J. S. HERON LTD
5 QUEENS CRESCENT
GLASGOW G4
TELEPHONE: 041-332 1883

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

DEFENCES AGAINST PERITONEAL INFECTION IN PATIENTS ON CONTINUOUS AMBULATORY PERITONEAL DIALYSIS

by

SHEILA J. MCGREGOR, B.Sc.

Being submitted for the degree of
Doctor of Philosophy in the Faculty of Medicine

University of Glasgow

November 1987

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PUBLICATIONS

Work described in this thesis is included in the following publications:

1. McGregor, S.J., Brock, J.H., Briggs, J.D. & Junor, B.J.R. (1987) Bactericidal activity of peritoneal macrophages from continuous ambulatory dialysis patients. Nephrology Dialysis Transplantation, 2, 104-108.

2. McGregor, S.J., Brock, J.H., Briggs, J.D., & Junor, B.J.R. (1987) Relationship of IgG, C3 and transferrin with opsonising and bacteriostatic activity of peritoneal fluid from CAPD patients, and the incidence of peritonitis. Nephrology Dialysis Transplantation, in press.

3. Brock, J.H., Mainou-Fowler, T. & McGregor, S.J. (1987) Transferrins and defence against infection. Annali dell' Istituto Superiore di Sanità (Roma), in press.

ABBREVIATIONS

Abbreviations used in the text included some of the commonly used abbreviations listed in the paper "Uniform requirements for manuscripts submitted to biomedical journals", British Medical Journal (1979), 1, 532. Other abbreviations used in this thesis included:

α	alpha
ADCC	antibody-dependent cellular cytotxicity
ANAE	α-napthyl acetate esterase stain
β	beta
CAPD	continuous ambulatory peritoneal dialysis
CR	complement receptor
DNCB	dinitrochlorobenzene
Fab	fragment obtained by papain hydrolysis of immunoglobulins
Fc	crystallisable fragment of immunoglobulins
FCS	foetal calf serum
g	gram or acceleration of gravity
HBSS	Hank's Balanced Salt Solution
HLA-DR	DR region associated human leucocyte antigen
H_2O_2	hydrogen peroxide
HVA	homovanillic acid
Ia	I-region associated antigen
Ig	immunoglobulin
IFN	interferon
IL-1	interleukin-1
M.W.	molecular weight
NAD	nicotinamide adenine dinucleotide

NADP	nicotinamide adenine dinucleotide phosphate
$OH^{\cdot}$	hydroxyl radical
O_2^-	superoxide anion
PBS	phosphate buffered saline
PDE	peritoneal dialysis effluent
PG	prostaglandin
ROI	reactive oxygen intermediates
SDS	sodium dodecyl sulphate
Tf	transferrin
v/v	volume per volume
w/v	weight per volume
>	greater than
<	less than
%	percentage
+ve	positive
-ve	negative

ACKNOWLEDGEMENTS

I would like firstly to express my indebtedness to my supervisor Dr. Jeremy H. Brock for his tremendous support, advice and unfailing help not only in writing this thesis but also during the three years I have worked for him. Thanks must also go to Professor D.M.V. Parrott who allowed me to carry out this work in her department.

In addition acknowledgement must be given to Dr. J.D. Briggs and Dr. B.J.R. Junor without whom this project would have been impossible. Their clinical expertise and help in organising sources of samples for testing were invaluable, not forgetting Dr. Brigg's financial support through the Western Infirmary Kidney Research Fund which allowed me to continue my work. The National Kidney Research Fund must also have recognition in this respect.

I am especially grateful to all the staff in the CAPD clinic who never once showed annoyance by my constant demands for "bags" no matter how busy they were. In particular, mention must be made of Nursing Sisters Caitriona Donald and Nan Morris as well as May Timms. The help given by the theatre staff of the Gynaecological Units of the Western Infirmary and Southern General Hospitals in supplying me with control samples was very much appreciated.

I must also take this opportunity to thank most sincerely all my friends and colleagues in the Department of Immunology and Bacteriology for their help and support including Dr. Tony Speekenbrink for his advice in statistical analysis.

Finally I would like to thank my parents for their encouragement and practical help in writing this thesis and my sister Riona for her work in typing this thesis.

SUMMARY

The peritoneal immune defences of patients on continuous ambulatory peritoneal dialysis (CAPD) were examined in order to determine whether any defects exist which may account for the susceptibility to peritonitis of some patients.

Overnight dwell peritoneal dialysis effluent (PDE) was obtained from uninfected CAPD patients and found to contain up to 10^7 leucocytes per bag. The average differential cell count was 70% macrophages, 21% lymphocytes, 6% neutrophils and 3% eosinophils. The number of cells isolated decreased with time on CAPD, perhaps due to thickening of the membrane or trapping of cells by the formation of adhesions in the peritoneum.

The ability of the peritoneal macrophages from CAPD patients to ingest and kill Staphylococcus epidermidis was examined. This organism was chosen because it is the major cause of peritonitis in CAPD patients. In most cases the macrophages were able to efficiently phagocytose and kill opsonised S.epidermidis in vitro. However when these cells were compared to normal controls they were found to be defective in their intracellular killing ability. Although no overall correlation was found between the degree of phagocytosis or intracellular killing and susceptibility of patients to peritonitis, cells from two patients with a high incidence of peritonitis did show abnormally poor ingestion and/or killing. This suggests that in some patients defective phagocytic activity may be a

contributing factor in determining susceptibility to peritonitis.

The ability of peritoneal macrophages to produce H_2O_2 and express class II major histocompatibility antigen (HLA-DR) were examined in order to provide information on the activation or maturation state of cells from CAPD patients. Both these parameters were decreased compared with normal controls. However, the degree of H_2O_2 release and HLA-DR expression by the peritoneal macrophages from CAPD patients was similar to that of blood monocytes. These results, in conjunction with those of the bactericidal assays suggest that the cells isolated from the peritoneum of CAPD patients are relatively immature macrophages and resemble blood monocytes. Peritoneal macrophages from patients commencing CAPD released more H_2O_2 and showed a greater proportion of HLA-DR positive cells than those from patients established on CAPD or normal control peritoneal macrophages, indicating that patients may have an initial inflammatory response. This may possibly be caused by the recent implantation of the catheter.

The levels and activities of the serum proteins IgG, C3 and transferrin (Tf) in the PDE were examined as they are believed to have important roles in defence. Levels of all these proteins in PDE were only 1-2% of those in serum, and when the levels were compared with each other and with total protein levels significant correlations were found in each case. The levels of these proteins in PDE were independent of those in the corresponding patients' sera, suggesting that entry of serum proteins into the peritoneal cavity does not involve a specific transport process but

depends on the permeability of the peritoneal membrane. There was also an inverse correlation between the level of total protein in PDE and length of time on CAPD which again implies that changes may occur in the permeability of the peritoneal membrane.

A positive correlation was found between the opsonising capacity of PDE and the IgG or C3 concentration, and there was an inverse correlation between the opsonic activity of PDE and frequency of peritonitis. These results strongly suggest that the levels of opsonins in the peritoneum are sub-optimal in CAPD patients.

The bacteriostatic activity of Tf in PDE was examined by measuring the in vitro growth of S.epidermidis in cell-free PDE. More growth of the bacteria occurred in PDE than in sera or normal peritoneal fluid, and in PDE the growth rate correlated inversely with the Tf concentration. Addition of extra Tf to the PDE reduced the growth of S.epidermidis, but had no effect when added to serum or normal peritoneal fluids. An inverse correlation was also found between the degree of reduction in growth of S.epidermidis on addition of extra Tf and the original level of Tf in the PDE. Overall, these results indicate that the levels of Tf in PDE provide sub-optimal inhibition of extracellular bacterial multiplication.

A longitudinal study was carried out on a group of CAPD patients who were examined for up to 9 months from the day of commencement of dialysis. These patients showed a general decline in the levels and activities of the cellular and humoral defence mechanisms in the peritoneum with time on CAPD. The decline was particularly prominent

in the first 4-6 months.

Overall, the findings indicate that the immune defences of the peritoneum of patients on CAPD are sub-optimal. The peritoneal cavity of the CAPD patient can therefore be considered an immunocompromised site and this should be taken into account in the management of these patients.

CHAPTER I

LITERATURE REVIEW

INTRODUCTION

The immune system of man is an extremely complex network of mechanisms, which have been developed in order to provide many ways in which to defend ourselves against pathogenic micro-organisms. These include both innate mechanisms, ie those we are essentially born with, and acquired defence mechanisms, which enable us to mount specific immune responses. As most infections enter the body via epithelial surfaces the exterior defences of the body are of importance. These include the skin covering the body surface, the lysozyme action of most secretions, the mucus and cilia of epithelial membranes and the acidic pH of the stomach.

Among the innate defences, soluble factors of the blood, such as the complement system and cellular factors, such as blood and tissue phagocytes are very important. In addition, non-specific inflammation at the site of infection is an essential part of the host defence.

Acquired immunity consists of antibodies secreted by differentiated B-lymphocytes, and cell-mediated immunity by specific T-lymphocytes. These systems are activated in response to antigenic stimulation.

Often the innate defence mechanisms and acquired immune systems interact together against invaders. Antibodies produced by differentiated B cells help phagocytes to recognise their targets and T cells produce lymphokines which stimulate activation of phagocytes. Also macrophages and other antigen-presenting cells play an

important part in the recognition of foreign bacterial antigens by lymphocytes and their subsequent stimulation.

A delicate balance therefore exists, with all arms of the immune system being dependent on each other for optimal activity. Any defect which may arise, in no matter what area, may severely compromise the system as a whole and leave a patient prone to infection.

1.1 CONTINUOUS AMBULATORY PERITONEAL DIALYSIS

Continuous Ambulatory Peritoneal Dialysis or CAPD represents a very effective form of renal dialysis for patients with chronic renal failure, which removes waste products (primarily urea and creatinine) from the blood by diffusion through the membrane of the peritoneum (Oreopoulos et al, 1980; Popovich et al, 1978).

Very simply, CAPD is a portable internal dialysis technique, which uses the continuous presence (24 hours a day, 7 days a week) of dialysis solution in the peritoneal cavity, except for periods of drainage and instillation of fresh solution 3-4 times per day. After each exchange, which occurs at 4-6 hour intervals or 8-10 hours overnight, the indwelling peritoneal catheter is capped, and the patient is free to participate in his usual daily activities. CAPD therefore exchanges the relatively long dialysis sessions of 4-6 hours, 3 days per week in haemodialysis, for three or four 30-45 minute interruptions of daily activities every day.

The peritoneal membrane which lines the abdominal cavity and participates in the diffusion of water and solutes during CAPD is composed, in the normal undialysed patient, of a compact surface layer of mesothelial cells with protruding nuclei and numerous microvilli (Di Paolo et al, 1986). This layer also has a network of capillary and lymphatic vessels. After prolonged CAPD, however, a reduction and subsequent disappearance of microvilli is seen, along with widening of intercellular junctions.

Popovich et al (1978) showed that CAPD can provide

acceptable or even better control of serum chemistry than other dialysis techniques, with resultant improvement in the patient health. Being continuous, CAPD patients have steady state chemistries, preventing the symptoms and problems associated with rapid fluctuations in serum chemistry and body fluid volumes that occur with haemodialysis (Popovich et al,1978) eg minimal cardiovascular stress.

CAPD is particularly suited to diabetics, as excellent control of blood sugar is achieved by intraperitoneal insulin, and it also has a favourable effect on the deterioration of retinopathy (Amair et al,1982). Popovich et al (1978) noted, however, that removal of small molecules (urea) with CAPD is only 60% as efficient as with haemodialysis. Removal of larger molecules, the so called middle molecules (MW 800-2000) with CAPD is, however, much more efficient compared with haemodialysis (Bergström et al,1979; Popovich et al,1978).

CAPD, in spite of its many advantages, has been overshadowed by a number of complications, the two most important of which are a high incidence of peritonitis (infection of the peritoneum), and a high rate of protein loss (10-20 g/day), particularly during peritonitis (Krediet et al,1986; Rubin et al,1981; Popovich et al, 1978). Rubin et al (1981) suggested that the increased protein loss may be due to alterations in peritoneal blood flow, which may affect membrane surface area or permeability. The problem of excessive protein loss can, however, be compensated by the ingestion of a high protein diet.

Peritonitis is still the major drawback of CAPD. About 70% of infections are caused by Gram positive organisms, such as Staphylococcus epidermidis, Staphylococcus aureus, and streptococci whereas Gram negative organisms account for about 15% of cases. The remaining peritonitis episodes are due to other organisms, including fungi (Smith et al,1986). If the infection is not adequately treated sufficient adhesions may occur to obliterate the peritoneal cavity, particularly if the organism is a fungus or a yeast (Coles,1985). Infection of the peritoneum may result from poor technique during bag changes and contamination with skin flora (Smith et al,1986; Vas,1981) and the fact that coagulase negative staphylococci, which are common skin commensals, are responsible for most infections, supports this conclusion. Nevertheless, some patients are unusually susceptible to peritonitis (Tsakiris et al,1986; Vas,1981), and this suggests that impairment of host defence mechanisms may be involved. Although the mechanisms of this increased susceptibility are not clear, deficiencies in various cellular immune functions have been implicated (Alobaidi et al,1986; Tsakiris et al,1986; Goldstein et al,1984), as well as inadequate levels of serum proteins (Keane et al,1984; Verbrugh et al,1983). It remains to be determined whether CAPD induces defects in the normal host defences of the abdominal cavity and whether these changes are important in the pathogenesis of peritonitis and it is this aspect with which the work reported in this thesis is concerned.

As mentioned above staphylococci, major commensals of man have consistently proved the commonest cause of peritonitis in CAPD patients (about 40% due to S. epidermidis and 20% to S. aureus). All staphylococci are Gram positive cocci that produce catalase which can be sub-divided by their capacity to produce or not produce the enzyme coagulase which clots rabbit plasma. S. aureus are coagulase positive whereas S. epidermidis are coagulase negative. S. aureus can also be distinguished from S. epidermidis by the presence of protein A which binds to the Fc portion of immunoglobulins, and by its ability to produce a large variety of toxins, haemolysins and leucocidins which play a major role in its pathogenicity (Gemmell, 1984).

S. epidermidis, often previously dismissed as culture contaminants, are assuming greater importance as true pathogens. There is now considerable evidence to show that coagulase-negative staphylococci are the major cause of infections of prosthetic devices and intra-vascular catheters (Archer, 1985). Virtually all of the S. epidermidis infections are hospital-acquired, and multiply antibiotic resistant which probably reflects the selection pressure of widespread antibiotic use in the hospital. Vancomycin is one of the few antibiotics effective against these organisms (Bailie et al, 1987). Colonisation of patients and hospital staff with antibiotic resistant S. epidermidis precedes infection with these organisms. S. epidermidis is also the most common cause of bacteremia among patients receiving immunosuppressive therapy.

Its pathogenicity may be related to its ability to

adhere to and grow on polymer surfaces such as catheters and by the production of an extra-cellular slime substance (Gray et al, 1984). This slime inhibits the proliferative responses of lymphocytes and possibly opsonisation of bacteria.

Protection of the peritoneum from microbial pathogens is believed to depend on three mechanisms. The first is a simple removal mechanism by which the intraperitoneal circulation of fluid carries particles to the diaphram, and hence via the lymphatics to the thoracic duct and eventually to the blood stream (Dunn et al,1985; Hau et al,1979). Bacteria can be demonstrated in the lymphatics within minutes after their inoculation into the peritoneum (Dunn et al,1985; Hau et al,1979). Of interest here is the finding that bacteremia is uncommon in peritonitis associated with CAPD (Duwe et al,1981; Vas,1981). This contrasted with an incidence of over 30% in surgically induced peritonitis (Vas,1981). Although this may be related to microbiological differences, it is possible that peritoneal dialysis may interfere with the normal mechanisms of lymphatic absorption and elimination of bacteria by impeding the passage of pathogens into the systemic circulation. Such a defect in normal lymphatic function was suggested by Duwe et al (1981) to result from mechanical factors associated with the instillation of large volumes of fluid into the abdominal cavity. It is not known what contribution altered lymphatic function makes to the development of peritonitis in CAPD.

Secondly, protection depends on a killing mechanism in which host phagocytes, about 80% of which are normally macrophages (Alobaidi et al,1986; Verbrugh et al,1983) act as effector cells (Dunn et al,1985; Verbrugh et al, 1983). Although macrophages, lymphocytes and polymorphonuclear leucocytes are found within the peritoneal cavity, the predominance of macrophages at the start of the bacterial infection led to the conclusion that these cells represent

the first line of host defence in the elimination of bacteria during the initial stages of infection (Dunn et al, 1985). They are largely replaced by polymorphonuclear neutrophils as the predominant phagocytic cell during episodes of peritonitis (Rubin et al, 1980; Hurley et al, 1977).

The final mechanism involved is sequestration due to fibrin formation which leads to direct trapping of micro-organisms as well as to the formation of adhesions between surfaces, both of which physically isolate invading micro-organisms (Dunn and Simmons, 1982; Hau et al, 1979). Hau and Simmons (1978) also found, however, that this may impair bacterial clearance from the peritoneum, thereby promoting the development of clinical peritonitis. The protective role of fibrin is therefore controversial. Interference of phagocytic function appears to account for the defect, underlining the importance of these cells in protecting the peritoneum from infection.

1.2 MONONUCLEAR PHAGOCYTES

The mononuclear phagocytes arise primarily from a population of phagocytic cells that originate in the bone marrow (promonocytes), are then transported by the blood stream as immature monocytes and finally migrate into the various tissues of the body where they differentiate into functional resident tissue macrophages (van Furth et al, 1979; van Furth and Cohen, 1968).

The origin and kinetics of these cells have been investigated in several animal species (most extensively in the mouse). In man the study of mononuclear phagocytes has been much more limited and has mainly concerned blood monocytes. It is highly probable, however, that the mononuclear phagocyte system identified in animal studies is also applicable to man.

Macrophages are extensively found throughout the body including the peritoneal, alveolar and pleural spaces, where they are generally free to move around, and in less motile, fixed forms in the spleen, liver and lamina propria of the gastrointestinal tract (van Furth, 1980).

The macrophages that occur under normal physiological conditions are called resident macrophages and are distinct from those that appear with inflammatory stimulation (exudate macrophages). Whereas the monocytic origin of the exudate macrophage is generally accepted (Daems, 1980; van Furth, 1980; van Furth and Cohen, 1968), the origin of the resident macrophages is still disputed.

1.2.1. Origin of Resident Macrophages

According to van Furth (1980), all macrophages, both under steady state (resident) and inflammatory conditions (exudate) are assumed to derive from blood monocytes and any phenotypic differences noted among macrophages are usually attributed to differences in their maturational state or local adaptation to the environment (Kaplan and Gaudernack,1982; Bar-Eli et al,1981; Simon et al,1977). This was confirmed by Parwaresch et al (1981), using esterase isoenzyme patterns when they showed that prolonged culturing of blood monocytes results in a gradual transition of their pattern to that of resident macrophages.

However although kinetic studies support the view that resident macrophages are monocyte derived (van Furth and Cohn,1968) doubts have still remained (Daems and De Bakker,1982; Daems,1980; Volkman,1976) mainly because of the differences in peroxidatic activity between blood monocytes and resident macrophages. A solution to this problem was explained, however, by Beelen and Fluitsma (1982), when they clearly showed both kinetically and cytochemically that resident macrophages are recruited from blood monocytes via the transitional stage of exudate-resident macrophages. This does not, of course, exclude the possibility that very occasionally a resident macrophage may be capable of division. The opposing view was that there are two functionally divergent types of macrophages i.e. resident macrophages and monocytes derived macrophages that differ in origin (Daems and De Bakker,1982; Daems,1980; Volkman,1976). Daems and De Bakker (1982)

suggested that peritoneal resident macrophages are derived from locally proliferating cells which themselves arise from specific stem cells in the bone marrow, differing from the stem cells of the monocyte/granulocyte series.

The origin of the resident macrophages is still therefore controversial and not resolved conclusively.

1.3 PROPERTIES OF MONONUCLEAR PHAGOCYTES

The cells of the mononuclear phagocyte system are involved in many areas, including defence against micro-organisms, and in scavenging dead or damaged cells or debris. They are also important as secretors of biologically active compounds, and play an important role in the regulation of haematopoiesis and iron metabolism. Macrophages possess a number of cell surface receptors and antigens which are involved in a variety of functions.

1.3.1. Defence Against Micro-organisms

1.3.1.1. Macrophages as Phagocytic Cells

Engulfment and killing of micro-organisms is an essential step assumed by macrophages in the defence against infection. Phagocytosis can be divided into three stages: attachment, ingestion, and killing of micro-organisms.

Attachment and ingestion can be mediated through Fc and complement receptors or by the non-specific binding of particles to glycoproteins on the macrophage membrane, which bind to carbohydrate constituents of the bacterial cell walls or cell membranes. These mechanisms are discussed in detail in section 1.3.4.

On ingestion of the molecule or particle the phagosome normally fuses with lysosomes in the cell forming a phagolysosome. Lysosomes are membrane-bound vesicles containing a variety of enzymes that are capable of degrading ingested debris (Jacques and Bainton,1978).

Most micro-organisms are destroyed within a few minutes, and within 2 hours extensive degradation may occur. Oxygen intermediates (O_2^-, H_2O_2, $OH^\cdot$) appear to be responsible for the initial killing of micro-organisms (see section 1.3.6.6).

The consequences of phagocytosis are different for intracellular and extracellular parasites. For the extracellular parasite, unequipped for intracellular survival, phagocytosis represents the end. The opposite is the case with intracellular parasites, as their defence mechanisms tend to be related to their ability to resist host microbicidal properties. For many extracellular bacteria effective antiphagocytic devices have evolved (reviewed by Horwitz, 1982), the most important of which is the antiphagocytic capsule. Other antiphagocytic determinants are the M protein cell surface antigen of group A streptococci, pili and the protein A of S.aureus which is known to bind the Fc region of Immunoglobulin G (IgG) from various species. Other ways for the bacteria to avoid phagocytosis is to kill the phagocyte itself by producing substances that are highly toxic to leucocytes eg streptolysins O and S, and streptococcal β-haemolysins.

1.3.1.2. Macrophages as Cytotoxic Cells

Macrophages can kill neoplastic cells by non-phagocytic mechanisms. When appropriately activated macrophages selectively and efficiently lyse neoplastic cells in a contact-dependent, non-phagocytic process requiring several days termed macrophage mediated tumour cytotoxicity (Adams and Nathan,1983). Macrophages can also

bind and lyse antibody-coated target cells via Fc receptors. This is known as antibody dependent cellular cytotoxicity (ADCC) and was demonstrated by Conkling et al (1982) in human peripheral blood monocytes and alveolar macrophages. The ability of macrophages to destroy tumour cells in a non-specific way makes it possible to envisage a role for macrophages in immunological surveillence of tumours (Fernandez-Cruz et al,1985).

1.3.2. Removal of Old and Damaged Cells and Debris

Changes in erythrocyte surface membranes caused by immunoglobulin (Ig) coating, physical or chemical injury, surface carbohydrate alterations or aging results in erythrophagocytosis by the mononuclear phagocyte system (Maruta and Mizuno,1971) as well as clearing of normal debris. Through this ability to catabolise red blood cells, the mononuclear phagocytes also play an important role in iron metabolism. The major part of the iron derived from degraded haemoglobin is released to the plasma and bound by Transferrin (Tf) which carries it to the bone marrow for erythropoiesis (Brock,1985). However in a variety of conditions such as acute and chronic infections and chronic inflammatory diseases a type of mild anaemia occurs, which seems to be due at least in part to an impaired release of iron from the phagocytic mononuclear cells of the reticuloendothelial system (Roeser,1980).

1.3.3. Regulation of Haematopoiesis

The formation of granulocyte and monocyte colonies in culture is dependent on substances with specific colony stimulating activity (Metcalf,1982; Golde and CLine,1972). Control of marrow stem cell proliferation may depend on production of prostaglandins (PG) by macrophages which limits stem cell proliferation, and is produced in response to raised levels of colony stimulating activity (Kurland et al,1978). Macrophages therefore control the proliferation of their own and other progenitor cells through both positive and negative feedback systems.

1.3.4. Macrophage Cell Surface Receptors

Optimal phagocytosis of opsonin-coated micro-organisms and particles is mediated mainly by receptors for either the Ig Fc domain or low molecular weight fragments of the complement component C3 (Newman et al,1985; Silverstein and Loike,1980). This response is highly specific and localised, each step of phagosome formation and fusion being directed by sequential engagement of opsonin and receptor, a process termed the "zipper hypothesis" of phagocytosis (reviewed by Silverstein and Loike,1980).

Membrane receptors provide the essential recognition function required for control of macrophage responses to changes in their cellular environment. Passwell et al (1980) also showed that the binding of Fc fragments to Fc receptors on human cells induced selective secretion of some proteins but at the same time decreases the synthesis and secretion of others.

In addition to receptors for Ig and complement the

macrophage surface bears receptors for a variety of hormones, including insulin (McKeever and Spicer, 1980) and other proteins such as fibronectin (Wright and Meyer,1985) and lactoferrin (van Snick and Masson,1976).

Macrophage plasma membranes also show lectin-like receptors for carbohydrate which may play a crucial role in recognition and endocytosis of glycoproteins and micro-organisms (Haeffner-Cavaillon et al,1982; Glass et al,1981; Weir,1980; Weir and ögmundsdóttir,1977).

Receptors for colony stimulating factor have also been found on macrophages (Guilbert and Stanley,1980). This receptor through the binding of colony stimulating factor not only initiates proliferation, but induces morphological changes such as spreading in mature macrophages and differentiation along the monocyte-macrophage lineage in immature myeloid stem cells (Metcalf,1982).

1.3.4.1. Receptors for Immunoglobulins

Macrophages express distinct plasma membrane receptors for different isotypes of Ig. Newman et al (1985) showed that human peritoneal macrophages possessed receptors for the Fc portion of IgG, while Melewicz et al (1982) detected Fc receptors for IgE on human peripheral blood lymphocytes and monocytes. Receptors for the Fc portion of IgA have also been found on human monocytes and polymorphonuclear leucocytes, which may be involved in defence of the mucosal surfaces (Fanger et al,1980) and evidence for the presence of specific IgM receptors on macrophages was provided by Uher et al (1981).

Green et al (1985) showed that Fc receptors are

probably transmembrane glycoproteins, which are synthesised in the rough endoplasmic reticulum and transported intracellularly to the plasma membrane. Binding of Ig to its receptors can modulate cell behaviour dramatically. For example, cross linking of the IgE receptors triggers explosive degranulation of mast cells and basophils, releasing preformed mediators with important physiological consequences (Metzger et al,1986; Metzger,1983). IgE receptors can also mediate the endocytosis of small IgE complexes (Isersky et al,1983) and human monocytes lyse IgE-coated erythrocytes (Melewicz et al,1981), and parasites in vitro (Joseph et al,1978).

