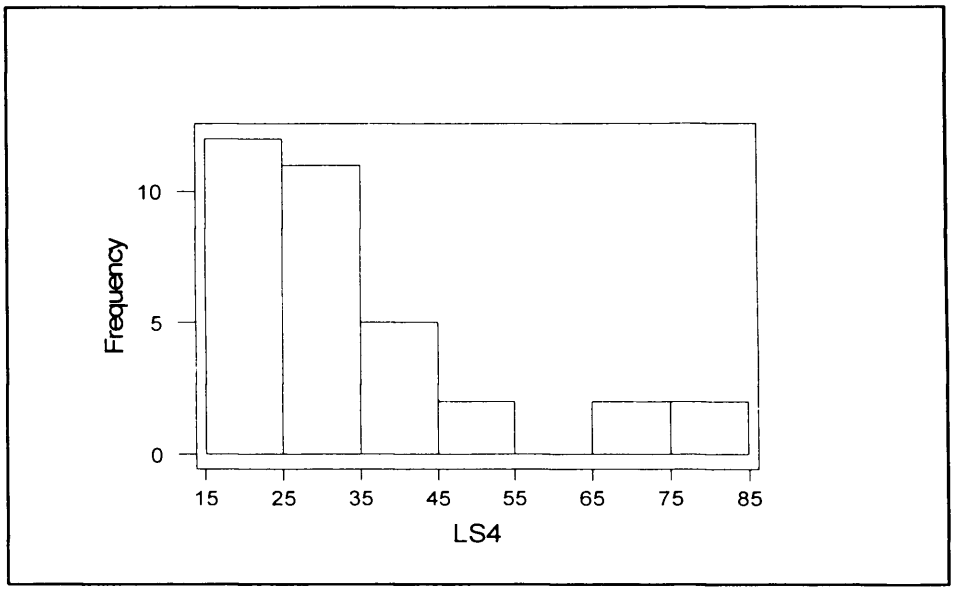

Figure 4.16. *Frequency histogram for Left site 4 (LS4). This has a much skewed distribution with outliers towards higher values. The majority of values cluster are between 15-35 PU.*

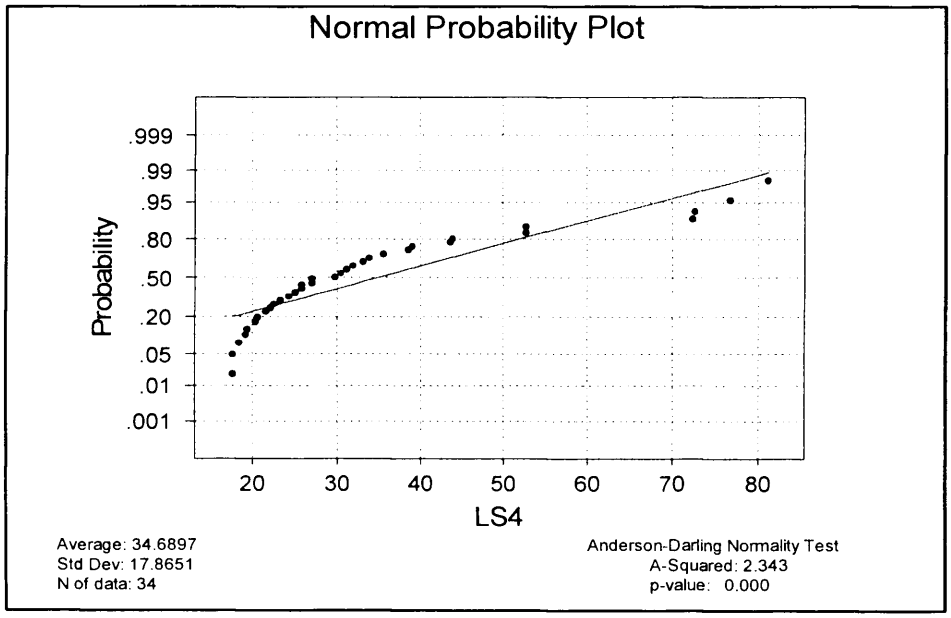

Figure 4.17. *Anderson-Darling probability plot for Left site 4. Again skewing to the low PU values with great deviation from the normal, leads to a very high A^2 value of 2.34 with a probability of <0.0001. This means the distribution is best analysed by non-parametric testing.*

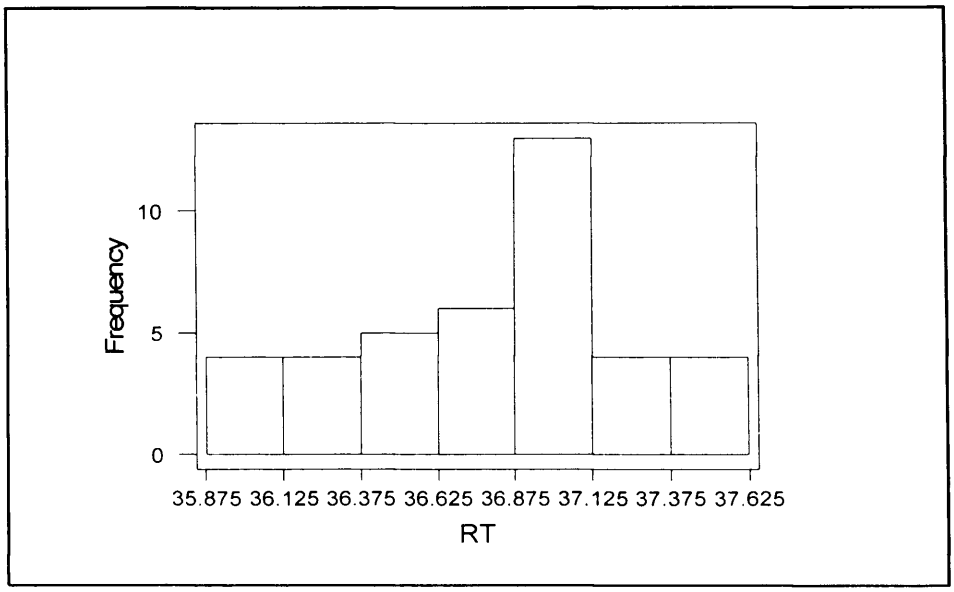

Figure 4.18. *This plot shows that body temperature at the Right TM (RT) is within normal range. Over this range, no correlation with LDF values was found.*

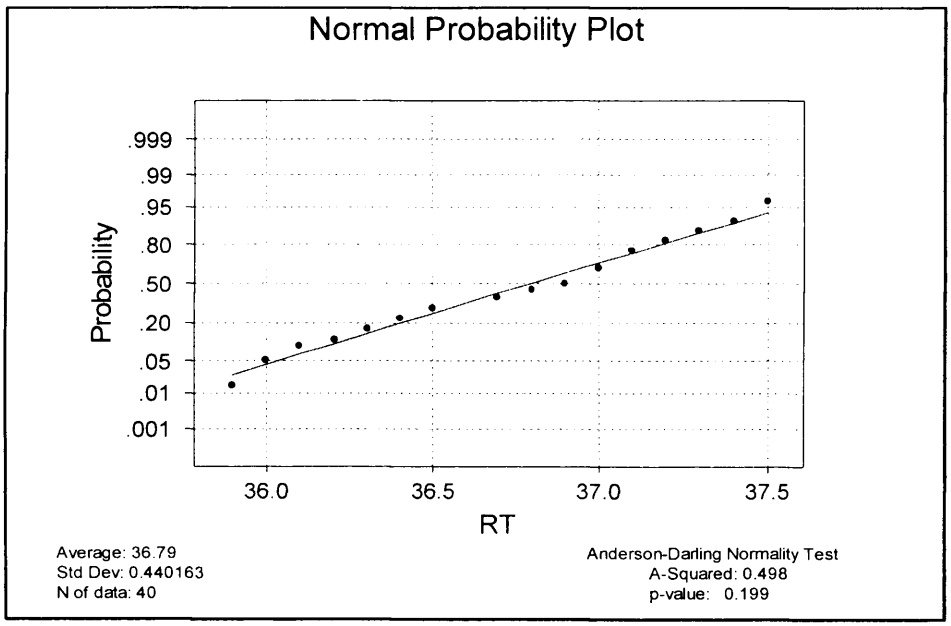

Figure 4.19. *The normal probability plot for body temperature*

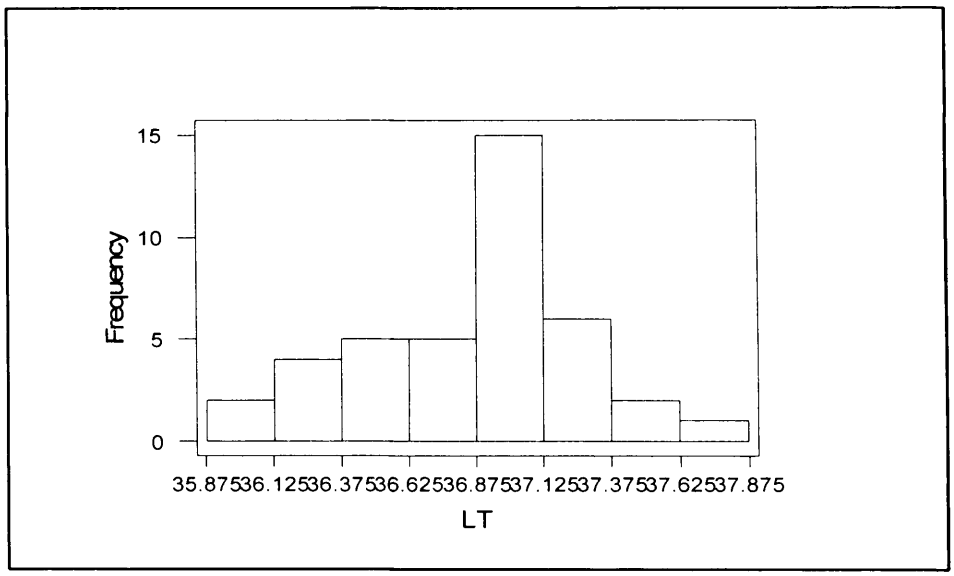

Figure 4.20. *This plot shows that body temperature at the Left TM (LT) is within normal range. Over this range no correlation with LDF values was found.*

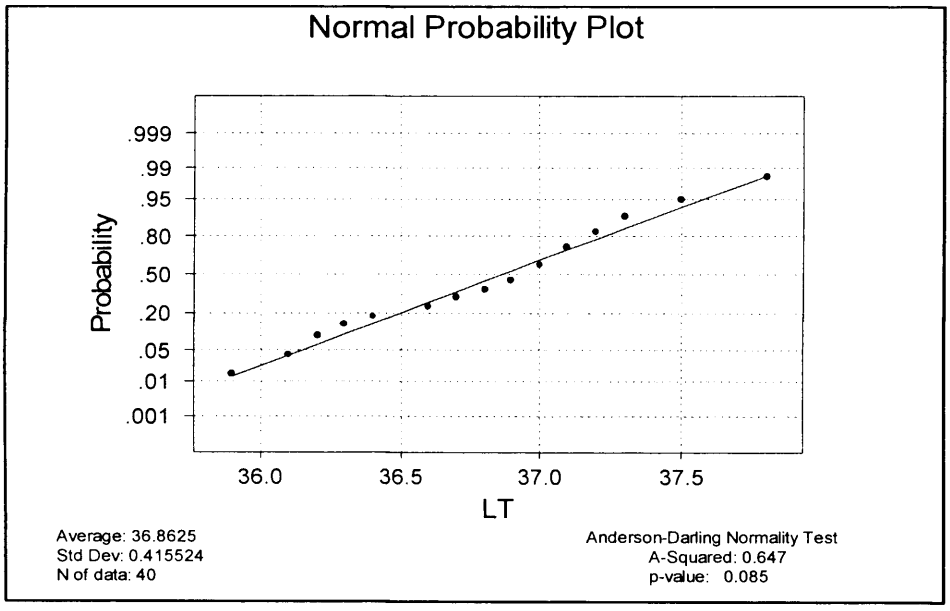

Figure 4.21. *The normal probability plot for body temperature*

Table 4.1 Summary of statistical measures at four sites

Site	***Median***	***Variability range (as 10^{th}-90^{th} percentile)***	***Sites 1-4 Site 1 median ratio***	***Median variability ratio***	***Sites 1-4 Site 1 variability ratio***
1	58	57	1	1.02	1
2	71	68	1.23	1.04	1.19
3	112	47	1.93	2.38	0.82
4	29	36	0.5	0.8	0.63

Table 4.1. *Shows the average values between ears for each of these measures. The figures give measures of absolute and relative variability*

Table 4.1 summarises the main statistical parameters for each measurement site. These include averaged median and variability range values for both ears. These are then used to describe proportionate changes in LDF signal between the sites. This table shows that there is a simple site order of 3:2:1:4 in terms of median blood flow measurement. The pattern in LDF variability at each of these sites was not found to not match this and was 2:1:3:4. This difference is likely to reflect the underlying structural variation as well as real differences in blood flow between the sites. The median/variability ratios and site 1-4/site 1 variability ratios in Table 4.1

also reflect this difference. This is discussed in more detail in Chapter 7.

In Table 4.2, the Summary of Anderson-Darling test measures at the four sites are given with commentary on the appearance of the plots in both ears. This table shows that there is no clear pattern to the distribution or appearance of the LDF data. The sites in both ears have different markedly A-squared values. The only site with a clear similarity in distribution is site 4 which is binomial in appearance. The physiological significance of this is discussed in Chapter 7. It is proposed to reflect again the contribution made by structural heterogeneity and the large variation in control of thermoregulatory blood flow.

Table 4.2 Summary of Anderson - Darling test measures at the four recording sites.

Site	***Right Ear Anderson Darling A^2 value (p-value)***	***Appearance of Right ear Frequency distribution plot***	***Left Ear Anderson Darling A^2 value (p-value)***	***Appearance of Left ear Frequency distribution plot***
1	0.73 (0.05)	Low PU skewed normal with high PU outliers	0.34 (0.48)	Flattened normal with a few high PU values
2	0.249 (0.76)	Slightly high PU skewed normal	1.02 (0.01)	Normal with high PU spread
3	0.73 (0.05)	Sharply peaked normal distribution with scattered outliers	1.138 (0.005)	Sharply peaked normal distribution with lower PU scattered outliers
4	2.54 (0.0001)	Highly skewed to low PU values with high value tail near binomial distribution	2.34 (0.0001)	Highly skewed to low PU values with high value tail near binomial distribution

Table 4.2. *Summaries of the Anderson darling values for deviation from a normal distribution and a brief description of the appearance of the frequency histogram. This shows that apart from Site 4 there was a considerable variability in appearance of their distribution.*

4.2 Correlation of blood flow between sites:

Comparison between blood flow at site 1-4 was carried out in both ears using Spearman rank correlation test.

Comparison was carried out as follows between:

Site 1 Vs: site 2; site 3; site4.

Site 2 Vs: site 3; site 4.

Site 3 Vs: site 4.

In no case was any significant correlation value found between blood flow in different sites.

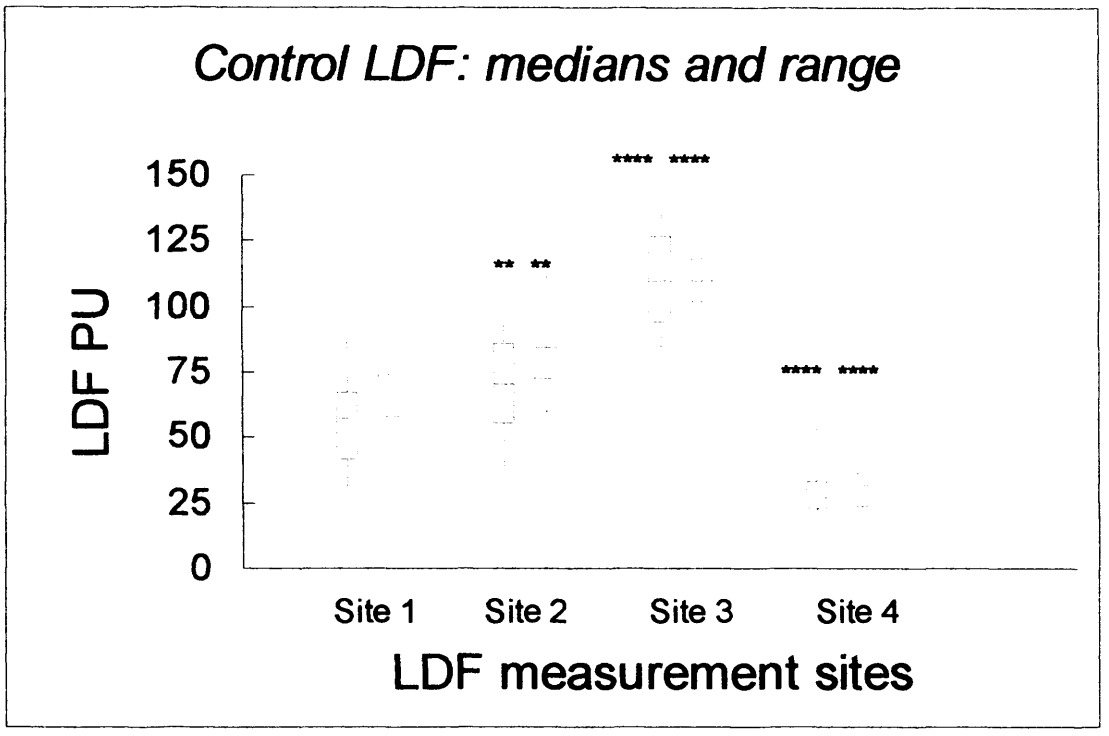

Figure 4.22 *shows the medians and range for LDF at each of the different sites in control subjects. Using site 1 as the comparator, significant differences were evident between sites 2, 3 and 4. Symbols for P<0.01 ** P<0.00001 *****

4.3 Comparison between sites

Visual inspection of the fours sites in both ears shows that there were clear differences between the four sites. The relative statistical significance of these differences was established using Friedman's ANOVA with *post hoc* testing using Wilcoxons test. Friedman ANOVA returned a highly significant value of S = 80.78 (df = 3 P < 0.0001).

For the right side, using Site 1 as the principle comparator group *post hoc* testing showed that there were highly significant differences between this site and sites 2 to 4 ($P<0.01–0.00001$). Comparison between right ear sites 2 and 3 also yielded a highly significant difference of $P<0.00001$. This clearly shows that there are very distinct variations in blood flow in the external auditory meatus and tympanum.

Similarly for the left ear, Friedmans ANOVA returned a value of $S = 78.7$ ($df = 3$, $P<0.0001$). Wilcoxons test for sites 1 vs. 2 3 and 4 all returned highly significant values between $p<0.01 – 0.00001$. Again sites 2 and 3 were also significantly different ($P<0.00001$).

4.4 Age differences:

For all sites in both ears, the relationship of blood flow and age were analysed using Spearman rank correlation test. No significant correlation was found in any site between age and blood flow.

4.5 Sex differences:

Comparison between sexes was carried out for each of the four sites in left and right ears. This was done using the Mann Whitney test. No significant differences were evident between the sexes.

4.6 Intrasubject variability:

For this measure, data was obtained from 13 subjects. Comparison of LDF PU values from each site was carried out using Wilcoxon's test. In no case was there any evidence of apparent change between the first and second measurement.

Chapter 5

LDF measurement of blood flow in the external auditory meatus of otitis externa patients

5.1 Patients Demographics:

This group consisted of 20 patients, 13 males and 7 females. The median age of the group was 43 years, with range between 19 – 70 years. Patients were chosen on the basis of them clinically presenting with bilateral otitis externa. All patients were diagnosed as having bilateral diffuse otitis externa. Of this group, one patient also presented with eczema of the ear canal and skin of the face.

As with the control group measurements were obtained from the four sites in each ear. This was carried out prior to any treatment. In some cases, the patients requested that measurement was stopped as the procedure felt uncomfortable. The testing was stopped immediately in these cases.

5.2 site 1

Figures 5.1- 5.4 show the frequency histogram and probability plot of RS1 and LS1. In figure 5.1, the majority of values lie between 40- 110 PU, with a median value of 65. The very noticeable outlier with a very high value of 390 PU was obtained from the patient presenting with eczematous otitis externa. Apart from this one value the range on LS1 is also broadly comparable. The presence of the outlier changes the relative scaling of the frequency histogram on the right side X-axis, but the overall appearance of the distribution, given the low number of observations (n=18 and 16) were comparable.

5.3 site 2

The plots for RS2 and LS2 in the otitis externa patient group (Figure 5.5 -5.8) again show an elevation of range when compared to the control group. Medians for the RS2 and LS2 were directly comparable

at 111 and 114 PU respectively. This compares with 73 PU for RS2 and LS2 in the control group (Figure 4.6 - 9). Notwithstanding the low numbers in this group the frequency histogram did appear to be different, with a block appearance in RS2 between 50 – 150 PU. LS2 in contrast showed a peak at 75 – 125 PU. This along with the high Anderson-darling values justifies the use of non-parametric statistical analysis.

5.4 site 3

Figures 5.9-12 illustrate the distribution for RS3 and LS3. The median values are both closely comparable at 189 and 185 PU for right and left sides respectively. The 25 – 75th % clusters of PU values in both ears fall between 170 – 210 PU. However, the pattern of outliers in both ears was different and was reflected in the appearances of the frequency histograms (Fig. 5.9 and 5.11). These PU values are again greater than the control RS3 and LS3 median values of about 112 PU.

5.5 site 4

The effect of otitis externa on blood flow is perhaps more striking as seen in the tympanic membrane. The medians for this site were 69 and 80 PU at RS4 and LS4 respectively. This is about a three fold increase in medians above the control median value of about 25 PU. In comparison with the other sites the relative increases was much lower, i.e. between 30-70 %.

The range of values in the right ear went from 22-212 PU, with the outlier of 212 PU coming from the patient with eczematous otitis externa. In comparison with the controls the pattern of distribution in RS4 appears to be stretched out, with no apparent clustering. This contrasts somewhat with LS4 which did show tendency to cluster at 60-90 PU as shown in figure 4. Again, the low Anderson-darling probability values ($P < 0.05$ in both ears) allied with the appearance of the frequency histogram supports the use of non-parametric statistics.