Apart from the well known attachment and internalisation of ligand-bound particles (Silverstein and Loike,1980), interaction of macrophage IgG-Fc receptors with immune complexes or opsonised particles also leads to secondary events including antibody-mediated cytolysis of target cells (Conkling et al,1982), the release of inflammatory agents (Humes et al,1980), as well as neutral proteases (Ragsdale and Arend,1979). On lymphocytes, Fc receptors appear to be involved less in endocytic and cytotoxic functions than in modulating antibody production (Neauport-Sautes et al,1979).

Human leucocytes express at least two different Fc receptors specific for IgG; a low avidity receptor which is found on tissue macrophages and neutrophils and a high avidity Fc receptor found on blood monocytes and macrophages (Kurlander et al,1984). The low avidity receptor is believed, from the work of Unkeless (1986), to be primarily for immune complex clearance in vivo. The

high avidity receptor, in contrast, may be involved in the transport of immunoglobulins .

Ligand binding can modulate the plasma membrane expression of Fc receptors in macrophages by altering the receptor's pathway of intracellular transport during endocytosis. Uptake of multivalent Ig immune complexes leads to a large irreversible loss of surface Fc receptors due to their delivery to and degradation in the lysosomes (Mellman and Plutner,1984; Mellman et al,1983). Receptors tagged with a monovalent ligand are also internalised but avoid transport to lysosomes by rapidly recycling to the cell surface (Mellman et al,1984). These findings suggest that cross-linking of adjacent Fc receptors to multivalent ligands may prevent recycling and provide the signal for transport to lysosomes. Interferon (IFN) was also found to alter the number of Fc receptors (Ezekowitz and Gordon,1986; Rhodes et al,1983; Vogel et al,1983), and these factors may therefore play an important role in the activity of phagocytes.

1.3.4.2. Receptors for Complement

Human blood monocytes and macrophages have at least two distinct membrane receptors - CR1 and CR3, that recognise primarily the C3b and iC3b fragments of C3 respectively (Newman et al,1985; Ross and Lambris,1982). In addition studies have suggested that under certain conditions monocytes may also express a CR2 receptor that is specific for the d region of iC3b (Inada et al,1983).

CR1, which binds C3b, is poorly expressed on resting, circulating phagocytic cells, but rapid "up regulation" is

seen with a variety of stimuli such as lymphokines (Joiner et al,1984). This mechanism for increasing the expression of CR1 could prepare chemotactically migrating cells for recognition of material that has been opsonised with C3b. The molecule binds particles coated with C3b to the phagocyte surface. The second C3 receptor, CR3, has a higher affinity for C3bi, a cleavage product of C3b, than for C3b. Recent studies showing that children deficient in the expression of CR3 are highly susceptible to bacterial infections (Dana et al,1984; Ross and Newman,1980) represent the best evidence that this receptor is important in phagocytosis.

Fc receptors are able to move freely within the plasma membrane and are thought to function independently of receptors for complement (Michl et al,1979; Griffin et al,1975). In contrast, studies by Kaplan et al (1978) indicate that unlike Fc receptors, the complement receptors on macrophages normally occupy a relatively fixed membrane distribution. This may be important as work by Griffin and Mullinax (1981) and Kaplan et al (1978) suggested that for a receptor to promote particle ingestion it must be able to move within the plane of the plasma membrane.

Ehlenberger and Nussenzweig (1977), and Mantovani et al (1972) suggested that C3 and IgG may have separate roles in phagocytosis. IgG, through its Fc fragment, directly stimulates particle ingestion, but is relatively inefficient at inducing particle binding. On the other hand C3 primarily mediates the binding of the particle via complement receptors. A marked synergy exists beween C3 and IgG in inducing phagocytosis. Depending on the

experimental conditions, the presence of particle-bound C3 can reduce by 100 fold the amount of IgG required to produce particle internalisation (Ehlenberger and Nussenzweig,1977). This suggests that the role of C3 in opsonisation is mainly one of establishing contact between particle and phagocyte.

Another important finding is that although the quantity of ingestion mediated by the Fc receptors is greater in activated than in nonactivated macrophages, virtually all Fc receptors of both resident, elicited and activated macrophages mediate attachment and ingestion (Bianco et al,1975). With complement receptors there is, however, a qualitative difference in function in activated and nonactivated macrophages i.e. resident peritoneal macrophages bind C3b-coated particles via the corresponding receptor, but do not ingest them, whereas elicited and activated macrophages can both bind and ingest such particles. Phagocytosis of complement-opsonised particles therefore occurs among activated populations of macrophages (Griffin and Mullinax,1985,1981; Bianco et al,1975) and may therefore be a marker for macrophage activation (Edelson,1981). The ability of human peritoneal macrophage CR1 and CR3 receptors to mediate phagocytosis was also shown to be maturation dependent (Newman et al,1985). Furthermore, in human macrophages interaction with fibronectin causes receptors for C3 to avidly promote phagocytosis in the absence of IgG (Wright and Meyer,1985). The activation of complement receptors by fibronectin occurs rapidly, is completely reversible and is not accompanied by a change in the number of C3b or C3bi

receptors (Wright et al,1984). C3 receptors on phagocytic cells may therefore exist in two states, one that leads to attachment without phagocytosis and a second one that, like the IgG-Fc receptor, mediates ingestion as well as attachment (Bianco et al,1975).

1.3.5. HLA-DR Expression

Macrophages play an important role as accessory cells in the initiation and regulation of specific immune responses by presenting antigen to T cells (reviewed by Unanue et al, 1984 and Unanue,1981). The membrane glycoproteins that appear to be critically involved in this antigen presentation are the I-region-associated antigens in rodents (Schwartz et al,1978) which are equivalent to the human-leucocyte antigens (HLA-DR) in man. These molecules are glycoproteins consisting of two noncovalently linked polypeptide chains of 34000-36000 daltons (alpha chain) and 24000-28000 daltons (beta chain; reviewed by Accolla et al,1984 and Lafuse and David 1984) which are recognised by the T cell receptor (reviewed by Kronenberg et al,1986 and Haskins et al,1984) on helper T cells in conjunction with the antigen. Ziegler and Unanue (1981) provided direct evidence that a macrophage-antigen processing event is essential to T cell recognition of the antigen. During this process the antigen is partially catabolised by the macrophage and then presented with the Ia to the T cell receptor leading to subsequent proliferation of antigen-specific T cells. The levels of Ia positive macrophages can be markedly increased in response to local immunological stimulation, indicating

that this population is sensitive to environmental regulation (Beller et al,1980). This amplification of the Ia positive macrophages in rodents and man is regulated by lymphokines produced by activated T cells (Stastny and Nunez,1984; Beller and Ho,1982; Scher et al,1982; Steeg et al,1982) now known to be gamma IFN (Gershon et al,1985; Sztein et al,1984; Basham and Merigan,1983; Steeg et al,1982).

Data from Calamai et al (1982), Beller and Unanue (1981), and Steinman et al (1980), indicate that there is an interconversion of Ia positive and Ia negative phenotype, and the presence or absence of Ia does not demarcate a stable subset of macrophages. Calamai et al (1982), for example, showed that all macrophage precursors generated macrophages with the potential to express Ia, as 100% of colonies contained Ia positive cells after stimulation by T-cell lymphokine. This suggests that there are not separate and divergent lineages of Ia positive and Ia negative macrophages but, as found by Scher et al (1982), Ia positive and Ia negative macrophages derive from a potentially common Ia negative stem cell.

Studies of HLA-DR antigen expression on macrophages and macrophage precursors (monocytes) in man have been less extensive than those in mice and sometimes divergent or conflicting results have been reported. According to Ng et al (1982) and Albrechtsen (1977) only a subpopulation of monocyte/macrophages in man express HLA-DR. Others (Smith and Ault,1981; Raff et al,1980) have concluded that probably all such cells were positive, although large graduations in the intensity of fluorescence were noted.

Smith and Ault (1981), also showed that peritoneal macrophages from CAPD patients stained at the time of isolation showed a similar level of HLA-DR expression to that found with purified blood monocytes. During short term culture of both monocytes and peritoneal cells, a 2-3 fold increase in the average amount of surface HLA-DR per cell was seen, which after 12 hours reached a plateau and diminished only slightly thereafter.

More recent work by other groups has not solved this question of the degree of HLA-DR expression by mononuclear phagocytes. Costabel et al (1986), Clerici et al (1984), Sztein et al (1984) and Golder and Doe (1983) found 45-94% of human macrophages expressed HLA-DR whereas Razma et al (1984) found much lower levels (21%). Possible reasons for these conflicting results will be discussed in Chapter 4.

PG, which are the well known mediators of inflammatory reactions, also function as important modulators of Ia expression. They markedly inhibited Ia expression on murine macrophages previously stimulated by lymphokines (Snyder et al,1982). Kunkel et al (1986) also showed that PG could suppress murine macrophage Ia antigen expression, but it appeared to have little effect on the expression of HLA-DR antigen by human monocytes and macrophages.

Gruner et al (1986) found decreased expression of HLA-DR antigens on human peripheral blood mononuclear cells after one day of culture with opsonised zymosan. This decrease was prevented by β carotene, a scavenger of reactive oxygen intermediates (ROI), or by superoxide dismutase. ROI are formed at inflammatory sites and during phagocytosis by macrophages/monocytes in the course of the

respiratory burst (see section 1.3.6.6; Rossi et al, 1986; Babior, 1984). The effect of ROI on HLA-DR expression was verified using mononuclear cells from patients with a heterozygous form of chronic granulomatous disease, which are deficient in the production of ROI because of a genetic defect in the enzyme NAPDH oxidase (Gallin et al,1983). No alteration in HLA-DR antigen expression after culture with opsonised zymosan was shown (Gruner et al,1986). The effect of ROI on HLA-DR expression is thought to be due to their ability to interfere with the quantity and spectrum of arachidonate metabolite production, some of which influence Ia expression (Snyder et al,1982).

1.3.6. Macrophages as Secretory Cells

Macrophages secrete a large and diverse range of substances. The enzyme content and secretory activity of mononuclear phagocytes are related to the degree of cellular differentiation and to the effects of exogenous stimuli.

1.3.6.1. Digestive Enzymes

Lysozyme, which mediates digestion of the peptidoglycan of bacterial cell walls, is a well known and major secretory product of macrophages. It is secreted continuously and all populations of macrophages produce and secrete large amounts of lysozyme irrespective of their state of activation, phagocytosis or exposure to lymphokines (Gordon,1980; Gordon et al,1974).

The selective release of lysosomal acid hydrolases eg ß-glucuronidase and acid phosphatase by mononuclear

phagocytes occurs in response to numerous exogenous stimuli (Page et al,1978). Resident peritoneal macrophages secrete lysosomal hydrolases at low levels, whereas macrophages obtained from inflammatory exudates or stimulated by lymphokines contain and secrete increased amounts of lysosomal hydrolases (Tanner et al,1984; Soberman and Karnovsky,1981; Schnyder and Baggiolini,1980).

The third category of digestive enzymes secreted by macrophages are the neutral proteases, including collagenase, elastase and plasminogen activator. Elicited or activated macrophages, on triggering, synthesise greatly increased amounts of neutral proteases compared with resident macrophages (Gordon, 1980; Schnyder and Baggiolini,1980).

1.3.6.2. Interleukin-1

Mononuclear phagocytes are a major source of Interleukin-1 (IL-1) and release it in response to a wide variety of stimuli, including viruses, bacteria and fungi. It participates in immune responses by inducing cell differentiation, antibody secretion, proliferation and lymphokine production (Kampschmidt, 1984; Staruch and Wood,1983; Smith et al,1980).

1.3.6.3. Arachidonic Acid Metabolism

Macrophages are a major source of these important immunoregulatory molecules, in particular prostaglandins (PG) and their release from macrophages may occur in response to a number of different stimuli eg immune complexes, lymphokines, endotoxin or opsonised bacteria (Pawlowski et al,1983; Gemsa,1981; Goodwin and Webb,1980;

Humes et al,1977). They appear to be able to act as negative feedback signals in those immune responses that require macrophage-lymphocyte interactions (Gemsa,1981; Goodwin and Webb,1980) eg by inhibiting IL-1 production.

1.3.6.4. Interferon

Interferons (IFNs) are a heterogeneous family of proteins. Type I or viral IFN of which mononuclear phagocytes are significant producers (Neumann,1982) is induced by virus infection or bacterial stimulation. Type II, (immune IFN or gamma IFN) is induced by specific antigens, mitogens or other stimuli and is the predominant form produced by stimulated lymphocytes (reviewed by Trinchieri and Perussia,1985).

IFN is known to enhance the capacity of macrophages to secrete H_2O_2 and to kill intracellular parasites as well as increasing HLA-DR antigen expression (Gershon et al,1985; Wilson and Westall,1985; Sztein et al,1984; Nathan et al,1983). It also increases the number of Fc receptors (Ezekowitz and Gordon,1986; Rhodes et al,1983, Vogel et al,1983) which would probably enhance phagocytosis as a result. It is therefore a major macrophage activating factor, which is able to regulate the immune response through its direct effect on mononuclear cells (reviewed by Virelizier and Arenzana-Seisdedos,1985).

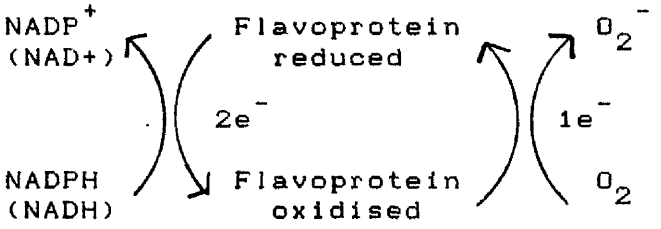
1.3.6.5. Other Secretory Products

Macrophages secrete multiple components of the complement system (Bentley et al,1981), including C1q, C2, C4, C3, and C5 which means that they generate all the components necessary for local opsonisation of particles.

1.3.6.6. Reactive Oxygen Intermediates

In response to stimulation macrophages are capable of releasing reactive oxygen intermediates such as hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and superoxide anion (O_2^-) (Rossi et al,1986; Babior,1984).

Activation of membrane-bound NADPH (or NADH) initiates this reaction in which oxygen is reduced to superoxide (O_2^-):



The latter converts to H_2O_2 either spontaneously or by the superoxide dismutase-catalysed reaction

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

H_2O_2 may further react to produce the hydroxyl radical ($OH^{\cdot}$) which is thought to have an important role in cell damage and killing (Babior,1984).

The production of microbicidal O_2 metabolites is enhanced markedly in macrophages that have been activated by the presence of infection in the host (Takemura and Werb, 1984; Johnston,1981), and sometimes in those elicited by injection of inflammatory agents (Johnston,1981). This capacity to respond to stimulation with increased release of reactive O_2 metabolites appears to play an essential role in the increased microbicidal capability of activated macrophages (Nathan et al,1983,1979; Johnston,1981).

It is now known that these in vivo adaptive changes are mediated by lymphokines, in particular gamma IFN

(Wilson and Westall,1985; Nathan et al,1983) which is released by specifically sensitised T-lymphocytes (reviewed by De Maeyer-Guignard and De Maeyer,1985; Schreiber et al,1983). In vitro induction of microbicidal activity does not require contact with sensitised lymphocytes and is non-specific. Once established, the activated macrophages can kill not only the bacterial species that led to activation, but also unrelated ones.

The mechanism of H_2O_2 killing is not yet clearly defined, but it is thought to involve the cells ability to oxidise thiol groups in enzymes and to cause bond breakage in proteins and DNA, leading to lethal alterations in cell metabolism (Crapo 1977; Michelson, 1977).

1.4. SERUM PROTEINS AND THEIR ROLE IN ANTIMICROBIAL ACTIVITY

1.4.1. Immunoglobulin and Complement

Phagocytic cells recognise micro-organisms by complex processes, the most important of which is opsonisation i.e. the coating of micro-organisms with molecules which facilitate phagocytosis, of which Ig and complement are the most important.

Immunoglobulins, or antibodies are induced when the host's lymphoid system comes into contact with immunogenic foreign molecules (antigens). Five distinct classes of Ig molecules are produced in man, namely IgG, IgM, IgA, IgD, and IgE. These differ from each other in size, charge, amino acid composition and carbohydrate content. There is also considerable heterogeneity within classes. IgG (MW 150000) is the major Ig in normal human serum accounting for over 70% of the total Ig and is the main antibody discussed in this thesis.

Complement is a system of interacting proteins, the components of which interact in an orderly sequential fashion giving rise to a cascade analogous to that of the clotting system, in that activation of each component (except the first) depends upon activation of the prior component or components in the sequence (reviewed by Joiner *et al*, 1984).

Opsonisation of bacteria may occur by three different mechanisms. Firstly specific antibody, namely IgG alone

may act as an opsonin, or specific IgG or IgM may induce complement activation via the classical pathway of C1, C4, and C2. Finally the alternative pathway of C3 activation provides a nonspecific mechanism of opsonisation (Keane and Peterson,1984; Winkelstein,1973).

Most specific antibodies to bacteria are directed against surfaces or cell-wall components such as the peptidoglycan (Peterson et al,1978). The antibody combines with the surface antigen of the bacterium through the antibody combining sites located in the $F(ab')_2$ portion of the molecule. The Fc portion of the molecule, which is critical to its function as an opsonin is then free to attach to specific receptor sites on the surface of the phagocyte (Newman et al,1985) and complete the bridge between bacterium and phagocyte. In the case of complement this bridge is formed by C3 components and its receptors. Fibronectin can also be involved in phagocytosis by enhancing ingestion of already opsonised particles bound to the Fc or complement receptors (Wright and Meyer,1985; Pommier et al,1983).

Two major pathways of complement activation are recognised. The classical pathway is generally activated by the interaction of antibody of the appropriate class and subclass. In man IgG 1,2 and 3 and IgM are capable of activating the classical pathway (reviewed by Joiner et al, 1984). A single IgM molecule can fix complement, whereas two closely spaced IgG molecules are required. IgA, D and E have no complement binding capacity (Ishizaka et al,1966).

A second major pathway of complement activation has

been termed the alternative pathway. This is activated by binding of C3 or C3b, to certain types of surface. This pathway can be activated by a number of bacterial organisms and particles in the absence of specific Ig (reviewed by Joiner et al,1984) and can provide a non-specific defence system for the host. All gram positive cell walls appear to activate the alternative pathway efficiently in non-immune serum, leading to the deposition of C3b on the bacterial cell wall (Verbrugh et al,1980).

1.4.2. Transferrin

Transferrin (Tf) is a serum glycoprotein of 80000 molecular weight, the main function of which is iron transport (Morgan,1981). Most of the iron is delivered to erythroid precursors for the synthesis of haem. Small but significant quantities of iron are also delivered to non-erythroid cells, especially those which are rapidly dividing (Brock and Mainou-Fowler,1983).

However another important property of Tf is its antimicrobial activity. All micro-organisms, with the possible exception of lactobacilli (Archibald,1983), require iron for growth. Hence Tf, by virtue of its high affinity for iron, can retard microbial growth by restricting the availability of this element. Tf may therefore have an important role in defence against infection (Brock,1986).

Schade and Caroline (1944;1946) first discovered that specific iron binding proteins, present in human blood and in egg whites would inhibit the growth of certain bacteria. They concluded that the proteins, now known as Tf in blood

(Holmberg and Laurell,1945; Schade and Caroline,1946) bound iron so tightly that bacteria were unable to obtain a sufficient amount of the metal for growth. Addition of iron was found to overcome this bacteriostatic effect of Tf. The enhanced susceptibility of animals injected with iron to infection is now well recognised and can be seen with a number of different organisms eg Escherichia coli (Bullen et al,1968), Clostridium welchii (Bullen et al,1967), S.aureus (Gladstone and Walton,1971) and Klebsiella (Miles et al,1979). In situations where iron is not readily available, bacteria normally acquire this metal by synthesising high affinity, low molecular weight iron chelators (siderophores; Finkelstein et al,1983; Neilands,1981). Schade (1963) also showed in vitro that many strains of S.epidermidis failed to grow in serum without additional iron and their growth was inhibited by Tf. Miles et al (1979), however, suggested that only a minority among the environmental bacteria can take advantage of the decreased resistance associated with such increased iron levels but this minority is likely to include the more virulent strains. Iron compounds were also found to abolish the antibacterial effects of body fluids in vitro (Weinberg,1984; Griffiths,1983; Bullen,1981).

Thus Tf, like IgG and C3 is an important factor to examine in the PDE of patients on CAPD, as any deficiencies may contribute significantly to defects in the defences of the peritoneum.

1.5 AIMS OF THESIS

The work presented in this thesis aims to examine the peritoneal immune defences of patients on CAPD. In particular the activity of the cells with regard to their ability to deal adequately with possible pathogens is investigated and their activation or maturation state is studied by examining their ability to release H_2O_2 and express HLA-DR. In addition this work examines the levels and activities of serum proteins in PDE, in particular IgG and C3, and relates these to the opsonic capacity of PDE, as inadequate opsonic activity may impair the efficiency of the phagocytic cells. Determination of the Tf levels and measurement of *in vitro* growth of bacteria in the PDE is also investigated, as defective Tf-mediated bacteriostatic activity of PDE would increase the chances of invading pathogens establishing themselves prior to mobilisation of phagocytic defences, and thus giving rise to peritonitis. This work also examines the relationship of these various cellular and humoral activities with the incidence of peritonitis, both in individual patients and by a 9 month longitudinal study of selected patients.

CHAPTER 2

CHARACTERISATION OF HUMAN PERITONEAL CELLS

2.1 INTRODUCTION

Macrophages are the first line of cellular defence against microbial invasion of the peritoneum in the CAPD patients. However, the removal of large numbers of cells each day from the peritoneum by the dialysis routine results in a rapid turnover of cells. Replenishment by cells other than by the normal resident peritoneal population may immuno-compromise the immune system of the peritoneum and leave it prone to infection. Indeed, Goldstein et al (1984) suggested that the peritoneal macrophages from CAPD patients are relatively immature bone-marrow derived cells which might therefore have reduced activity.

Much of the work described in this thesis seeks to identify any functional defects in these recently arrived cells which might impair the local antimicrobial activity in some patients, leaving them prone to peritonitis. It also seeks to examine the effect the dialysis procedure has on the overall activity of cells in the PDE and compare these with the resident peritoneal mononuclear phagocyte population normally found in the peritoneum.

Before such studies could be undertaken it was necessary to establish the quantitative and qualitative characteristics of cells in the peritoneal dialysate of uninfected CAPD patients.

In this chapter the peritoneal cell populations of CAPD patients have therefore been characterised and compared with normal human peritoneal cells.

2.2 MATERIALS

2.2.1. Culture Medium

This consisted of RPMI 1640 culture medium with 25mM Hepes (Flow, Irvine, U.K.) containing 5% heat inactivated (56^{o}C, 30 min) foetal calf serum (FCS : Flow).

2.2.2. Reagents for α-Napthyl Acetate Esterase Stain

(i) Fixative Formol calcium solution consisting of 10% w/v formaldehyde and 1% w/v $CaCl_2$ was used.

(ii) Phosphate Buffer(0.06M, pH 5) This consisted of a ratio of 98.5: 5 of KH_2PO_4 (9.08 g/l) : $Na_2HPO_4.2H_2O$ (11.88 g/l) respectively. The buffer was sterilised by autoclaving.

(iii)Hexazotised Paraosaniline This contained equal volumes of two solutions which were prepared as follows :

Solution A : 5 ml of concentrated HCl was added to 20 ml of distilled water to which had been previously added 1g of parasoaniline (Sigma: Poole, Dorset, U.K.). This solution was gently warmed to dissolve the parasoaniline, allowed to stand at room temperature to cool and finally filtered. The filtrate was then stored in the dark at 4^{o}C.

Solution B : This was a freshly prepared solution of 4% $NaNO_2$ in distilled water.

(iv)α-Napthyl Acetate Esterase (ANAE) Stain A 2.4 ml volume of hexazotised parasoaniline was added to 40 ml of

the phosphate buffer. Next a freshly prepared solution of 10 mg of α-napthyl acetate (Sigma) in 0.4 ml acetone was added and the pH raised to 5.8 with 2N NaOH. The stain was filtered before use.

2.3 PATIENTS AND CONTROLS

2.3.1. Patient Samples

Samples were obtained from a total of 35 patients (19 females, 16 males) on CAPD (Table 1). Their ages ranged from 23 to 76 years and they had been on CAPD for times ranging from 1 day to 4 years. Fifteen of the patients were on the Fresenius safe-lock system and the remainder on the Travenol System II. A total of 76 samples were obtained (Table 2). Peritonitis was diagnosed by the medical staff of the Western Infirmary by the criteria of abdominal pain, cloudy PDE, positive growth culture of micro-organisms and a dialysate white cell count of greater than $100/mm^3$ in which neutrophils predominated. Although these criteria are suitable for the actual diagnosis of peritonitis, no indication is given as to the types and numbers of cells found before and after its clinical diagnosis which it is essential to know in order to ensure that infected samples are not used as examples of cells found in the normal uninfected PDE. Other criteria for excluding infected samples may therefore have to be used to overcome this problem, and this is discussed further in section 2.5.1.

2.3.2. Normal Controls

Normal peritoneal cells were obtained from 8 healthy women under going laparoscopy for sterilisation.

TABLE 1: Details of Patients from whom Peritoneal Cells were isolated for Characterisation.

PATIENT INITIALS	SEX	AGE (YRS)	MONTHS ON CAPD	SYSTEM	DIAGNOSIS
A.Ba	female	58	40	F*	Polycystic Kidneys
A.Bl	male	70	9	T+	Glomerulonephritis
A.Ca	female	75	7	F	Glomerulonephritis
W.Ch	male	48	9	T	Diabetic Nephropathy
R.Co	female	39	39	F	Glomerulonephritis
W.Co	male	33	0	T	Unknown
I.Cu	female	68	7	T	Pyelonephritis
C.Fe	male	53	1	T	Diabetic Nephropathy
A.Fu	female	42	1	F	Unknown
M.Ge	female	60	41	F	Hypertension
B.Go	male	23	16	T	Diabetic Nephropathy
N.Ja	male	37	19	F	Diabetic Nephropathy
I.Jo	male	37	6	T	Glomerulonephritis
W.Ka	male	46	0	T	Glomerulonephritis
R.Le	male	55	2	T	Diabetic Nephropathy
M.McA	female	51	46	F	Hypertension
M.McC	female	55	19	F	Analgesic Nephropathy
A.McD	male	64	20	F	Glomerulonephritis
J.McF	male	48	45	T	Glomerulonephritis
C.McG	female	62	6	T	Analgesic Nephropathy
L.McL	female	55	7	F	Pyelonephritis
M.McV	female	54	7	F	Polycystic Kidneys
P.McV	male	61	0	T	Pyelonephritis
A.Me	female	36	18	T	Glomerulonephritis
T.Mo	male	45	1	T	Glomerulonephritis

TABLE 1: cont.

PATIENT INITIALS	SEX	AGE (YRS)	MONTHS ON CAPD	SYSTEM	DIAGNOSIS
A.My	female	42	27	T	Polycystic Kidneys
S.Pa	female	52	5	F	Polycystic Kidneys
C.Pa	female	68	46	F	Analgesic Nephropathy
M.Re	female	63	4	F	Diabetic Nephropathy
R.Ro	male	39	1	T	Unknown
I.Sc	female	49	36	T	Analgesic Nephropathy
M.Sm	female	60	1	T	Polycystic Kidneys
W.St	male	76	6	T	Unknown
A.Wa	female	49	1	F	Polycystic Kidneys
W.Yo	male	44	21	T	Diabetic Nephropathy

* F= Fresenius safe-lock system

+ T= Travenol System II

TABLE 2: Differential White Cell Counts and Peritonitis Rates in the PDE of Dialysis Patients.

PATIENT INITIALS	MONTHS ON CAPD	NUMBER OF CELLS IN PDE $\times 10^{-5}$	PERCENTAGE OF MACRO-PHAGES	LYMPHO-CYTES	NEUTRO-PHILS	EOSINO-PHILS	FREQUENCY# OF PERITONITIS
+A.Ba	40	<5	-	-	-	-	0.3
+	41	<5	-	-	-	-	0.3
+	42	<5	-	-	-	-	0.3
A.Bl	9	<5	-	-	-	-	2.7
	10	<5	-	-	-	-	2.4
A.Ca	7	45	78.0	20.0	2.0	0.0	6.9
	9	24	71.3	21.8	4.6	2.3	5.3
	13	56	71.9	15.5	9.1	3.5	5.5
+W.Ch	9	320	60.8	15.5	8.1	15.6	1.3
	11	203	62.0	15.2	9.8	13.0	2.2
R.Co	39	<5	-	-	-	-	0.0
W.Co	0	292	64.3	18.6	7.8	9.3	0.0
*	1	12000	36.5	5.5	58.0	0.0	12.0
	2	141	71.0	17.1	2.4	9.5	6.0
	2	96	64.8	26.0	3.8	5.4	6.0
I.Cu	7	16	76.0	22.2	1.8	0.0	1.7
C.Fe	1	140	76.9	22.3	0.8	0.0	12.0
A.Fu	1	52	88.4	11.6	0.0	0.0	0.0
+M.Ge	41	<5	-	-	-	-	5.3
B.Go	16	35	61.3	24.2	14.5	0.0	0.0
N.Ja	19	<5	-	-	-	-	0.0
	22	<5	-	-	-	-	0.5
I.Jo	6	60	66.7	23.6	4.7	5.0	6.0
W.Ka	0	70	70.3	25.8	3.0	0.9	0.0
R.Le	2	20	69.8	23.9	3.8	2.5	12.0
	3	25	85.0	14.0	1.0	0.0	8.0
+M.McA	46	960	13.0	6.0	79.4	1.6	2.1
+	47	480	21.7	19.6	28.3	30.4	2.0
+	49	203	15.9	7.3	75.4	1.4	2.0

TABLE 2 cont.

PATIENT INITIALS	MONTHS ON CAPD	NUMBER OF CELLS IN PDE $\times 10^{-5}$	PERCENTAGE OF MACRO-PHAGES	LYMPHO-CYTES	NEUTRO-PHILS	EOSINO-PHILS	FREQUENCY# OF PERITONITIS
M.McC	23	<5	-	-	-	-	0.5
+	19	168	37.0	17.8	8.2	37.0	0.6
+A.McD	20	<5	-	-	-	-	2.4
+	23	<5	-	-	-	-	2.1
J.McF	45	<5	-	-	-	-	2.9
*	46	960	44.2	3.4	52.4	0.0	2.9
	49	<5	-	-	-	-	2.7
C.McG	6	26	75.6	18.4	3.5	2.5	0.0
	9	172	60.6	13.5	14.8	11.1	2.7
+	11	384	45.6	22.6	16.1	15.7	3.3
	12	<5	-	-	-	-	3.0
L.McL	7	15	77.7	20.6	1.7	0.0	5.1
M.McV	7	112	61.4	21.0	6.2	11.4	5.1
+	11	104	39.3	29.7	15.3	15.7	4.4
P.McV	0	72	63.6	20.4	14.2	1.8	0.0
	1	114	52.2	13.9	32.2	1.7	0.0
	2	43	50.3	42.1	5.9	1.7	0.0
*A.Me	18	52500	27.9	7.0	58.1	7.0	4.0
	19	136	61.1	30.0	7.3	1.6	3.8
	20	12	65.4	24.6	10.0	0.0	3.6
T.Mo	1	24	75.0	23.0	2.0	0.0	0.0
	3	310	49.5	15.8	25.4	9.3	0.0
A.My	27	<5	-	-	-	-	0.4
	30	<5	-	-	-	-	0.4
	33	<5	-	-	-	-	0.4
S.Pa	5	<5	-	-	-	-	9.6
	6	32	77.6	18.3	4.1	0.0	8.0
+C.Pa	46	<5	-	-	-	-	0.5
+	47	<5	-	-	-	-	0.8
+	48	<5	-	-	-	-	0.8
+	49	<5	-	-	-	-	0.7

TABLE 2: cont.

PATIENT INITIALS	MONTHS ON CAPD	NUMBER OF CELLS IN PDE $X10^{-5}$	PERCENTAGE OF MACRO-PHAGES	LYMPHO-CYTES	NEUTRO-PHILS	EOSINO-PHILS	FREQUENCY# OF PERITONITIS
*M.Re	4	19000	20.0	5.0	75.0	0.0	6.0
^	5	460	58.4	16.7	23.8	1.1	4.8
	6	76	64.8	28.5	4.4	2.3	4.0
	12	120	71.7	11.9	15.0	1.4	4.0
	13	26	75.0	14.0	11.0	0.0	3.7
R.Ro	1	25	86.0	12.0	2.0	0.0	0.0
I.Sc	36	21	74.8	19.1	6.1	0.0	1.0
	37	<5	-	-	-	-	1.0
^M.Sm	1	48	32.3	8.7	54.7	4.3	12.0
o	2	132	34.3	16.9	48.8	0.0	6.0
*	3	58000	10.7	1.4	87.9	0.0	8.0
W.St	6	64	66.0	30.0	4.0	0.0	6.0
	6	77	64.0	21.0	15.0	0.0	6.0
A.Wa	1	84	68.3	18.0	4.3	9.4	0.0
	6	<5	-	-	-	-	0.0
W.Yo	21	<5	-	-	-	-	2.9
Mean	17	195	58.3	18.0	19.0	4.7	3.1
Minimum	0	<5	10.7	1.4	0.0	0.0	0.0
Maximum	49	58000	88.4	42.1	87.9	37.0	12.0

* Samples from patients with active peritonitis

^ Samples from patients recovering from peritonitis

o Samples from patients just before clinical diagnosis of peritonitis

+ Samples from patients with inflammatory or allergic reactions

- No differential cell count

The frequency of peritonitis was calculated by multiplying the total number of episodes experienced by each patient by 12 and dividing by the number of months on CAPD. Diagnosis of peritonitis was determined as described in section 2.3.1.

2.4. METHODS

2.4.1. Isolation of Peritoneal Cells

The fluid from the overnight CAPD exchange was spun in a M.S.E. Mistral centrifuge in 1-litre bottles at 500g for 15 minutes. The supernatant was removed and the cells resuspended in culture medium (2.2.1), washed by centrifugation at 250g and pooled. The cells were counted and viability determined by eosin exclusion. Peritoneal cells obtained by aspiration of fluid from the peritoneum of normal women (samples were normally 2-5ml) were centrifuged at 250g and the cells washed as above. If the pellet showed heavy contamination with erythrocytes these were lysed by suspending the cells in 1ml of distilled water for 1 minute followed by readjustment to isotonicity with a 10-fold excess of culture medium (2.2.1).

2.4.2. Leishman Staining

Peritoneal cells were collected and counted as described above. Approximately $5x10^4$ cells were deposited on clean glass slides in a Shandon cytocentrifuge at 800 r.p.m. for 90 seconds and then air dried. The cells were then fixed and stained by first adding undiluted Leishman Stain (Exogen Ltd., Clydebank, Glasgow, U.K.) for 2 minutes and then diluting the stain with 2 volumes of distilled water and leaving for 10 minutes. Preparations were then washed in tap water, air-dried, and mounted in DPX.

Different fields of view were examined in a Leitz-

Wetzlar light microscope (x200) and the percentage of each cell type calculated.

2.4.3. α-Napthyl Acetate Esterase (ANAE) Staining

Cytospin preparations of peritoneal cells were prepared as above. They were then fixed in formol calcium (2.2.2.i) at 4°C for 10 minutes and washed in tap water for 20 minutes at room temperature. ANAE activity was demonstrated by incubating with the ANAE staining reagent (2.2.2.iv) for 2 hours at 37°C. Cell preparations were then washed gently in running tap water for 10 minutes, left to dry at room temperature and then counterstained with 2% methyl green for 30 seconds. Finally, cells were washed in tap water, air-dried, and mounted in DPX. Slides were examined using the light microscope (x400) and the different cell types scored.

2.4.4. Statistical Methods

Spearman rank correlation was the only test used.

2.5. RESULTS

2.5.1. Differential White Cell Counts of Peritoneal Cells Obtained from CAPD Patients

Macrophages, lymphocytes, neutrophils, and eosinophils were classified using Leishman-stained preparations according to the normal criteria. Macrophages were identified by their size and typical bean-shaped nucleus, lymphocytes by their large round nucleus and scanty cytoplasm, and polymorphonuclear leucocytes by their multilobed nucleus. Neutrophils and eosinophils could easily be distinguished by the absence or presence, respectively, of staining of the cytoplasmic granules. Typical morphology of each cell type is shown in Figure 1.

Differential white cell counts of CAPD cell preparations were performed whenever possible on the 76 samples obtained from patients. Overall the mean percentage of each cell type was 58.3% macrophages, 18.0% lymphocytes, 19.0% neutrophils, and 4.7% eosinophils (Table 2). However, this was not a true representation of the cells found in the PDE of "steady-state" CAPD patients, as it includes several patients with on-going peritonitis, as well as a few who were recovering from peritonitis or just before its clinical diagnosis. In addition several PDE samples were obtained from patients with allergic/inflammatory reactions (Table 2). Since the purpose of this study was to examine the normal population of cells found in patients, it was important to establish criteria for classifying

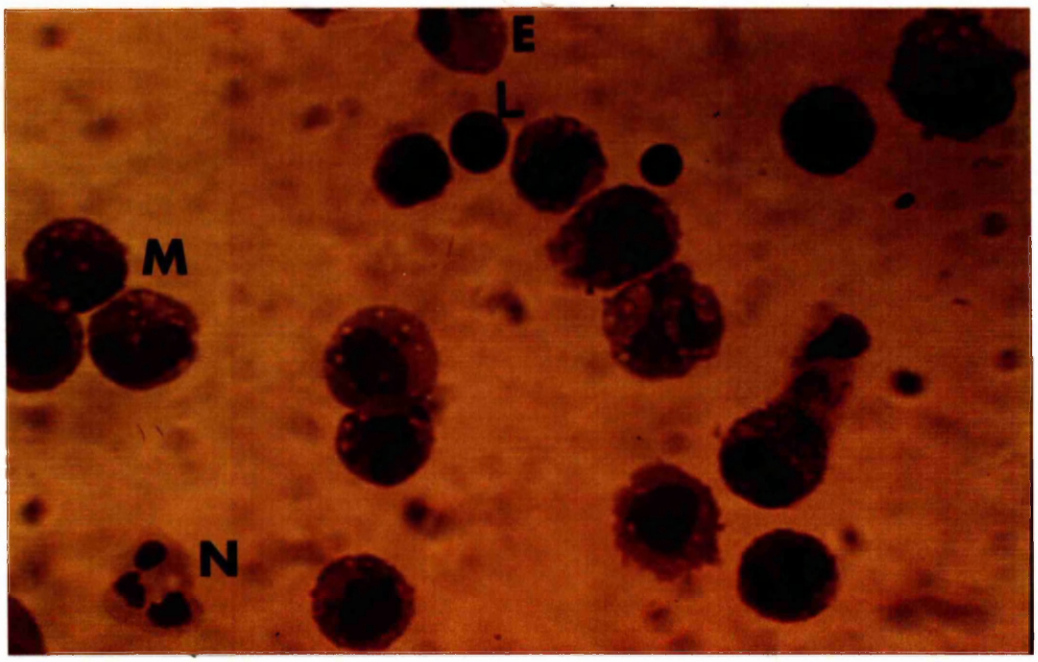


FIGURE 1: Peritoneal Cells from a CAPD Patient Stained with Leishman (x1600)

E : Eosinophil

N : Neutrophil

L : Lymphocyte

M : Macrophage

"steady-state" patients and avoid including cell populations which may have been altered by factors outside the normal dialysis routine.

When a bar chart was constructed showing the proportion of neutrophils in the PDE of dialysis patients (Figure 2), it was found that all except 2 of the samples with 15% or less neutrophils were from patients who were free from either peritonitis complications or hypersensitivity reactions to the dialysis routine or the dialysate itself. The 2 samples mentioned above were from a patient with sclerosing peritonitis and another who had had a recent catheter implanted. The proportions of eosinophils in these were 37.0% and 15.6% respectively. In view of this only samples containing <15% of both neutrophils and eosinophils were considered appropriate for further investigations of cellular activity of cells from normal CAPD patients.

When samples containing 15% or more of neutrophils or eosinophils were eliminated from those on which differential white cell counts were performed, 66% of samples remained. In these, the mean percentage of each cell type was 69.9% macrophages (range 50.3-88.4), 20.7% lymphocytes (range 11.5-40.1), 6.1% neutrophils (range 0-15.0) and 2.9% eosinophils (range 0-13.0).

Since the presence of non-specific esterase is highly characteristic of macrophages, seven samples were also stained with ANAE as well as with Leishman in order to establish that the Leishman was adequate for routine identification of cells (Table 3). Macrophages showed diffuse brown staining in their cytoplasm, while presumed

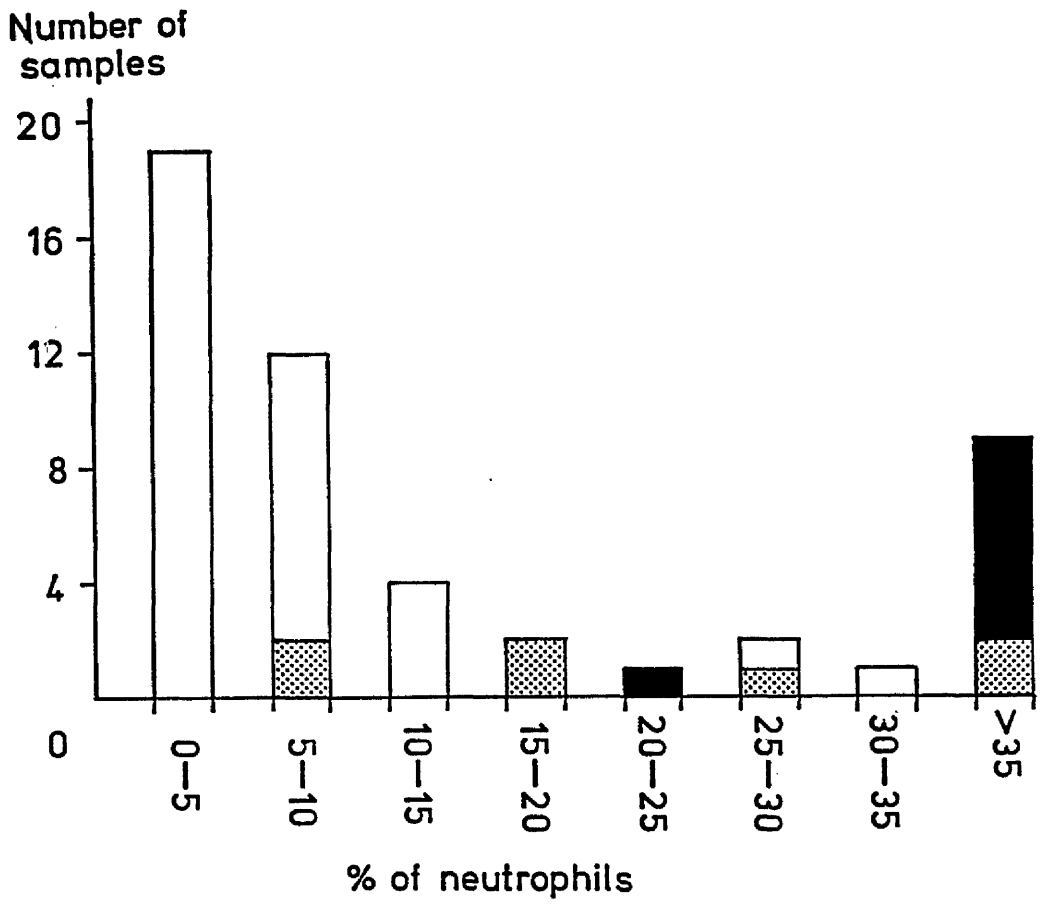


FIGURE 2: Proportion of Neutrophils in PDE.

Samples from patients with no diagnosed complications.

Samples from patients with non-infectious inflammatory or allergic reactions.

Samples from patients with peritonitis.

TABLE 3: Comparison of Leishman and α-Napthyl Acetate Esterase (ANAE) Staining of Peritoneal Cells from CAPD Patients.

	LEISHMAN STAIN PERCENTAGE OF*:			NON-SPECIFIC ESTERASE STAIN PERCENTAGE OF :		
PATIENT	MACRO-PHAGES	LYMPHO-CYTES	NEUTRO-PHILS	MACRO-PHAGES	T-CELLS	NEGATIVE STAINING
A.Ca	78.0	20.0	2.0	80.7	5.0	14.3
W.Co	36.5	5.5	58.0	32.7	4.1	63.2
A.Fu	88.4	11.6	0.0	89.1	6.4	4.5
R.Le	85.0	14.0	1.0	86.0	8.0	6.0
S.Pa	77.6	18.3	4.1	81.4	9.7	8.9
I.Sc	74.8	19.1	6.1	73.5	7.4	19.1
A.Me	65.6	24.4	10.0	70.0	9.9	20.1

* None of these samples contained any eosinophils.

T-lymphocytes contained small brown spots. The remaining lymphocytes (presumed B-cells) and all other cell types remained unstained (Figure 3a). Results showed that staining for non-specific esterase verified the Leishman stain (Table 3). For example, in Figure 3a (cells from patient W.Co) the percentage of ANAE-positive cells (excluding T-cells) was low (32.7%) as the sample contained large numbers of neutrophils and this compares well with the corresponding figure obtained with Leishman stain (Table 3, and Figure 3b; 36.5%). In contrast, the percentage of ANAE-positive cells (excluding T-cells) in Figure 4a (patient I.Sc) was 73.5%, which agrees well with 74.8% assessed by Leishman stain (Table 3, and Figure 4b).

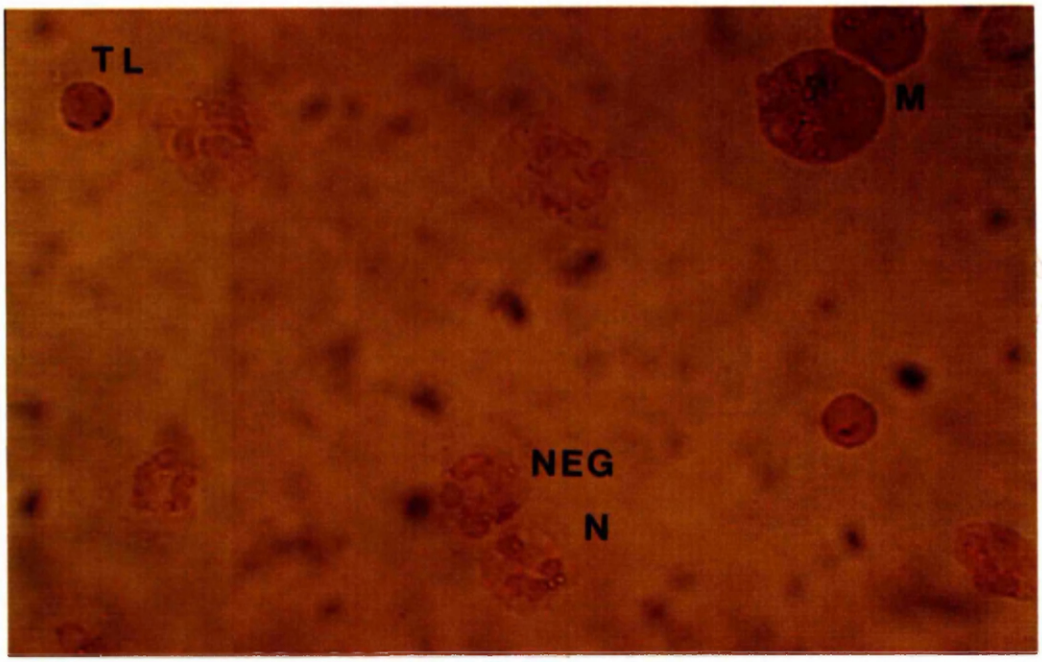
2.5.2. Eosinophilia

An interesting observation in several patients' samples was the presence of eosinophilia. Five patients, all with between 10^7-10^8 cells in their peritoneal effluent, had an increased average number of eosinophils (mean 22.7%) compared with the average of 4.7% found in all the samples (Table 2). An example of cells from an extreme case of eosinophilia is shown in Figure 5.

2.5.3. Differential White Cell Counts of Peritoneal Cells Obtained from Normal Individuals

An average of 91.4% of normal peritoneal cells were identified as macrophages by Leishman stain (Table 4, and Figure 6b). Neutrophils were absent, the remaining cells being lymphocytes. Three of the samples were also stained for non-specific esterase and the percentage positive

(a)



(b)

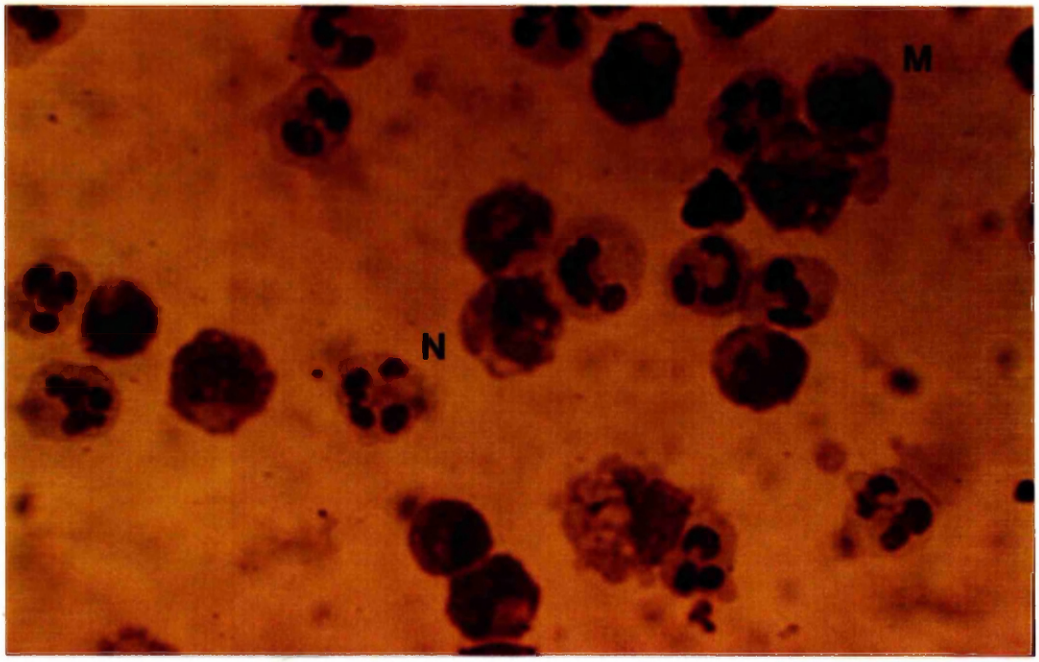


FIGURE 3: Peritoneal Cells from a CAPD Patient with Peritonitis. (x1600)

Stained with:-

(a) α-Naphyl Acetate Esterase (ANAE) Stain

(b) Leishman Stain

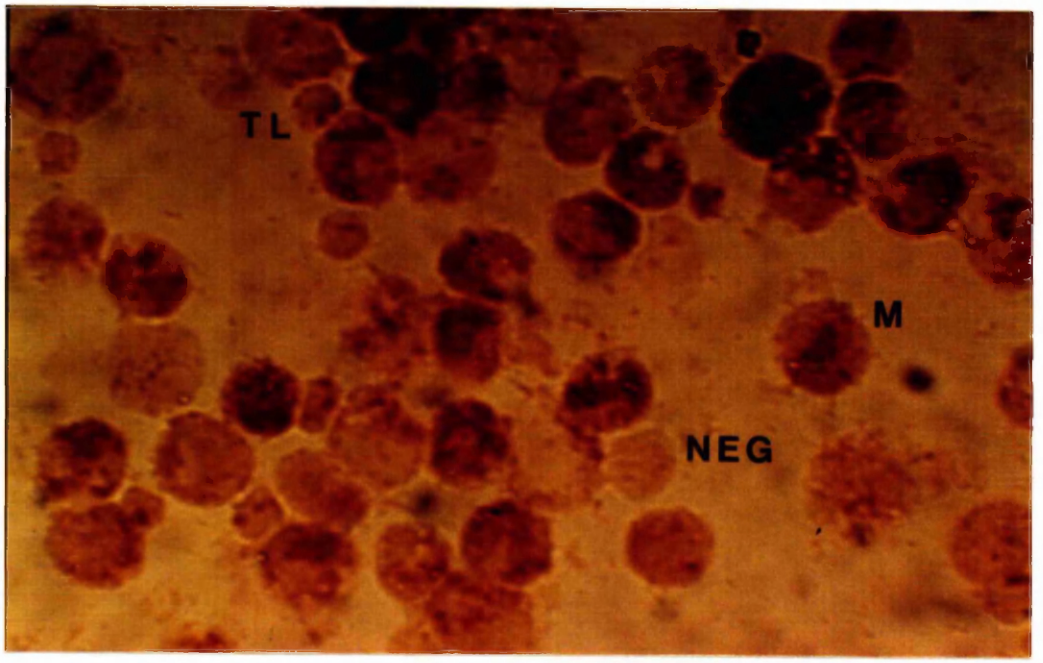
TL : T-lymphocyte

NEG : ANAE negative cell

M : Macrophage

N : Neutrophil

(a)



(b)

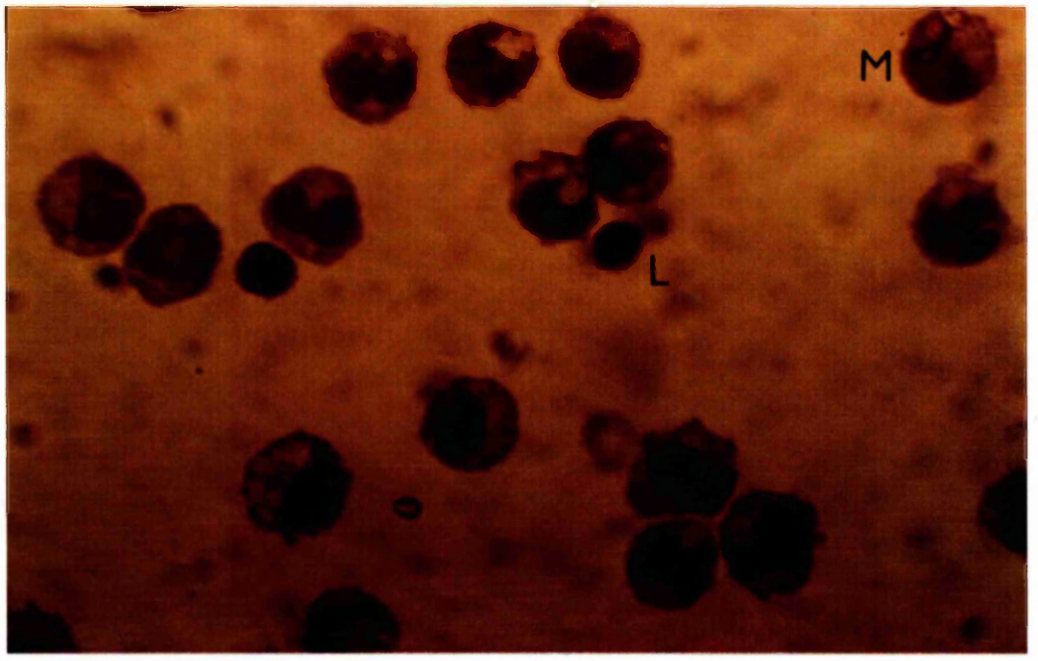


FIGURE 4 : Peritoneal Cells from a CAPD Patient. (x1600).