Otitis Externa Group:

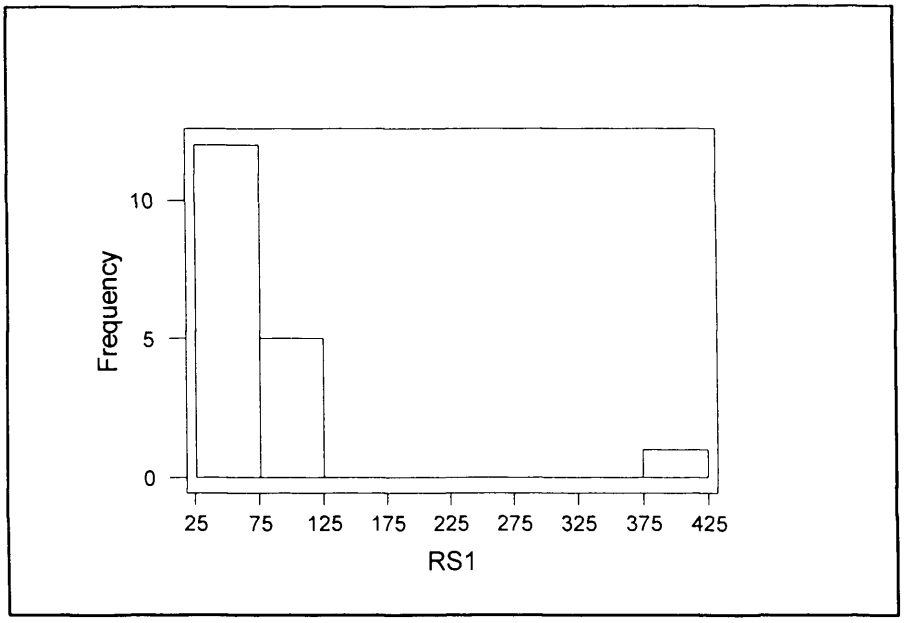

Figure 5.1. *Frequency histogram for right site 1 (RS1), because of outlier there is an apparent skew to the distribution. The very marked outlier was due to eczematous otitis externa.*

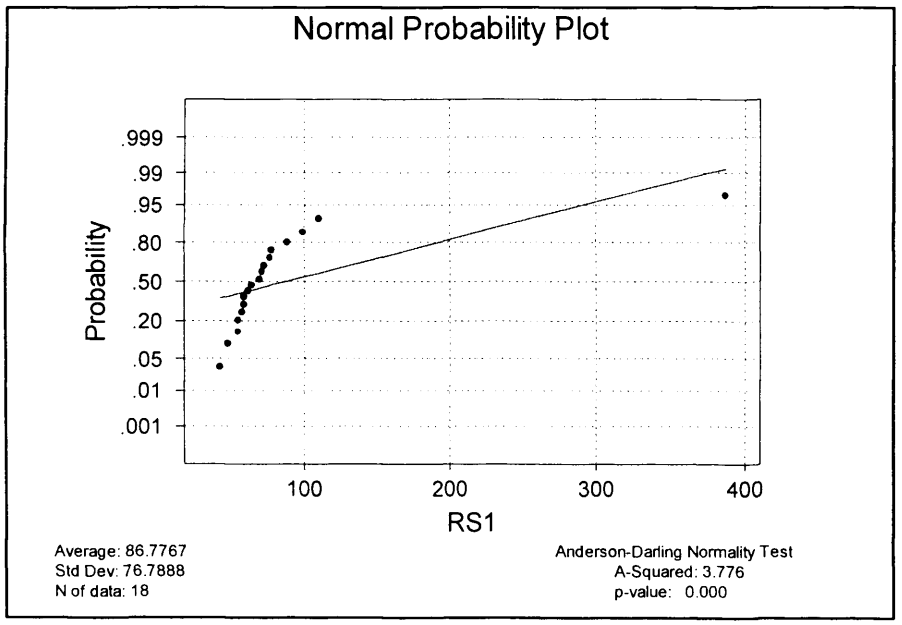

Figure 5.2. *Anderson-Darling probability plot for Otitis Externa (OE) for RS1. The A^2 value reflects the extreme outlier but the majority of the distribution is similar to control values.*

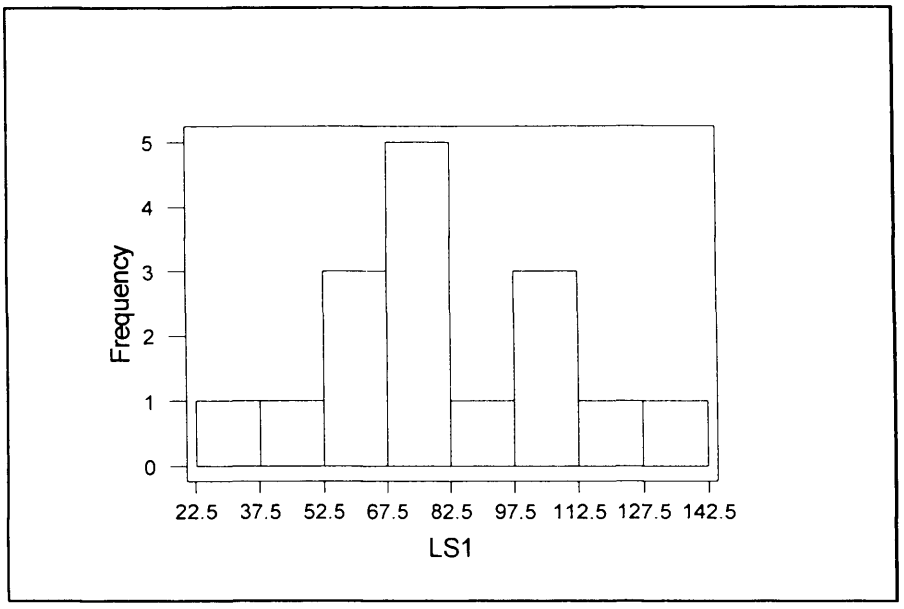

Figure 5.3. *Frequency histogram for OE LS1 (LS1). These values are more nearly normal though slightly higher than control PU values.*

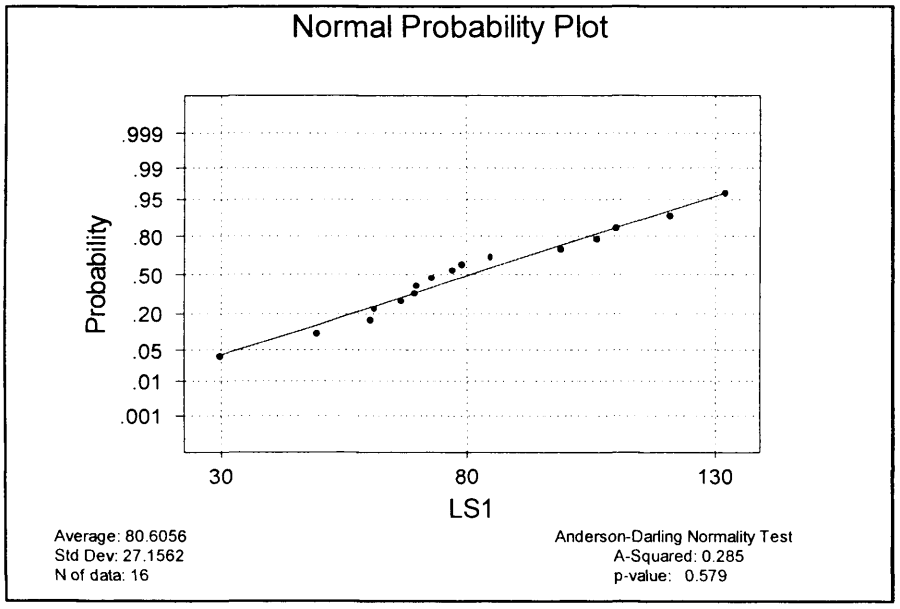

Figure 5.4. *Anderson-Darling probability plot for OE Left side 1 (LS 1). In contrast with the right ear, the low A^2 value and probability of 0.285 support a normal distribution.*

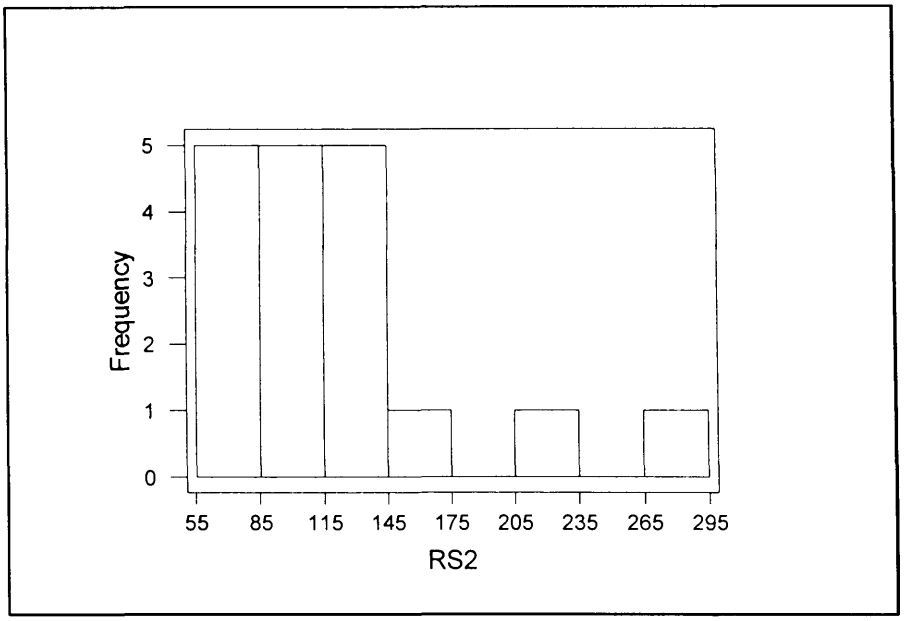

Figure 5.5. *Frequency histogram for OE RS2. These overall values are higher than control but mainly clustered to 80-140 PU.*

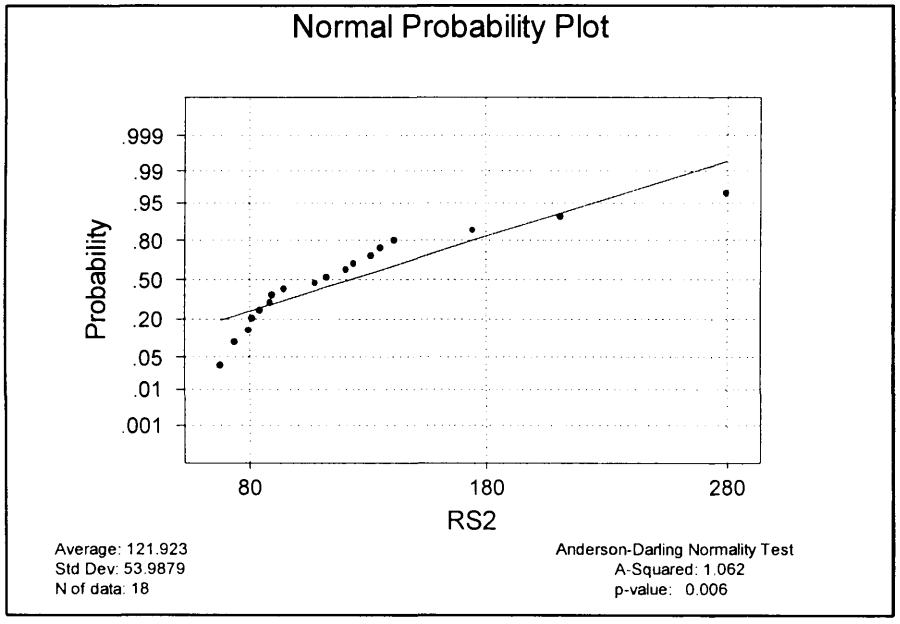

Figure 5.6. *Anderson-Darling probability plot for OE RS2. The A^2 value is 1.06 and probability of 0.006 shows the distribution is not normal.*

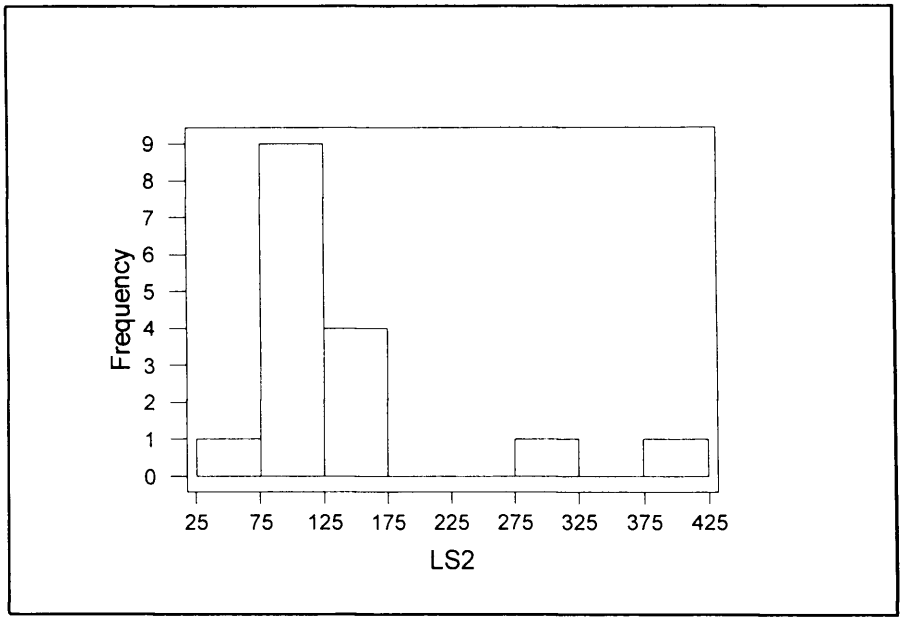

Figure 5.7. *Frequency histogram for OE LS2. These overall values are higher than control and clustered to 75-150 PU.*

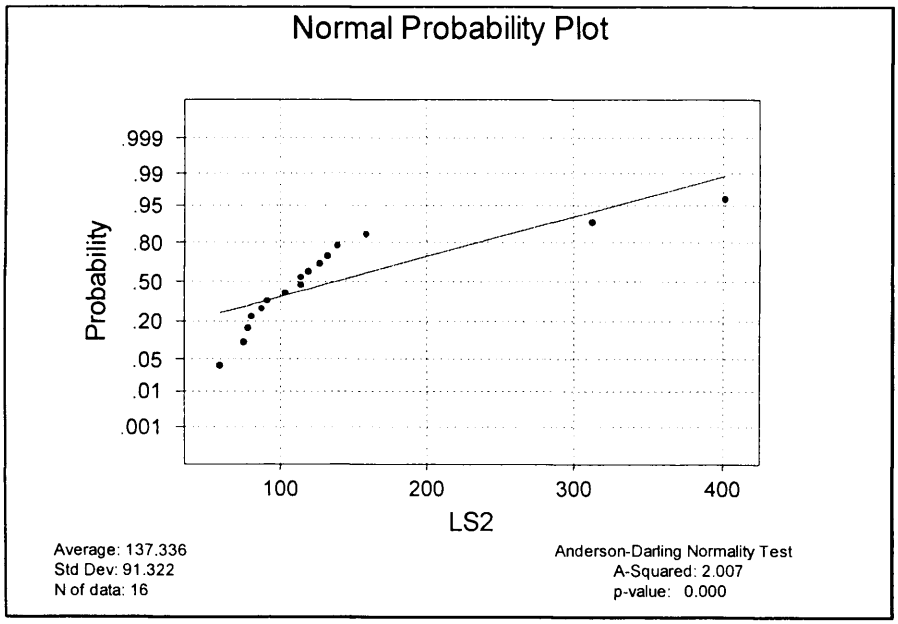

Figure 5.8. *Anderson-Darling probability plot for OE LS2. The A^2 value is 2 and probability of <0.0001 due to high value of outliers.*

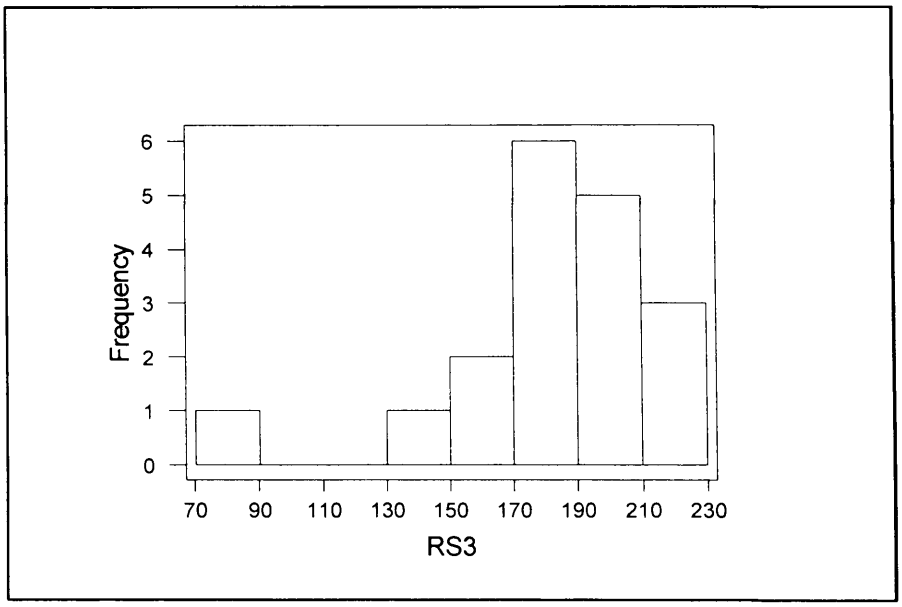

Figure 5.9. *Frequency histogram for OE RS 3. This has a skewed though partly normal appearance and is dissimilar to control RS 3 in appearance and range. The effect of OE is more marked here, the majority of values cluster between 170-225 PU.*

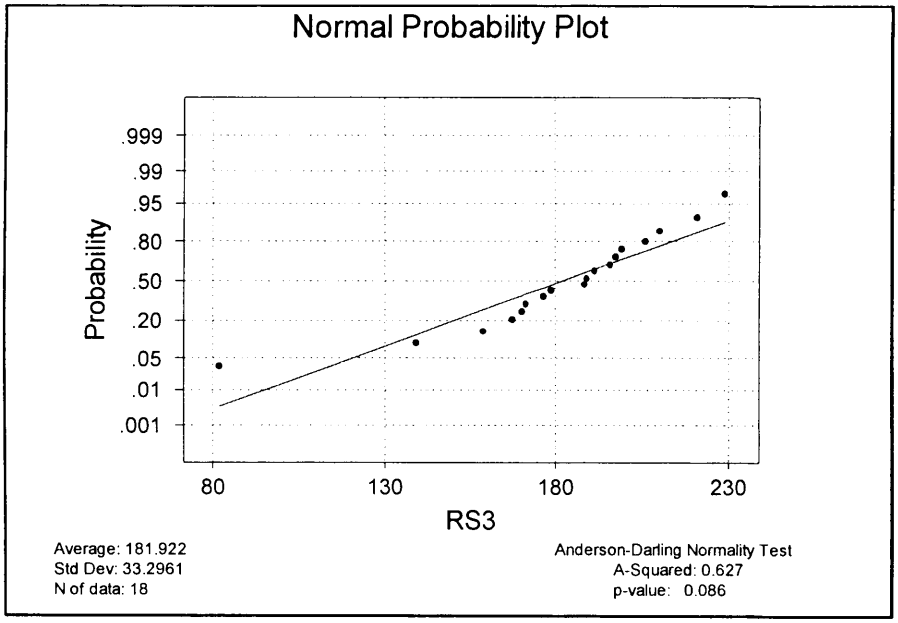

Figure 5.10. *Anderson-Darling probability plot for OE RS 3. Consideration of the plot yields a low A^2 value of 0.63 with a marginal probability of 0.09. This is not a typical normal distribution.*

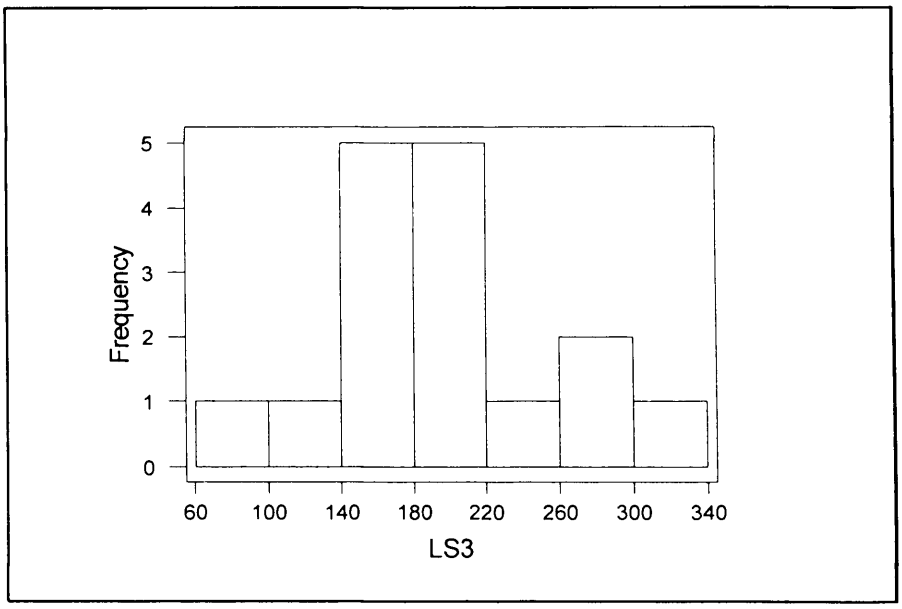

Figure 5.11. *Frequency histogram for OE LS 3. This has a partly normal appearance but dissimilar to control RS 3 in range. The effect of OE is more marked at this site here. The majority of values cluster between 140-220 PU with some outliers at both ends.*

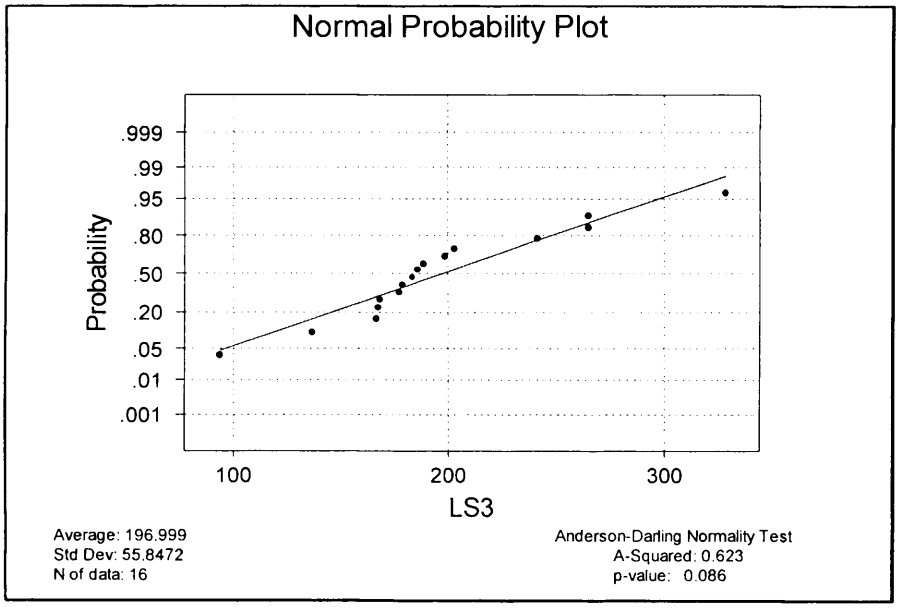

Figure 5.12. *Anderson-Darling probability plot for OE LS 3. Consideration of the plot yields a low A^2 value of 0.62 with a marginal probability again of 0.09.*