Stained with :-

(a) α-Naphyl Acetate Esterase (ANAE) Stain

(b) Leishman Stain

TL : T-lymphocyte

NEG : ANAE negative cell

M : Macrophages

L : Lymphocytes

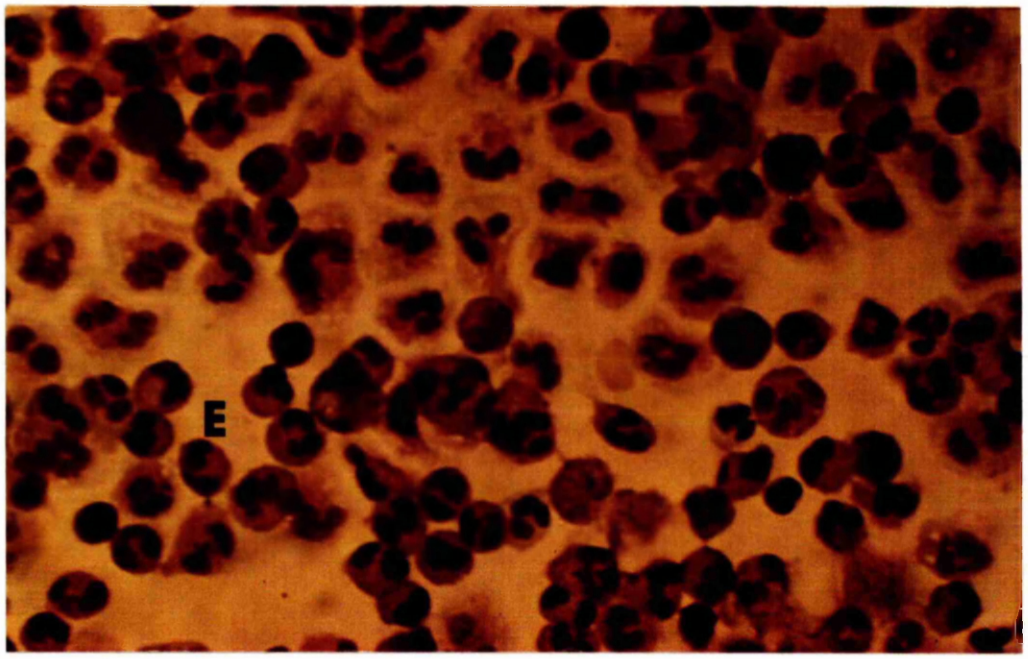


FIGURE 5 : Peritoneal Cells from a CAPD Patient with Eosinophilia. (x1600)

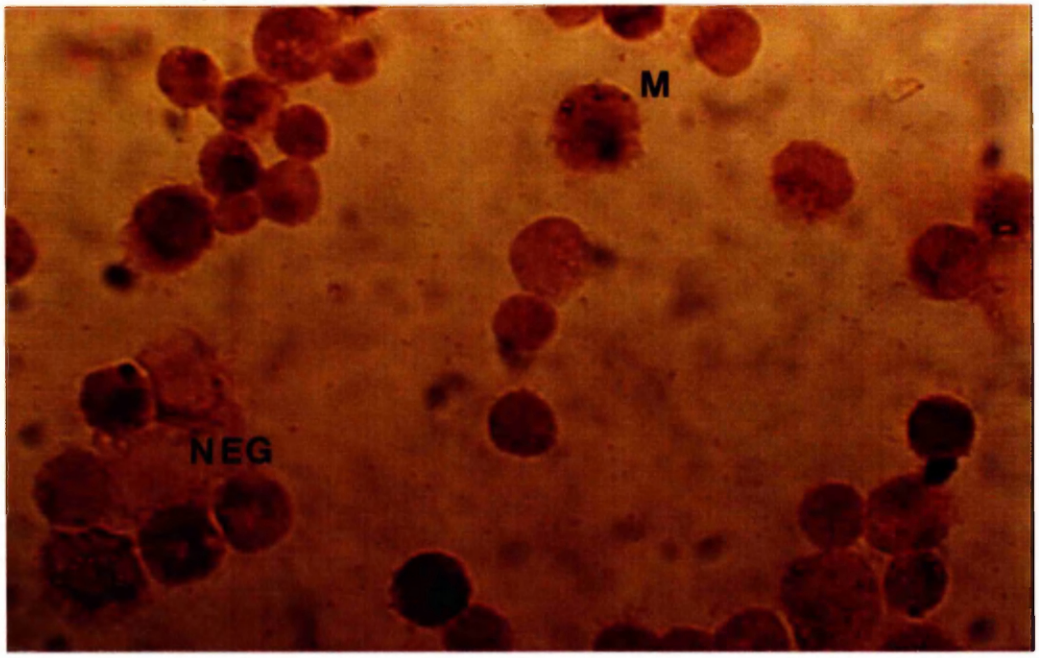
E : Eosinophil

TABLE 4: Comparison of Leishman and α-Napthyl Acetate Esterase (ANAE) Staining of Normal Peritoneal Cells

SAMPLE NUMBER (NORMAL PERITONEAL CELLS)	LEISHMAN STAIN PERCENTAGE OF :		NON-SPECIFIC ESTERASE STAIN PERCENTAGE OF :		
	MACRO-PHAGES	LYMPHO-CYTES	MACRO-PHAGES	T-CELLS	NEGATIVE STAINING
1	86.0	14.0	85.4	5.0	9.6
2	94.1	5.9	93.4	2.3	4.3
3	90.0	10.0	88.4	3.9	7.7
4	89.0	11.0	*	*	*
5	89.0	11.0	*	*	*
6	92.1	7.9	*	*	*
7	96.0	4.0	*	*	*
8	95.1	4.9	*	*	*
mean	91.4	8.6	*	*	*
mimimum	86.0	4.0	*	*	*
maximum	96.0	14.0	*	*	*

* Cell sample not stained for non-specific esterase.

(a)



(b)

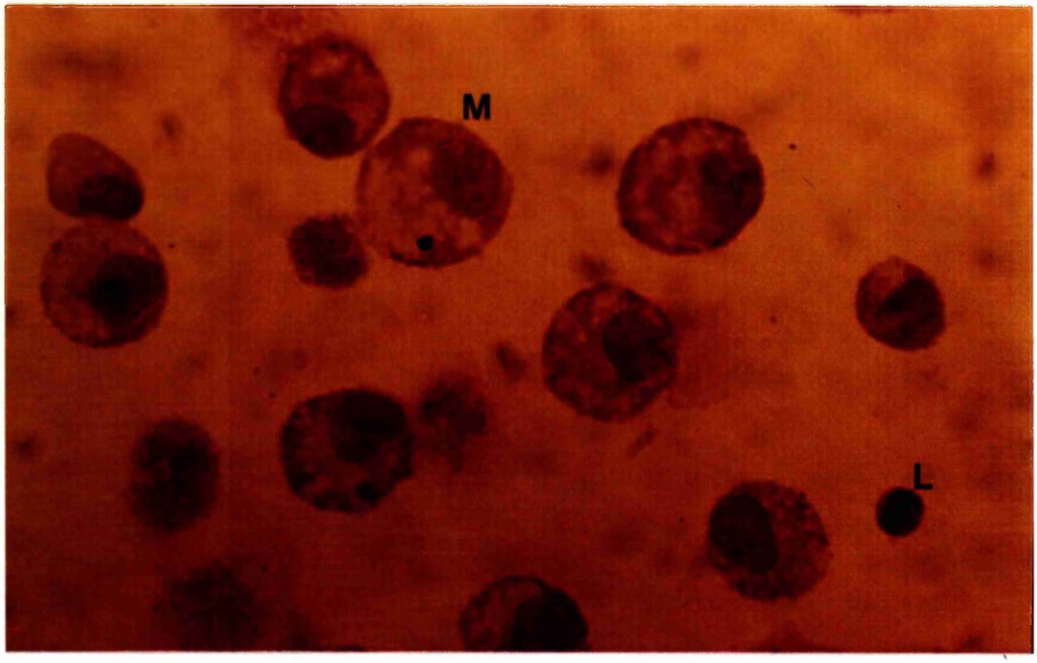


FIGURE 6 : Normal Peritoneal Cells. (x1600)

Stained with :-

(a) α-Naphyl Acetate Esterase (ANAE)

(b) Leishman Stain

NEG : ANAE negative cell

M : Macrophage

L : Lymphocyte

staining was found to be consistent with equivalent Leishman results (Table 4, and Figure 6a).

Overall therefore CAPD cells showed greater heterogeneity, both in terms of the types and proportions of the cells found and in macrophage size, than normal peritoneal cells, which tended to be relatively homogeneous.

2.5.4. Number of Cells Isolated from the PDE of Patients on CAPD

The yield of cells obtained from the PDE varied considerably from sample to sample, ranging from less than $5x10^5$ in many samples, to 10^9 cells in extreme cases. Most of the samples (61.8%) yielded a total of 10^6-10^8 cells (Table 2). Differences in cellularity and cell composition were particularly noticeable in dialysate obtained from the five patients with peritonitis. A rise in cellularity with neutrophilia were typical features (Table 2, and Figure 3b). Of the samples containing more than 10^8 cells, all were from patients with active peritonitis. Although the vast majority of samples which contained between 10^7 and 10^8 cells were from patients who did not have active peritonitis, the average proportion of macrophages in this group was 51.6% which was lower than that of 69.9% from samples with 10^7 or less cells.

In 34.2% of the samples, only low numbers of cells (less than $5x10^5$ cells per bag) were recovered from the overnight PDE, and there was a significant inverse correlation between the number of cells in the fluid and the length of time the patient had been on CAPD ($p<0.001$;

Figure 7). Twelve of the fifteen samples containing less than $5x10^5$ cells came from patients who had been on CAPD for 1 year or more. However, there was no significant correlation between cell number and the number of peritonitis episodes experienced by each patient per year (Figure 8).

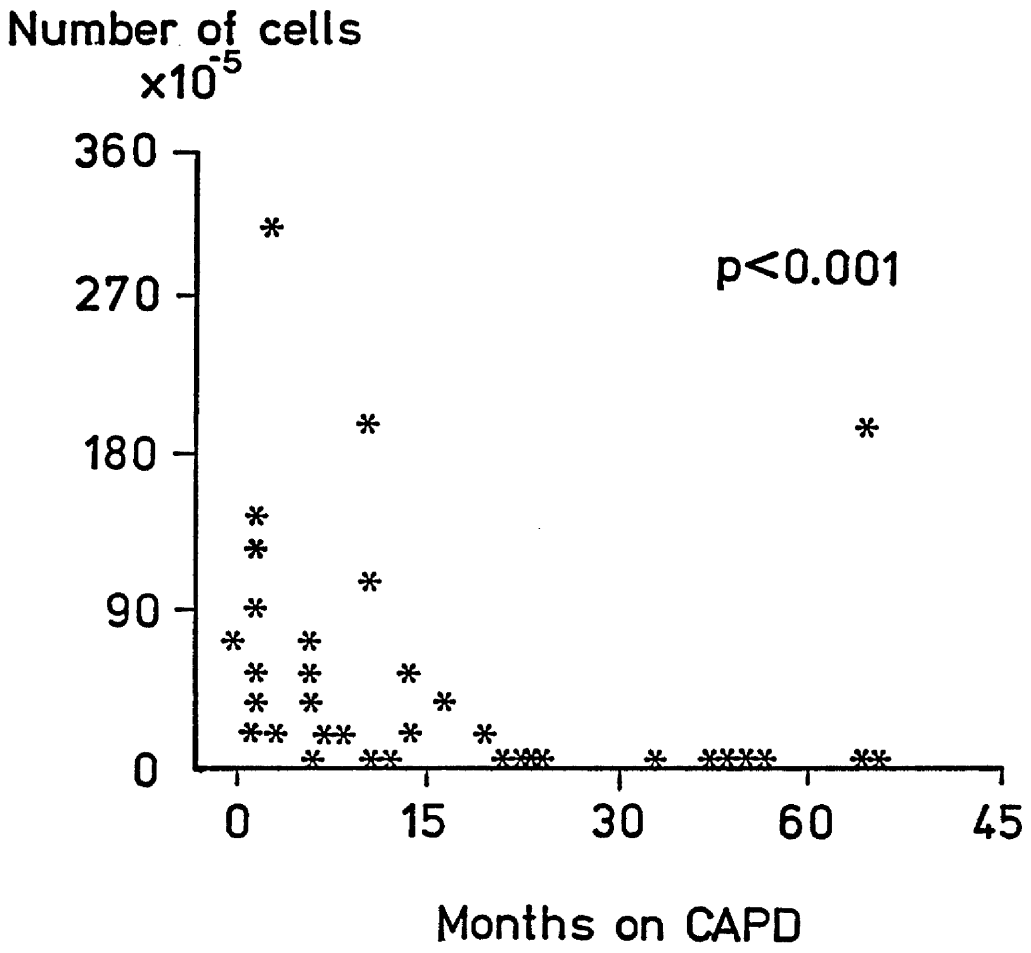


FIGURE 7 : Comparison of Length of Time on CAPD with the Total Number of Cells in the Peritoneal Dialysis Effluent

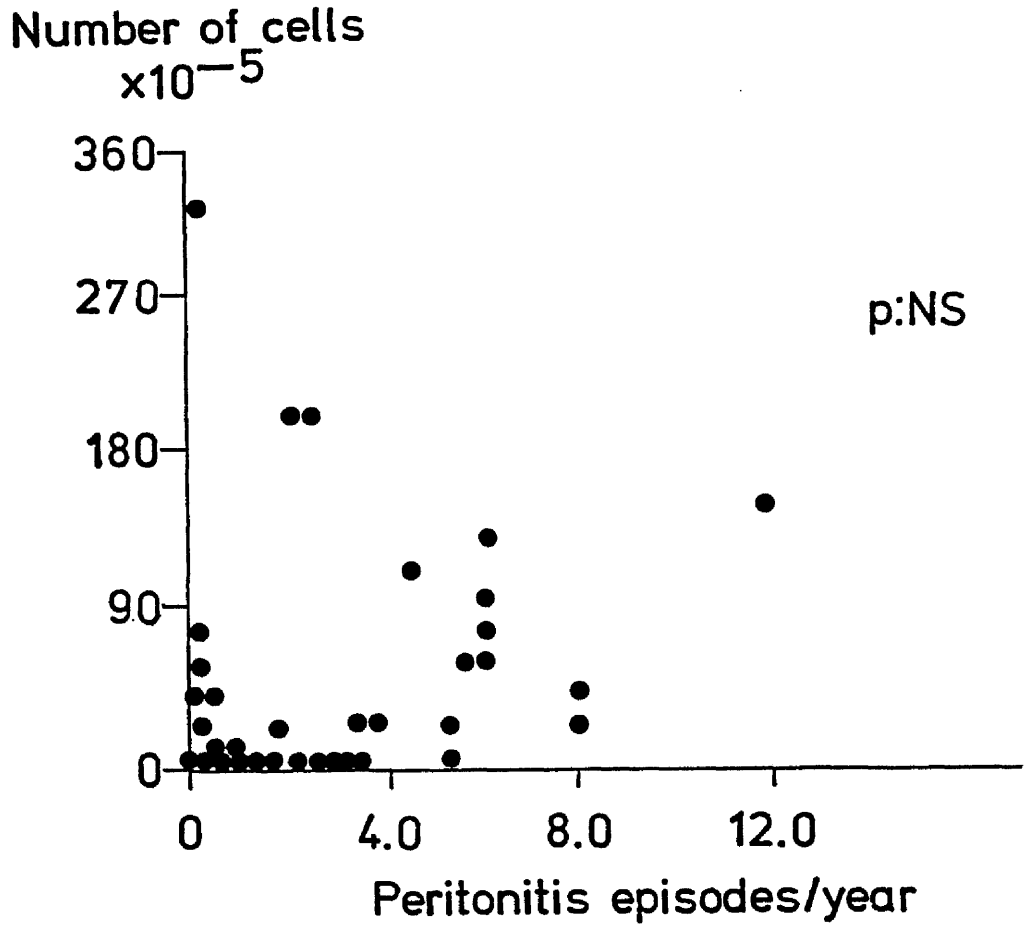


FIGURE 8 : Comparison of the Frequency of Peritonitis with Total Number of Cells Isolated from Peritoneal Dialysis Effluent

2.0. DISCUSSION

The characteristics and yield of cells isolated from the individual PDE of CAPD patients varied considerably. In terms of total cell numbers this ranged from less than $5x10^5$ to as high as 10^9 cells in extreme cases, the latter always being samples from patients diagnosed with peritonitis. Very high neutrophil levels were, in addition to raised cell counts, invariably associated with peritonitis as also shown by Hurley et al (1977) and Rubin et al (1980), and it was these cells which replaced the normal macrophage population as the major phagocytic cell in the peritoneum.

High eosinophil levels, possibly due to allergic or inflammatory reactions to either the dialysis solution or the dialysis procedure, were seen in five patients. Two of these had just received a replacement catheter following recurrent peritonitis, two had sclerosing peritonitis and one had changed to a different form of CAPD with different solutions, all of which may be associated with inflammation of the peritoneum. This has been previously noted by Digenis et al (1982) and Humayun et al (1981) at the start of CAPD in the case of catheter implantation. Possible explanations suggested include the body's first exposure to the peritoneal dialysis solutions with their unphysiologically elevated hydrogen ion and dextrose concentrations, dialysate additives (eg heparin, NaCl, KCl) or dialysate contaminants including particulate matter such as silica, talc and plasticizers released from dialysate

containers and tubings (Digenis et al, 1982; Humayun et al,1981; Spinowitz et al, 1981; Popovich et al, 1978; Lasker et al, 1975; Lee & Schoen, 1967). These substances may be irritant and/or allergic, either individually or in combination. Furthermore repeated inflammation in the abdominal cavity due to recurrent peritonitis or to irritants or particulate matter in the dialysate solutions, were thought to be the cause of sclerosing peritonitis (Shaldon et al, 1986; Lasker et al, 1975; Gandhi et al, 1972; Pauli et al, 1966).

However, the majority of PDE samples contained predominantly macrophages as shown also by Alobaidi et al (1986), Peterson et al, (1985), and Verbrugh et al (1984; 1983). On analysis of the results, a value of 15% for neutrophils and eosinophils was considered appropriate for excluding any sample that might have come from a patient with peritonitis from further investigation of its cellular activity. Indeed, from work undertaken by others the normal range of neutrophils found varied from 0-22% in one case (Verburgh et al, 1983), and from 0-9% (Verburgh et al, 1984) in another. The 15% value used here therefore seems in line with the above findings which give an average value of 15.5%.

In spite of the fact that quite a number of the PDE samples were discounted when this condition was imposed, it did ensure that the likelihood of analysing infected samples, or samples where the cellular activity may have been influenced by non-specific inflammatory agents, was greatly reduced. It was also important to exclude samples with raised polymorph values because it was primarily the

activity of the macrophage population that was to be examined, and some of the parameters could be affected by neutrophils or eosinophils.

Even when one considers only samples of PDE containing 15% or less polymorphs, the differential white cell counts of peritoneal cell samples from CAPD patients are still different from those of the normal cell population found in the peritoneum. In particular the average proportion of macrophages present in PDE and in normal controls were 69.9% and 91.4% respectively, a finding in line with that reported by Alobaidi et al (1986), Peterson et al (1985), Goldstein et al (1984) and Verbrugh et al (1983). This altered differential ratio of cells may severely compromise the normal defences of the peritoneum, even if their activity was unchanged, by upsetting the normal immune response mechanism of the peritoneum, thereby leaving it prone to infection. In particular, the flushing out of large numbers of cells each day may mean that the peritoneum may be replenished by immature cells as suggested by Goldstein et al (1984) leaving it severely short of mature macrophages which play such a large part in many of the immune responses of the body eg antigen presentation, antibody production, immunoregulation (Kampschmidt,1984; Unanue et al,1984; Staruch and Wood,1983; Unanue,1981). The greater size heterogeneity found in CAPD peritoneal cells, compared with normal peritoneal cells, could be the result of the replacement of peritoneal cells in CAPD patients by a very mixed population of monocytes all at different levels of maturation.

The inverse correlation found between the number of cells isolated from the PDE of patients and the length of time on CAPD might suggest that the cellular defence mechanisms in these patients would be severely affected by this cell shortage if they were of prime importance in protecting the patient from peritonitis. However no such increased susceptibility to peritonitis was shown in these patients, which brings into question the importance of these cells as the front line of the defence system in the peritoneum of CAPD patients. It is possible, however, that these patients' cells may become trapped by changes in the peritoneal membrane, and the number of cells in the fluid would represent only a small fraction of those present in the peritoneal cavity. Indeed, marked thickening and sclerosing of the peritbneal membrane (sclerosing peritonitis) has been noted in a number of patients (McWhinnie et al, 1986; Shaldon et al, 1986; Gandhi et al, 1980) who have been on CAPD for at least 1-2 years. The exact reason for this thickening, characterised by the deposition of a dense layer of fibrous tissue on the peritoneal surface which comes to resemble a fibrous band rather than an ultrathin membrane, is unknown, but as suggested earlier, repeated inflammation in the abdominal cavity due to recurrent peritonitis or to irritant in the dialysate solutions has been blamed (Di Paolo et al, 1986; Shaldon et al, 1986; Gandhi et al, 1980; Lasker et al, 1975; Pauli et al, 1966). The dialysate might also cause peritoneal irritation by virtue of its acidity (Gandhi et al, 1980), as initially the fresh dialysate has an acidic pH. The phenomenon of peritoneal thickening is of great

potential importance, since it may seriously limit the long term application of all forms of peritoneal dialysis.

In conclusion, it has been shown that macrophages are normally the predominant cells found in the PDE of patients without infections or inflammatory complications. In the following chapters the cell samples will be examined for a number of parameters related to their antimicrobial and immunological activity.

CHAPTER 3

DEVELOPMENT OF A RADIOMETRIC ASSAY FOR COMBINED MEASUREMENT OF INGESTION AND INTRACELLULAR KILLING OF *S.EPIDERMIDIS*

3.1 INTRODUCTION

To establish whether macrophages do indeed play an important role as a first line of defence against peritoneal infection in CAPD, it is necessary to establish whether they possess normal antimicrobial activity. This requires examination of the peritoneal cells isolated from the dialysis fluid of a large number of patients on CAPD. It was therefore necessary to develop a technique that would allow rapid evaluation of uptake and intracellular killing of *S. epidermidis* (the most frequent cause of peritonitis in CAPD patients) and which would permit processing of several samples per day if necessary.

A major difficulty in the quantification of phagocytosis over the years has been the lack of methods that distinguish precisely between micro-organisms attached to, or ingested by phagocytes. This distinction is particularly important when attempting to evaluate the capacity of phagocytes to kill ingested organisms.

Methods previously used for the evaluation of phagocytosis of micro-organisms include that of looking at stained smears, under light microscopy (Patterson and Youmans,1970; Chang,1969), or by phase-contrast microscopy (Gibbs and Roberts,1975; Swanson *et al*, 1974). These microscopical examinations are time consuming and liable to subjective error. Other approaches include the recovery of cell-associated microbes after attempts to remove or kill extracellular organisms by exposure to enzymes, antisera or antimicrobial agents (Verhoef *et al*,1977; Veale *et al*,

1976; Tan et al, 1971). Chemiluminescence can also be used as another measure of phagocytic activity. However this is an indirect method of measuring phagocytosis, as attachment of micro-organisms to the cells stimulates the plasma membrane, leading to activation of a respiratory burst (Easmon et al, 1980). This does not necessarily imply that the micro-organisms are subsequently phagocytosed, and chemiluminescence gives no indication as to the fate of the micro-organisms, i.e. whether they are killed or have survived.

Most techniques for estimating the microbicidal capacity of phagocytes require the separation of intracellular and extracellular organisms. When bacteria are used, the separation is usually achieved by differential centrifugation or destruction of the extracellular organisms. Radiometric assays, which use prelabelled bacteria to measure phagocytosis, still require a separation step, and bacterial death is determined by colony counting (Verbrugh et al, 1978) which is tedious and time consuming.

Bridges et al (1980) developed a radiometric assay based on an earlier method for measuring phagocytosis (Yamamura et al, 1977) but, in addition to this, also measured metabolic death of Candida albicans without involving any inconvenient plate counting or complicated separation steps. Lam and Mathison (1979) also modified Yamamura's method (Yamamura et al, 1977) to quantitate bacterial phagocytosis. Both methods easily distinguish between adherent or ingested micro-organisms. The basic principle of the assays was established by Yamamura et al

(1977), who demonstrated that incorporation of [^{3}H]-uridine into *C. albicans* was a good indicator of phagocytic function since:-

(i) a linear correlation was found between uridine incorporation and yeast number

(ii) Candida organisms surviving within phagocytic cells did not take up uridine from culture medium, and

(iii) phagocytic cells did not incorporate significant amounts of uridine in short term cultures.