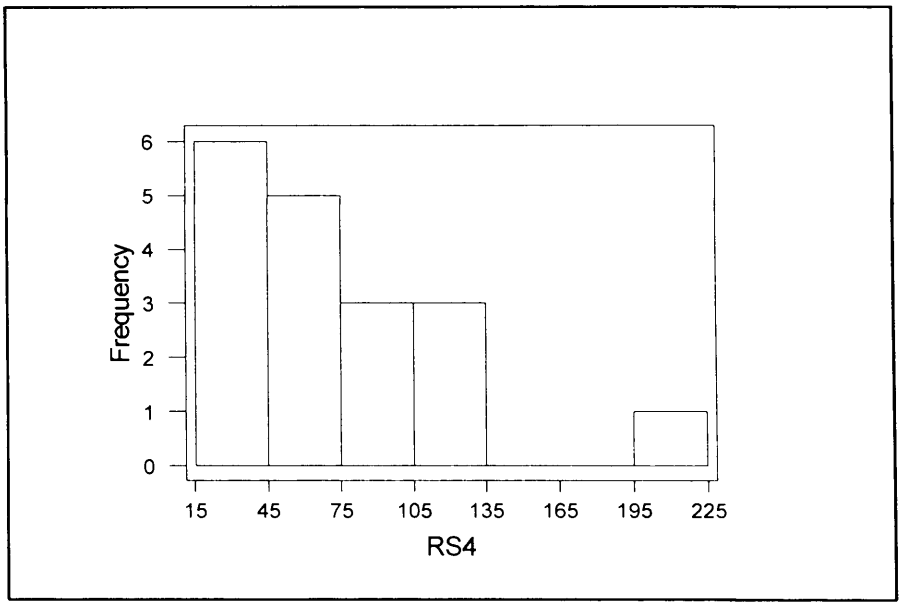

Figure 5.13. *Frequency histogram for OE RS 4. This has a clustered skewed appearance and is very extended compared to control RS 4 in range. The effect of OE is very marked at this site. The majority of values cluster between 140-220 PU, with some outliers at both ends.*

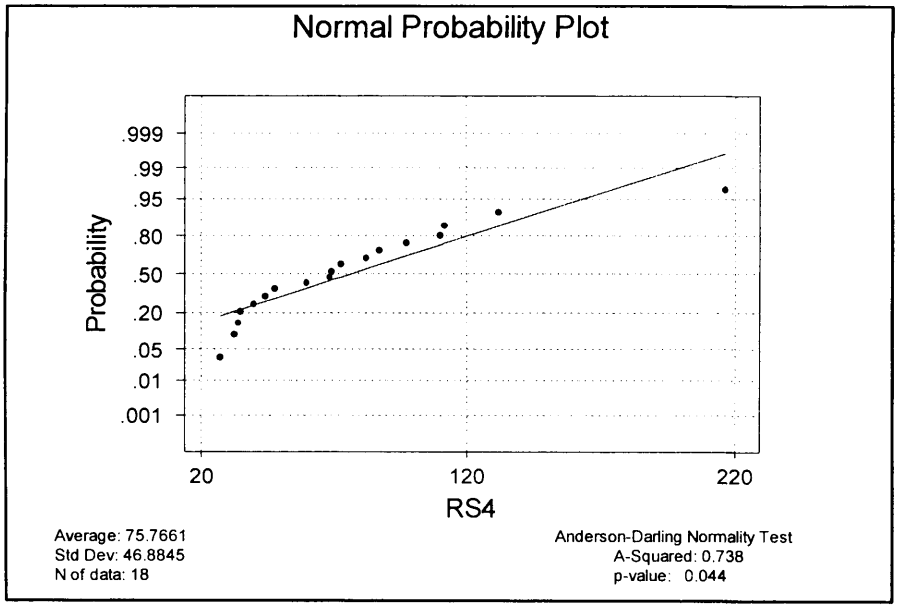

Figure 5.14. *Anderson-Darling probability plot for OE RS 4. Interestingly, the plot yields a relatively low A^2 value of 0.74. This yields a probability of 0.04, which is much less marked than in the controls. This supports the idea that OE causes a major shift in distribution.*

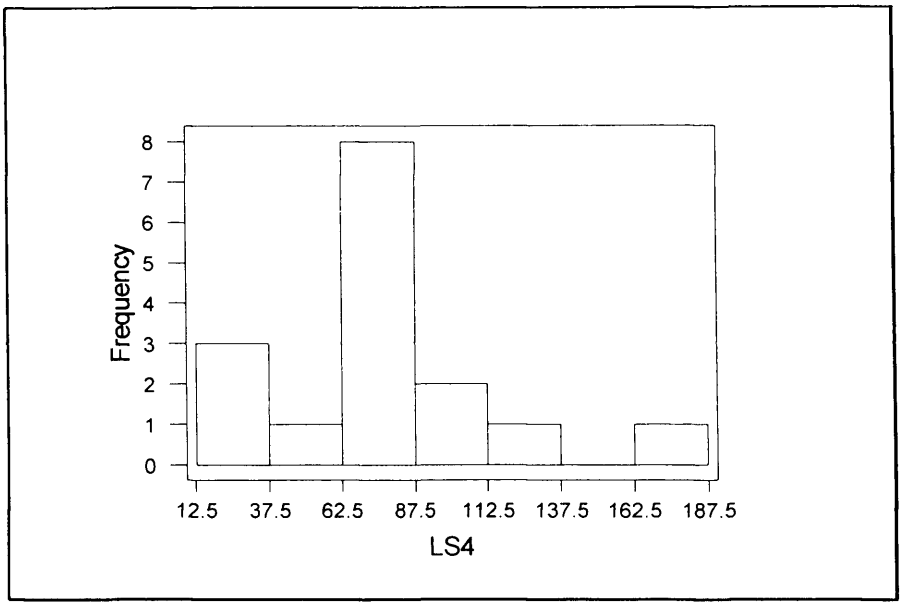

Figure 5.15. *Frequency histogram for OE LS 4. This has a much changed distribution compared to controls and is also very extended compared to in range. Again, the effect of OE is very marked. Values peak around 80-90 PU, with outliers scattered at both ends.*

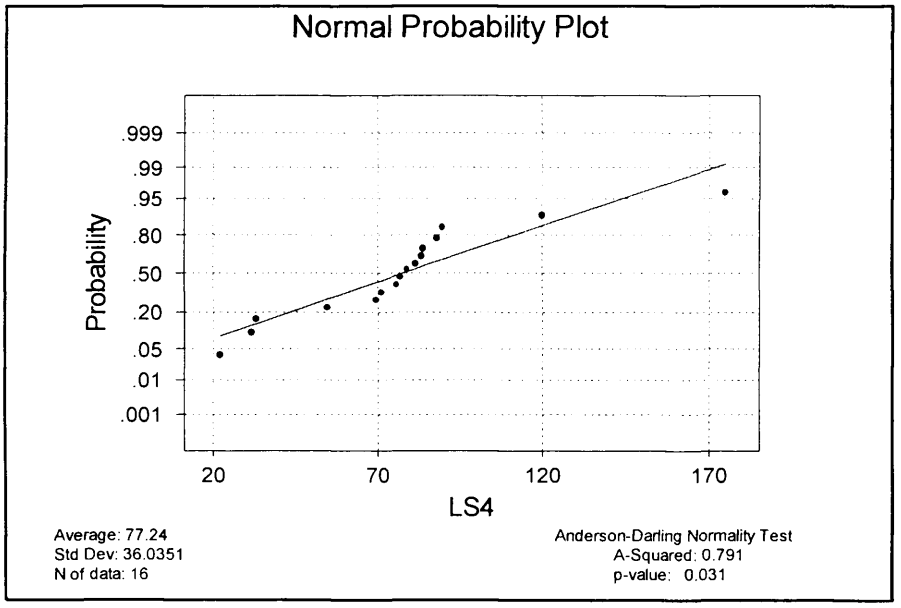

Figure 5.16. *Anderson-Darling probability plot for OE LS 4. Again, the plot yields a relatively low A^2 value of 0.8, with a probability of 0.03, which is much less marked than in the controls. This clearly supports the idea that OE causes a major shift in distribution.*

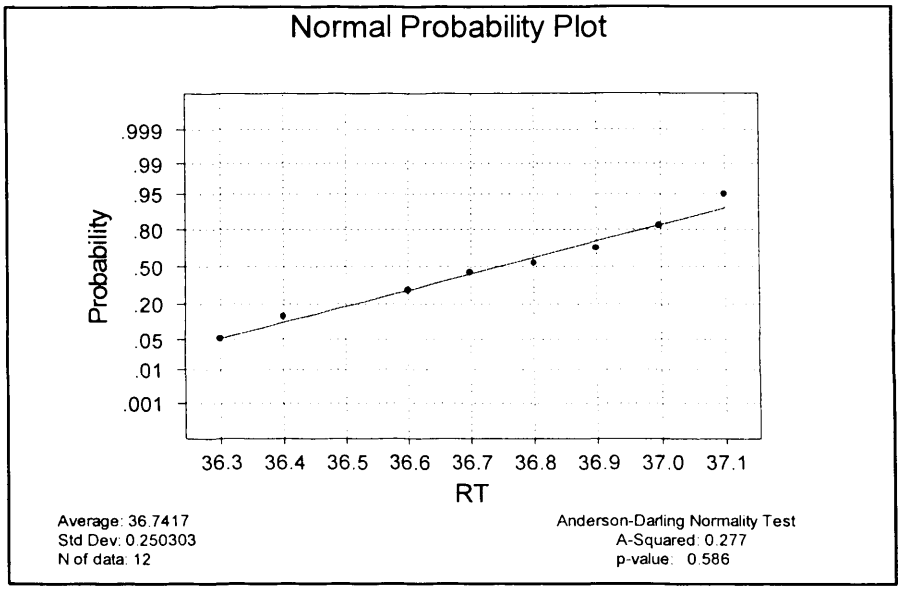

Figure 5.17. *This plot shows that body temperature at the Right TM (RT) in OE. This is within normal range.*

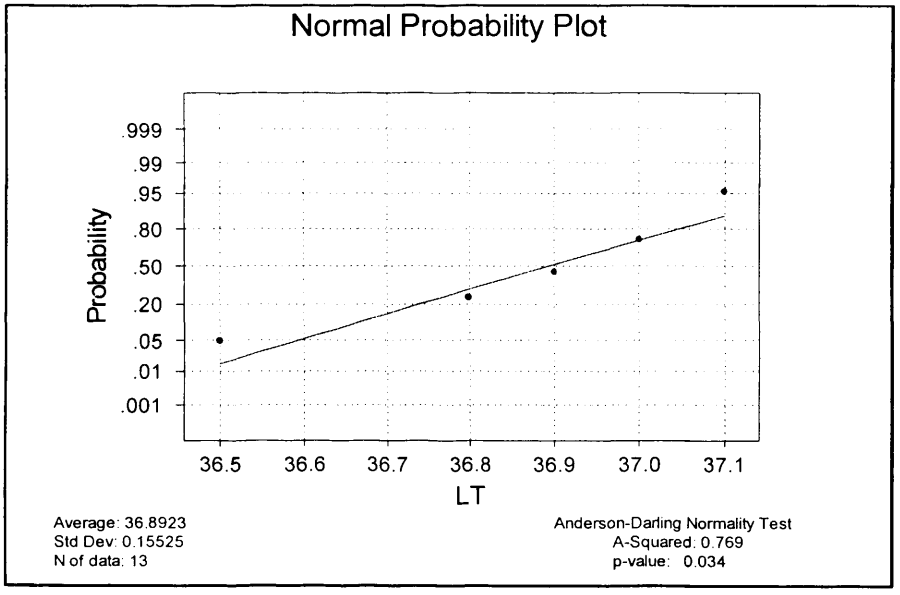

Figure 5.18. *This plot shows that body temperature at the Left TM (LT) in OE.*

5.6 Comparison of LDF in left and right ears in otitis externa group sites 1-4.

Figure 5.19 show the median with range plots for both control and otitis externa groups. Comparison of the otitis externa group left and right ears for each of the four sites by Wilcoxons test did not reveal any significant differences. This suggests, at least in the patients in this study that the severity of inflammation was comparable in both ears.

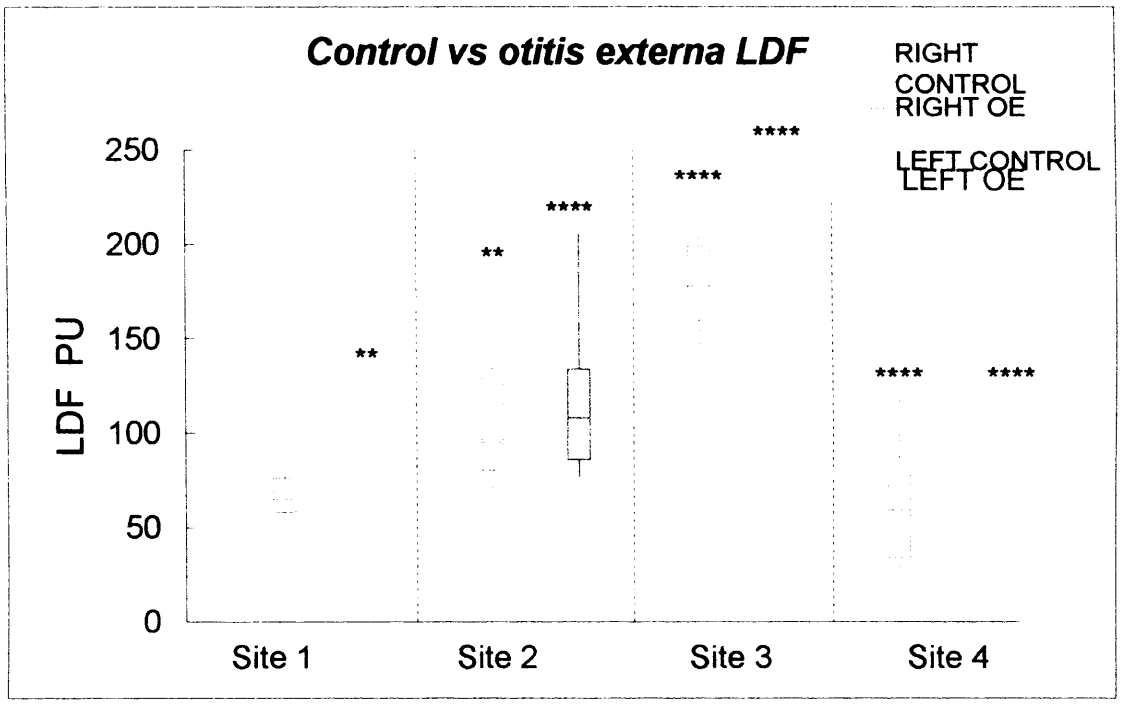

Figure 5.19. *A comparison of site medians and ranges in control and OE groups. The most marked changes are seen at site 3 and 4. Each site was compared against control subject data. Significance levels symbols for p<0.01 **, p<0.00001 *****

5.7 Comparison of Control LDF with Otitis externa: left & right ears sites 1-4.

It is clear from Figure 5.19 that the ranges of LDF PU values at all fours sites are different. This is most markedly seen at sites 3 and 4. Comparison of medians by Mann-Whitney, at the four sites in both ears, yielded highly significant p values (0.01- 0.00001). The only site not reaching significance at the $p<0.05$ level was right site 1, though this was borderline at $p=0.07$. In terms of percentage increase in median

LDF values, site 1 values increased by about 10-25%. For site 2 and 3 these values went up by 30-50 % and 60-65% respectively. At site 4, the relative increase was the most dramatic, by between 120-140%. This suggests that during otitis externa the tympanum shows proportionately the most severe inflammatory response.

Table 5.1 Summary of statistical measures at four sites

Site	Median	Variability range (as 10^{th}-90^{th} percentile	Site 1-4 Site 1 Median ratio	*Median* variability ratio	Site1 2 3 or 4 Site 1 Variability ratio
1	70	56	1	1.25	1
2	112	132	1.6	0.84	2.35
3	187	88	2.67	2.125	1.57
4	75	73	1.07	1.02	1.303

Table 5.1 *Shows the average values between ears for each of these measures. The figures give measures of absolute and relative variability.*

Table 5.1 summarises the changes in site median values and variability range. It also includes the different site median ratios and each site median/variability ratio and the site variability ratios with respect to site 1. These give further information about how blood flow at each site changes when compared with Table 4.1. As already described all

median values increase with respect to control medians, significantly so at the majority of sites.

The summary of 10^{th} – 90^{th} percentile range in comparison with Table 4.2 also shows that there is no change in this range for site 1. Compared to Table 4.2 the range does increase dramatically by factors between 1.8 to 2 at sites 2, 3 and 4. The relative greatest change seen at site 4. As there are two principle components to variability, that of static and dynamic, the increase must be due to increase in biological signal as opposed to the influence of static inclusions. Unless inflammation also leads to an increase in these factors but this is not considered to be likely.

Therefore, absolute variability in blood flow also increases with OE. The change in median ratio in the otitis externa group also shows that there is a change in site LDF ranking. In the controls this is 3:2:1:4. This now becomes 3:2:4:1 with site 4 greater by 7 % over site 1.

The individual site median/variability ratio is partly a measure of biological 'signal/ noise' and actual or 'real' variability in the blood flow signal. In other words it is a description of the tendency of how the overall signal measured tends to the median. In the case of site 1 and 4, the median variability ratio increases above that seen for control values and represents an increase in this tendency.

Table 5.2 Summary of Anderson - Darling test measures at the four recording sites

Site	Right Ear Anderson Darling A^2 value (p-value)	Appearance of Right ear Frequency distribution plot	Left Ear Anderson Darling A^2 value (p-value)	Appearance of Left ear Frequency distribution plot
1	3.78 (0.0001)	Very skewed PU by v.high outlier but does not affect median estimate Probably near normal	0.285 (0.58)	Ragged near normal
2	1.06 (0.006)	Skewed by three outliers with median not affected	2 (0.0001)	Normal with high PU spread
3	0.63 (0.09)	Low PU outlier causes skew - near normal distribution otherwise	0.62 (0.09)	Scattered distribution with central peak Looks near normal
4	0.74 (0.04)	Flattened binomial appearance with outlier	0.79 (0.03)	Scatter with distinct mid range peak more normal like

Table 5.2. *Summaries of the Anderson darling values for deviation from a normal distribution and a brief description of the appearance of the frequency histogram. This shows that compared to controls in Table 3.2, there were considerable changes in distribution, especially at site 4.*

At sites 2 and 3, there is a modest decrease in the ratio suggesting a relative increase in variability. The final measure of relative variability expressed as the variability ratio shows that at all three sites there is an increase in relative variability at sites 2 3 and 4 by about a factor of two. Again this increase must primarily reflect the contribution made by moving RBCs within the capillary bed.

Table 5.2 summarises the appearance of the distribution as described by the Anderson-Darling test and verbal description inferences are limited by the relatively low number of observations in the OE group. Generally sites 1 to 4 are affected by increased scattering of values. Again this justifies the use of non-parametric statistical analysis.

Chapter 6

Pre and postoperative LDF in myringoplasty patients

6.1 Patients' demographics

This group consisted of 18 patients, 6 females and 12 males. The median age was 55 years with the average range 19-70 years. A large majority (65%) fell between 54 – 62 years of age. The first part of this chapter presents the results on individual patients' basis. Any remarkable features about the patients are discussed.

Originally, the data from these patients was analysed using Friedman's ANOVA. The results from this analysis (Cook *et al.,* 2000) showed that the surgical procedure did not appear to result in any significant changes in blood flow following the procedure. The pre and post operative median values were comparable with the control group. However, it was considered clinically desirable to check each patient individually to establish if there were individual changes that may have been missed in a non-parametric analysis. This is important to carry out because whilst statistical analysis is very useful, the surgeon and clinician have to deal with patients on an individual basis. It is therefore important in clinical practice to look for the 'outliers' as it is invariably these patients that are more difficult to manage.

Consequently, each patient is reviewed individually here so that any unusual features in individual patients could be identified.

Case 1

This was a 59 years old male patient with right tympanic membrane perforation. Postoperative measurement was performed 5 months after surgery. Prior to surgery blood flow on the operated ear on sites 1-3 was low. Postoperatively values were elevated, although on sites 1 and 2 these values remain low.

In contrast, at site 4 preoperative blood flow value was out of range (90 PU), but was reduced by about 35% postoperatively to (58 PU). This postoperative value for site 4 still within the high normal range but greater than the 90^{th} % of our values. In the non-operated ear pre and postoperative PU values at site 1 were within the normal range. At sites 2, 3 and 4 preoperative values were low but recovered and came up near the normal range postoperatively.

Case 2

This was a 63 years old male patient with a right tympanic membrane perforation; follow up was carried out 5 months postoperatively. PU values at sites 1 and 2 were within normal range pre and postoperatively. At site 3 values were high but stable pre and postoperatively. At site 4 the preoperative PU value was high but fell postoperatively to normal range.

In the non-operated left ear sites 1, 2 and 4 were within the normal range and very stable pre and postoperatively. Site 3 was very high pre and postoperatively but was stable.

Case 3

This was a 40 years old male with a left tympanic membrane perforation. In both ears pre and postoperative values for sites 1, 2 and 4 were stable and within normal range. Interestingly, site 3 values in both ears were very high pre and postoperatively were outside the normal range by about 100%.

Case 4

This was a 27 years old female with a left tympanic membrane perforation, follow up carried out 6 months after her surgery. In both ears sites 1 and 2 were normal and very stable. In the left operated ear site 3 was very low preoperatively (51 PU), but postoperatively came back to within the normal range. The preoperative site 4 value at left ear was very high at (271 PU), but recovered to within normal (48 PU) postoperatively. This highly elevated blood flow values in the operated

ear may reflect the zealous use of cotton buds by the patient prior to her operation. In the non-operated, ear blood flow at sites 3 and 4 were slightly higher postoperatively, but not considered to be excessive or significant.

Case 5

This was a 70 years old male with a dry right tympanic membrane perforation. Follow up was performed 4 months postoperatively. All sites on the right operated ear show increase in the blood flow values, which is not remarkable in sites 1 and 2, in site 3 both values are outside the normal range both pre and post operatively. However, site 4 showed a very high increase in values from (26 PU) to (90 PU); i.e. an increase of 250%. Changes in all sites in the left ear are not remarkable. In this patient the tympanic membrane graft was congested at the time of measurement.

Case 6

This was a 56 years old male with right tympanic membrane perforation, follow up carried out 4 months postoperatively. Sites 1 and 2 values on the right operated ear were normal both pre and postoperatively. Sites 3 and 4 were very high both pre and postoperatively, but there is an evident reduction in values postoperatively. Site 3 in this ear has the highest value in the whole study of 830 PU.

This patient presented with a very congested ear canal prior to surgery subsequent to otitis externa and the frequent use of cotton buds to clean the ear canal. This high blood flow values did not resolve postoperatively to within the normal range of values, although the sites did not appear to be infected. In the non-operated left ear values were unremarkable at all sites.

Case 7

This was 47 years old male with left tympanic membrane perforation presented for follow up 6 months after surgery. In this case both ears showed unremarkable changes in blood flow values in all sites, which were within normal range both pre and postoperatively.

Case 8

This was a 58 years old female with a tympanic membrane perforation. Follow up carried out 6 months after surgery. Sites 1, 2 and 3 on the left ear showed increased values postoperatively but were within the normal range. Preoperatively site 4 was very high at 140 PU, which dropped to within the normal range (44 PU) postoperatively. All values in the right non-operated ear were not remarkable pre and post-operatively.

Case 9

This was a 62 years old female with a left tympanic membrane perforation. She had follow up 5 months after surgery. Sites 1, 2 and 3 on the left side showed little to moderate variability with increase in values postoperatively in the 3 sites, but were largely within normal range and were unremarkable.

Curiously, site 4 was normal prior to surgery at (28 PU), but postoperatively has risen to (138PU). This was near a fivefold increase in flow. In this patient the tympanic membrane graft was clearly inflamed and congested at the time of measurement.

The right ear showed slight increase in values for all sites postoperatively, which was unremarkable, as it was within normal range pre and postoperatively.

Case 10

This was a 37 years old male with a left tympanic membrane perforation, follow up carried out 6 months after surgery. Sites 1 and 2 were unremarkable in both ears pre and postoperatively. Site 3 in the left ear showed some increase postoperatively. In contrast site 4 was (160 PU) prior to surgery, which fell to (37 PU) post-surgery.

In the right ear site 3 was within the lower normal range both pre and post operatively. Site 4 on the right ear followed the same pattern as the left operated ear, preoperatively (123 PU) fell down to (28 PU) postoperatively.

Case 11

This was a 28 years old male with a dry left tympanic membrane perforation. Follow up measurements was carried out 6 months after surgery. All sites in the left ear were found to be within the normal range both pre and postoperatively. The right ear showed some variability at sites 3 and 4, but sites 1 and 2 were stable.

Case 12

This was a 54 years old male with a left tympanic membrane perforation and was reviewed 6 months postoperatively. Sites 1, 2 and 4 on the left operated ear were largely within normal range both pre and postoperatively. Site 3 showed high values both pre and postoperatively, above the 90^{th} percentile of the normal values, but still within the normal range of values.

In the right non-operated ear sites 1, 2 and 4 were within the normal range both pre and post-operatively. Site 3 on the right ear was below normal prior to surgery but returns to normal after surgery.

Case 13

Was a 37 years old male with a dry right tympanic membrane perforation. Follow up was carried out 6 months postoperatively. The right ear was unremarkable with all values in the 4 sites within the normal range both pre and postoperatively. In the left ear sites 1, 3 and 4 within normal range, but site 2 showed a preoperative high values, which came to within normal range postoperatively.

Case14

This was a 19 years old male with a right dry tympanic membrane perforation with follow up review carried out 6 months after surgery. Sites 1, 2 and 3 were unremarkable and within the normal range both pre and postoperatively. Site 4 was slightly high both pre and postoperatively above the 90^{th} percentile but within the normal range of values. The left non-operated ear showed some variability but within the normal values at all sites.

Case 15

This was a 57 years old female with dry right tympanic membrane perforation reviewed 6 months after surgery. Sites 1, 2 and 3 on the right ear showed no change, within the normal range. At site 4 values both pre and postoperatively were high, rising from (60 PU) prior to surgery to (87 PU) after surgery, with approximately 50% increase in the postoperative value.

Left ear showed some variability with a downward trend postoperatively at sites 1, 2 and 4, although all values still within the normal range at these sites. Site 3 preoperative value was missing.

Case 16

This was a 56 years old male with a dry right tympanic membrane perforation. Follow up measurement carried out 6 months after surgery. All values in the 4 sites on the right ear were within normal range both pre and postoperatively. On the left non-operated ear only the

postoperative measurements were available and all within the normal range.

Case 17

This was a 35 years old female with a left dry tympanic membrane perforation with follow up carried out 6 months after surgery. On the left operated ear, preoperative values at sites 1, 3 and 4 were above the 90^{th} percentile but postoperatively these values fell down within the high normal range. At site 2 the preoperative value was not available but the postoperative value within normal range as well.

In the non-operated right ear sites 1 and 4 were within the normal range both pre and postoperatively. Site 2 showed a high preoperative value, which came down to within the normal range postoperatively. Site 3 preoperative value was not available but postoperative value within the normal range.

Case 18

This was a 55 years old female with a dry right tympanic membrane perforation. Sites 1, 2 and 3 on the right ear showed some variability but all within the normal range postoperatively. Site 4 on the right ear was within the normal range both pre and post operatively. On the left non-operated ear preoperative measurement was not available, but all postoperative values in all sites within the normal range.

Myringoplasty Group.

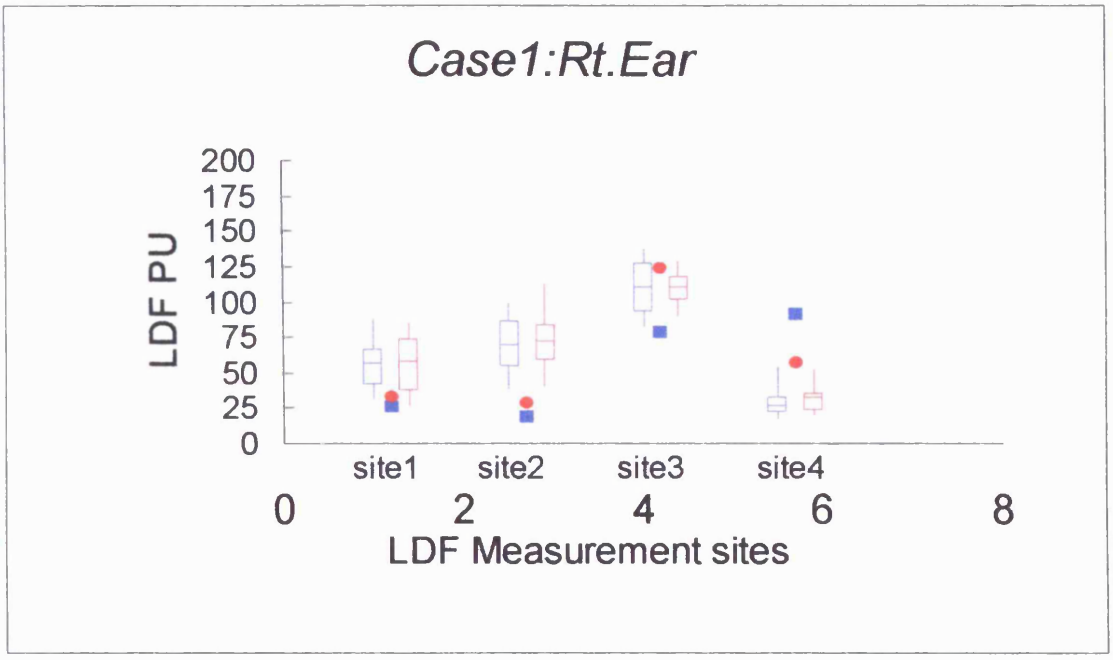

Figure 6.1. The *box and whisker plot control values for left and right ears are taken from Figure 4.22 showing the median 25th and 75th percentiles in the box. The limits of the 10th and 90th percentiles are shown as the upper and lower lines. The blood flow pre and post operatively for each site are shown as pre operative flow: blue squares. Post operative flow: red circles. This key applies to all Figures 6.1 to 6.36.*
Case 1 operated ear; whilst both pre and post op values are mainly low, these measurements were not remarkable. Site 4 PU values are high.

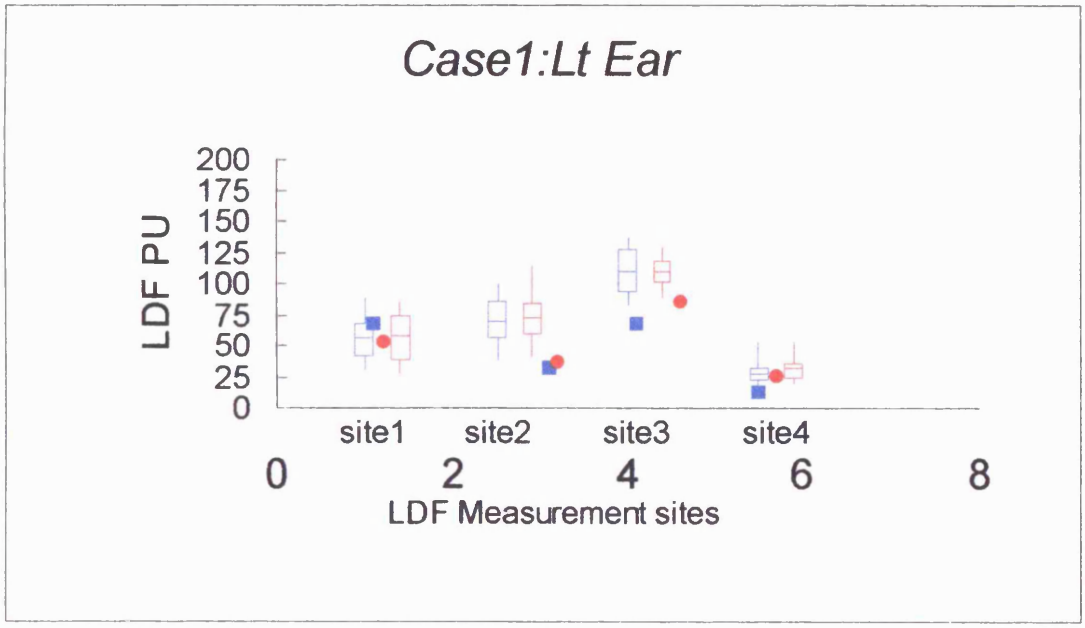

Figure 6.2. *Case 1 non-operated ear; whilst both pre and post op PU values were mainly low, these were not remarkably so.*

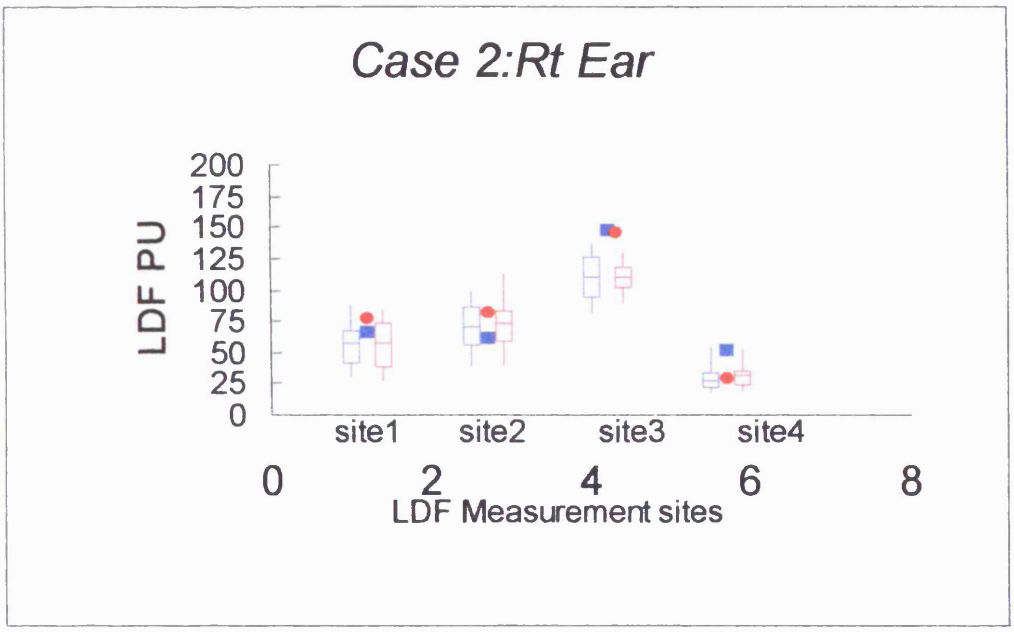

Figure 6.3. *Case 2 operated ear these measurements were not considered remarkable.*

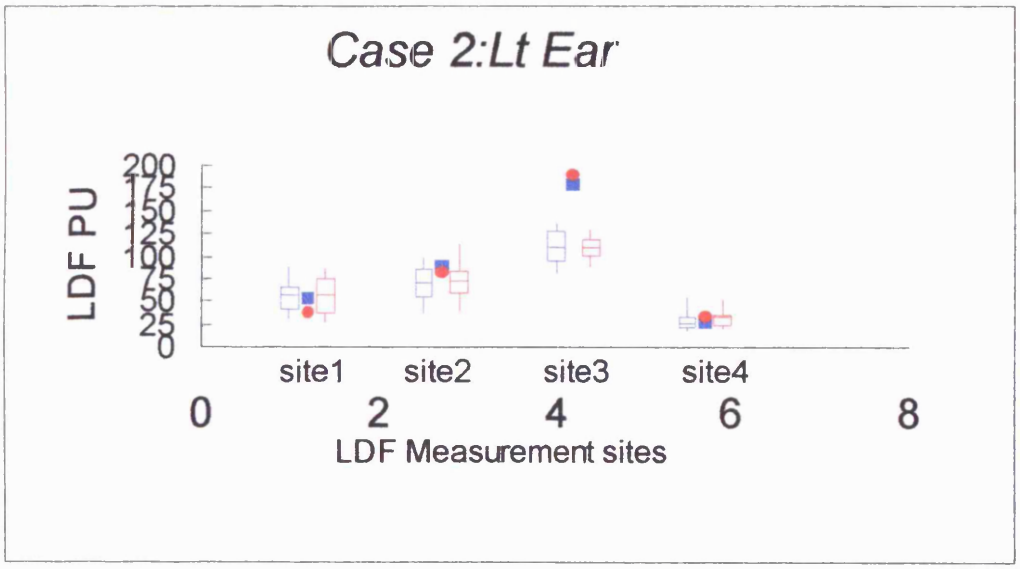

Figure 6.4. *Case2 non-operated ear with stable within normal values apart from site 3, was very high pre and postoperatively.*

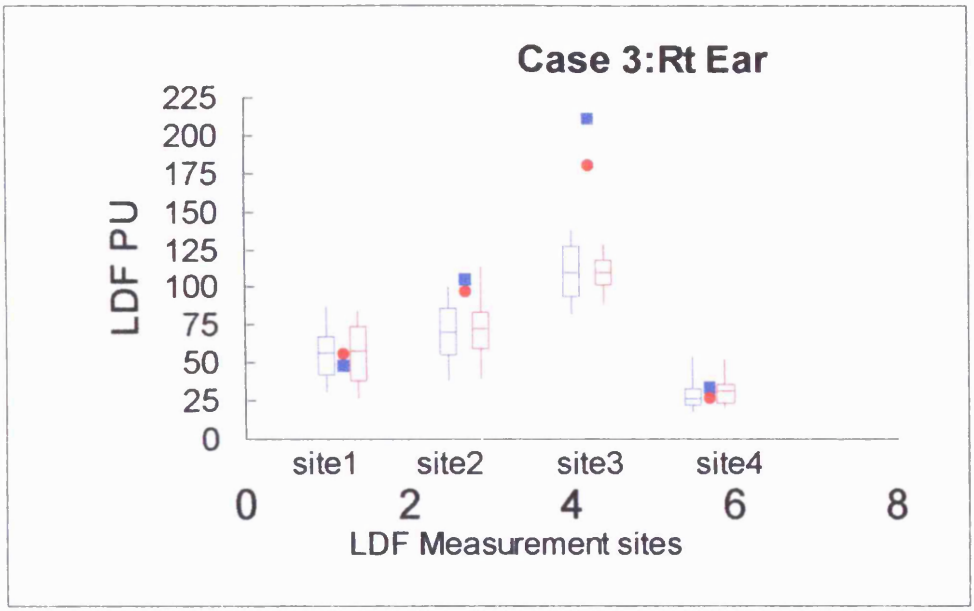

Figure 6.5. *Case 3 non-operated right ear with stable values at sites 1, 2 and 4, but very high values at site 3.*

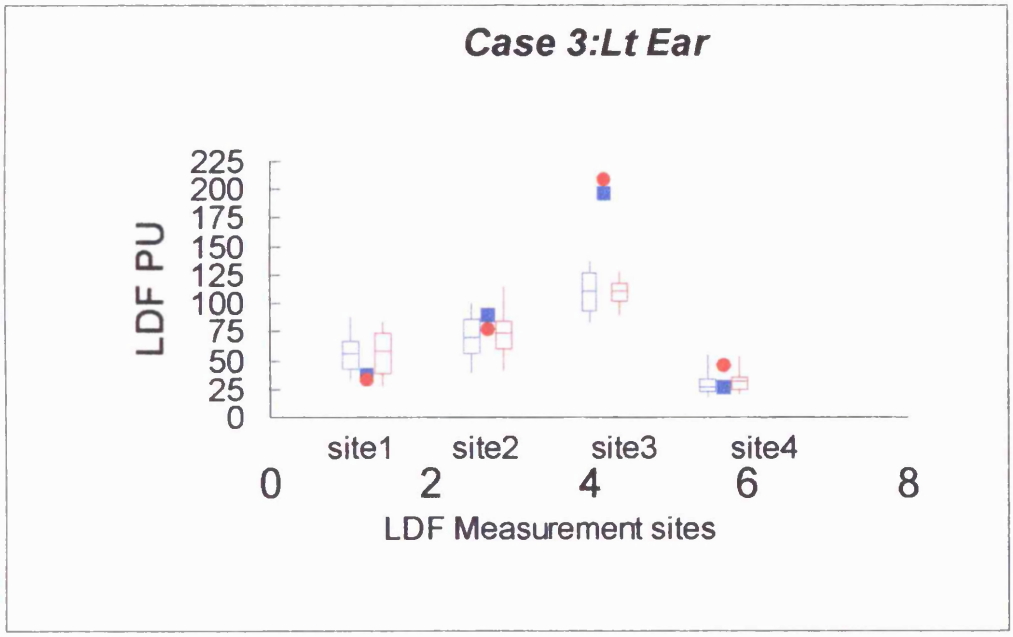

Figure 6.6. *Case 3 operated ear with similar appearance to right non operated ear. There were high but stable PU values at site 3.*

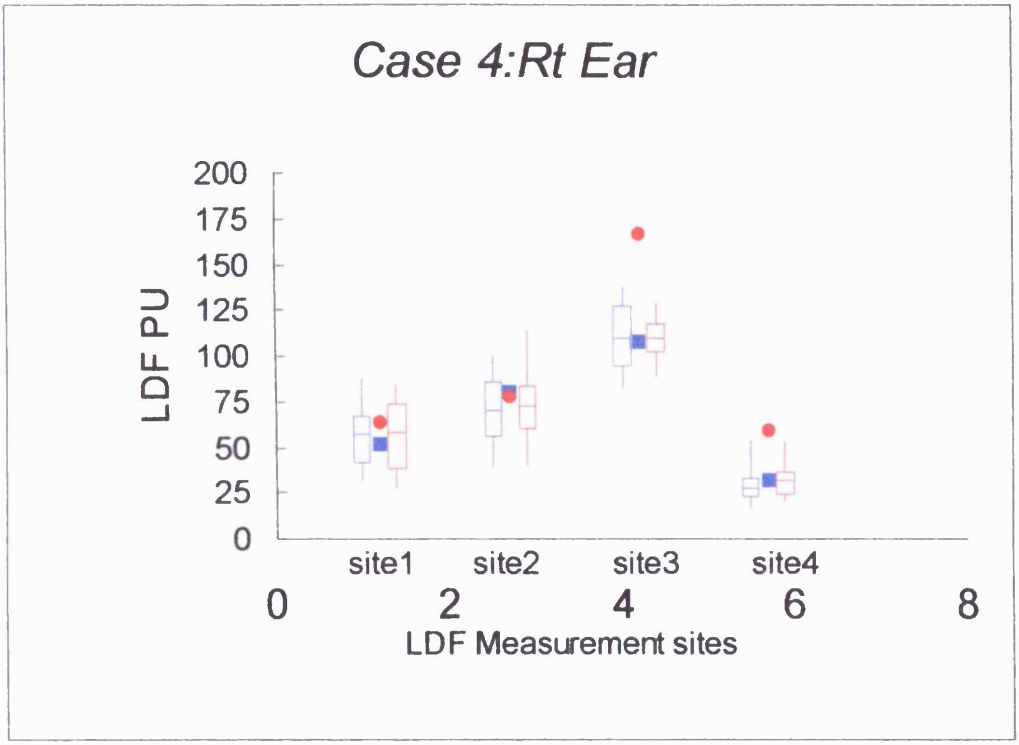

Figure 6.7. *Case 4 non-operated ear had stable values at sites 1, 2 and 4. There was only one higher postoperative value at site 3 which was well outside the 90th percentile.*

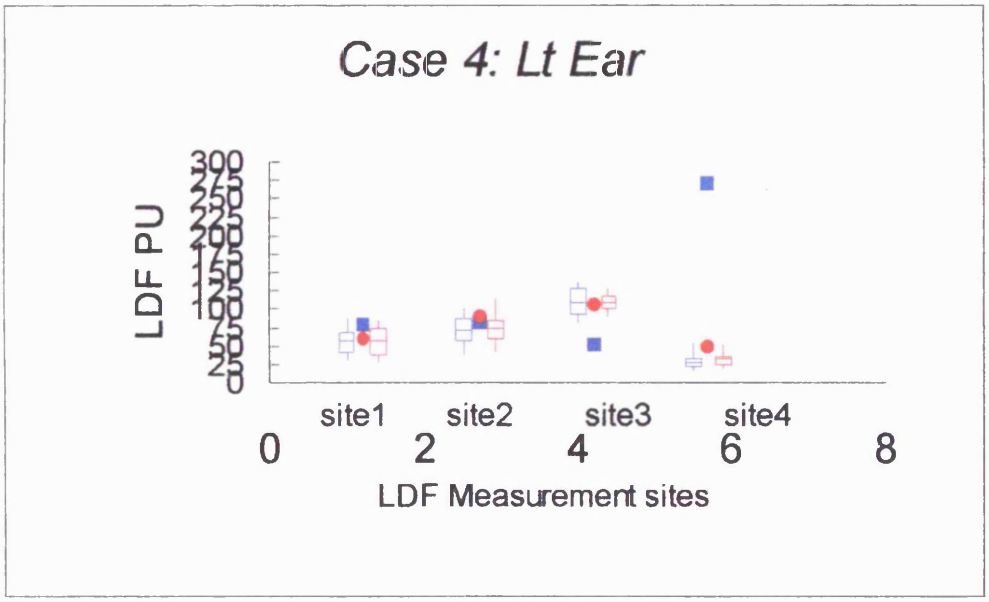

Figure 6.8. *Case 4 left operated ear with very low preoperative value at site 3, which increased to within normal range postoperatively. Also, a very high preoperative value at site 4 dropped down to within normal range after surgery.*

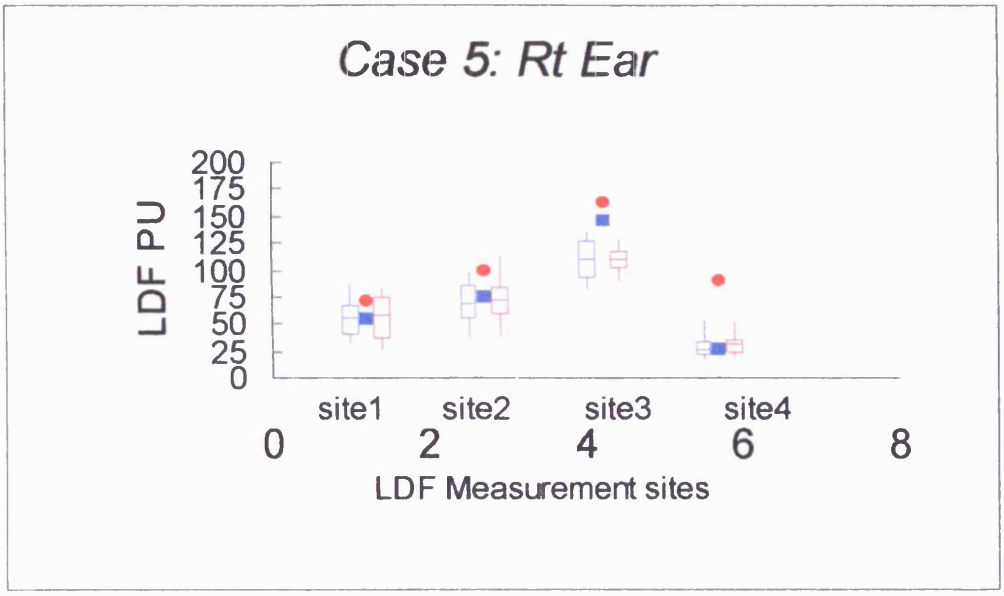

Figure 6.9. *Case 5 right operated ear with higher postoperative values at all sites. Postoperative values at sites 3 and 4 clearly appeared to be outside the normal value range.*