Phagocytosis can thus be determined by comparing the uridine incorporation into a fixed number of micro-organisms in the presence and absence of phagocytes. Intracellular killing can be determined in a similar manner by measuring the uridine incorporation after cell lysis, which results in ingested but still viable organisms being released, and thus able to incorporate uridine.

In this chapter, the development of a simple but accurate radiometric technique for measuring phagocytosis and killing of *S. epidermidis* based upon incorporation of [^{3}H]-uridine into viable micro-organisms is described. Although based on that of Bridges *et al* (1980), many of the parameters had to be adapted for use here, as their assay was developed for measuring phagocytosis and intracellular killing of *Candida albicans* by blood neutrophils and not of *S. epidermidis* by peritoneal macrophages. This method allows phagocytosis and intracellular killing to be measured simultaneously making it possible to determine whether increased survival of the micro-organisms is due to reduced ingestion or reduced ability of the cells to kill the ingested bacteria.

3.2 MATERIALS

3.2.1. Phosphate Buffered Saline (PBS)

This consisted of NaCl (8 g/l), KCl (0.2 g/l), Na_2HPO_4 (1.15 g/l) and KH_2PO_4 (0.2 g/l), (all Analar grade, BDH Chemicals Ltd., Poole, Dorset, U.K.). The PBS was then divided into 10ml aliquots and sterilised by filtration.

3.2.2. PBS - Glucose

This consisted of glucose (1 mg/ml) in PBS. Aliquots were sterilised by filtration and stored at $4^{o}C$.

3.2.3. Normal Human Serum (NHS)

Serum was obtained from the clotted blood of 30 normal individuals, pooled and aliquots stored at $-20^{o}C$.

3.2.4. Tritiated Uridine

The 5,6[^{3}H]-uridine used had a specific activity of 53 Ci/mmol (Amersham p.l.c., Amersham, U.K.).

3.2.5. Culture of Staphylococcus epidermidis

A strain of S. epidermidis isolated from the fluid of a CAPD patient with active peritonitis in the Western Infirmary, Glasgow, was used throughout this work. It was typed by Dr Anne Eastaway (Dept of Bacteriology, Western Infirmary, Glasgow) using the API-Staph typing system (API Laboratory Products Ltd., Hampshire, U.K.) which gave a 99.7% probability of identification as S.epidermidis. The organism was routinely cultured in meat extract broth and then transferred to a nutrient agar slope, which was subcultured weekly.

3.3 METHODS

3.3.1. Standard Turbidity Curve of S. epidermidis

In order to calculate the number of bacteria in a sample for use in the assay without having to plate out a sample overnight it was necessary to relate bacterial numbers to turbidity readings from a spectrophotometer.

A 1ml aliquot of an overnight culture of S. epidermidis in meat extract broth was added to 20ml of fresh broth and incubated at 37°C. After two hours of incubation, and at 45 minute intervals thereafter, 2ml aliquots of the suspension were removed and serially diluted up to 10^6 times with ice cold sterile saline. The number of viable bacteria was then determined by the plate counting method of Miles and Misra (1938). Plots of the number of viable bacteria with time were made and the log-phase determined (Figure 9). The E_{550} reading of the bacteria present in the remaining 1ml volume was measured against a meat extract broth blank on a LKB Ultrospec 4050 spectrophotometer. From these results, a plot of the E_{550} reading against the number of viable bacteria was made (Figure 10). This standard curve was used to estimate the number of viable bacteria at the beginning of each individual experiment.

3.3.2. Uridine Incorporation Into Bacteria and Cells

A 500µl aliquot of an overnight culture of S. epidermidis was incubated in 10ml of fresh broth for 2.5

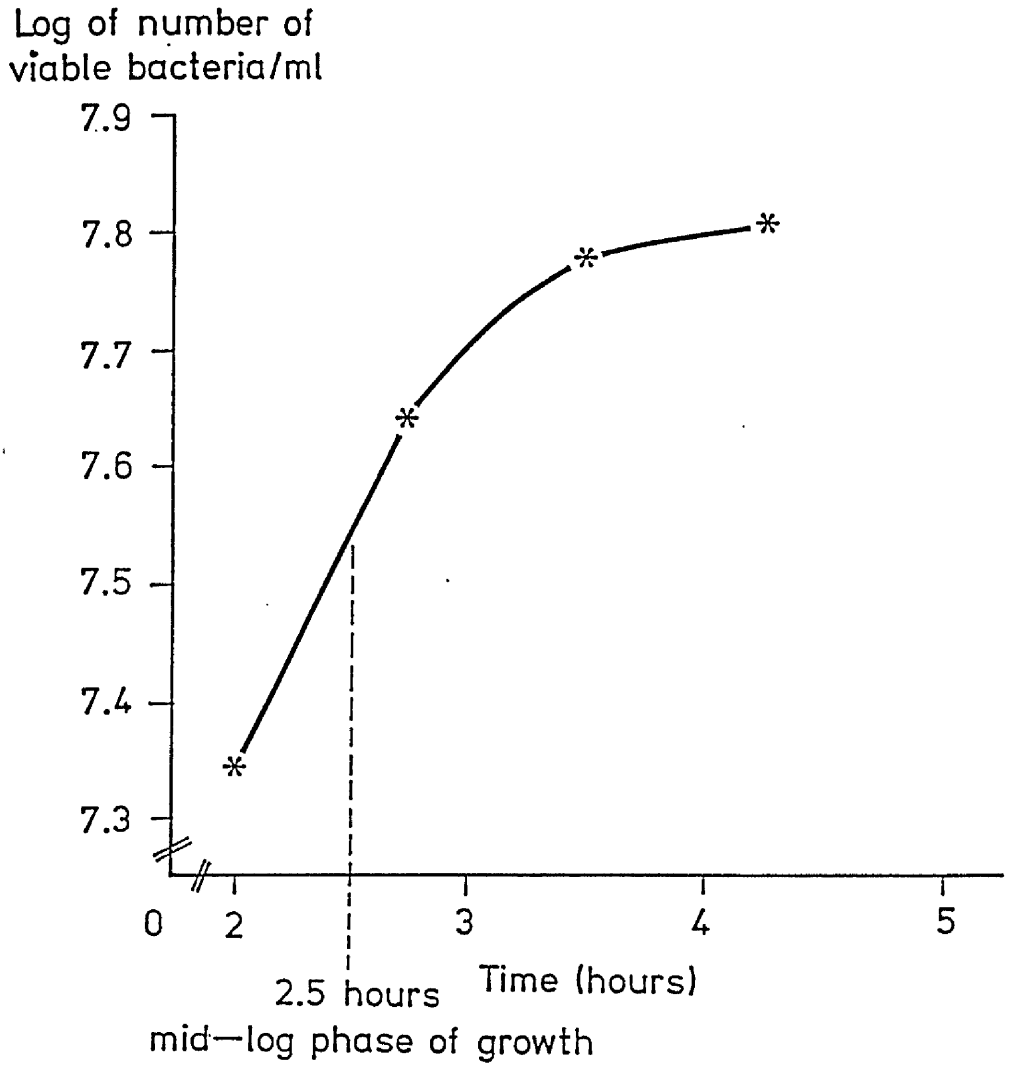


FIGURE 9: Growth Curve of S.epidermidis in Meat Extract Broth.

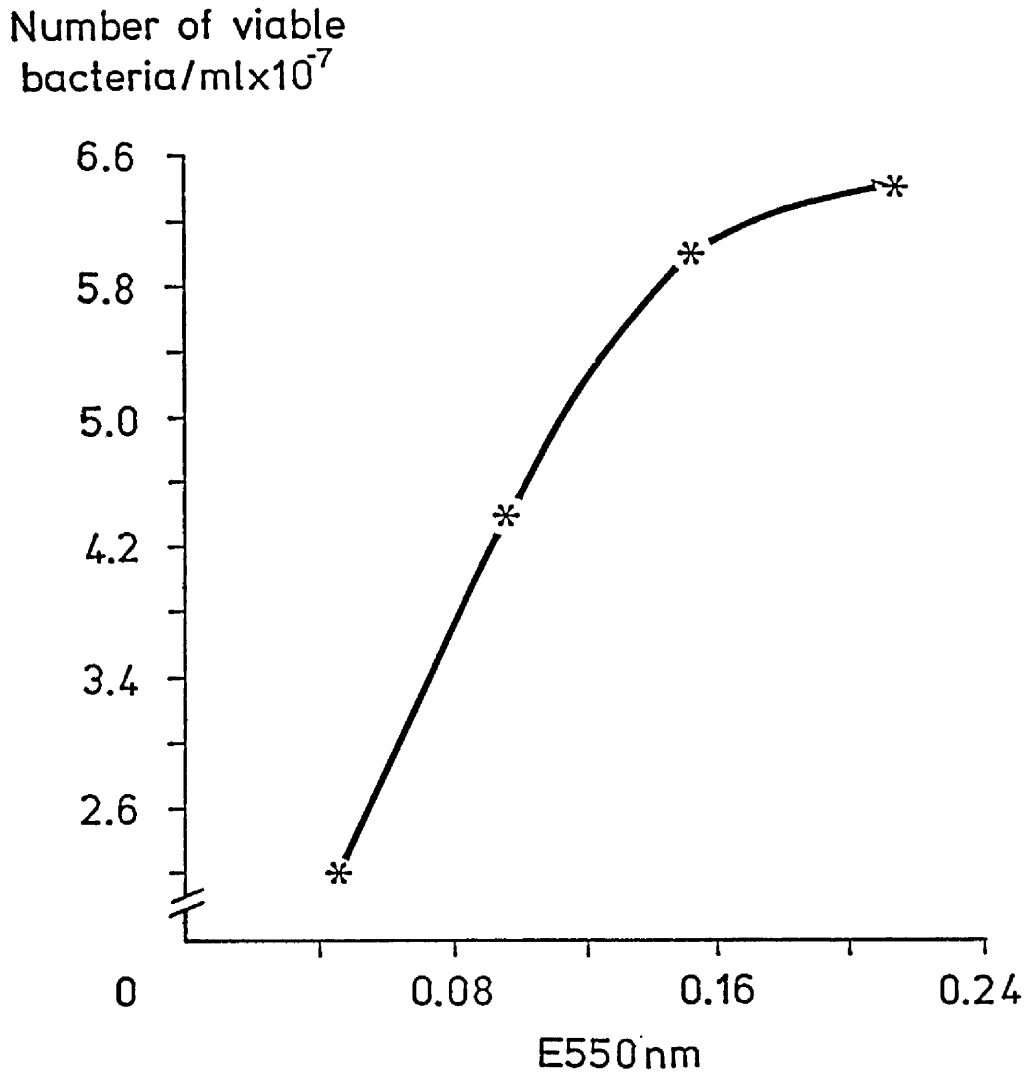


FIGURE 10: Plot of Number of Viable S. epidermidis Against Turbidity at 550nm.

hours as in 3.3.1. The bacteria were then washed 3 times in PBS and resuspended in 10ml of PBS-glucose. The turbidity of the suspension was measured as above, and the number of bacteria per ml calculated. Appropriate dilutions of bacteria, made up in culture medium (2.2.1) to 0.5ml, were then dispensed into 1.5ml plastic microvials to which 2µCi of [^{3}H]-uridine was then added. Control tubes containing similar concentrations of dead bacteria (heated at 100^{o}C for 15 minutes) were also set up to ensure that the uridine was being incorporated by specific metabolic activity rather than by passive absorption. All the tubes were then incubated for one hour at 37^{o}C on a blood tube rotator (Stuart, Croydon, U.K.), after which time the bacterial suspensions were harvested on a Millipore Millititre apparatus (Millipore UK Ltd., Harrow, Middlesex, U.K.). Each sample was split into 2x200µl aliquots and each was drawn by suction through a separate well of a Millititre 96-well plate fitted with a 0.4µm type STHA filter base. All wells were washed twice with 200µl of PBS and the filters cut out with a Millipore filter punch. The filters were dried and ^{3}H activity counted in a 4450 scintillation counter (Packard Instruments, Caversham, Berks, U.K.). A graph of bacterial number against uridine incorporation was then plotted (Figure 11). From the graph it is apparent that uridine incorporation increases linearly with the number of viable bacteria. In contrast uridine was not incorporated by the dead bacteria.

A similar experiment was carried out on the PDE cells isolated from patients on CAPD (2.4.1) to ensure that the cells could not incorporate significant amounts of uridine

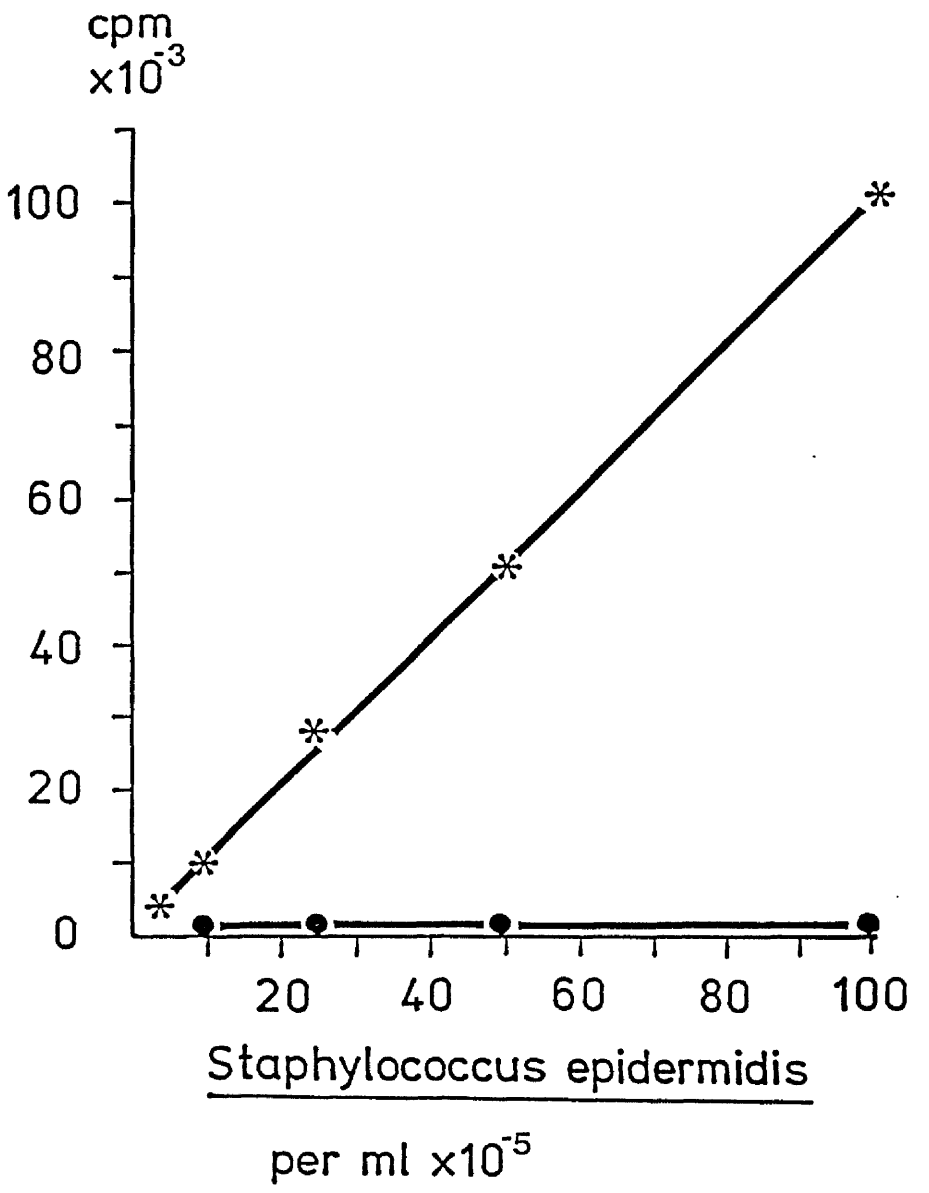


FIGURE 11: Incorporation of [^{3}H]-Uridine by S.epidermidis

* Viable S.epidermidis

● Dead S.epidermidis

cpm : counts per minute

and therefore interfere with the assay. A suspension of $5x10^5$ cells in 0.5ml of culture medium was incubated with 2µCi of [^{3}H]-uridine in 1.5ml plastic microvials. Tubes containing only culture medium were also set up. After one hour the tubes were harvested as described above. It was shown that although the incorporation of [^{3}H]-uridine by cells was usually greater than the counts obtained from controls containing culture medium alone, it was not high enough to greatly affect results (Table 5) as it was much lower than the incorporation by S.epidermidis, c.f. Figure 11. In any event [^{3}H]-uridine incorporation by the cells is taken into account when calculating results.

3.3.3. Viability of Cells

In order to ensure that the viability of the cells isolated from the PDE of CAPD patients (2.4.1) was maintained over the assay time, $5x10^5$ cells in 0.5ml of culture medium were rotated on a blood tube rotator for varying lengths of time (Table 6). No decrease in viability, determined by eosin stain exclusion, was observed during a 3 hour period (Table 6).

3.3.4. Preparation of Opsonised S.epidermidis

A 500µl aliquot of an overnight culture of S.epidermidis in meat extract broth was incubated in 10ml of broth in a water bath at 37°C for 2.5 hours. The bacteria were then pelleted at 1750g for 5 minutes, resuspended in 1ml PBS, and 1ml of NHS added to opsonise the bacteria. After incubation at 37°C for 15 minutes, the bacteria were washed three times and resuspended in 10ml of

TABLE 5: [^{3}H]-Uridine Incorporation by CAPD Peritoneal Cells.

EXPERIMENT NUMBER	* CPM IN SAMPLES CONTAINING: CAPD PERITONEAL CELLS	CULTURE MEDIUM ONLY
1	806	178
2	361	260
3	358	400
4	894	197
5	551	422
6	261	166

* CPM : counts per minute

TABLE 6: Viability of Cells From PDE Samples After Various Lengths of Time.

LENGTH OF TIME CELLS WERE ROTATED (MIN)	PERCENTAGE VIABILTY OF CELLS FROM PDE SAMPLE NUMBER		
	1	2	3
0	98.9	100.0	96.0
30	90.0	97.6	96.3
60	94.3	95.1	94.1
90	96.7	94.3	100.0
120	90.9	97.6	90.7
150	97.7	100.0	93.3
180	97.8	94.7	95.3

PBS containing 1% glucose (3.2.2). The turbidity of the suspension was measured at 550nm in a spectrophotometer, and the number of bacteria per ml calculated from the turbidity curve (Figure 10). The suspension was then adjusted to $8x10^5$ bacteria per ml.

3.3.5. Preparation of Cells

Peritoneal cells were prepared as previously described (2.4.1) and resuspended in culture medium (2.2.1).

3.3.6. Incubation of Cells With Bacteria and Subsequent Separation of Intracellular and Extracellular Bacteria.

Appropriate numbers of cells in 250µl aliquots (3.4.2) were dispensed into 1.5ml plastic microvials along with a suspension of $2x10^5$ S.epidermidis in 250µl of culture medium. Control tubes were set up containing either bacteria or cells only, and the volume made up to 0.5ml with culture medium. The tubes, which were set up in triplicate, were than incubated at 37^oC on a blood tube rotator.

After one hour the tubes were removed and each suspension split into two aliquots, one for measuring percentage killing and the other percentage ingestion. All the tubes containing cells were then spun at low speed (250g) for 5 minutes in order to pellet the cells, but leave the bacteria in suspension. The supernatants were removed and those from the control tubes, containing cells only, were discarded. The supernatants from the tubes containing both cells and bacteria were transferred to new tubes and spun at high speed (1750g), along with the

control tubes containing only bacteria, to sediment the organisms. All supernatants were discarded. The precipitates were then processed as described in section 3.4.4.

3.4 RESULTS

3.4.1. Variables in the Phagocytosis Assay

The basic principle of the assay was described by Bridges et al (1980), who studied the ability of human blood neutrophils to ingest and kill Candida albicans, and by Lam and Mathison (1979) who measured uptake, but not killing, of S.aureus by human neutrophils. Some of the parameters in the present work were based on the earlier studies. However, these used different organisms, and blood neutrophils rather than peritoneal macrophages. It was therefore necessary to optimise a number of parameters for the particular conditions of this study.

3.4.2. Cell:Bacteria Ratio

Initially, experiments were carried out using different ratios of cells:bacteria. A cell:bacteria ratio of 2:1 was found, in preliminary experiments, to result in 60-90% ingestion in most cases (Table 7). At a 4:1 ratio, ingestion tended to be close to 100% with little variation between samples, while at a 1:1 ratio only a small proportion of the bacteria were ingested. High cell:bacteria ratios were also impractical as they required larger numbers of cells than were generally available. A 2:1 ratio of cells:bacteria was therefore used throughout the assays.

TABLE 7: Ingestion of S.epidermidis by Different Ratios of Cells:Bacteria

RATIO OF CELLS:BACTERIA*	PERCENTAGE INGESTION OF S.EPIDERMIDIS BY PERITONEAL CELLS FROM CAPD PATIENTS : EXPERIMENT NUMBER			
	1	2	3	4
4:1	92	94	96	100
2:1	63	71	90	88
1:1	20	17	32	Not Done

* A suspension of $2x10^5$ S.epidermidis in 250µl of culture medium was used throughout the experiments.

3.4.3. Lysis of Cells to Release Intracellular Bacteria

In order to measure the bactericidal activity of the cells it was necessary to ensure that effective lysis of the cells occured so that any ingested but still-viable bacteria would be released and have access to the [^{3}H]-uridine when added. For this Bridges *et al* (1980), who worked with Candida, used deoxycholate, but this was unsuitable for use with bacteria as it dissolves components of bacterial cell walls. Instead the effect of Triton X-100 (BDH Chemicals Ltd.) on cellular and bacterial viability was tested. Aliquots of cell suspension or *S.epidermidis* of known concentrations were pelleted and resuspended with 100µl of various dilutions of Triton X-100 in distilled water (Table 8). The cells were then vortexed and left for two minutes, after which the detergent was diluted with a five-fold excess of culture medium (2.2.1). Cell viability was determined by eosin exclusion, and bacterial viability by [^{3}H]-uridine incorporation as described in section 3.3.2. Triton X-100, at a concentration of 0.01% was found to give 100% lysis of cells (Table 8), but unlike higher concentrations, did not significantly affect viability of *S. epidermidis*. A 0.01% solution of Triton X-100 was therefore used to lyse cells. Lam and Mathison (1979) who worked with neutrophils used distilled water, but in the present studies this was found to leave an average of over 40% of the cells still intact (Table 9).

TABLE 8: Effect of Triton X-100 On Viability of S.epidermidis and Peritoneal Cells.

CONCENTRATION OF TRITON X-100 (%)	% OF PERITONEAL CELLS VIABLE	VIABILITY OF BACTERIA (% OF CONTROL WITHOUT TRITON X-100)
0.1	0	22
0.01	0	93
0.001	10	104
0.0001	19	109
NONE	80	100

TABLE 9: Survival of Peritoneal Cells After Exposure to Distilled Water.

SAMPLE NUMBER	% OF VIABLE PERITONEAL CELLS AFTER 1 MINUTE IN 100µl OF DISTILLED WATER
1	35
2	12
3	41
4	52
5	50
6	40
7	56
AVERAGE	41

3.4.4. Procedure for the Measurement of Ingestion and Intracellular Killing of Bacteria.

To the cell pellets in the tubes to be used for assaying killing, obtained as described in section 3.3.6, 100µl of 0.01% Triton X-100 in distilled water was added, the tubes vortexed and the liquid added to the corresponding bacterial pellet. To ensure uniform conditions, 0.01% Triton X-100 was also added to the bacterial pellet from the bacteria-only control tubes to be used for the intracellular killing part of the assay. All the tubes were vortexed and left for two minutes to allow the cells to lyse, and then 380µl of culture medium (section 2.2.1) was added to each tube.

The tubes used to measure ingestion (section 3.3.6) were treated in a similar manner, except that the medium was added first and used to recombine the cells and bacteria, 0.01% Triton X-100 being added afterwards. This was done to allow for any possible effect of Triton X-100 on subsequent bacterial growth; once diluted with medium (section 2.2.1) it was no longer sufficiently concentrated to lyse the cells. Finally 20µl of [^{3}H]-uridine (2µCi) was added to each tube and these were then rotated at 37^{o}C for one hour.

After one hour the cultures were harvested (section 3.3.2). The percentage ingestion and killing were calculated using the following formula, the appropriate controls being used in each case:

$$\text{percentage ingestion or killing} = \frac{A + B - C}{A + B} \times 100$$

where, A,B and C are the average counts in tubes containing bacteria only, cells only, and bacteria and cells respectively. Variation in counts between triplicate samples was normally very good and well centred about the mean (Table 10).

3.4.5. Comparison of Radiometric Method With Conventional Colony-Counting Technique.

The accuracy of the assay method as finally devised and described above was tested by comparing the results obtained by [^{3}H]-uridine incorporation with those using a conventional colony-counting technique. For this an aliquot of each sample was removed and serially diluted with ice-cold saline for plate counting by the method of Miles and Misra (1938) immediately prior to passing the remainder through the filter (3.3.2). Four samples of cells from CAPD patients, in which the cells varied in their ability to ingest and kill S.epidermidis, were used, and in all cases good agreement was found (Table 11).

TABLE 10: Variation in Counts Per Minute After [^{3}H]-Uridine Incorporation Between Triplicate Samples.

TYPE OF SAMPLE	EXAMPLES OF COUNTS PER MINUTE (CPM)	MEAN (CPM)	% VARIATION ABOUT MEAN
Peritoneal cells and Bacteria	2472 2525 2289	2429	± 5.8
Bacteria only	8277 8225 8297	8266	± 0.5
Peritoneal cells and Bacteria	6010 5883 6199	6031	± 2.8
Bacteria only	42006 43010 44078	43031	± 2.4
Peritoneal cells and Bacteria	4781 4828 4541	4717	± 3.7
Bacteria only	14436 15317 15045	14932	± 3.3

TABLE 11: Comparison of Ingestion and Killing of S. epidermidis by Human Peritoneal Cells Using the [^{3}H]-Uridine Incorporation Assay or Colony Counting.

SAMPLE NUMBER	% INGESTION		% KILLING OF INGESTED BACTERIA	
	[^{3}H]-URIDINE INCORPORATION	COLONY COUNTS	[^{3}H]-URIDINE INCORPORATION	COLONY COUNTS
1	64.4	62.6	67.7	63.1
2	78.1	78.4	79.4	79.8
3	93.1	92.6	93.1	94.6
4	70.0	69.7	100.0	99.6

3.4.6. Summary of Complete Assay Procedure

1. Opsonised *S.epidermidis* were prepared as previously described in section 3.3.4.
2. The peritoneal cells, after isolation from PDE (2.4.1), were resuspended in culture medium (2.2.1) at 1.6 x 10^6 cells per ml.
3. The method for the incubation of cells with bacteria, and the subsequent separation of intracellular and extracellular bacteria was as described in section 3.3.6. A 2:1 ratio of cells to bacteria was used throughout ie 250µl of the cell suspension at 1.6 x 10^6 cells per ml was incubated with 250µl of bacterial suspension at $8x10^5$ bacteria per ml.
4. Cells were lysed to release intracellular bacteria using 100µl of 0.01% Triton X-100. Details of the method used are as described in section 3.4.4.