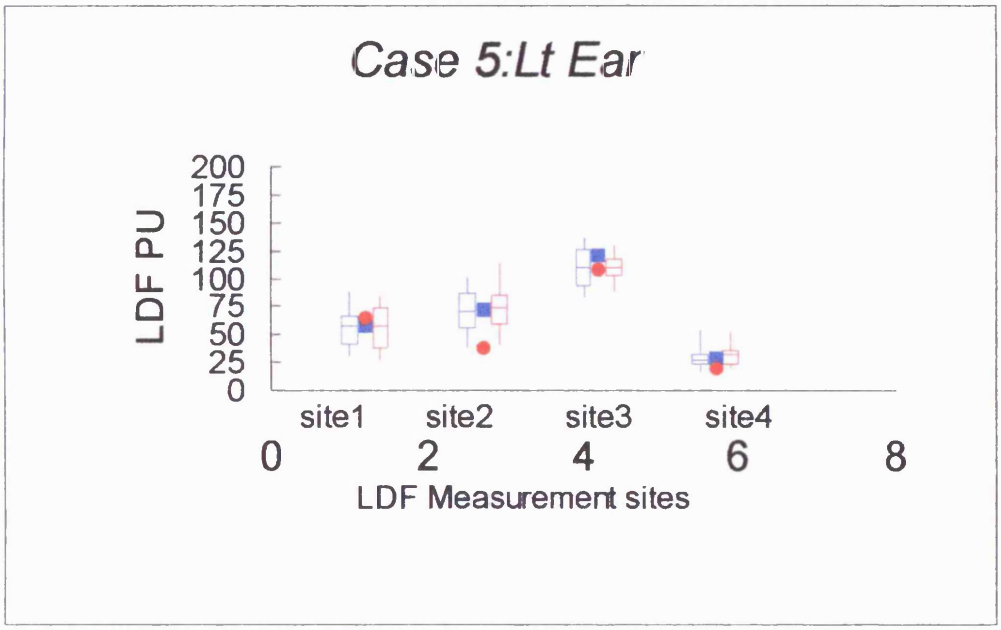

Figure 6.10. *Case 5 left non-operated ear with unremarkable changes at all sites. All values pre and postoperatively were within the normal control range.*

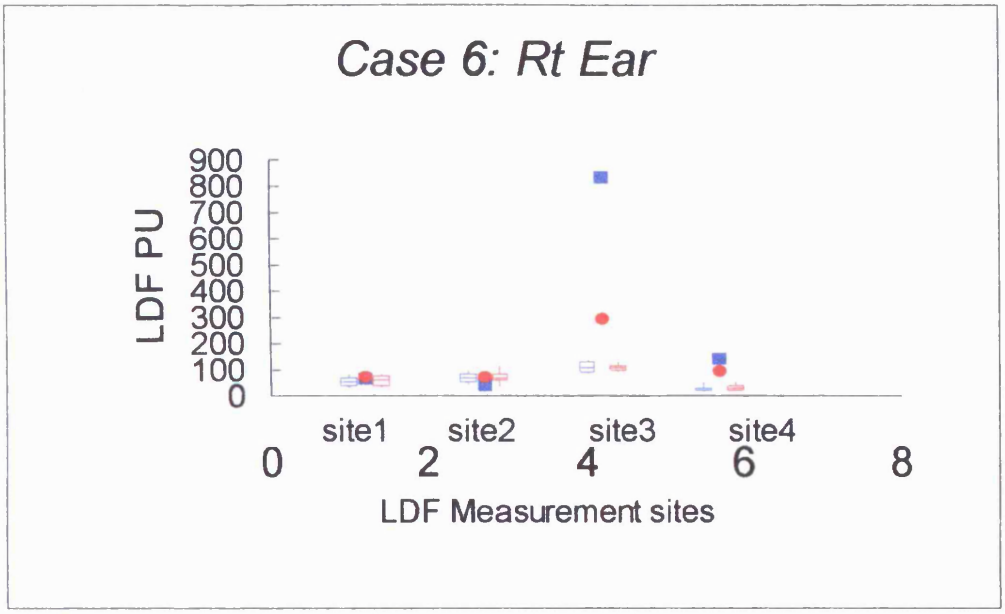

Figure 6.11. *Case 6 right operated ear with stable values at sites 1 and 2. Very high preoperative values at sites 3 and 4 came down after surgery. However, these were, still outside the normal range. The site 3 preoperative value is the highest value in this study.*

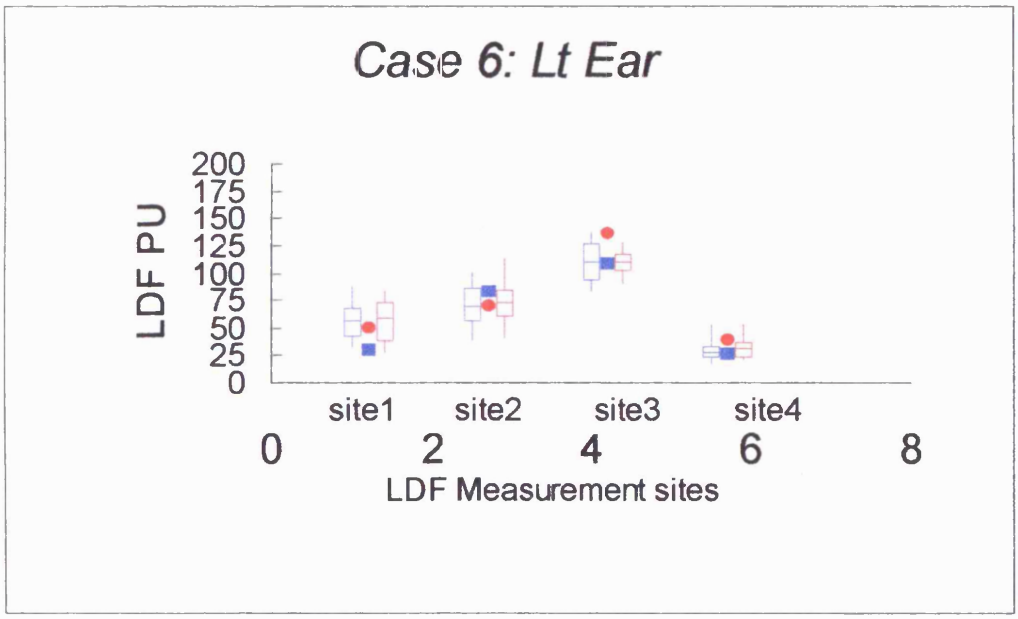

Figure 6.12. *Case 6 non-operated ear with unremarkable value changes at all sites.*

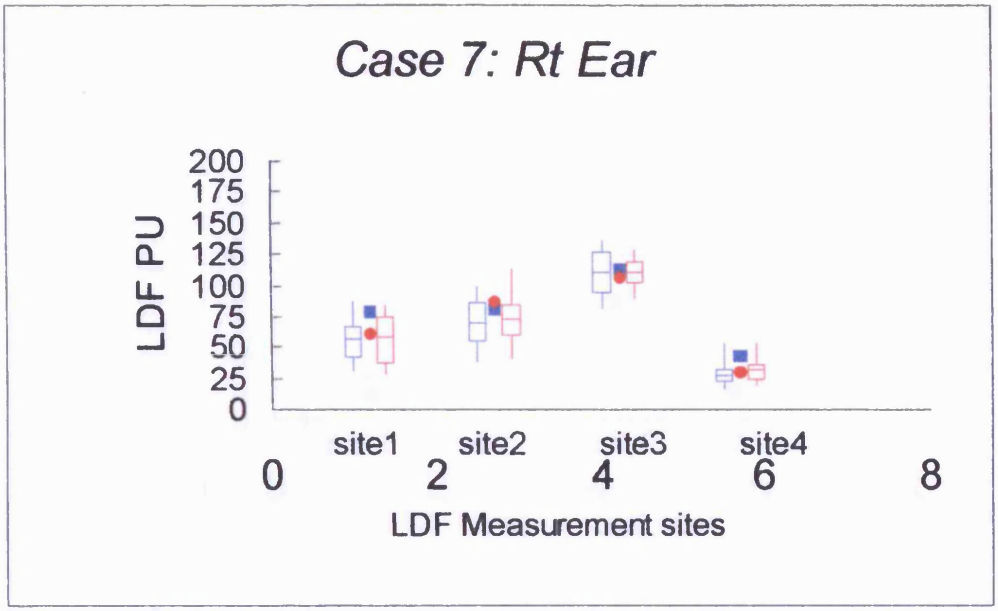

Figure 6.13. *Case 7 non-operated right ear had stable within normal range values at all sites both pre and postoperatively*

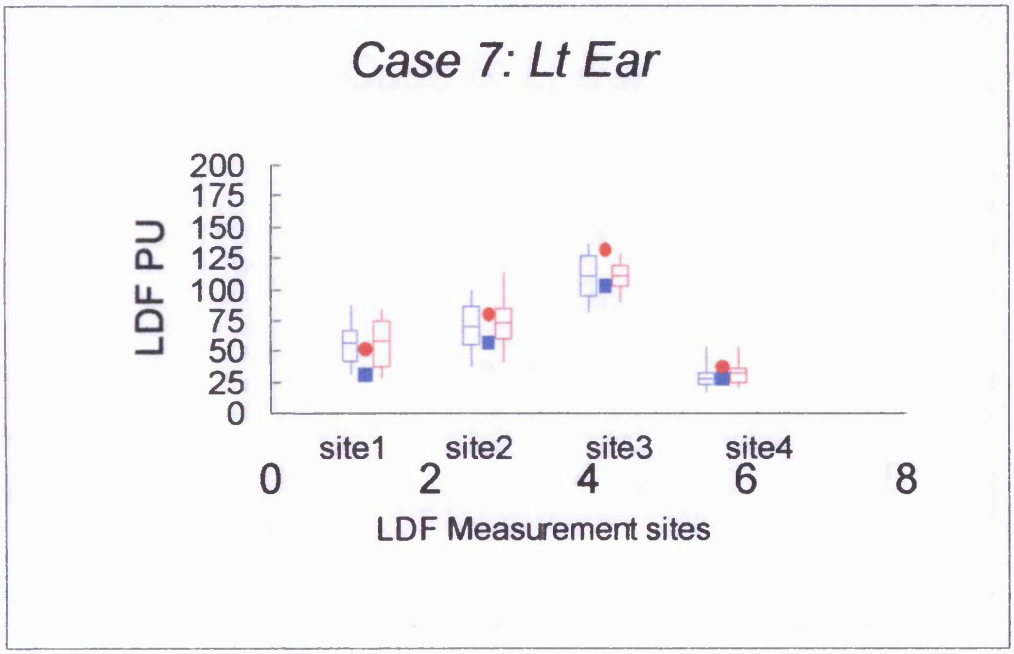

Figure 6.14. *Case 7 operated left ear with higher values at all sites after surgery. Both pre and post operative values were within the normal range.*

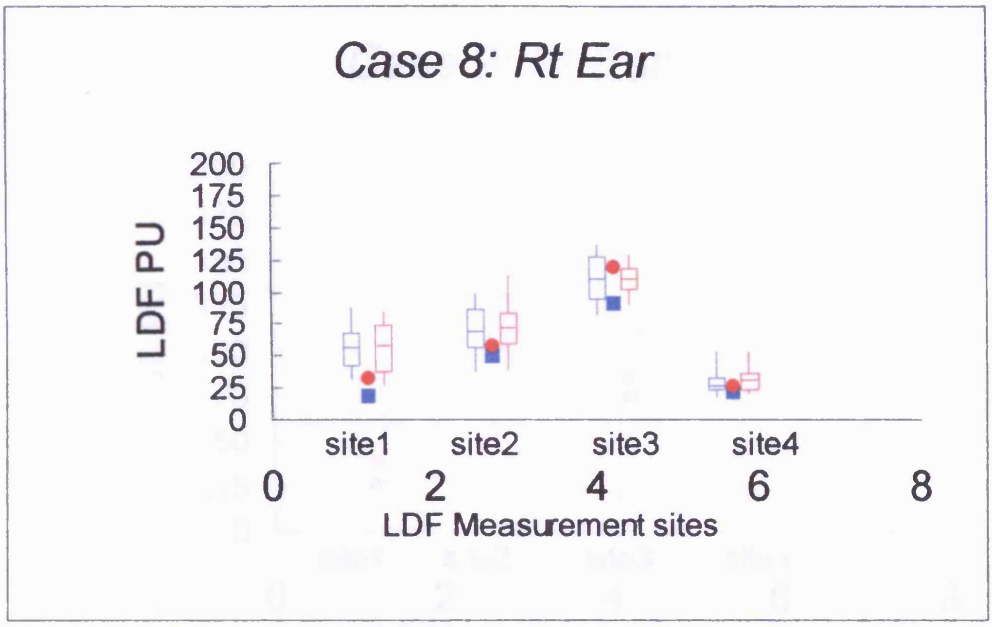

Figure 6.15. *Case 8 non-operated ear with stable values mainly within the normal range.*

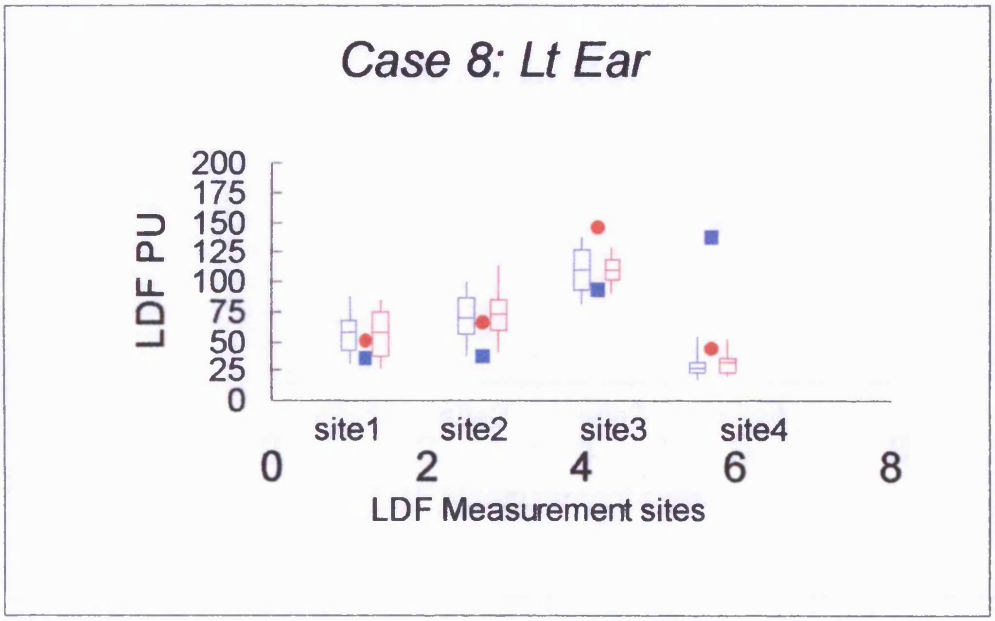

Figure 6.16. *Case 8 left operated ear with higher but within normal range, postoperative values at site 1, 2 and 3. Only site 4 shows a postoperative drop from a very high preoperative value that then returns within the normal range of values as well.*

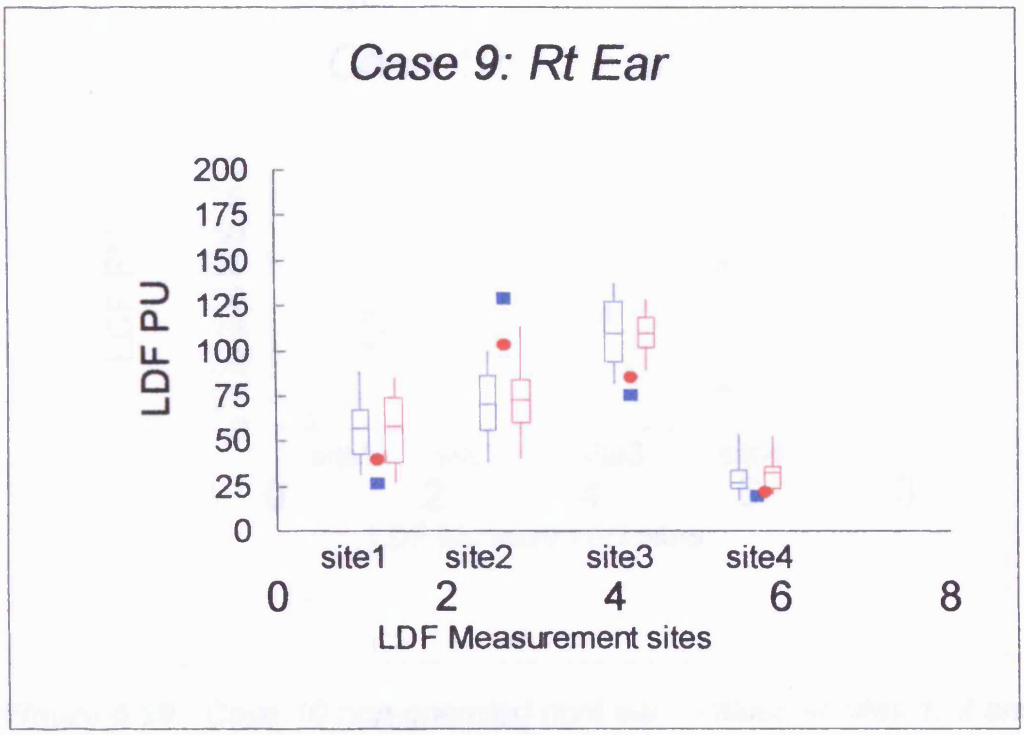

Figure 6.17. *Case 9 with right non-operated ear with non-remarkable change in values both pre and post operatively.*

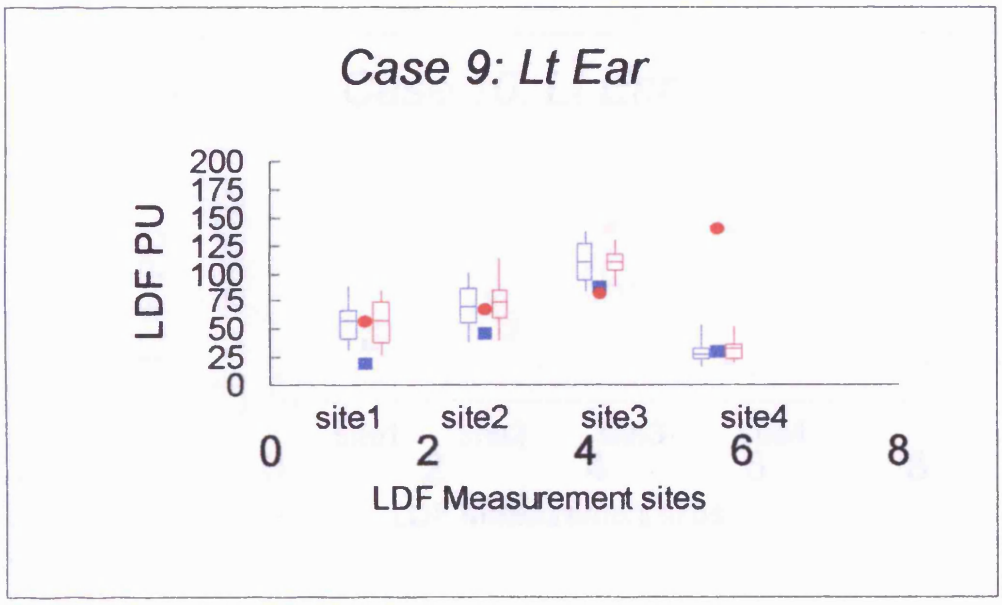

Figure 6.18. *Case 9 left operated ear. There was little variability in values at sites 1 and 2. At site 4 there was a clear postoperative inflamed congested TM graft, with a fivefold increase in flow.*

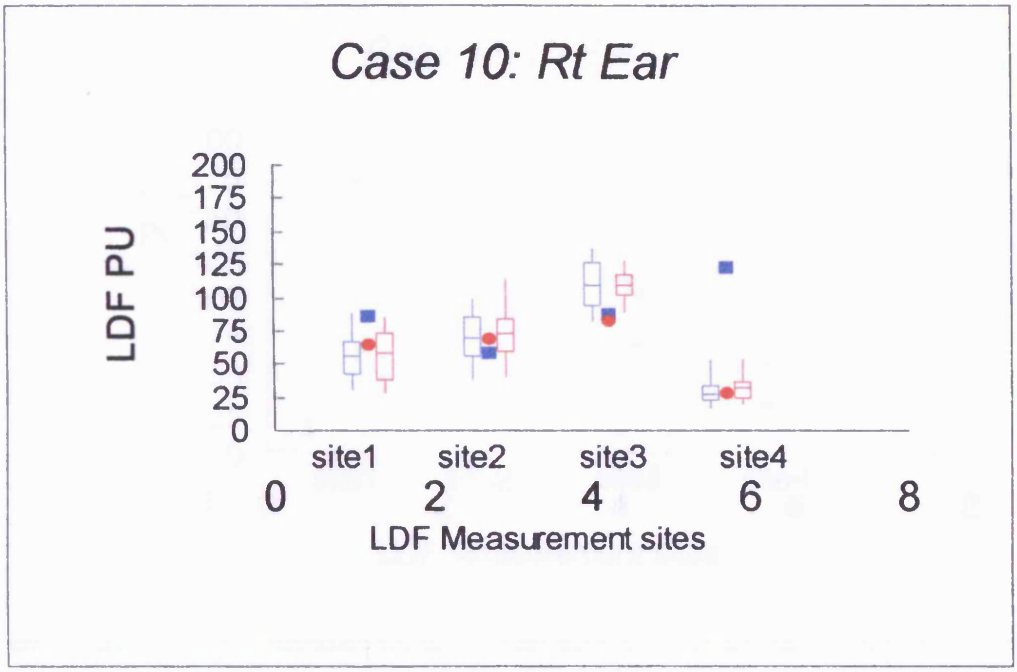

Figure 6.19. *Case 10 non-operated right ear. Values at sites 1, 2 and 3 were within the normal range. At site 4, following surgery the very high preoperative value returned to within the normal range of values.*

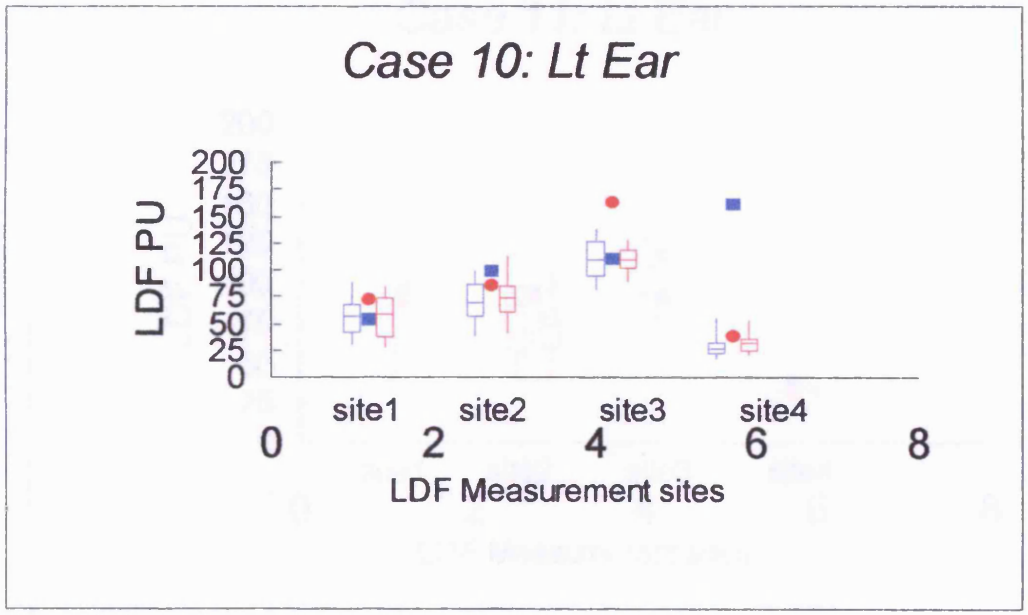

Figure 6.20. *Case 10 left operated ear showed unremarkable changes at sites 1 and 2. At site 3, there was an increase in the postoperative flow value. Site 4 showed similar changes postoperatively as the right ear.*

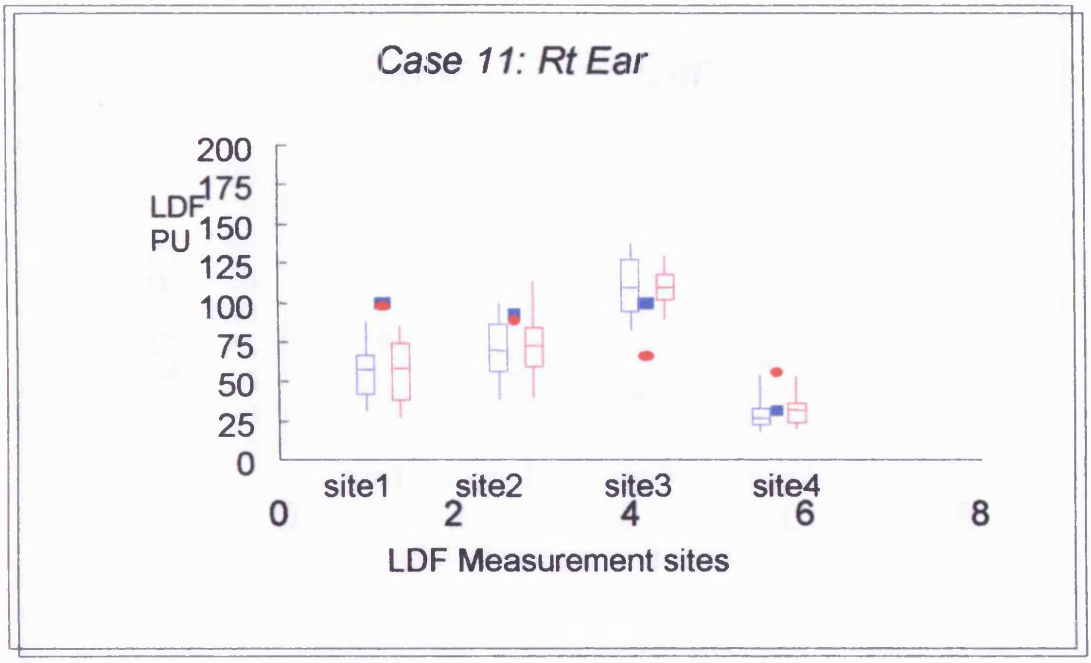

Figure 6.21. *Case 11 right non-operated ear. There were stable values at sites 1 and 2. At sites 3 and 4 there was some pre and post operative unremarkable variability.*

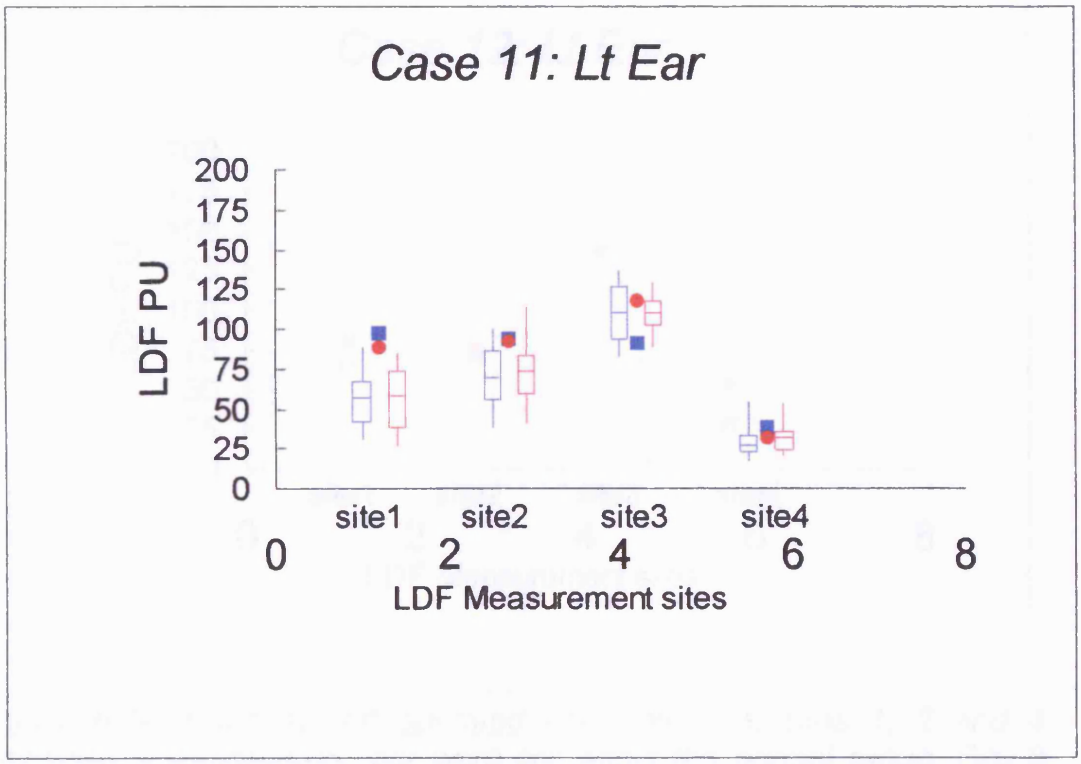

Figure 6.22. *Case 11 left operated ear. All pre- and post-operative values appear within the normal range of values.*

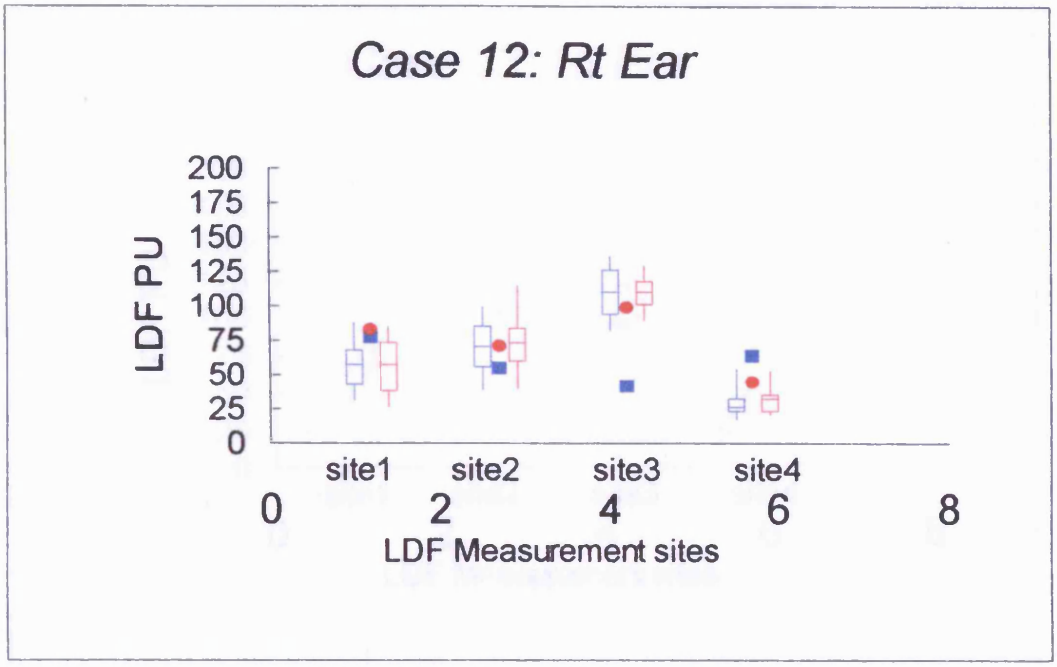

Figure 6.23. *Case 12 right non-operated ear. Flow values were stable and within normal at sites 1, 2 and 4. At site 3, the very low preoperative value returned to within the normal range after surgery.*

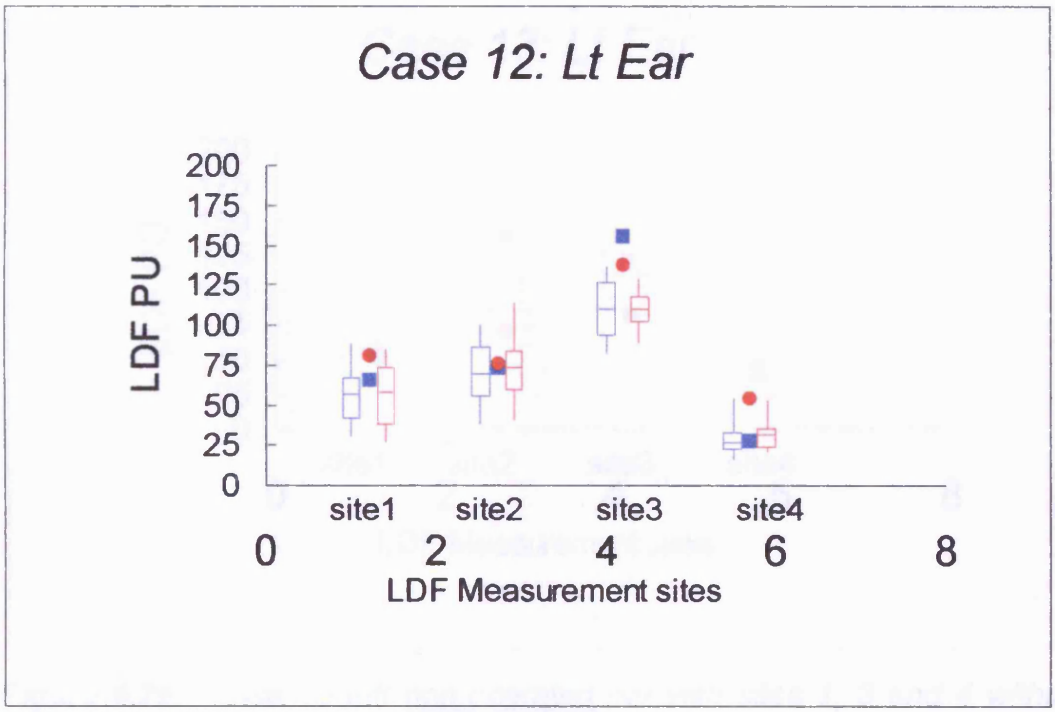

Figure 6.24. *Case 12 left operated ear. Values at sites 1, 2 and 4 increased postoperatively, but were still within the normal range. Site 3 showed high pre and postoperative values, with some slight decrease postoperatively.*

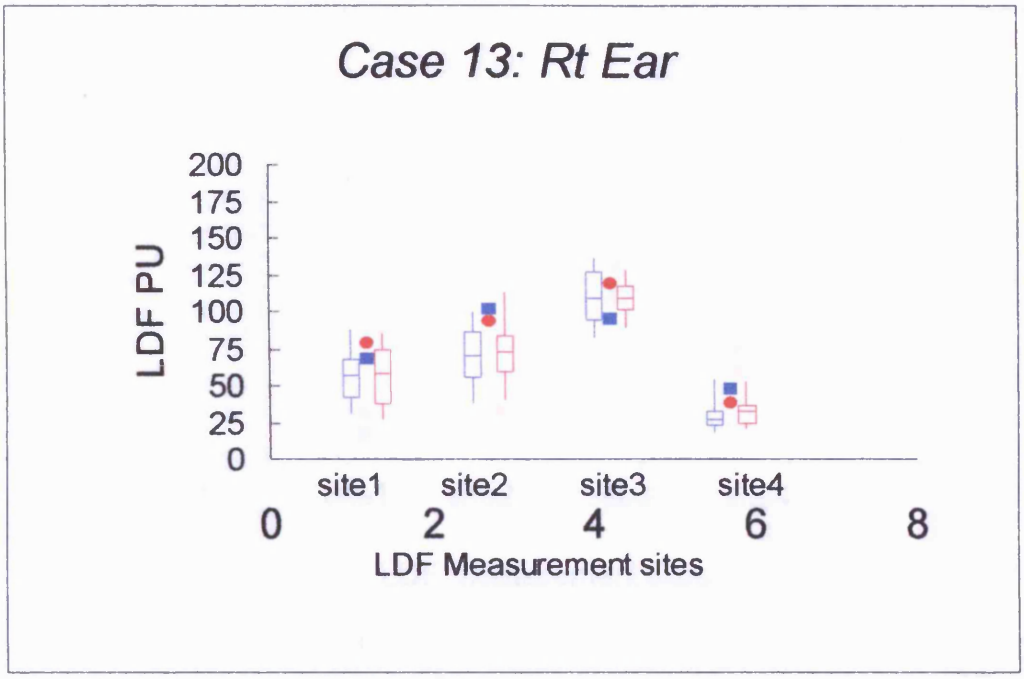

Figure 6.25. *Case 13 right operated ear. Unremarkable changes at all sites were seen both pre and post operatively.*

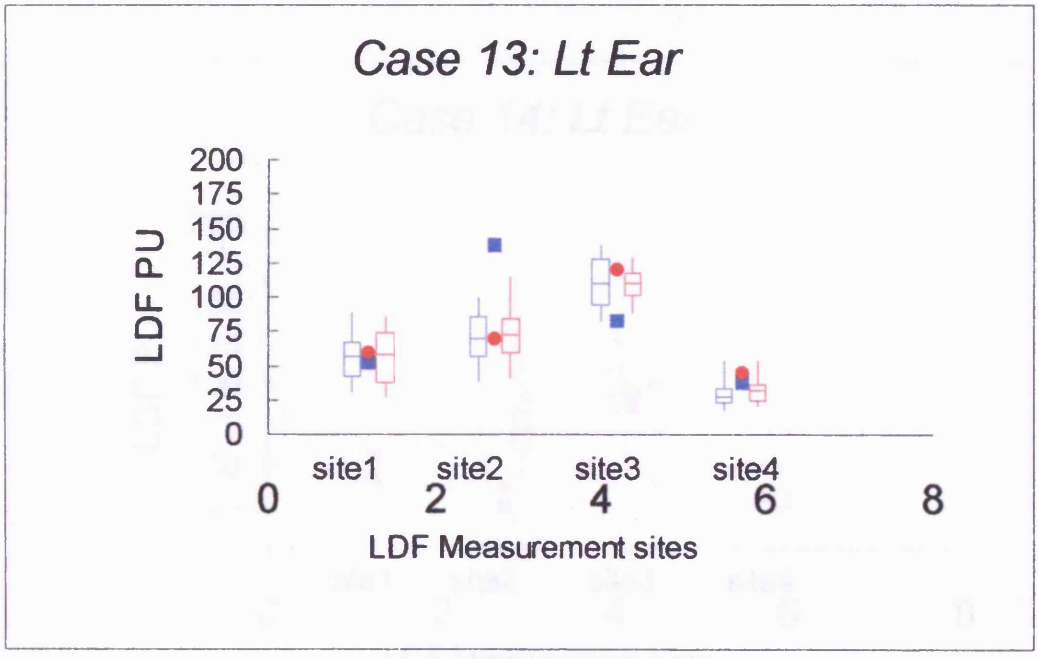

Figure 6.26. *Case 13 left non-operated ear with sites 1, 3 and 4 within normal range of values both pre and postoperatively. Preoperatively, the high values at site 2 had dropped down to within normal range postoperatively.*

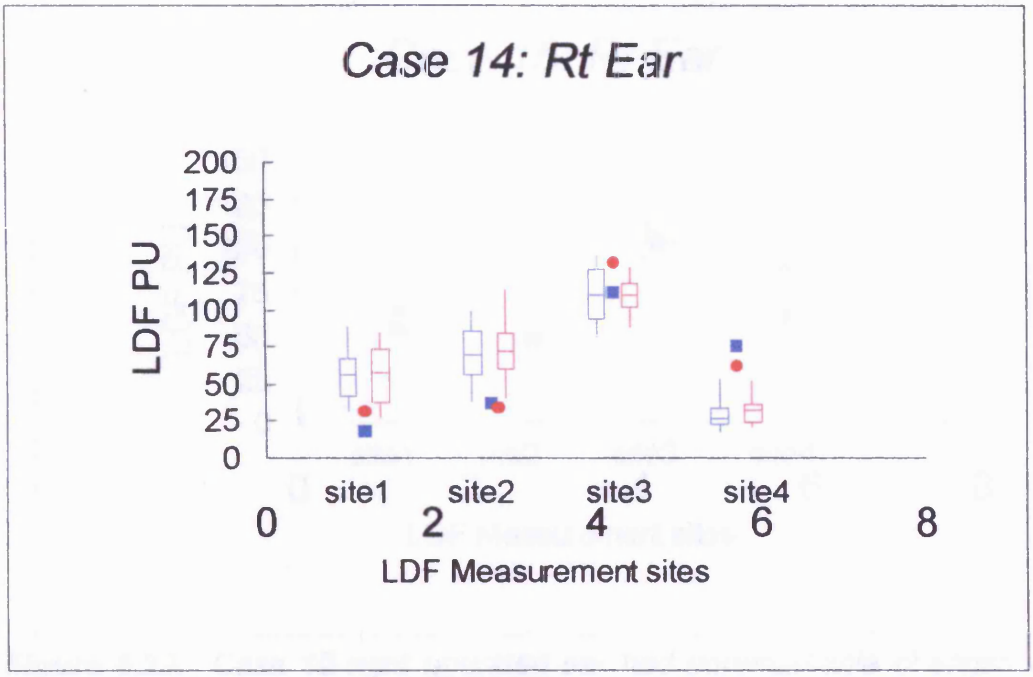

Figure 6.27 *Case 14 right operated ear showed unremarkable changes at sites 1, 2 and 3. Site 4 exhibited high values both pre and postoperatively.*

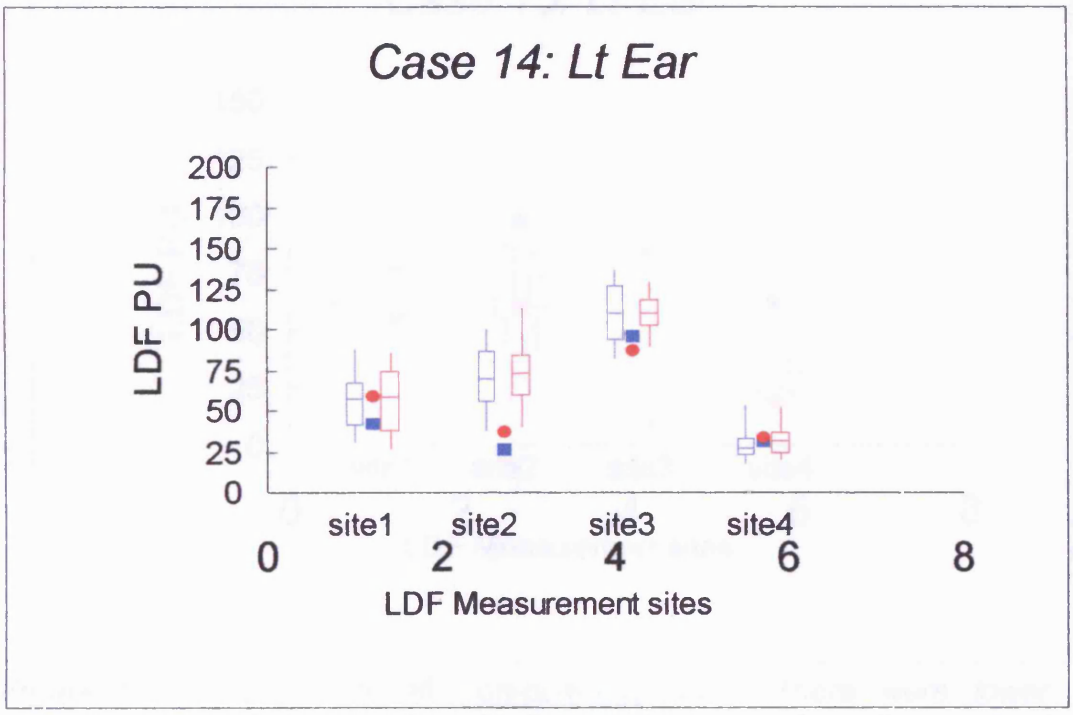

Figure 6.28. *Case 14 left non-operated ear exhibited some variability within the normal range at all sites.*

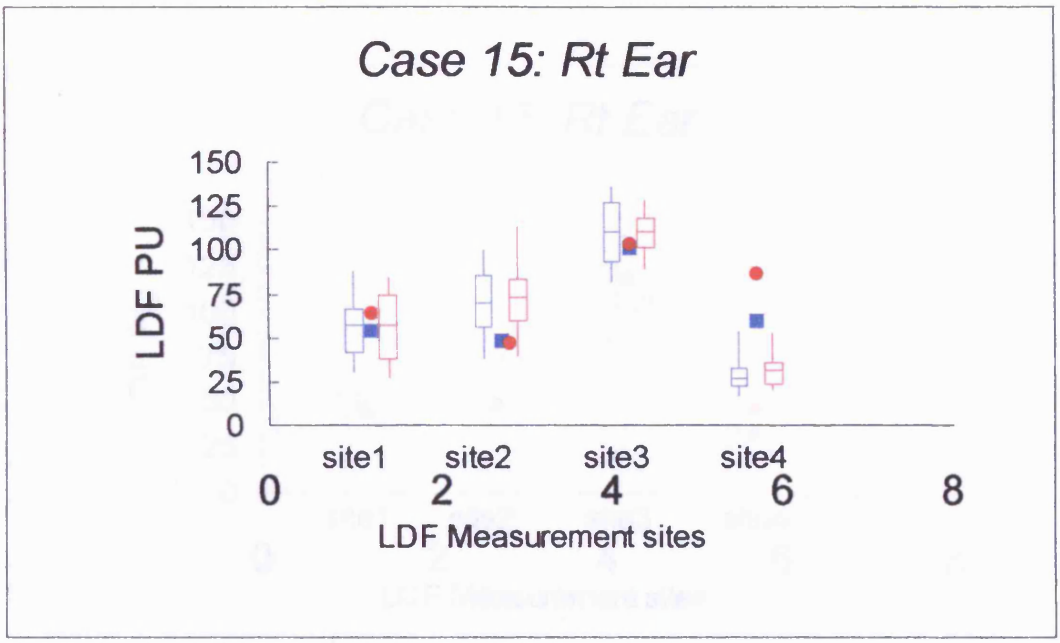

Figure 6.29. *Case 15 right operated ear had unremarkable changes at sites 1, 2 and 3. Site 4 shows high pre and post operative values, with an approximately 50% increase in postoperative flow values.*