3.5 DISCUSSION

In order to evaluate the ingestion and intracellular killing of S. epidermidis by peritoneal cells isolated from the PDE of patients on CAPD, an assay had to be developed which would be both accurate and simple enough to be carried out routinely. It is also important to establish if their activity is reduced compared with normal peritoneal cells, and whether this reduced activity, if any, could account for the increased susceptibility of some patients to peritonitis. With this in mind a radiometric assay has been developed based on that described by Bridges et al (1980) but with modification of a number of parameters.

The method developed is simple, convenient, accurate and fairly inexpensive in terms of money and time which are essential requirements if the assay is to be carried out routinely for monitoring patients' cells. It also does not involve tedious colony-counting used to determine bacterial viability in previous methods (Verburgh et al,1978) or microscopical examination of slides (Patterson and Youmans,1970; Chang,1969) which is time-consuming and subject to error.

Unlike Lam and Mathison (1979), water was found to be unsatisfactory for lysis of cells. Instead a 0.01% solution of Triton X-100 was used, a concentration which while lysing cells did not adversely affect the bacteria. Deoxycholate, used by Bridges et al,(1980) is unsuitable as it dissolves components of bacterial cell walls.

Accuracy and reliability of the assay were tested by comparing results obtained from the radiometric assay with the conventional colony-counting technique. In all cases good agreement was found. There was also little variation between triplicate samples.

The technique described here therefore allows rapid evaluation of phagocytosis and bactericidal activity of cells from patients on CAPD. Both are measured simultaneously, making it possible to determine whether increased survival of the micro-organisms is due to reduced ingestion or reduced ability of cells to kill the ingested bacteria. Unlike many methods (Verbrugh et al, 1978; Verhoef et al, 1977; Tan et al, 1971) the problem of precisely distinguishing between attachment or ingestion of micro-organisms does not arise as only uningested bacteria have access to the [^{3}H]-uridine which is not taken up by the cells.

This asssay described here has been used in the following chapter to evaluate the activity of peritoneal cells from patients on CAPD compared with those cells from normal individuals. Possible differences in activity between patients and controls, and within patients may provide an explanation for the increased susceptibility of some patients to peritonitis.

CHAPTER 4

FUNCTIONAL PROPERTIES OF HUMAN PERITONEAL MACROPHAGES

4.1. INTRODUCTION

Recently there has been increasing interest in peritoneal defense mechanisms, aiming at clarifying the root causes of peritonitis in CAPD patients, and identifying characteristics of patients with high infection rates (Diskin et al,1983; Rubin et al,1983; Verbrugh et al, 1983). Work by Tsakiris et al (1986) on the skin reaction of CAPD patients to dinitrochlorobenzene (DNCB) suggested that intrinsic differences may exist in the host immune response among CAPD patients. They showed that patients with strong DNCB responses have a better chance of remaining free of peritonitis for longer than those with poor responses. This may indicate differences in the actual peritoneal defence mechanism within patients, and may contribute to the increased susceptibility to peritonitis found in some patients. The DNCB skin test has the advantage over those using recall antigens of not testing immunological memory, since it is a new antigen to which patients will not have been exposed to (Bramwell et al,1985; Watson et al,1979).

Immunosuppressive effects of uraemia have been reported, and have frequently been attributed to inhibitory or toxic factors retained in the serum of uraemic patients- the so-called middle molecules (Funck- Brentano et al,1975; Touraine et al,1975). An improvement in cell mediated immunity (CMI) during CAPD compatible with the removal of uraemic substances that inhibit T-lymphocyte functions has been observed (Young et al,1986; Giangrande et al,1984;

Giacchino et al,1983). However, while improvement in CMI may increase resistance to fungal, viral and intracellular bacterial infections such as mycobacteria it is unlikely to confer any clinical advantage against peritonitis by normal extracellular Gram-negative or Gram-positive bacteria. In addition, as Young et al(1986) suggested, a depletion of specific enzymes, vitamins or trace elements cannot be excluded and this could have an important effect on the immune response.

While there is evidence that CAPD patients have impaired immune responses, there is little information as to the possible defects in specific mechanisms, and in particular of the resident mononuclear phagocytic cells which may be of prime importance in the protection of the peritoneum from infection. Any defects in these cells may greatly impair host resistance, and increase the possibility of organisms establishing themselves, thus giving rise to peritonitis. In general it has been shown that patients with defects in their phagocytic cells suffer from recurrent and severe infections, not infrequently with a fatal outcome (White and Gallin,1986; Stossel,1974). Phagocytic dysfuncton most commonly occurs secondary to acquired diseases such as rheumatoid arthritis, diabetes mellitus, malnutrition or certain viral infections (White and Gallin,1986). In view of this it seems important and appropriate to examine the antimicrobial activity of the peritoneal cells from patients on CAPD to determine whether these cells are also defective.

Further information about the activity of these peritoneal cells may be provided by examining their ability

to release H_2O_2 as H_2O_2 levels are believed to be related to the bactericidal activity of the cells and to their maturational or activational state (Takemura and Werb,1984; Nathan et al,1983,1979; Johnston 1981). This appears not to have been investigated in CAPD patients. In general patients whose phagocytes lack the ability to generate reactive oxygen derivatives, such as those with chronic granulomatous disease, have impaired microbicidal activity which emphasises their importance in killing bacteria (Gallin et al,1983; Holmes et al, 1967,1966).

Macrophages also play a key role in the development of specific immune responses by processing antigens, which requires the presence of HLA-DR antigens. Indeed membrane expression of human Ia-like molecules (HLA-DR) by macrophages is critical for the induction of T-cell responses to foreign antigens (reviewed by Unanue et al,1984 and Unanue,1981). Considerably less information is available about HLA-DR expression on human mononuclear phagocytes compared with the mouse and in particular it is not known whether patients on CAPD show any abnormalities in this respect. Such information could be of relevence to the immune status of CAPD patients, as low levels of HLA-DR expression on macrophages may hamper initiation of specific immune responses. Therefore abnormalities in the properties of macrophage function may inhibit not only the non-specific phagocytic role of macrophages, but might also contribute to an overall lowering of the whole immune response.

HLA-DR expression, like H_2O_2 release, may be related to the maturational or activational state of the CAPD

patients' peritoneal macrophages, as it is widely known that cells activated through gamma IFN have raised levels of HLA-DR and H_2O_2 release compared with those in the resting state (Gershon et al,1985; Wilson and Westall,1985; Sztein et al,1984; Basham and Merigan,1983; Nathan et al,1983). It was therefore considered worthwhile to examine HLA-DR expression and H_2O_2 release by peritoneal cells from CAPD patients to provide information about their functional ability which may be related to their state of activation.

The work reported in this chapter was therefore aimed at examining the characteristics of the CAPD cells in regard both to their role in peritoneal defence against infection and to their relationship to other human mononuclear phagocytes. This involved:-

(1) examining their bactericidal activity and comparing it with that of normal human peritoneal cells. It was also important to relate these findings to the frequency of peritonitis to determine whether patients' cellular activity could be correlated with their susceptibility to infection.

(2) measuring the levels of H_2O_2 released by the peritoneal macrophages from the patients on CAPD and comparing their activity with that of peritoneal macrophages from the first exchange PDE of patients commencing CAPD, normal peritoneal macrophages and of blood monocytes.

(3) determining the expression of HLA-DR by mononuclear phagocytes from the same sources as in (2).

4.2. MATERIALS

4.2.1. Ethylenediamine-tetra-acetic Acid (EDTA) Glycine Buffer.

This consisted of 0.1M glycine (BDH Chemicals) and 0.025M EDTA (Koch-Light Laboratories Ltd., Colnbrook, Berk, U.K.). The pH was adjusted to 12 with NaOH.

4.3. PATIENTS AND CONTROLS

In order to study the phagocytic and bactericidal activity of peritoneal cells from patients on CAPD, 28 peritoneal cell samples from 18 patients were analysed along with 8 normal peritoneal cell samples by the assay procedure described in chapter 3. Details of each of these patients and controls, and the samples tested, were given in chapter 2 (Tables 1,2 and 4). To study H_2O_2 release by peritoneal macrophages from patients on CAPD, peritoneal cell samples from 10 patients commencing CAPD and 22 from patients established on CAPD were analysed. Details of these patients are given in Tables 12 and 13. In addition 9 normal peritoneal macrophage samples from women undergoing laparoscopy and 7 peripheral blood monocyte samples from normal controls were also tested. HLA-DR expression was analysed using peritoneal macrophages from 12 patients commencing CAPD and 18 patients established on CAPD (Tables 14 and 15). In addition 17 normal peritoneal macrophage samples and 9 blood monocyte samples from normal controls were tested.

TABLE 12 : Details of Patients whose Peritoneal Macrophages from the First Exchange PDE were Used to Measure Release of H_2O_2.

PATIENTS' INITIALS	SEX	AGE (YEARS)	ORIGINAL DIAGNOSIS
H.Ca	Female	53	Polycystic kidneys
V.De	Male	39	Pyelonephritis
M.Fo	Female	74	Glomerulonephritis
J.He	Male	63	Analgesic Nephropathy
M.Il	Female	55	Glomerulonephritis
H.Le	Female	56	Glomerulonephritis
M.Mc	Female	66	Diabetic nephropathy
M.Mi	Female	25	Glomerulonephritis
J.Sh	Male	45	Glomerulonephritis
P.Sm	Male	18	Unknown

All patients were commenced on the Travenol Disconnect System.

TABLE 13: Details of Patients Established on CAPD whose Peritoneal Macrophages were used to Measure Release of H_2O_2.

PATIENTS' INITIALS	SEX	AGE (YEARS)	MONTHS ON CAPD	ORIGINAL DIAGNOSIS
M.An	Female	77	2	Analgesic nephropathy
J.Be	Female	30	15	Glomerulonephritis
B.Ca	Male	64	12	Hypertension
R.Ca	Female	40	60	Glomerulonephritis
R.Co	Female	40	15	Glomerulonephritis
W.Co	Male	32	10	Hypertension
A.Cr	Male	32	6	Obstructive uropathy
P.Cr	Male	60	17	Renal artery thrombosis
W.Do	Male	73	1	Neurogenic bladder following whiplash injury
J.Fl	Female	52	6	Diabetic nephropathy
R.Fu	Male	71	4	Glomerulonephritis
I.Jo	Male	39	28	Glomerulonephritis
W.Ka	Male	47	16	Glomerulonephritis
R.Ke	Male	49	12	Glomerulonephritis
H.Mc	Female	21	9	Relux nephropathy
M.Mc	Female	56	10	Polycystic kidneys
W.Mc	Male	69	4	Prostatic obstruction
M.Mu	Female	72	15	Unknown
W.Ra	Male	65	3	Diabetic nephropathy
W.Ro	Male	57	6	Analgesic nephropathy
P.We	Female	66	12	Analgesic nephropathy
M.Wh	Female	67	3	Hypertension

All patients were on the Travenol Disconnect System except for R.Ca who was on Fresenius.

TABLE 14: Details of Patients whose Peritoneal Macrophages from the First Exchange PDE were Used to Determine the Proportion of HLA-DR Positive Cells.

PATIENTS' INITIALS	SEX	AGE (YEARS)	ORIGINAL DIAGNOSIS
C.Ba	Male	39	Glomerulonephritis
R.Da	Female	46	Diabetic nephropathy
J.Ha	Female	53	Polycystic kidneys
R.Ha	Male	75	Obstructive uropathy
J.He	Male	63	Analgesic nephropathy
R.Ho	Female	68	Renal artery thrombosis
H.Il	Female	55	Glomerulonephritis
R.Ke	Male	49	Glomerulonephritis
S.Mc	Female	48	Reflux nephropathy
J.Ra	Male	55	Obstructive uropathy
J.Sh	Male	45	Glomerulonephritis
M.Wh	Female	67	Hypertension

All patients were commenced on the Travenol Disconnect System.

TABLE 15: Details of Patients Established on CAPD whose Peritoneal Macrophages were Used to Determine the Proportion of HLA-DR Positive Cells.

PATIENTS' INITIALS	SEX	AGE (YEARS)	MONTHS ON CAPD	ORIGINAL DIAGNOSIS
J.Be	Female	30	15	Glomerulonephritis
A.Ca	Female	77	27	Glomerulonephritis
B.Ca	Male	64	12	Hypertension
J.Co	Male	31	21	Glomerulonephritis
W.Co	Male	32	7	Hypertension
W.Do	Male	73	10	Neurogenic bladder following whiplash injury
J.Fl	Female	52	6	Diabetic nephropathy
B.Go	Male	24	31	Diabetic nephropathy
H.Il	Female	55	3	Glomerulonephritis
A.Ka	Female	64	2	Polycystic kidneys
W.Ka	Male	47	16	Glomerulonephritis
R.Ke	Male	49	12	Glomerulonephritis
R.Lo	Male	47	11	Pyelonephritis
C.Mc	Male	55	3	Diabetic nephropathy
D.Mc	Female	64	26	Analgesic nephropathy
W.Mc	Male	69	4	Prostatic obstruction
A.Me	Female	37	20	Glomerulonephritis
A.My	Female	44	46	Polycystic kidneys

All patients were on the Travenol Disconnect System except for A.My who was on the Travenol System II.

4.4. ISOLATION OF CELLS

4.4.1. Peritoneal Macrophages

CAPD and normal peritoneal cells were isolated as described in section 2.4.1.

4.4.2. Monocytes

Blood mononuclear cells were isolated by layering 2 volumes of heparinised blood mixed with an equal volume of PBS over 3 volumes of separating mixture (Lymphoprep, Nycomed U.K. Ltd., Birmingham, U.K.). The samples were then centrifuged at 400g for 30-40 minutes. The cells were removed from the interface layer by a Pasteur pipette, washed twice with PBS and resuspended in culture medium (2.2.1.).

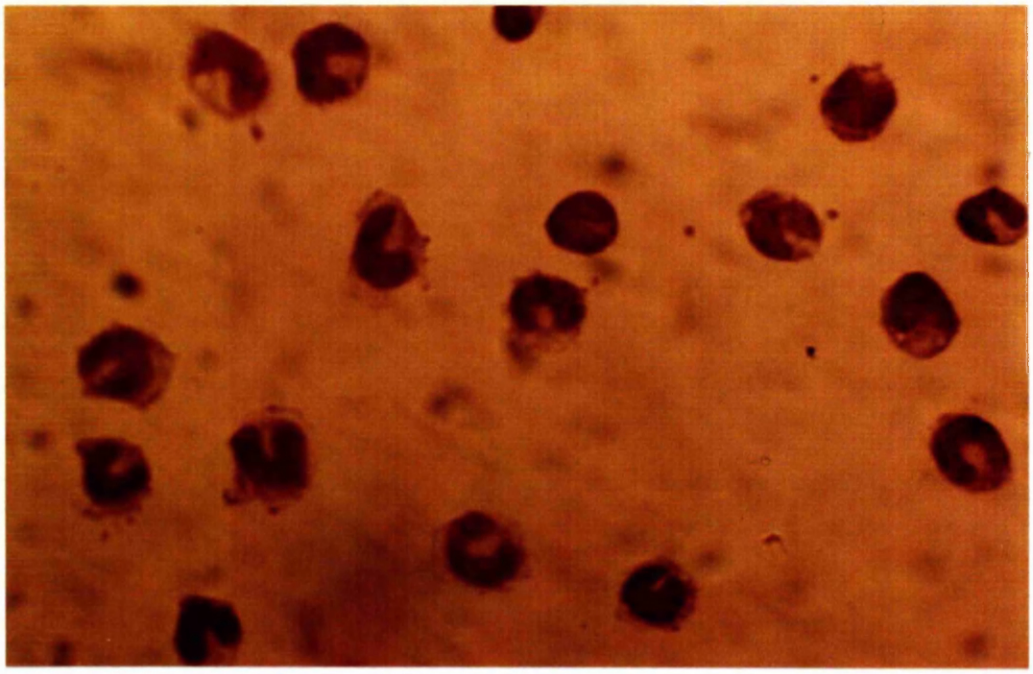
All cell samples tested contained 15% or less neutrophils or eosinophils when stained with Leishman (2.4.2.). Cell viability was always greater than 95% as determined by eosin exclusion.

4.5. METHODS

4.5.1. Differential Staining of Adherent Cells

Adherent cell cultures were prepared by adding 1ml of a 10^6 cell/ml suspension in culture medium (2.2.1.) to sterile round glass coverslips (16mm diameter : MacFarlane Robson, Glasgow, U.K.) placed in 25 square well plastic culture dishes (Sterilin Ltd., Feltham U.K.). These were then incubated for 2 hours at 37^oC in 10% CO_2. Non-adherent cells were removed with a Pasteur pipette and adherent cells washed twice with medium. Enumeration of non-adherent cells in a haemocytometer revealed that approximately $4\text{-}5x10^5$ cells had attached to the coverslips in each well. Monolayers were allowed to dry at room temperature before staining. Differential cell counts of the adherent cell samples were determined routinely using Leishman stain (2.4.2.) and some preparations were also stained with ANAE (2.4.3.). Only adherent cultures where more than 97% of cells were identified as macrophages and where contamination with either neutrophils or lymphocytes did not exceed 2% were used for determining H_2O_2 release or HLA-DR expression. Examples of cells from all four sample types used for measuring H_2O_2 release and HLA-DR expression are shown in Figures 12-15.

(a)



(b)

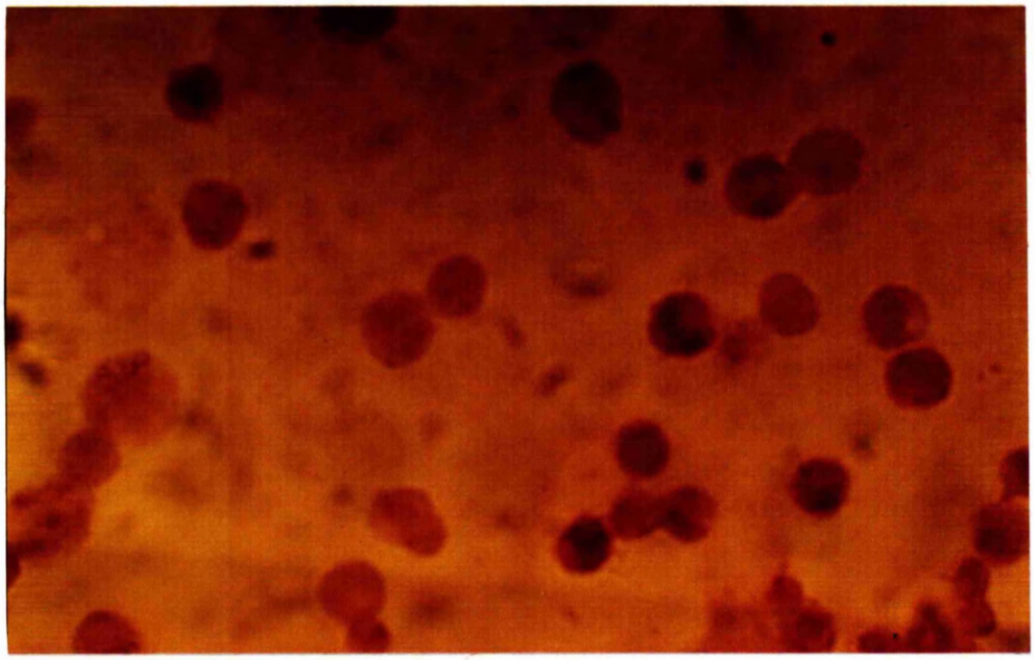
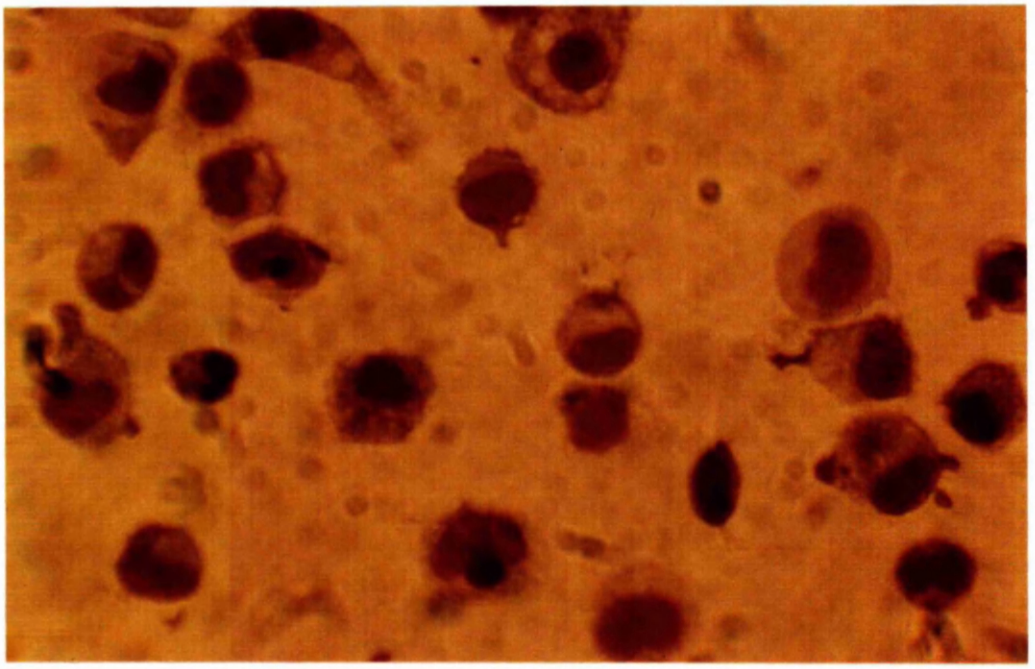


FIGURE 12

(a) Peritoneal macrophages from patients established on CAPD, stained with Leishman (x1600)

(b) Peritoneal macrophages from patients established on CAPD, stained with ANAE (x1600)

(a)



(b)

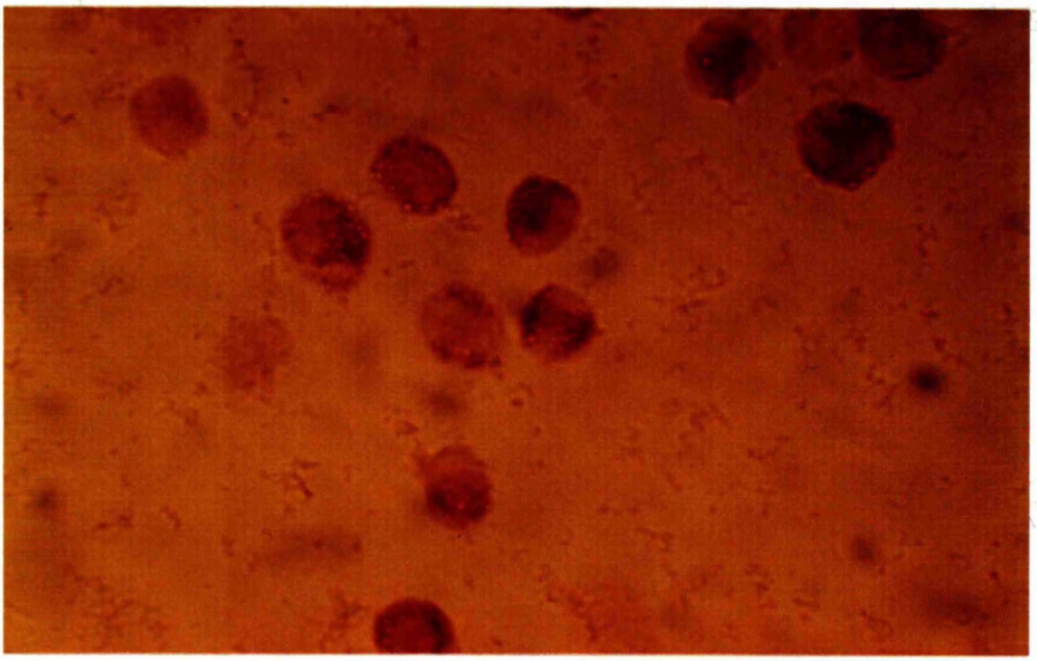
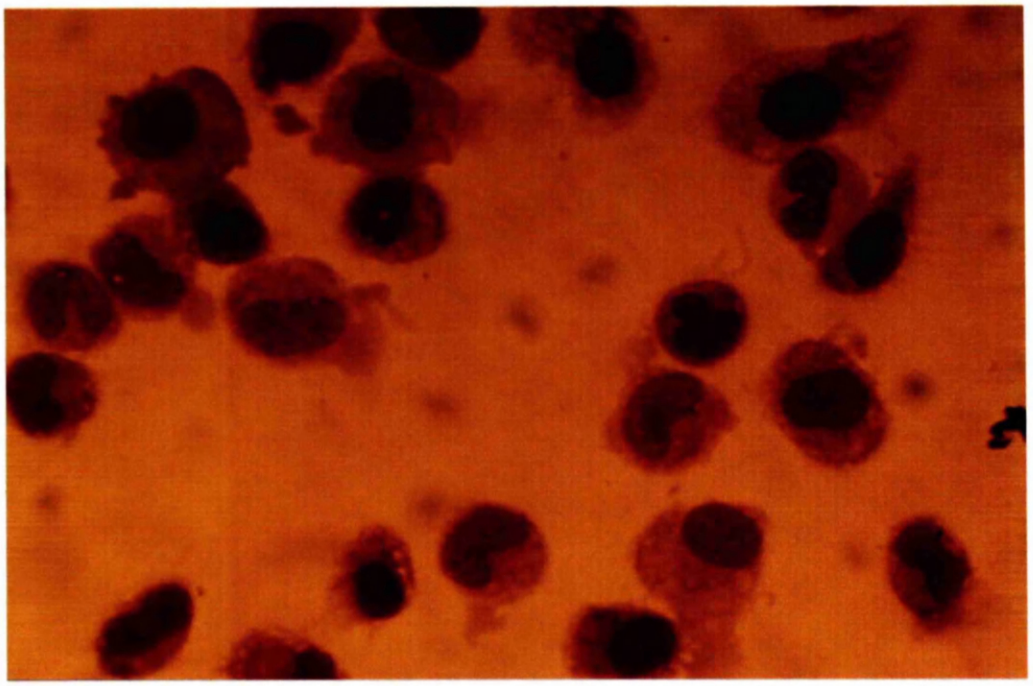


FIGURE 13

(a) Peritoneal macrophages from patients commencing CAPD, stained with Leishman (x1600)

(b) Peritoneal macrophages from patients commencing CAPD stained with ANAE (x1600)

(a)



(b)