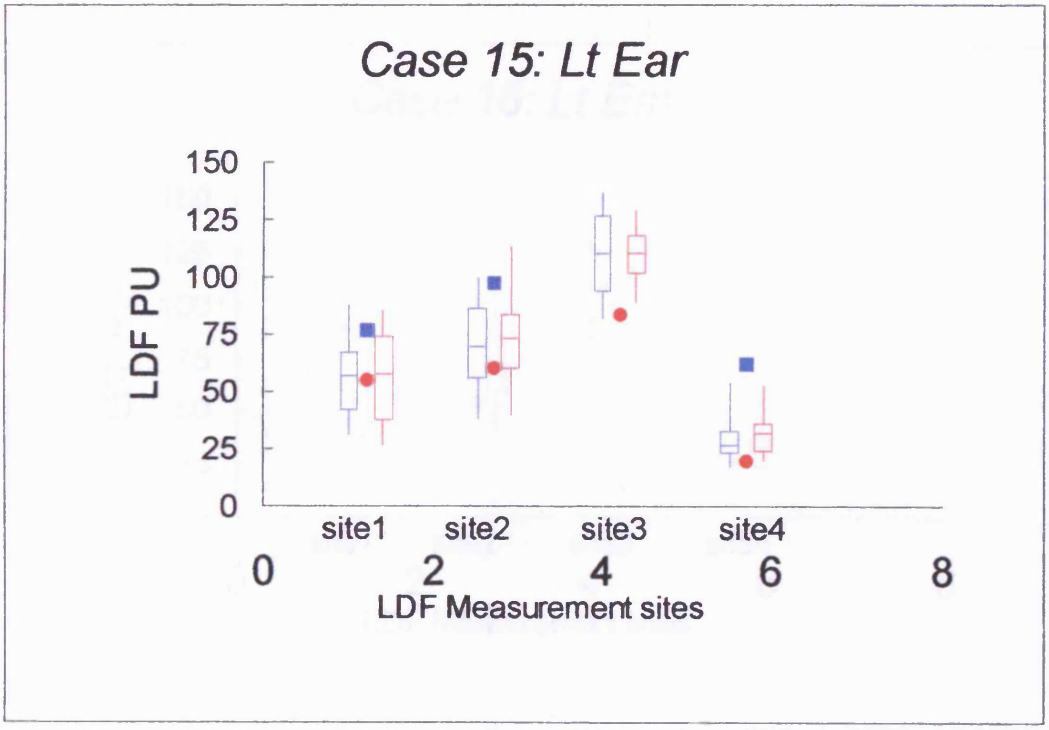

Figure 6.30. *Case 15 left non-operated ear. There were lower postoperative values at sites 1, 2 and 4, although both pre and postoperative values within normal range. Preoperative site 3 value is missing.*

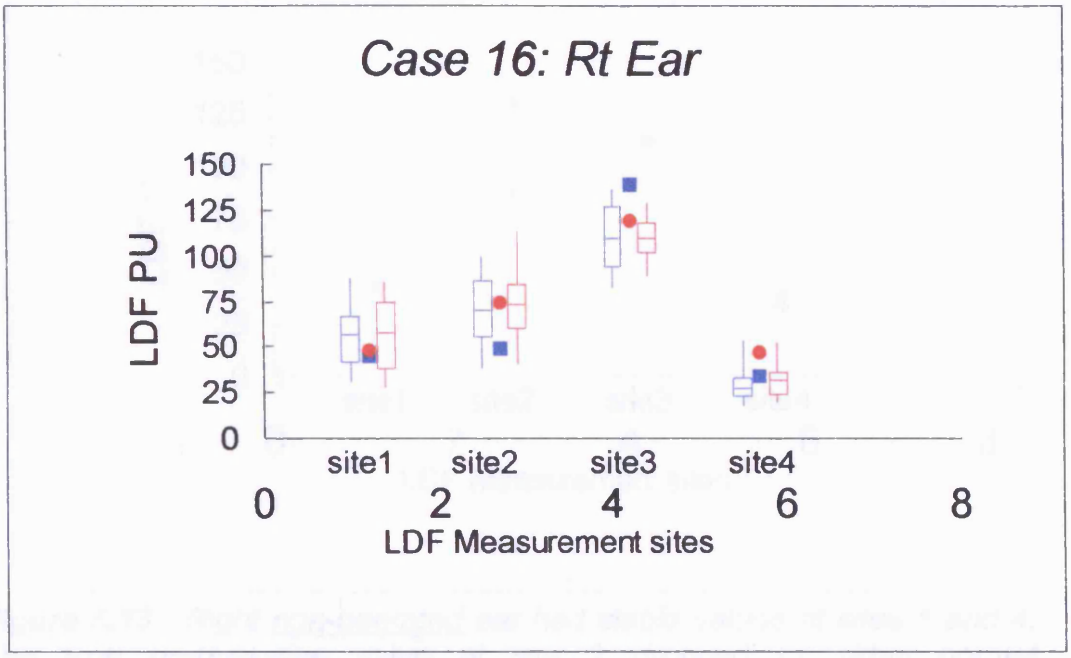

Figure 6.31. *Case 16 right operated ear. Both pre and postoperative values were within the normal range of values.*

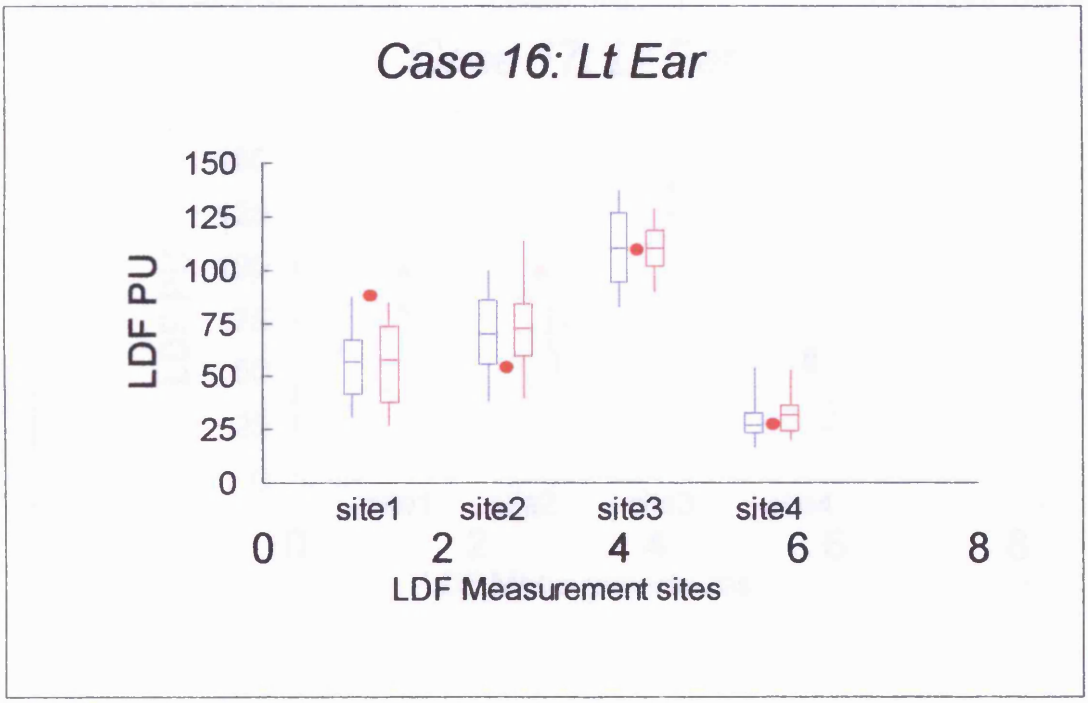

Figure 6.32. *Case 16 left non-operated ear with only postoperative values, all within normal range.*

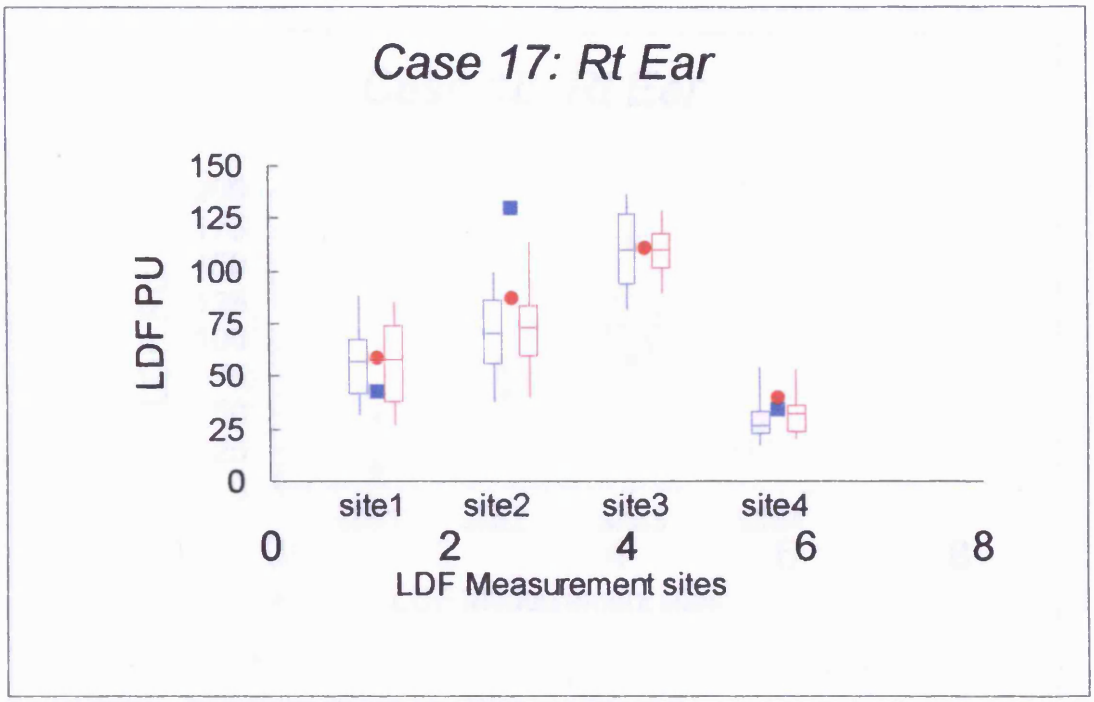

Figure 6.33. *Right non-operated ear had stable values at sites 1 and 4. The high preoperative value at site 2 dropped to within normal postoperatively. At site 3 only the postoperative which was within normal range value was available.*

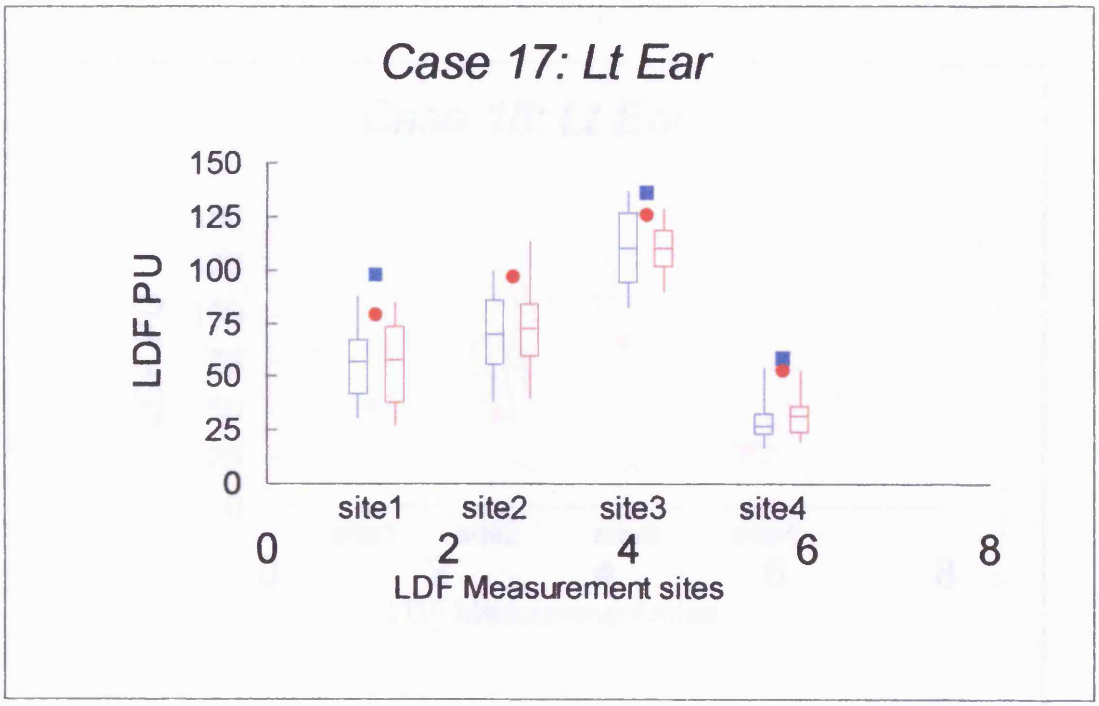

Figure 6.34. *Case 17 left operated ear had high preoperative values. These dropped to within the normal values range postoperatively. Only the postoperative value for site 2 was available and this fell within the normal range as well.*

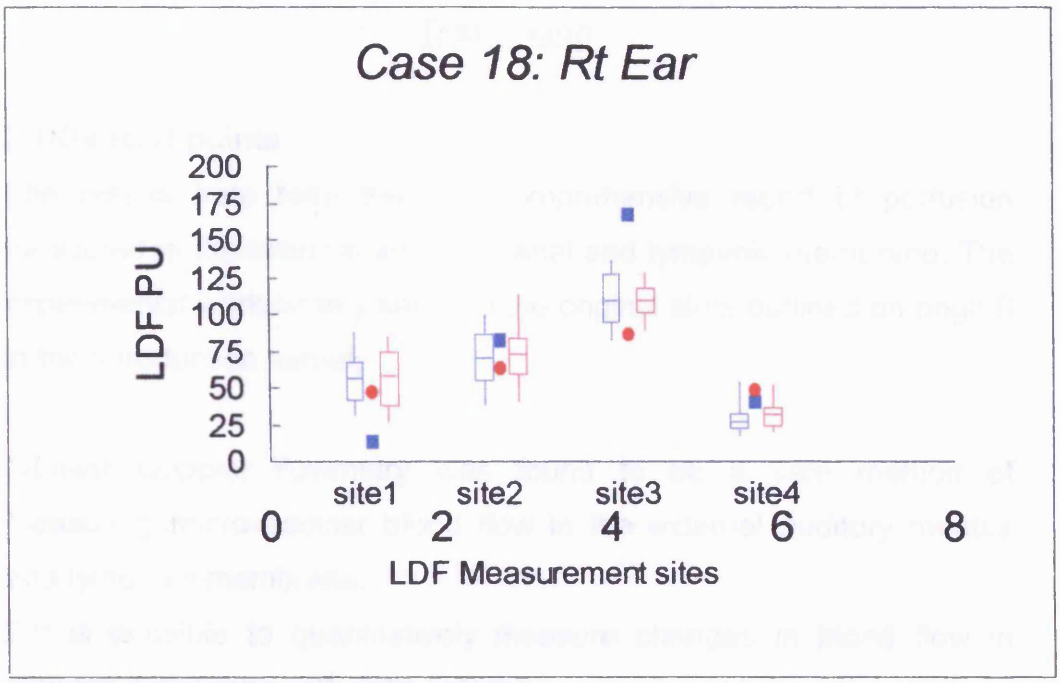

Figure 6.35 *Case 18 right operated ear. All postoperative values fell within the normal range. Site 1 showed an increase post operatively to within normal range. In contrast site 3 decreased to within the normal range.*

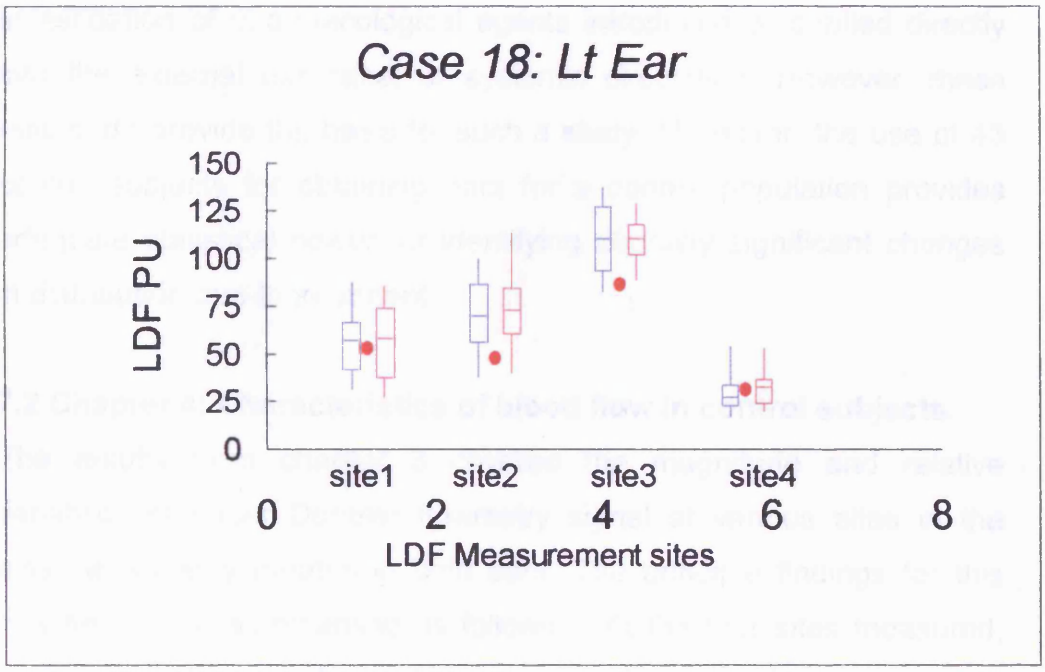

Figure 6.36. *Case 18 left non-operated ear. All postoperative values were within normal range. The preoperative values were not available.*

Chapter 7

Discussion

7.1 General points

The results here form the first comprehensive report of perfusion measured in the external auditory canal and tympanic membrane. The experimental work largely satisfied the original aims outlined on page 6 in the introduction namely:

1-Laser Doppler flowmetry was found to be a safe method of measuring microvascular blood flow in the external auditory meatus and tympanic membrane.
2-It is possible to quantitatively measure changes in blood flow in patients presenting with otitis externa.
3-It was quantitatively established in a patient by patient examination what changes in blood flow occurred following myringoplasty.

Of necessity, this first study was limited in that it did not involve investigation of pharmacological agents introduced or applied directly into the external ear canal or systemic circulation. However, these results do provide the basis for such a study. Moreover, the use of 43 control subjects for obtaining data for a control population provides adequate statistical power for identifying clinically significant changes in distribution due to treatment.

7.2 Chapter 4: Characteristics of blood flow in control subjects.

The results from chapter 3 covered the magnitude and relative variability of Laser Doppler flowmetry signal at various sites in the external auditory meatus in both ears. The principle findings for this chapter can be summarised as follows. At the four sites measured, there was a clear significant difference in LDF values. This gave rise to a clear ranking of order of site3: site2: site1: site4. These sites also have substantial differences in their pattern of distribution and relative

degrees of variability and spread of data. The possible causes of this are discussed in more detail below.

Some of the usual fundamental sources of variability in biological systems were not found to contribute to this variability. That is the age, sex and body temperatures (as measured at tympanic membrane) were not associated with any differences in LDF. There was also no evidence of any significant difference for each site between the two ears. Furthermore, there was no correlation within an individual between LDF at the four separate sites. This last finding would suggest that each of the four sites are probably subject to separate autonomic control factors, or at the very least, the other factors affecting variability are sufficiently independent to smear out any single control factor.

It was considered surprising that age, sex and body temperature were not at least weakly correlated with differences in blood flow. The results here do not necessarily mean there is no change with these parameters. Indeed, it could be possible that a greater number of subjects would be required to detect any change. But against this, the literature for LDF blood flow in the skin at other locations, also reported that there was no correlation between these factors and blood flow (Bircher *et al.,* 1994; Winsor *et al.,* 1989). This lake of correlation reported for other skin sites implies that the biological relevance of non correlation for the sites within the ear canal would also be the appropriate one.

7.3 Intrasubject variability

The measure of intrasubject variability was limited by the number of control subjects (13/43) who agreed to undergo a second measurement. However, no significant changes were seen within this group over the time period measured. This suggests that the signals measured were quite stable over the morning/afternoon time period of 4-6 hrs.

7.4 Variability of LDF values of blood flow measured at different sites: contributory factors.

The principle two sources of variation in LDF signal are most likely to come from two sources. The first one is the passive decrement due to absorption or scattering for both incident and reflected photon path as summarised in (Table 7.1).

Table 7.1 Summary of possible photon paths at sites 1-4.

Possible Photon path	***LDF Signal contribution***	***Comments***
Scattered in tissue not reflected	Nil	All sites
Scattered in tissue and reflected but from stationary structure (including stationary RBC)	Nil	All sites
Scattered in tissue and reflected back from moving RBCs or rouleaux of RBCs	+	All sites
Scattered in tissue and reflected back from more than one moving RBC	+	All sites but less likely in TM due to lower capillary density
Scattered and reflected back from underlying tissue plane e.g. bone reflecting off moving RBC or RBCs and passing to detector.	+	Sites 1-3 but not site 4 as no underlying tissue plane

Some absorption would probably take place, though to differing degrees, in all tissue types. Scattering would be contributed to by macrocellular structures would be the thick epidermis, the hair follicles, sebaceous glands, sweat glands and ducts and dermal collagen in the dermis. That is, anywhere where there was an abrupt change in optical density e.g. the interface between air and water (Rendell *et al.,* 1989).

The other source of variation would be a 'true' variation in terms of contribution by the number of moving RBCs that would yield a Doppler shifted signal. In turn this variation would be due to two causes. First, the capillary bed density within the detector volume of about 1 mm^3 (Karanfilian *et al.,* 1984; Slaaf *et al.,* 1990; Nilsson *et al.,* 1980; and Bollinger *et al.,* 1991). Secondly, the actual number of moving red blood cells within any given tissue volume or length of capillary. The former contribution in an individual would not usually change in normal skin, but the latter would vary physiologically.

In skin of the normal ear canal, the capillary RBC flow rate would be expected to be affected by a number of separate resistances offered by the arteriolar and metarteriolar smooth muscle tone. At the capillary level, flow would be determined by the precapillary smooth muscle sphincters of the metarteriolar network (Rhodin, 1967; McCuskey, 1971).

The overall flow in the detector volume would also be determined by the proportion of capillaries open to flow. As cited in the introductory section 2.4, on skin elsewhere on the body surface only 20-30 % of capillaries may be open at any one time, though this in turn reflects the degree of involvement necessary for skin thermoregulation (Granger, 1998).

Normally, the proportion of skin blood flow concerned with thermoregulation is about 90 %, with only 10 % usually required for nutrition and waste removal (Fagrell, 1984; Nitzan *et al.*, 1988).