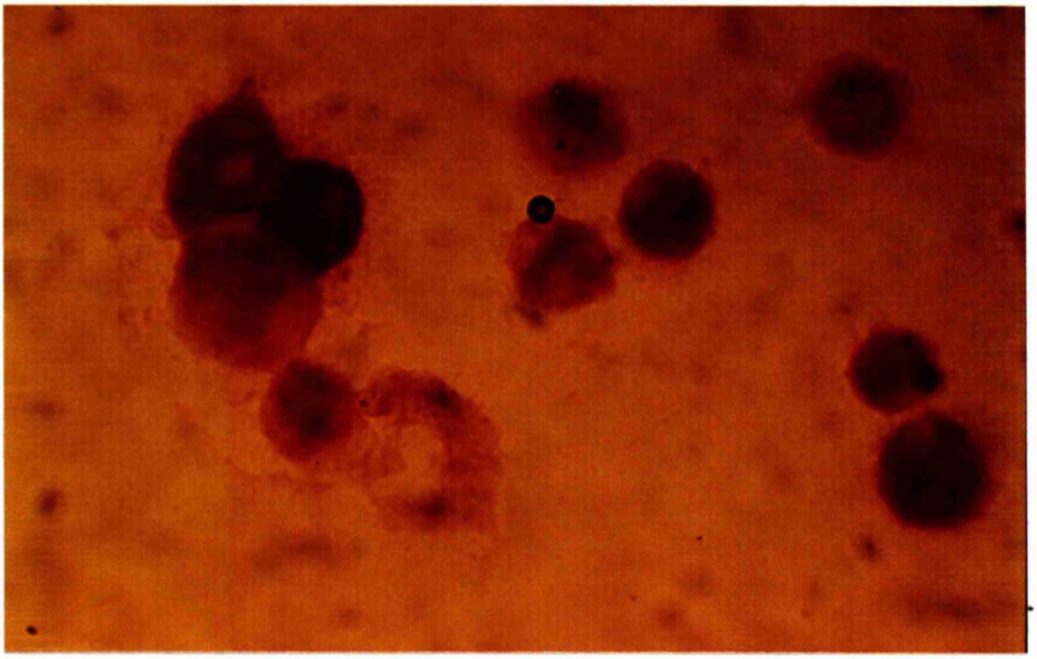
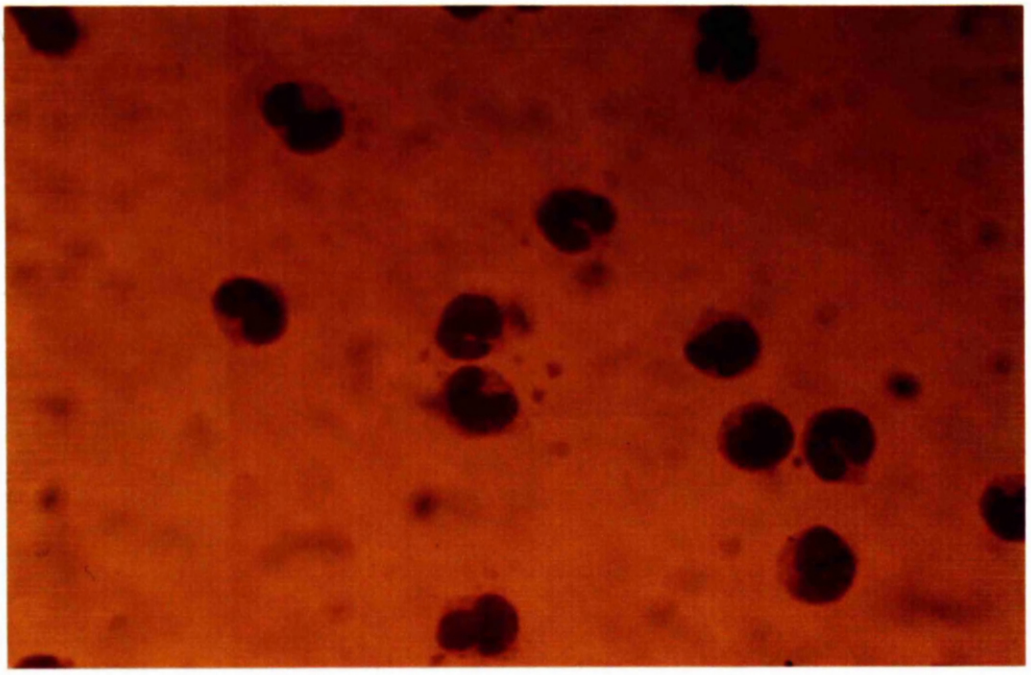


FIGURE 14

(a) Normal peritoneal macrophages stained with Leishman (x1600)

(b) Normal peritoneal macrophages stained with ANAE (x1600)

(a)



(b)

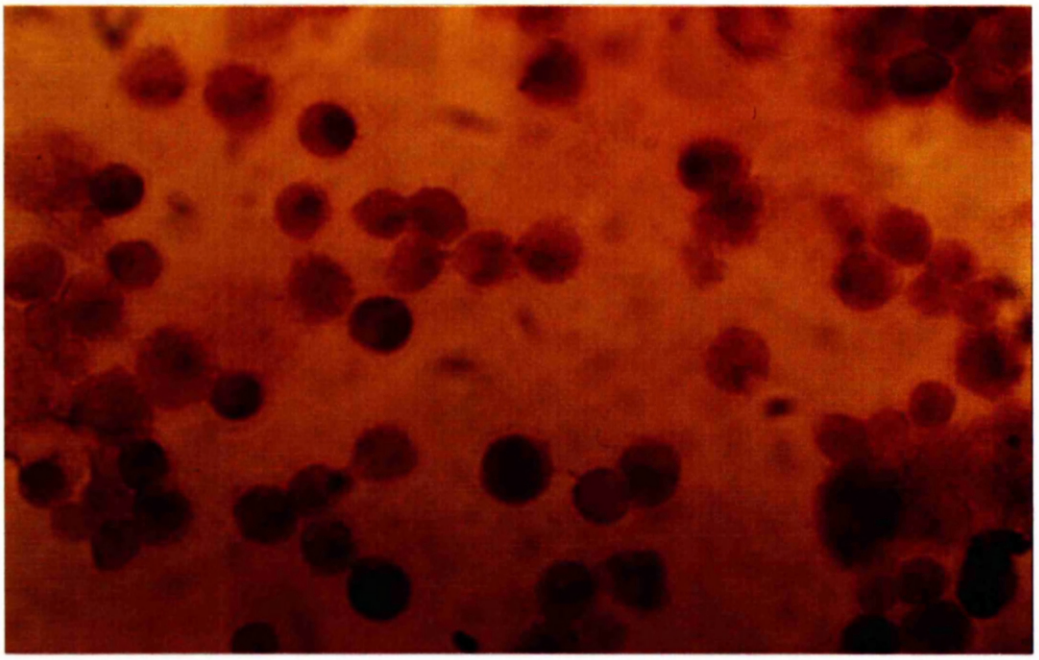


FIGURE 15

(a) Normal blood monocytes stained with Leishman (x1600)

(b) Normal blood monocytes stained with ANAE (x1600)

4.5.2. Measurement of H_2O_2 Release and Estimation of DNA Content of Adherent Cells.

Cultures were established at $2x10^6$ cells in 1ml of culture medium (2.2.1.) in Linbro flat-bottomed 24-well tissue culture plates (Flow Laboratories). After 2 hours at 37^oC in 10% CO_2, monolayers were washed twice with warm Hanks Balanced Salt Solution without phenol red (HBSS; Gibco, Paisley, U.K.) to remove non-adherent cells and fresh HBSS added.

The method of Ruch et al,(1983) was employed for determining H_2O_2 release, which depends on the oxidation of homovanillic acid (HVA) to a fluorescent dimer, the oxidation being mediated by horse radish peroxidase. The HVA oxidation product (a measure of H_2O_2 release) was determined using a Perkin-Elmer model 1000 fluorimeter with excitation at 312nm and emission measured at 420nm. A stock suspension of $20mg_{\Lambda}^{/ml}$ zymosan (Sigma) in PBS was prepared and washed twice in PBS. From this, freshly opsonised zymosan for use as the triggering agent for H_2O_2 release was prepared each day by incubating one volume of the suspension with an equal volume of pooled normal human sera (3.2.3) for 15 minutes at 37^oC, and centrifuged at 2000g for 10 minutes. The pellet was washed twice in HBSS and resuspended at a final concentration of 5mg/ml in HBSS.

The HVA-peroxidase solution consisted of 400µM HVA (Sigma) in HBSS containing 4 units/ml of horse radish peroxidase (Sigma). The solution was prepared every week and stored in aliquots at 4^oC.

Following removal of non-adherent cells from macrophage monolayers, 1ml HBSS, 0.5ml opsonised zymosan

and 0.5ml of HVA-peroxidase were added to each well, and incubated at 37°C for 1 hour. Control wells were also set up with no zymosan to measure the spontaneous release of H_2O_2. Supernatants were then transferred to conical bottomed plastic tubes, centrifuged at 1800g for 5 minutes to pellet any loose cells or cell debris and the resultant supernatants transferred to glass tubes. To stop the reaction 0.25ml of EDTA-glycine buffer (4.2.1.) was added, the tubes vortexed and H_2O_2 assayed by fluorimetry. A standard curve was obtained using varying concentrations of H_2O_2 (Boots Ltd., Nottingham, U.K.). The fluorimeter blank consisted of 1.5ml HBSS, 0.5ml HVA-peroxidase and 0.25ml of EDTA-glycine buffer.

DNA was determined according to the method of Cesarone et al (1979). After removal of the cell supernatants above, 200µl of 0.05% (w/v) sodium dodecyl sulphate (SDS; BDH Chemicals) was added to the macrophages in each well of the plate. They were then freeze-thawed once to complete lysis of the cells. Following addition of 1ml PBS each lysate was mixed and transferred to a conical bottomed plastic tube. These were then centrifuged at 900g for 5 minutes after which 600µl was transferred to glass tubes containing 4.4ml PBS. Each tube received 20µl of 7.5×10^{-4}M bis-benzimide (Hoechst 33258; Sigma) and was then vortexed before incubation in darkness at room temperature for 30 minutes. The DNA content of each tube was subsequently assayed by fluorimetry (excitation 364nm, emission 448nm) using a standard curve (Figure 16) prepared from calf thymus DNA (Sigma). The fluorimeter blank consisted of 4.9ml PBS plus 100µl 0.05% SDS and 20µl of bis-benzimide.

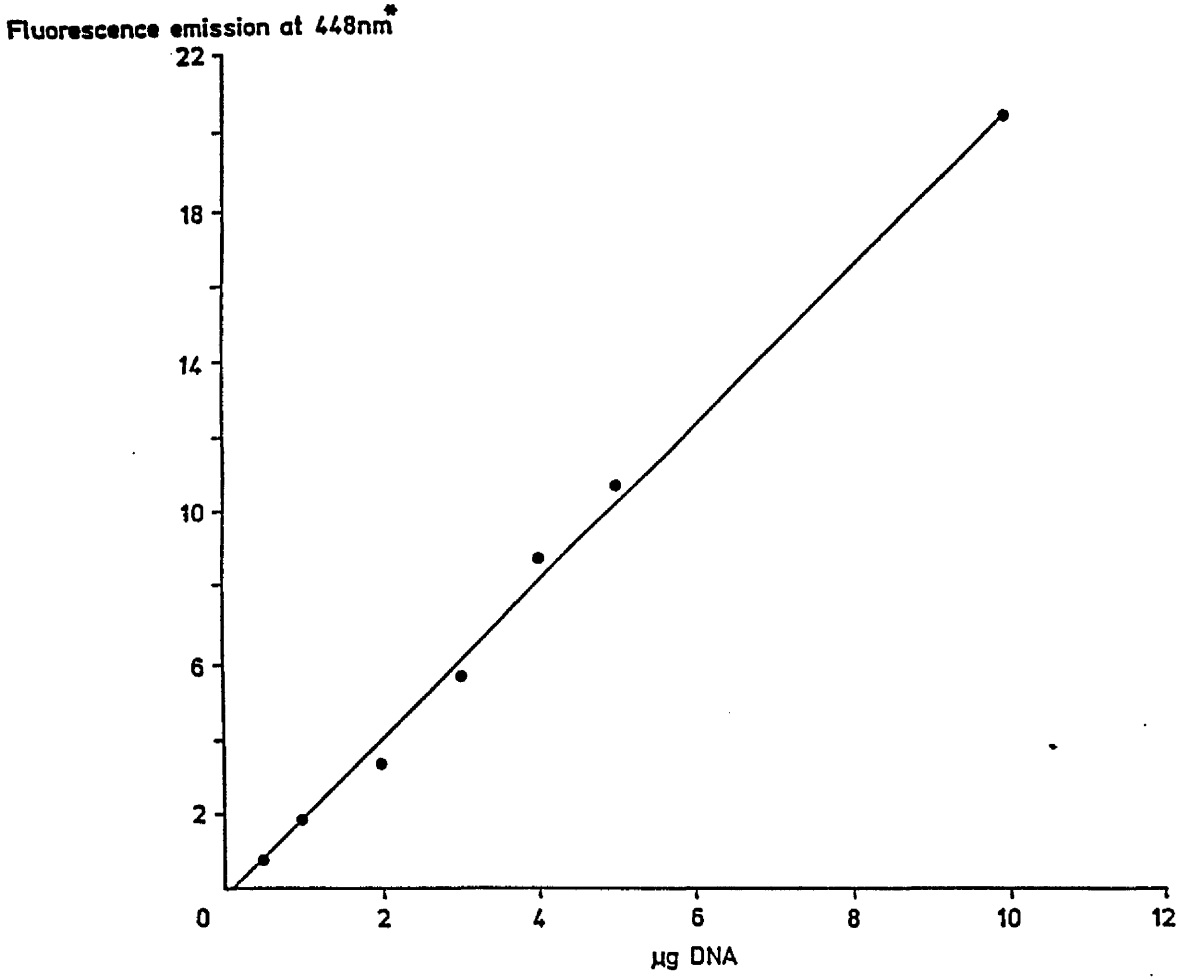


FIGURE 16 : Standard Curve for Measurement of DNA

* Excitation 364nm

The results both for H_2O_2 release and DNA content were means of duplicates, in which variation did not exceed 10% of the mean. Results were expressed as $\mu m H_2O_2$/10 µgDNA.

4.5.3. Expression of HLA-DR Antigen by Human Macrophages and Monocytes.

Human macrophages/monocytes isolated from blood, normal peritoneal fluid and CAPD fluid were established as monolayers on circular glass coverslips (4.5.1.). After adherence, cell monolayers were fixed for 10 minutes with a 2.5% solution of formaldehyde in PBS (ie 1 volume of 40% v/v stock formaldehyde in 15 volumes PBS) at room temperature. Cells were then washed for 5 minutes with PBS and stained by direct immunofluorescence with one drop of a 1:3 dilution of a phycoerythrin-conjugated monoclonal antibody to human HLA-DR antigen (Becton Dickinson, Laboratory Impex Ltd., Twickenham, Middx., U.K.).

After 30 minutes in the dark, cultures were washed for 5 minutes in PBS. Coverslips were then removed from the wells, mounted with PBS-glycerol (4 parts PBS:1 part glycerol) and the edges sealed with nail varnish. Initially cells were preincubated for 30 minutes with normal mouse serum to mask any Fc receptors to which the monoclonal antibody might have adhered. No difference was found with or without the masking step, and so this was discontinued. The cells were examined under oil in a Leitz Ortholux fluorescence microscope with a Fluorotar objective. Staining of HLA-DR was standardised using the HLA-DR negative and positive cell lines K562 and B-JAB repectively. The K562 is a transformed human erthyroid

precursor cell line, which lacks the HLA-DR antigen. It was kindly supplied by Dr. M.J. Lesko, Dept. of Immunology, Western Infirmary, Glasgow. The HLA-DR positive cell line, B-JAB, is a human lymphoma cell line which was provided by Dr. W. Cushley, Dept. of Biochemistry, University of Glasgow. Staining of the B-JAB cell line was performed by Dr. A. Hughes.

The cells were observed using excitation wavelengths of 550nm. A minimum of 200 cells were counted and scored as positive only if a sharp peripheral fluorescence was observed.

4.5.4 Statistical Analysis

Spearman rank correlation and sum of ranks were used as appropriate.

4.6. RESULTS

4.6.1. Uptake and Killing of S.epidermidis by Peritoneal Cells.

In most cases the peritoneal cells from the CAPD patients were efficient at ingesting and killing opsonised S.epidermidis (Table 16). The majority were able to ingest over 80% of the test culture from patients and in only 2 samples (A.Ca and L.McL) were less than 60% of the organisms ingested. Intracellular killing of the ingested organisms was normally >80% with again only 2 samples, from patients A.Ca and I.Sc, giving <60% killing. Seven of the 8 cell samples obtained from normal individuals ingested >80% of the organisms and for the remaining sample (sample number 1, Table 17) the figure was 68%. In all these samples the intracellular killing was virtually 100% (Table 17). Overall, the peritoneal macrophages from normal individuals were no more efficient at ingesting S.epidermidis than were the cells from CAPD patients ($p>0.05$), but they were significantly better at killing the ingested organisms ($P<0.001$).

4.6.2. Relationship Between Phagocytic Activity of Cells and Frequency of Peritonitis in CAPD Patients.

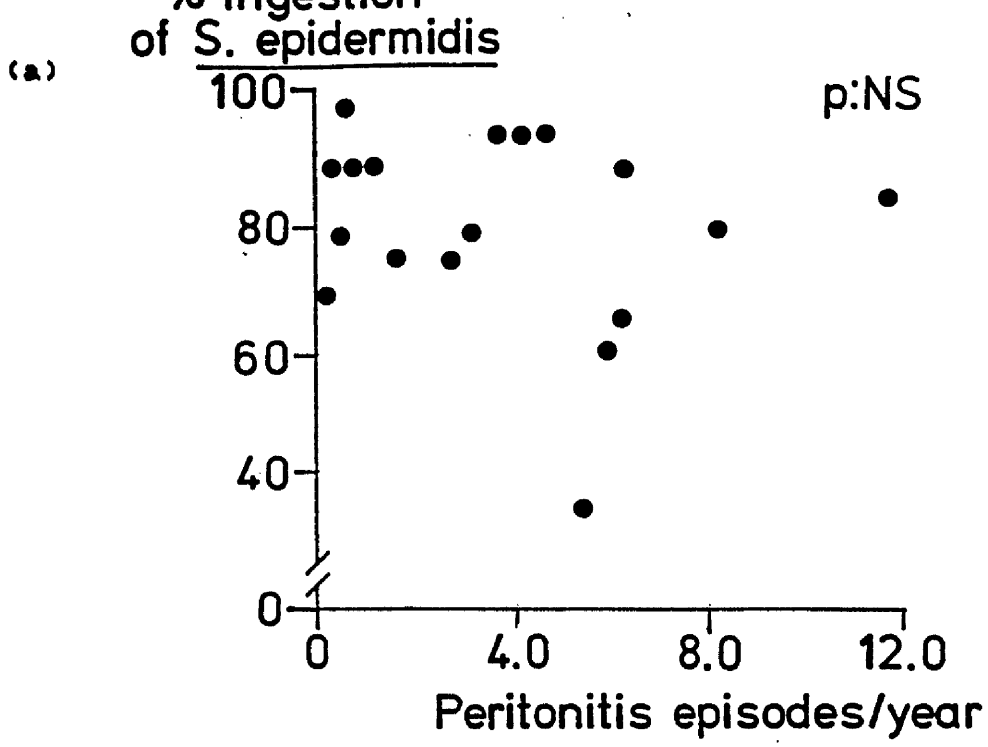
There was no correlation between the degree of ingestion or of intracellular killing by cells from CAPD patients with the frequency of episodes of peritonitis (Figure 17). However, in Table 16 it can be seen that cells

TABLE 16 : Ingestion and Intracellular Killing of S.epidermidis by CAPD Peritoneal Cells

CAPD PATIENTS' INITIALS	% OF S. EPIDERMIDIS INGESTED	% OF INGESTED S.EPIDERMIDIS KILLED	FREQUENCY OF PERITONITIS (see Table 2)
A.Ca	32.1	48.0	6.9
	64.7	97.8	5.3
	61.4	73.3	5.5
W.Ch	70.2	100.0	1.3
	74.5	80.5	2.2
W.Co	98.2	95.9	0
	75.4	80.6	6.0
	64.4	67.7	6.0
C.Fe	83.1	97.5	12.0
A.Fu	78.1	79.4	0
B.Go	97.8	90.5	0
W.Ka	70.6	78.2	0
R.Le	90.1	80.6	12.0
	81.4	62.0	8.0
A.Me	80.2	93.0	3.8
	93.7	91.2	3.6
T.Mo	91.0	94.5	0
M.Re	87.4	88.4	4.0
	62.8	87.7	4.0
	95.0	99.2	3.7
C.McG	85.1	97.4	0
	81.8	84.6	2.7
L.McL	36.9	88.6	5.1
M.McV	93.6	92.3	4.4
P.McV	88.5	82.6	0
I.Sc	72.9	57.2	1.0
W.St	90.4	88.2	6.0
A.Wa	89.2	94.2	0
Median	81.6	88.3	3.8
Lower quartile	70.3	79.7	0
Upper quartile	90.3	94.4	5.9

TABLE 17 : Ingestion and Intracellular Killing of S.epidermidis by Normal Peritoneal Cells.

NORMAL PERITONEAL CELL SAMPLE NUMBER	% OF S.EPIDERMIDIS INGESTED	% OF INGESTED S.EPIDERMIDIS KILLED
1	67.8	98.5
2	89.6	96.3
3	93.4	100.0
4	86.0	100.0
5	96.9	100.0
6	98.5	99.9
7	84.7	99.6
8	90.0	99.9
Median	**89.8**	**99.9**
Lower quartile	**85.0**	**98.8**
Upper quartile	**96.0**	**100.0**



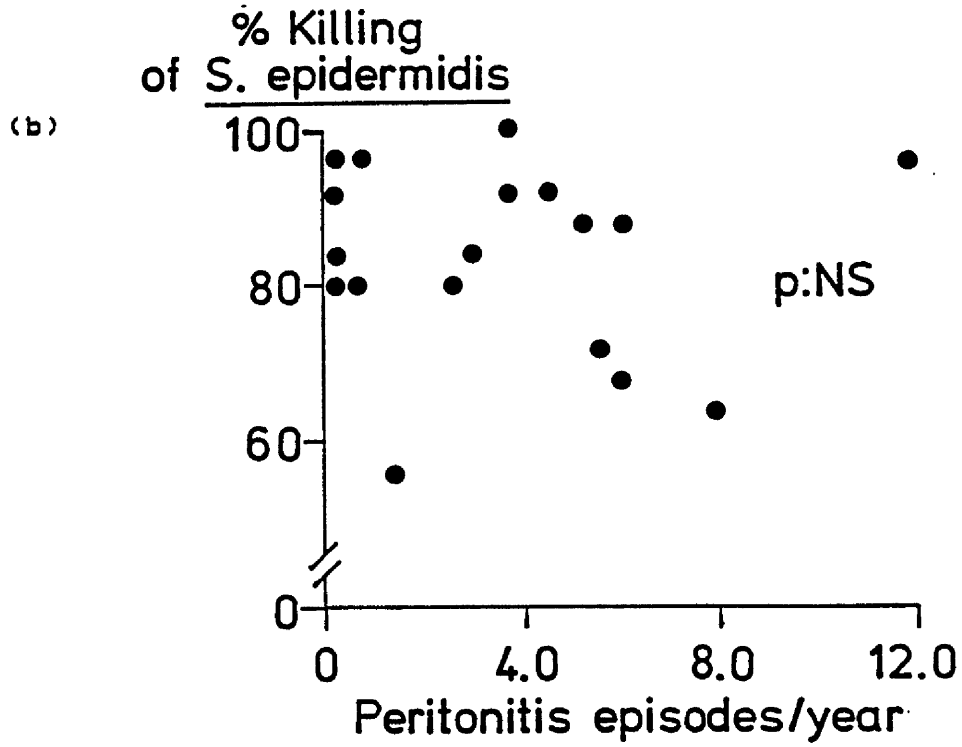


FIGURE 17 :

(a) Comparison of Ingestion of S.epidermidis with Frequency of Peritonitis (Data from Table 16).

(b) Comparison of Intracellular Killing of Ingested S.epidermidis with Frequency of Peritonitis (Data from Table 16).

obtained from one patient (A.Ca) with a high incidence of peritonitis (6.9 episodes per year on average) showed a low level of ingestion, and similar results were obtained with two subsequent samples from the same patient over a six month period, the average ingestion being 53±15% for the three samples. In two of these, intracellular killing was also low (48% and 73% of the ingested bacteria). The cells in a single sample obtained from another patient (L.McL) with a high incidence of peritonitis (5.1 episodes per year on average) also showed very poor ingestion (37%), though intracellular killing was normal. Thus although in most cases peritoneal macrophages from CAPD patients appear to efficiently ingest and kill S.epidermidis it is possible that in isolated patients this function may be impaired and this could be a cause of the patient being more prone to peritonitis.

It should be noted that for statistical reasons only one sample from each patient was used to relate bactericidal activity to frequency of peritonitis. Where more than one sample was tested the last sample was used in each case.

4.6.3. H_2O_2 Release by Peritoneal Macrophages and Monocytes

Peritoneal cell samples from the PDE of 10 new and 22 established CAPD patients as well as 9 normal peritoneal cell samples from women undergoing laparoscopy for sterilisation, and 7 normal peripheral blood monocyte samples were analysed for their capacity to produce H_2O_2, with and without stimulation with opsonised zymosan.

The amounts of H_2O_2 produced both on stimulation with

zymosan and spontaneously is shown in Table 18. A significantly greater amount of H_2O_2 was released by macrophages from PDE of new CAPD patients, compared with all the other cell types examined ($p<0.005$). Release of H_2O_2 by normal peritoneal macrophages was also significantly higher than that from macrophages from established CAPD patients and from blood monocytes ($p<0.01$). However no significant difference in H_2O_2 release was found between peritoneal macrophages from established CAPD patients and normal blood monocytes. The amount of H_2O_2 released spontaneously by all cell types was very small (Table 18).

4.6.4. Expression of HLA-DR Antigen by Human Peritoneal Macrophages and Monocytes.

Peritoneal cell samples obtained from the PDE of 12 new and 18 established CAPD patients, as well as 17 normal peritoneal cell samples and 9 normal blood monocyte samples were stained with anti HLA-DR monoclonal. Results are shown in Table 19.

Considerable variation in the level of HLA-DR expression between different samples was seen. The intensity of the staining also varied extensively within a sample, from virtually nothing through faint surface staining to well defined peripheral rings. As a consequence of this, cells were initially only classified as positive if an intense well-defined peripheral ring was observed. These are the cells labelled "strong" in Table 19. An example of the staining is shown in Figure 18.

However the proportion of HLA-DR positive monocytes

TABLE 18 : H_2O_2 Release by Peritoneal Macrophages and Monocytes.