This division of flow is likely to be reflected in the skin of the ear canal, but it is possible that the proportion of capillaries actually open for temperature control is greater than the 30% figure cited above. This is because the ear canal would be expected to form a reasonably stable thermal environment - at least from site 2 inwards, due to it partial enclosure.

This would be expected to result in temperatures at or near core body temperature at the skin surface. Therefore this would lead to more capillaries being used for allowing flow to lose heat. Indeed, the fact that the thermal environment of the TM reflects that of the body core temperature has lead to its choice for temperature measurement using infra red thermometers (Fraden and Lackey, 1991; Rhodes and Grandner, 1990; Edge and Morgan, 1993). However, no studies have been carried out to indicate the proportion of capillaries that are in the open/closed state in the ear canal.

The capillary itself does not have the ability to vary resistance to flow as there is no smooth muscle component in their structure (Granger, 1998; Michel, 1984). Increase or decrease in LDF signal will be related to: (i) the proportion of capillaries actually opened to any blood flow; (ii) in those, which are open, the number of RBCs passing through per unit time.

7.5 Site medians and variability in LDF values possible explanation.

The simple site order of 3:2:1:4 in terms of median blood flow measurement were 112:71:58:29. The pattern in LDF variability did not match this and was 2:1:3:4 with actual 10^{th} - 90^{th} percentile ranges 68:57:47:36. The possible reasons for these patterns are proposed in terms of thermoregulation and density of skin inclusions. Given that the relative component of blood flow for nutrition and waste removal is relatively low (10%) it is not considered to make a major contribution to blood flow variability measured here (Fagrell, 1984).

There is no published data on the relative density of the thermoregulatory capillary beds at each site, although it would be reasonable to assume that the capillary bed density would not be dramatically different over sites 1-3. Site 4, is anatomically distinct from these three sites and has a significantly different function to these other sites, being uniquely involved in the transmission of external acoustic stimuli. It is therefore, considered separately from sites 1 to 3.

Site 1, is about 3-5 mm thick in the adult, with the majority of LDF signal is generated in the first mm of skin depth. As site 1 is external compared to sites 2 and 3 more heat would be expected to be lost at this site. All experiments were conducted in an ambient temperature of 22°C. At this temperature, it would be expected that local vasocontrol would be likely to down regulate blood flow compared to sites 2 and 3. Hence, this would partly explain the lower median LDF value. The macrocellular structures at this site contributing to scatter and absorption include those listed above. These structures would be expected to contribute to the variability of signal, which was the second highest.

Site 2 is the outer third of the ear canal; the skin again is about 3-5 mm deep. This location had the second highest median LDF value being about 23% greater than site 1. This site differs from site 1 and 3 in that it also contains the ceruminous glands that secrete ear wax (Alvord and Farmer, 1997). In terms of temperature control, site 2 is partly enclosed and heat dissipation would be consequently expected to be reduced. This would lead to a higher external temperature that would act a signal to increase blood flow for heat removal (Fagrell, 1984). This would in turn result in greater capillary blood flow in an attempt to increase heat loss at this site. Therefore, this could partly explain the greater median LDF value over site 1.

Site 2 had the greatest variability range, which was about 20% greater than the range for site 1. This could possibly be explained in terms of the greater number of macrocellular inclusions, including those components of the ceruminous gland within the first mm of skin depth, contributing to scatter and absorption.

Site 3 is the deep part (inner 2/3) of the ear canal and the skin depth is typically about 1-2 mm (Alvord and Farmer, 1997). Importantly, there is no sweat, sebaceous or ceruminous glands at this location and there are no hair follicles. As this the deepest of the three sites the effect of thermal retention would be greater here and this would be expected to contribute to a greater stimulus to more marked capillary blood flow. Indeed, the median flow here was nearly 95 % greater than at site 1. There is also the possibility that the underlying well vascularized periosteum at this site also contributes to the LDF signal although this would need to be tested in separate experiments. The lower density of macrocellular inclusions at site 3 may also account for the lower variability range which was about 20 % lower than at site 1.

Site 4, is considered separately here to site 1-3 as it is anatomically distinct from these sites. It is also, the most distinct in terms of the degree of LDF signal skewedness as seen in normal subjects (Figures 3.14-17). The Anderson-Darling values of 2.55 and 2.34 for right and left ears respectively reflect this marked skewedness.

In the TM, Figure 1.8 shows that there are no macrocellular inclusions, i.e. sebaceous glands, ceruminous glands or hair follicles responsible for abrupt changes in tissue density. The structures in the TM are all laminated in a horizontal plane to the beam. Therefore, there would be expected to be less opportunity for absorption and scattering.

The tympanic membrane does not have an underlying tissue layer, with light signal simply passing through the tympanic membrane into the middle ear. This means that, there is no opportunity for back reflected

light from underlying tissue layers to then collide with moving RBCs to yield a Doppler shifted signal at the detector.

In terms of vascular anatomy the tympanic membrane has a much lower density of capillaries (Imanishi *et al.*, 1997; Triana *et al.,* 1990). This would go some way to explaining the lower median value, which was about 50% of site 1.

In explaining the LDF in terms of temperature regulation this also is a better reflection of regulation of capillary bed flow at actual body temperature for the reason stated above. That is, that the infrared signal arising from the TM is used as a measure of body temperature as its own temperature reflects more closely body core temperature (Edge and Morgan, 1993).

In comparison with the other three sites, site 4 shows a distinct skewing of signal so that the median falls nearer the lower end of the range in a near binomial type distribution. Whilst sites 1 to 3 were not strictly normal in their appearance they did not have the appearance of the TM distribution. This suggests that in the normal TM blood flow is regulated nearer the lower end of the operating range. At the other sites it would appear to operate nearer the midpoint of the normal operating range.

As mentioned above, the variation in LDF signal is most likely to reflect the effect of non physiological passive scattering and the physiological variability in temperature regulation via skin capillaries. The experiments presented here could not easily distinguish between these two proposed sources of variability. However, it is assumed here that the LDF ranges reasonably reflect the variation in physiological control of the arteriolar blood flow; operation of the precapillary sphincters; and the proportion of capillaries open to flow.

With regards to thermoregulation at these sites this tells us that, there is a considerable dynamic operating range at these sites, when the variability is normalised relative to the median value (see Table 4.1, column 5). It was also shown that the LDF at none of these sites correlated with body temperature. This would perhaps suggest that, very local control of microcirculation at these specific sites is closely correlated with the whole body temperature control mechanism.

7.6 Comparison of LDF in external ear canal with previous reports at other sites: The absence of standardisation

Direct comparison with the results obtained from other body sites is difficult and not fully justified. This is for a number of reasons first outlined in the introduction. The primary reason is the lack of a recognised physical standard for measurement of cutaneous blood flow or blood flow in general (Tenland *et al.,* 1983; Marszalek, 1996). This is in part because of the diversity of instrumentation used. For example the dimensions of the probe on these instruments are not identical and the skin depth to which they recruit the LDF signal is not standardised. If a probe was to sample over a 10% greater volume then this would be able to integrate that signal from a vascular bed existing in that volume.

The signal sampling and processing also differ so that, for example, increasing the signal processing bandwidth gives rise to a larger output signal (Obeid *et al.,* 1990). These instruments do not all use the same hardware and the differences in the way the LDF signal is translated into a greater or lesser output. The literature largely reflects this problem of comparability between and within sites by the variety of ranges used for in the scaling used to represent arbitrary blood flow units.

Consequently, it would seem that for any group utilising the technique for clinical use they first have to gather their own control data for a given measurement site (or sites). They would then have to utilise this against reasonably well defined patient groups e.g. diabetes whose

severity of condition at least falls within with a recognised clinical grading system. This then allows the technique to be used as a technique in diagnosis. In reading the literature, however, this does not appear to have been comprehensively carried out with clinical studies often being carried out on very small (less than 10) groups of subjects or patients (Schops *et al.,* 1987). It appears that much of the literature presents studies in which large changes in cutaneous skin flow occur without much real consideration of use of testing in experimental sample groups that are reasonably statistically well defined.

It would seem that there would be grounds for larger well designed studies to address these issues, notwithstanding the difficulties in standardisation. Support for such an enterprise is manifest in the large literature on cutaneous blood flow cited at over 1500 papers on cutaneous blood flow (Leahy *et al.,* 1999).

7.7 Comparison of variability.

There absence of universal standardisation in terms of expressing cutaneous flow or perfusion in absolute units present difficulties, However, it should still be possible to present results in such a way that means or medians standard deviations or percentile ranges can be expressed in a proportionate manner to allow normalised comparison of the mean/median vs. the measure of variability as done in Tables 4.1and 5.1 in this study. What were again apparent from the literature, were the poor reporting of basic statistical properties that would allow these comparisons. This is really necessary in order to attempt to draw out the various factors that may be responsible for the variability measure.

One report that did provide some information about this was by Winsor *et al.,* (1989). This study reported the effects of room temperature on relative cutaneous flow in the toe. At a room temperature of $25^{O}C$, the median value of 17 arbitrary units and a 10^{th}–90^{th} percentile range of 5-33 arbitrary units in control subjects. This gives a median variability

ratio of 17/28 = 0.607. This normalised value is lower than any of the sites measured in this study and would be most easily explained by the fact that the big toe is anatomically dissimilar to the ear canal, with larger variations in epidermal thickness to be expected.

Only two reports that could be found, that measured LDF in the external ear canal and both these measured flow at the TM. The first by Schops *et al.*, (1987) was a short report based on five patients. They simply reported that blood flow could be measured using LDF at the TM. The other report by Das *et al.,* (1997) used a Vasamedics instrument with a 2mW 780nm beam and 2mm probe tip in 50 patients. The LDF PU scaling was about reduced by about a factor of 20 in comparison with this study.

The distribution of flow rates was however, comparable with those seen in this study. The mode indicated a Poisson like distribution with the low PU value mode. The relative range of values i.e. about a four fold range of values from highest to lowest was apparent.

In summary, the results here provide control values of blood flow in the ear canal. The estimates of flow rate distribution have not been reported before.

These results can therefore be used in establishing significant treatment on vascular sufficiency and function in other patient groups. For example these results could be used for establishing the limits of normality (10^{th} to 90^{th} percentiles) in blood perfusion for diabetics, who often present at ENT clinic. This group is clinically well recognised for having altered blood flow to the skin (Russell *et al.*, 1993).

It could also be used in detecting early onset changes, for example, in patients presenting with suspected infection such as otomycosis. More generally, it could be used to monitor postoperative recovery of vascular function in any patients who had undergone surgical

procedures involving the external ear canal. This would include meatoplasty for external auditory canal stenosis or atresia, benign or malignant osteomas and exostosis.

7.8 Chapter 5: Characteristics of blood flow in patients with otitis externa.

As was expected, it was clear from the results here that the principle response of the skin to infection was increased blood flow. Importantly in this case, the increase in blood flow is not related to any attempts to control temperature, but to increase the delivery of components of the immune system to the infection site.

Due to the nature of the inflammation, the passive optical properties of the skin are likely to be affected to some degree. This is due to the increased permeability of tissues that accompany inflammation. This is accompanied by increases in water content and presence of white blood cells in the tissue matrix (Yoshikai, 2001). This could conceivably affect the scatter and absorption of the photons although review of literature of the effects did not reveal any detailed consideration of this issue. It was also outside the scope of this study to address this more general problem.

In the case of otitis externa LDF values, the increase in signal is not directly related to temperature regulation changes, but is primarily due to a variety of immune signalling processes. These act to increase blood flow to deliver a those components of the immune system (WBCs etc.) to effectively deal with the infection. In otitis externa this infection penetrates to the dermis, with the depth being variable and dependent on the severity of infection.

Apart from the evident increase in blood flow at all sites there was also a change in the ranking of site by median blood flow. In otitis externa this now became site3: site2: site4: site1. The rank change between 4

and 1 reflected the proportionally greatest increase in LDF signal at site 4.

At site 1 the overall increase in LDF of about 20% was the least marked. This shows that the infection of the ear canal has an effect on blood flow externally. Interestingly at site 1 there was virtually no effect on variability as measured by the 10^{th}– 90^{th} percentile range. This would indicate that those factors affecting scatter and absorption did not show any change.

At site 2, there was an overall increase in median blood flow of about 57% the second highest absolute and proportionate increase, showing that infection has a physiologically greater effect on blood flow than site 1. Interestingly, there is also a substantial increase in variability independent of outliers. This is about a 95% increase over that in the control possibly reflecting in part changes in the optical properties in the tissue. However, the major part is likely to reflect a real physiological increase due to greater recruitment of capillaries into an open state allowing a greater flow.

Similarly, at site 3 blood flow increased by about 65%, although variability was marginally less marked than at site 2, increasing by about 85%. Presumably, this lesser decrease than at site 2 could be explained in terms of the absence of dermal inclusions, and hence reflecting a more physiological response.

At site 4 median flow increased very significantly and proportionately by about 160%. The most extreme outlier reached a value of 218 PU (seen in the patient with eczema) which is an increase of 750% over the control median. This is a very high value for the TM which has the least dense capillary bed and is comparable with very high flow rates in the other sites. Again the variability as defined by the 10^{th}–90^{th} percentile range also increased dramatically by just over 100%.

In summarising the effects of LDF at these three sites it appears that the severity of the inflammatory response increases the deeper the site within the canal. The absolute increase was greatest at site 3, but the greatest proportionate change was seen at site 4 on the TM.

These changes show, especially when the outliers that go up to 400 PU that there is a very considerable functional reserve for supporting blood flow in the capillary bed of the upper dermis.

As with estimates of flow in the control population there was no evidence in the literature of studies that had investigated the effect of infection in the ear canal. There is a considerable literature on the LDF measurement of blood flow in inflammation and dermatopharmacology extensively reviewed by Bircher *et al.,* (1994) and Eun (1995). The majority of papers reviewed appear to cover causes of inflammation other than infection being biased towards allergic response and ultraviolet reactions. Many of the citations use the small groups of patients as active controls against which to assess the effectiveness of dermatopharmacologic treatments (eg Hammarlund *et al.,* 1989, 1990, 1991*).* The studies reviewed by Eun (1995) have had considerable clinical relevance in providing efficacy ranking for steroid and antihistamine treatments.

Eun (1995) and Winsor *et al.,* (1989) commented on the limitations on separate control and patient groups by commenting on the large variability at some sites, making it difficult to detect significant changes in flow with treatment. In terms of relative mean increase in flow with exposure to skin irritants, Eun (1995) reported four fold increases in LDF flow. This was in response to 5% nickel sulphate and 20ug intradermal injection of bradykinin respectively. This compares to the more modest range of increases in flow seen here at sites 1-3 with a maximal median increase of 160% for site 4. Interestingly, the outlier with the highest values of 750% is well above this reported four fold increase with a potent vasodilator

Because of the difference in the sites measured from, it is difficult to infer if the values referred to in this study and in these other studies represent the maximal extent of the use of functional reserve in the capillary system for supporting infection induced flow in the ear canal. This would require an absolute measure of the number of capillaries open at a given measurement site by other techniques. In addition the partially enclosed environment of the ear canal would lead to higher temperatures as discussed above. Winsor *et al.,* (1989) showed that at the big toe going from 26°C to 36°C caused a near 20 fold increase in flow. But again this comparison has to be viewed with caution due to the very different epithelial thickness in the toe compared to sites 1-3, which are appreciably thinner. This would suggest however that there were a greater number of capillaries one per unit volume of the capillary bed.

In summary these results show that flow increases during infection. Additionally, there is a large range of values from the lower end of the norm (about 20 PU) to the upper end of the infected ears (about 400 PU) that covers a near 20 fold range in flow. This gives an approximate estimate of the operating range for blood flow in the external canal.

Clinically, this work is of considerable relevance to both dermatology and otology. The external ear canal is a common infection site as far as the whole body surface is concerned. It is an especially common condition for presentation in otology clinics and in general practice. Because of this, it is perhaps surprising that no previous studies using LDF to look at blood flow at this location have been carried out.

7.9 Chapter 6: Characteristics of blood flow in pre and post operative myringoplasty patients.

Myringoplasty is a common procedure in Otological practice; When the TM fails to heal spontaneously, surgery is often necessary to close the perforation (Saadat *et al.,* 2001). Again as with otitis externa this

makes study of external ear canal blood flow in this patient group pertinent. The data from these patients was firstly analysed statistically using Friedman's ANOVA. The results from this analysis were presented in part at a meeting of the ORS in 2000 (Cook *et al.,* 2000). These results showed that the surgical procedure did not appear to result in any significant changes in blood flow following the procedure. Additionally, the pre and post operative median values were comparable with the control group.

This evidence that the myringoplasty procedure was not having any effect on blood flow was re-examined in Chapter 6, because it was felt that the patients in the myringoplasty group might not have shared a sufficiently homogenous profile. It was therefore decided to examine these patients on a case by case basis. For example, some may have presented with congested TM or an accompanying mild otitis externa.

Another factor prior to analysis of the data that could have been taken as a potentially biasing factor was the median age of this group. The median age of these patients was 55 compared to 41 in the control group. Following analysis of the control group data it did not appear that age was a factor affecting blood flow in the ear canal, but it was another secondary factor contributing to the decision to consider each patient individually.

As far as the myringoplasty procedure is concerned it is usually a commoner procedure in the 20-40 year age range. The sample of patients in this study did not appear to reflect this. The reason for this age bias was that the patients approached to take part in this study agreeing to be measured pre and post operatively were generally older. Younger patients who had originally agreed to take part in the study did not attend for post operative measurement.

A step that was taken to minimise heterogeneity in this patient group was to select patients with a dry TM perforation. This was done to

avoid problems with altered blood flow in evidently inflamed and discharging TMs. Whilst this was carried out it was possible that recruitment of patients with mildly infection and inflammation could have been recruited. It was evident from a number of patients both pre and post operatively that some patients had elevated blood flow. Some also had depressed flow.

For example Cases 2, 3, 4, 6, 8, 9 and 10 had at least one site with very high LDF values well outside the normal range. Site 3 seemed to be the most frequently affected. Cases 1, 4, 8, 9 11, 12, 14 and 18, also, showed evidence of lower LDF values typically at site 1.

The elevated flows occurred in ears that did not appear to be infected. What may have been a factor was the use of cotton buds in attempts to self cleans the ears. This was reported in at least two patients 4 and 6 and it was a possible factor in the other cases. Self cleaning is quite a common habit that often leads to inflammatory damage to the canal and the TM. Some patients use a range of implements such as keys or paper clips as aids to cleaning and these would be expected a greater potential risk of causing damage to the canal. The undesirability of avoiding attempts at self cleaning was made clear to these patients.

Overall then the conclusion from the study on this patient group when considered on an individual basis remains unchanged from the earlier report by Cook *et al.,* (2000). This is that myringoplasty does not lead to any long term changes in blood flow in the external ear canal and TM.

Direct comparison of the myringoplasty procedure with other types of graft surgery is not really justified. This is because the graft itself consists of connective tissue fascia to provide a template or support for epithelial and mucosal re-growth from the edges of the TM to close the perforation.

7.10 Proposals for future work

There are a number of potential applied and basic science projects that could be developed further from this work. Some of these are suggested below.

7.10.1 Applied Projects

Diabetes is known to affect peripheral blood flow due to neuropathy that affects control of supply (Nathan, 1993). These changes in flow also lead to secondary changes in clearance of interstitial tissue fluid which can lead to oedema (Hilz *et al.,* 2000). In turn this makes the skin more susceptible to infection. This is reflected in the number of diabetic patients presenting at clinic with otitis externa (Pedersen and Rosborg, 1997).

At its most severe the infection can be necrotising or malignant, spreading through bone to the brain. It would be of both clinical and basic interest to recruit a group of diabetic patients with widely varying degrees of diagnosed severity of diabetes both with and without otitis externa.

Firstly, it would be of particular interest to measure blood flow in those without otitis externa to establish the change (an expected decrease) with the diagnosed severity of the condition. This would provide a further marker of the severity of the diabetic condition. It would then be interesting to see how the changes in blood flow related the likelihood of developing otitis externa. Within this patient group it would be then possible to establish whether the vascular response to infection followed the same pattern as was seen in control subjects. This would provide information about how compromised the vascular response to infection might be altered by the diabetic condition.

Testing of pharmacological agents on blood flow especially in patients with external ear canal infection is an obvious area for study. As with other studies on the skin, the efficacy of various pharmacological

steroids for reducing inflammation and pain could be tested. Similarly the effectiveness of antibiotic preparations alone and in combination with steroids could be investigated.

Another applied project would concern the design of hearing aids in affecting blood flow in the external canal. The design of many hearing aids leads to inadequate aeration of the canal, which in turn can lead to an increased risk of infection in the canal. This is not an uncommon problem seen in ENT clinics, particularly in elderly patients. In this case, the effect of wearing a particular hearing aid for a given period on blood flow and its relationship to discomfort or irritability could be examined.

This type of study would also be applicable to the effects of long term wearing of earpieces in the canal. The wearing of discrete earpieces in the canal is now very common through use of personal music players and mobile phones. In some users, these earpieces are also in place over many hours of use. Another project would be the investigation of the changes in blood flow in the TM in patient presenting with otitis media with effusion.

7.10.2 Fundamental Projects

In terms of more fundamental science it would be interesting simply to investigate blood flow in this area in more detail. Perhaps the most obvious one that comes to mind would be simply tracking circadian changes in perfusion in this region. This possibility was put forward to subjects in this study, but only a few were willing to take part in such a study. Consequently, this was not taken further.

As mentioned earlier the external ear canal is a common infection site in the population but often presents in children. Hence, a developmental study establishing the patterns of blood flow in the ear canal with respect to age would be justified. This investigation could

also be extended then to not only children with otitis externa, but to those presenting with glue ear.

The effects of acoustic stimulus on the physiology of TM blood flow would be another fundamental project of relevance. This is because the main task of the TM is to affect the first stage of physical transmission from air: fluid within the cochlea. Acoustic energies impinging on the TM vary tremendously in their energy. At 0 dB SPL this is about 10^{-16} W up to 10^{-4} W at 120 dB SPL. Whilst this process is passive at higher intensities, there would be expected to result in sinusoidal displacements of the TM of dimensions that would possibly affect the physiology of the TM vascular supply and of the constituent cells. This may not be of any immediate clinical relevance but as the TM plays a central role in transduction of acoustic stimuli, it is of considerable physiological interest.

7.11 Limitations of the application of LDF in Otology

The limitations of the development of use of LDF in otology are broadly the same as in the rest of other clinical disciplines. That is whilst there has been a large experimental interest in the technique; this interest has not resulted in its application as a diagnostic tool in the clinical setting. This is somewhat surprising as semi-quantitative measure of skin and organ perfusion has interest across many branches of medicine. This probably arises from the absence of clear standards in instrumentation. Review of the literature also showed that there appeared to be an absence of systematic study of what constitutes the properties of perfusion in the microcirculation of the normal population. In part, this study has attempted to redress that imbalance and the current limitations should not rule out the careful development of the future use of this tool for experimental work. For example, the finding here of the scale of increase of perfusion in otitis externa would be of interest to many otologists.

The future success of this technique in clinical application then requires the instrumentation companies, clinicians and researchers to come together. These parties then need to decide: what the specific clinical needs are; what further developments in the current technology are necessary; what specific rigorously designed projects need to be undertaken to provide the baseline for clinical use. With this starting LDF could then be considered for integration as a routine tool in clinical diagnosis.

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