	PERITONEAL MACROPHAGES FROM:						NORMAL BLOOD MONOCYTES	
	PATIENTS ESTABLISHED ON CAPD		NEW CAPD PATIENTS		NORMAL PERITONEAL CELLS			
ZYMOSAN	+	-*	+	-*	+	-*	+	-*
NUMBER OF SAMPLES	22	13	10	10	9	7	7	5
H_2O_2 RELEASE (µm/10µg DNA):								
MEDIAN	6.4	ND	13.4	0.3	10.6	ND	7.1	ND
LOWER QUARTILE	5.0	ND	12.3	ND	9.2	ND	6.3	ND
UPPER QUARTILE	8.6	0.2	14.8	1.8	11.4	ND	7.7	ND

* Only determined when enough cells were available in each sample

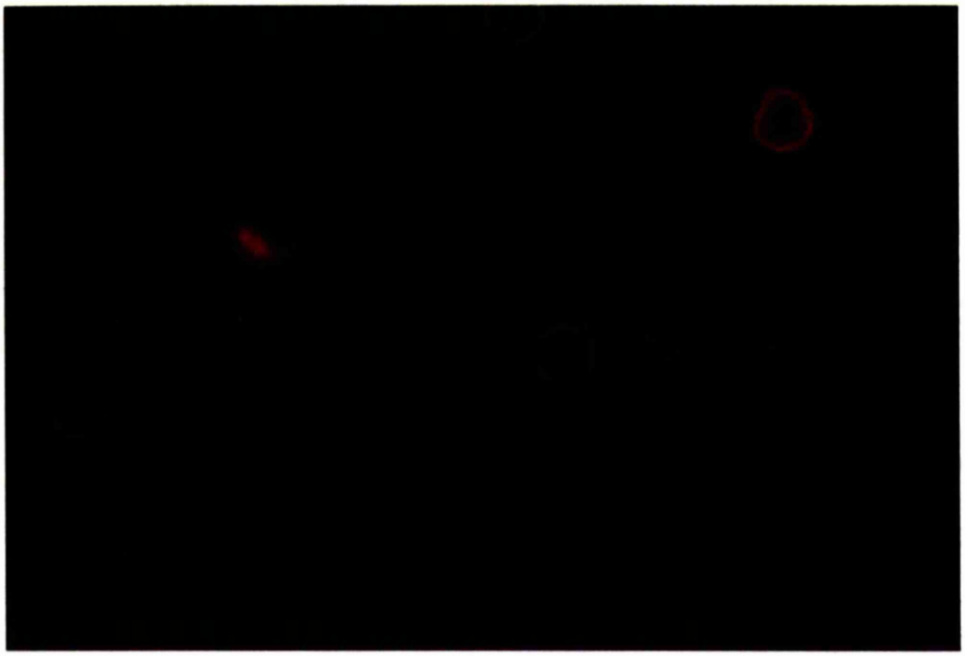
ND= not detectable

TABLE 19 : Expression of HLA-DR by Peritoneal Macrophages and Monocytes.

	CAPD PERITONEAL MACROPHAGES FROM:							
	PATIENTS ESTABLISHED ON CAPD		NEW CAPD PATIENTS		NORMAL CONTROLS		NORMAL BLOOD MONOCYTES	
	STAINING INTENSITY:							
	STRONG	FAINT*	STRONG	FAINT	STRONG	FAINT	STRONG	FAINT
NUMBER OF SAMPLES	18	8	12	6	17	9	9	7
% OF HLA-DR +VE CELLS:								
MEDIAN	38	60	68	84	59	88	39	68
LOWER QUARTILE	34	56	60	78	48	74	36	64
UPPER QUARTILE	49	64	81	90	62	90	46	70

* The designation of HLA-DR positive macrophages was extended to include not only those cells with strong peripheral staining but also those which were faint. Such a recount was only performed on a representative proportion of macrophage samples. See text for relevance of these results.

(a)



(b)

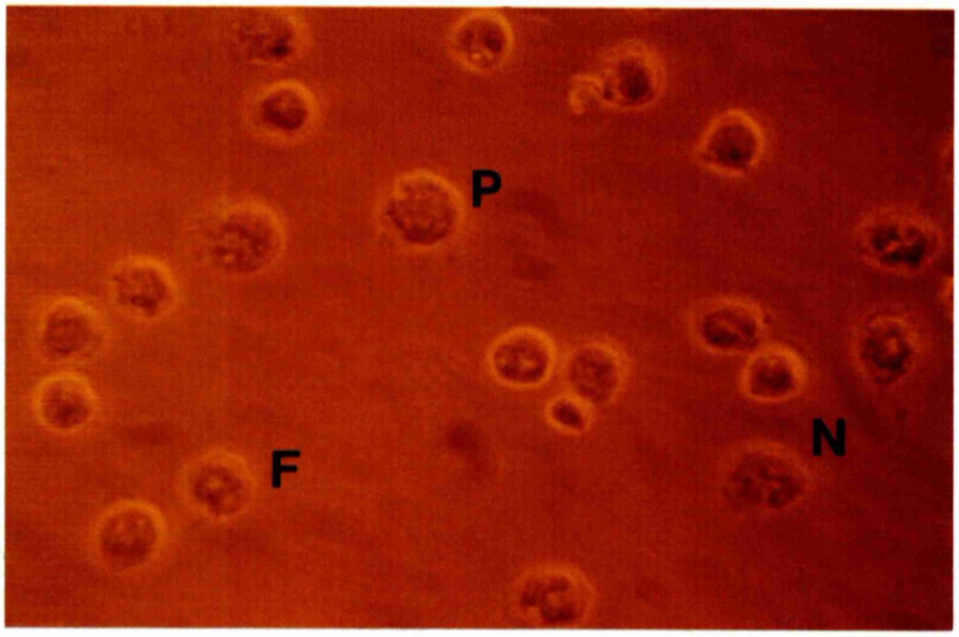


FIGURE 18 :

(a) Staining of CAPD peritoneal cells with a phycoerythrin-conjugated monoclonal antibody to HLA-DR antigen.

(b) Same field as (a) seen under phase-contrast

P — positive cell with sharp peripheral cell fluorescence
F — faint fluorescence
N — negative cell

thus obtained was considerably less than the 60%, 70% or virtually 100% positivity quoted by others (Volk et al,1985; Ng et al,1982; Smith and Ault,1981). In order to account for this difference some of the samples were re-examined, with the classification of HLA-DR positive cells being extended to include those cells with faint staining. These figures are also shown in Table 19. As a result, the proportion of HLA-DR positive monocytes was nearly doubled from a median of 39% to 68%, a figure more in line with those reported previously. The proportion of HLA-DR positive cells therefore depends to a large extent on the criteria used for classifying cells as either positive or negative.

Nevertheless, when only the strongly-staining cells were counted a significantly greater proportion of HLA-DR positive cells were found in macrophages from PDE of new CAPD patients compared with all the other macrophages ($p<0.01$). Expression of HLA-DR by normal peritoneal macrophages was also greater than in macrophages from established CAPD patients or blood monocytes ($p<0.01$). The proportion of HLA-DR positive cells in peritoneal macrophages from patients established on CAPD and in blood monocytes did not differ significantly. If however this classification was extended to include faint staining as well as strong, the proportion of HLA-DR positive cells was found to be greater in normal blood monocytes than in peritoneal macrophages from patients established on CAPD.

4.7. DISCUSSION

Investigation of the activity of cells from the peritoneum of patients on CAPD was undertaken in order to detect any defects which might possibly account for the increased incidence of peritonitis observed with certain patients. It was also important to compare the properties of these peritoneal cells with normal peritoneal cells to give an indication of possible effects of the CAPD process itself on the immune system of the peritoneum.

4.7.1. Bactericidal Activity of CAPD Peritoneal Cells

The peritoneal cells, the majority of which were macrophages, in the PDE of uninfected CAPD patients were able in most cases to efficiently phagocytose and kill opsonised S.epidermidis. However, when compared with peritoneal macrophages obtained from normal individuals undergoing laparoscopy, the cells from CAPD patients were significantly less efficient at killing ingested organisms, although there was no difference in the degree of ingestion. Lamperi and Carozzi (1986a) also found deficiencies in the killing ability of some CAPD patients' peritoneal macrophages. These workers, however in contrast to the results reported here, also found that cells from CAPD patients with high peritonitis rates were significantly less able to kill bacteria than those from normal subjects. The reason for this difference is not clear, although the bactericidal assays employed were different. The reduction in bactericidal activity of the

peritoneal macrophages observed by Lamperi and Carozzi (1986a) correlated directly to their ability to release PG suggesting that the cells in the peritoneum of CAPD patients are in an inflammatory environment, their cellular activity being suppressed by the PG which is a well known regulator of inflammation (reviewed by Gemsa,1981).

The reduced ability of CAPD peritoneal cells to kill ingested bacteria may be due to the immaturity of the macrophages obtained from CAPD patients as they are thought to be recently arrived and relatively immature cells, whose microbicidal mechanisms may not be fully developed. Goldstein et al (1984) found that peritoneal cells from CAPD patients exhibited a significantly lower uptake of eicosanoid precursors (ie arachidonic acid) compared with normal peritoneal macrophages, a finding which suggests immaturity as incorporation of these fatty acid precursors appears to increase with cell maturity (Pawlowski et al, 1983; Scott et al, 1980). In addition the absence of ecto-5' nucleotidase (Goldstein et al,1984) suggests that these peritoneal cells are blood-derived monocytes which have not yet differentiated into macrophages, as peripheral blood monocytes in humans lack detectable quantities of this enzyme (Edelson, 1981) but accumulate large ammounts as they mature into macrophages either in vivo or in vitro. The continuous removal of 30-40 million peritoneal cells daily may stimulate bone marrow replacement of resident cells at an excessive rate, so that instead of the peritonea of patients on CAPD containing mature tissue macrophages, they may instead be populated by cells more akin to immature blood monocytes. The increased

chemotactic activity of these cells shown by Goldstein et al (1984), may again reflect this immaturity as Wilkinson (1982) has previously shown that young mononuclear cells elicited by an inflammatory stimulus are more chemotactially active than are their older equivalents in the form of resident macrophages.

An alternative explanation for the decreased bactericidal activity of CAPD patients' peritoneal macrophages could relate to the effect the dialysis fluid may have on their function. CAPD involves constant contact with commercial dialysis solutions which when instilled are relatively hypertonic and acidic (Duwe et al,1981). Before the fresh dialysate approaches isotonicity and physiological pH, the cells may be significantly damaged. Duwe et al (1981) found that commercial dialysis fluid suppressed the activity of peripheral blood leucocytes as measured by chemiluminescence, phagocytosis and bacterial killing. Suppression was found to be due to the low pH and high osmolality of the fluid. Alobaidi et al (1986) also found that PDE suppressed peritoneal macrophage function, although the effect decreased with increasing dwell time. How important this finding is to the results reported here is debatable as Alobaidi et al (1986) also found that overnight PDE, from which all the cell samples here were obtained, did not affect cellular function. It is possible however that the initial hour after each exchange, before the PDE has had time to adjust, may severely hamper the activity of the phagocytes and elimination of any contamination at this point may be inadequate. Patients may therefore be particularly at risk each time an exchange is

performed.

Even if cellular activity were normal the large fluid volume may disrupt the usual intraperitoneal flow and clearance of bacteria from the peritoneum (Dunn et al,1985; Duwe et al,1981; Vas,1981; Hau et al, 1979). This failure of the normal transport mechanism may mean that antigen will not be delivered to the lymph nodes, which is essential for the initiation of specific immune responses and the resultant effective elimination of pathogens from the peritoneum. In addition it is possible that the antigen presenting function may be defective due to the immaturity of the peritoneal macrophages, or the suppressive effect of the PDE (Duwe et al,1981) may also severely inhibit the mounting of these specific immune responses. Experiments by Duwe et al (1981), and Verbrugh et al (1983), also suggest that the concentration of peritoneal macrophages in PDE may not be sufficient to handle bacteria that gain entrance to this site. In vivo, less than 100 phagocytic cells per mm^3 of blood (as in granulocytopenic patients) is well known to predispose patients to bloodstream infections. Only during peritonitis is this number reached in CAPD patients.

4.7.2. Relationship of Microbicidal Activity of CAPD Peritoneal Cells to Frequency of Peritonitis.

Overall there was no correlation between either ingestion or killing of S.epidermidis by macrophages from CAPD patients and frequency of peritonitis, indicating that an intrinsic defect in phagocyte function is not likely to be a major cause of increased susceptibility to peritonitis. However, in two cases (patients A.Ca and

L.McL), cells from patients with a high incidence of peritonitis did show reduced ability to ingest and/or kill S.epidermidis, suggesting that in some patients defective phagocytic activity may be a contributing factor. Indeed, recurrent or persistent Staphylococcal infections seen in some patients due to intraleucocyte sequestration of bacteria, (Buggy et al,1984) may possibly result from defective intracellular killing.

4.7.3. Ability of Cells to Release H_2O_2

In view of the importance of reactive oxygen intermediates in the process of intracellular killing and as a marker of the activation state of the cells, the amount of H_2O_2 released by peritoneal macrophages from CAPD patients and controls was examined. Blood monocytes from normal controls and peritoneal macrophages from patients established on CAPD appear to be analogous in terms of their ability to release H_2O_2 which would support the previous suggestion in section 4.7.1 that the CAPD cells are relatively immature. Alternatively, as CAPD patients have a tendency to anaemia (personal communication; J.D. Briggs), the lower than normal H_2O_2 levels released by CAPD peritoneal macrophages might be due to insufficient metabolically active intracellular iron (Thompson et al,1986) which is believed to be involved in O_2 metabolism (eg as a component of cytochromes; Brock and Mainou-Fowler,1986). Low O_2 tension in the peritoneal fluid, possible due to the adverse effect of the large fluid volume as suggested by Duwe et al (1981), may also impair H_2O_2 production, an effect which may not necessarily

be alleviated after two hours in culture.

In contrast, the greater amount of H_2O_2 released by normal peritoneal macrophages compared with those from patients established on CAPD agrees with their increased bactericidal activity, reflecting their increased maturity. The increased H_2O_2 production by peritoneal macrophages from new CAPD patients, compared with normal peritoneal macrophages, which in theory should be analogous, may be due to activation of the CAPD peritoneal macrophages. This may possibly be brought about indirectly via IL-1, which is produced in response to steroids such as estrogens (Flynn, 1986) released by an inflammatory reaction to the catheter implantation a fortnight before. IL-1 will in turn stimulate production of lymphokines (Kampschmidt,1984; Staruch and Wood,1983) such as gamma IFN, which is known to increase H_2O_2 production by cells (Wilson and Westall,1985; Nathan et al,1983). An alternative cause of the increased H_2O_2 release by peritoneal macrophages from new CAPD patients could be factors secreted by polymorphs (Bird et al,1984). Polymorph supernatants, lysates, and their respective ultrafiltrates were found to enhance the chemiluminescence responses of normal macrophages. The peritoneal cells from these new CAPD patients contained 15.5% of neutrophils compared with 3.8% in patients established on CAPD.

In contrast to the results reported here, Peterson et al (1985) found that CAPD patients' peritoneal macrophages generated greater chemiluminescence than normal resident peritoneal macrophages, presumably reflecting their increased ability to produce O_2^-. A possible

explanation for this may be that chemiluminescence measures an earlier step in the chain of the respiratory reaction than H_2O_2 release and therefore O_2^- levels may not correspond to H_2O_2 levels. Secondly unlike the H_2O_2 assay described in this chapter, which was performed on adherent cells, the chemiluminescence was performed on cells in suspension. As a result the average polymorph contamination of the CAPD patients' cells in the study of Peterson et al (1985) was 6% compared with 1% in the present work. This is important as MacGowan et al (1983), have shown that each polymorph produces approximately four times the chemiluminescence of every macrophage and therefore any polymorph contamination might artificially raise results. In addition, Peterson et al (1985), were comparing CAPD cells containing 6% polymorphs, with suspensions of normal peritoneal cells in which the polymorph content was only 2%. In the studies reported here, there was less than 2% neutrophil contamination in each case (4.5.1).

4.7.4. HLA-DR Expression by Peritoneal Macrophages and Monocytes.

The antigen presenting function of CAPD peritoneal macrophages, as well as their maturational or activational state, was investigated indirectly via the proportion of HLA-DR positive cells.

Large differences exist in the percentage expression of HLA-DR found on mononuclear phagocytes by different groups. Costabel et al (1986), found that more than 90% of alveolar macrophages expressed HLA-DR whereas Razma et al (1984), found much lower levels (21%). Some groups were

unable to detect HLA-DR negative subsets among human peripheral or peritoneal macrophages (Smith and Ault, 1981) while other studies have found negative cells (Ng et al, 1982). This discordance may be due to the different sensitivities of the methods used. Costabel et al (1986) used immunoperoxidase which was more sensitive than direct immunofluorescence used by Razma et al (1984), and direct immunofluorescence used in this work. Costabel et al (1986) may therefore have classified as positive cells that expressed only small amounts of HLA-DR antigen on their surface. Secondly Razma et al (1984) studied adherent macrophages after 24 hours culture rather than macrophage preparations made immediately after isolation. Cells in culture may alter their surface marker characteristics. Differences in the reactivity and specificity of different anti HLA-DR antibodies used by different groups may also account for discrepancies between the results of different groups. Clerici et al (1984) showed that variable results were obtained for HLA-DR expression depending on the monoclonal antibody used, suggesting that not all anti-HLA-DR antibodies recognise the same antigenic determinant.

Peritoneal macrophages from new CAPD patients were found to express a greater proportion of HLA-DR positive cells than macrophages from established CAPD patients, normal peritoneal macrophages, or blood monocytes. Again, as with H_2O_2 release, activation or stimulation of these cells with possible IL-1 production may be the cause. Flynn (1986) has shown with murine cells that the activation of Ia expression correlated with IL-1 production. This is also probably mediated through gamma

IFN, which is well known to increase HLA-DR expression on cells (Gershon et al,1985; Sztein et al,1984; Basham and Merigan,1983).

The similarity in the degree of HLA-DR expression by peritoneal macrophages from patients established on CAPD and by blood monocytes (also shown by Smith and Ault,1981), may, as with the amount of H_2O_2 released by these cells, again suggests that these CAPD macrophages are indeed immature cells closely similar to monocytes. Smith and Ault (1981) showed a 2-3 fold increase in expression of HLA-DR on culture of both blood monocytes and CAPD patients' peritoneal cells, which also suggests that the CAPD cells are immature monocyte-like macrophages, which mature on culture to resemble resident tissue macrophages.

If, however, the CAPD peritoneal macrophages from established patients were more like chronic inflammatory cells than monocytes, as suggested by Beelen et al (1986), then the decreased staining for HLA-DR compared with other cell types could be explained by the dampening effect of PG, which is released during inflammation (Humes et al,1977). Snyder et al (1982), have shown that PG markedly inhibited Ia expression on macrophages previously stimulated by lymphokines. PG may provide a physiological dampening mechanism that in concert with the positive stimulation of the T-cell lymphokines regulate the levels of Ia expression in a given population. Reduction of HLA-DR expression has also been suggested to be due to ROI which are released at inflammatory sites (Gruner et al,1986) and this might occur in the peritoneum of CAPD patients. ROI achieve their effect by influencing PG synthesis (Gruner

et al, 1986).

When the criteria used to classify cells as HLA-DR positive was extended to include not only those cells with bright peripheral staining, but also those with faint fluorescence, the average proportion of positive staining for monocytes was higher than that for the CAPD cells, which also may suggest that HLA-DR levels are suppressed in CAPD patients' peritoneal macrophages. It would also have been of interest here to examine blood monocytes from CAPD patients and compared HLA-DR expression on these with that found on the normal blood monocytes to see whether HLA-DR expression is depressed generally in patients on CAPD. Lamperi and Carozzi (1986a) found peritoneal macrophages from a group of high peritonitis rate CAPD patients released larger amounts of PG and lesser amounts of IL-1 than cells from CAPD patients with low peritonitis rates or from normal subjects. This could explain why these patients' cells show reduced HLA-DR expression. This also suggests that the normal equilibrium state which exists between the positive and negative signals is disturbed in some of these CAPD patients, who may overproduce PG and as a result suppress their immune responses. However the importance of PG in the regulation of HLA-DR expression has recently been called into question by the finding of Kunkel et al (1986), who reported that while PG did indeed depress Ia/HLA expression in murine cells no effect was found with human cells. It must also be noted that Goldstein et al (1984), indicated that CAPD cells had morphological characteristics of resting rather than stimulated mononuclear phagocytes, based on the lack of

membrane ruffling and characteristic organelle content, suggesting again that PG may not be responsible for the reduced HLA-DR expression on CAPD cells.

The suppression of HLA-DR antigen in CAPD patients might be beneficial, in that it could prevent or limit presentation of altered self determinants or unrevealed hidden antigens arising during tissue destruction by inflammatory effector mechanisms. The benefits of this decreased HLA-DR level may, however be outweighed by the simultaneous decrease in the peritoneal defences of these CAPD patients as reduced expression of HLA-DR in addition to functionally defective antigen presenting cells may severely compromise any mounting of specific immune responses. Indeed such a decrease in immune responsiveness has been noted in certain CAPD patients on challenge with DNCB (Tsakiris et al,1986).

In summary therefore it appears that at least some of the cellular activities of CAPD peritoneal cells are reduced in comparison with the population found in the normal peritoneum. This is shown both by their reduced bactericidal activity and by a decreased ability to release H_2O_2. There is also a reduced expression of HLA-DR antigen. These differences are probably caused primarily by the immaturity of the CAPD cells rather than by actual defects in their activity. This immaturity will weaken not only their importance in phagocytosis but may also impair other macrophage functions, such as interactions with T-lymphocytes. It is also clear that the peritoneal macrophages from new CAPD patients are not analogous to normal peritoneal macrophages but appears to have undergone

some kind of activation or stimulation, perhaps due insertion of the catheter.

CHAPTER 5

LEVELS AND FUNCTIONS OF IgG, C3 AND TRANSFERRIN IN PERITONEAL FLUID

5.1 INTRODUCTION

Up to this point the thesis has focused on the intrinsic properties of the CAPD macrophages, however efficient bactericidal activity of phagocytic cells generally requires that the micro-organisms be opsonised with molecules that facilitate their ingestion. Of these, IgG and C3, which represent the traditional heat-stable and heat-labile opsonising elements respectively, are generally considered to be the most important (Keane and Peterson,1984; Johnston and Stroud,1977; Stossel,1974; Winkelstein,1973).

The contribution of opsonic molecules to host defences against infection has been demonstrated in a variety of diseases (Johnston and Stroud,1977; Stossel,1974; Winkelstein,1973) and any quantitative deficiency of opsonins can seriously compromise the bactericidal function of phagocytic cells (Keusch et al,1982; Lew et al,1979). Indeed it has been suggested that infection of the peritoneal cavity by Gram-negative organisms in patients with alcoholic cirrhosis and ascites is related to a deficiency of heat-labile opsonins in ascitic fluid secondary to decreased complement levels (Simberkoff et al,1978; Fromkes et al,1977).

The frequent fluid changes involved in CAPD means that not only phagocytic cells, but also soluble proteins are constantly removed from the peritoneal cavity in the peritoneal dialysis effluent (PDE) and replaced by a large quantity of fresh fluid which is completely devoid of

both proteins and cells. This may adversely affect the normal antimicrobial activity of the peritoneal cavity. Indeed, it has been shown that the bactericidal activity of peritoneal macrophages and blood leucocytes suspended in PDE was impaired due to suboptimal levels of opsonins (Verbrugh et al, 1984; Verbrugh et al,1983), which means that even if the phagocytic activity of the peritoneal macrophages from CAPD patients were intact, (as appears to be the case from results reported earlier in this study), inadequate levels of serum proteins may severely undermine the defences of the peritoneum. Hence, while effective phagocytosis early in the course of bacterial invasion may limit the spread of bacteria and prevent ongoing infection, ineffective phagocytosis, due to inadequate levels of opsonins, may lead to uncontrolled bacterial multiplication and overwhelming infection.

Another serum factor involved in defence against infection is the iron transport protein transferrin (Tf), which binds any available iron and thereby helps to prevent bacterial growth, as iron is an essential nutrient for most bacteria (Brock, 1986). It may be that the dilutional effect mentioned above reduces the concentration of Tf in CAPD fluid to a level where it is no longer able to provide bacteriostatic activity.

It is generally believed that the proteins present in the dialysate, including IgG, C3 and Tf, mainly originate from the blood by passage through the peritoneal membrane, although local synthesis of some proteins by cells in the peritoneum has been suggested (Dulaney and Hatch,1984; Kowalewski et al,1971). Some authors reported elevated

albumin to globulin ratios in dialysate compared with those in serum (Gordon and Rubini,1967) suggesting some restriction to the passage of high molecular-weight proteins. Others were unable to find such differences in peritoneal protein clearances, and instead suggested that protein entered the peritoneum by free diffusion (Blumenkrantz et al, 1981).

In the light of this, the present study was undertaken to determine the concentration and antimicrobial activities of IgG, C3 and Tf in PDE from patients on CAPD and compare them with normal peritoneal fluid and sera, both from normal controls and CAPD patients. Finally, in order to ascertain whether any of these factors are of clinical significance with respect to susceptibility of patients to peritonitis, each of the above was related to the frequency of episodes of peritonitis experienced by each patient.

5.2 MATERIALS

5.2.1. Peritoneal Fluid Samples

Overnight dwell (10-12 hours) PDE was obtained from a total of 44 patients on CAPD, details of whom are given in Table 20. One or two samples were obtained from each patient, with at least two weeks between samples in the latter case. Only PDE samples where peritonitis had not occurred within three weeks either side of the date of the sample were used in this study. Three weeks was considered to be an adequate period for normalisation, as no sample taken outwith this period was found to contain more than 15% of neutrophils or more than 10^8 total cells in PDE (c.f. section 2.5.1). Samples of PDE were spun at 250g for 5 minutes to separate the cells and then stored in frozen aliquots. Normal peritoneal fluid, which was obtained by aspiration from 8 healthy women undergoing laparoscopy for sterilisation, was similarly treated.

5.2.2. Serum Samples

Serum samples from 17 CAPD patients were obtained at the same time as peritoneal fluid and stored at $-20^{\circ}C$ (see Table 20). Normal human serum (NHS) was obtained from healthy volunteers and stored at $-20^{\circ}C$. Some samples were pooled.

TABLE 20 : Details of Patients Used in Monitoring the Levels and Activities of Serum Proteins in PDE.

PATIENTS' INITIALS	SEX	AGE (YRS)	MONTHS ON CAPD	SYSTEM[+]	ORIGINAL DIAGNOSIS
R.Ad	male	59	21	T	Pyelonephritis
M.Ba	female	55	7	F	Glomerulonephritis
A.Ba	female	59	42	F	Polycystic Kidneys
A.Bl	male	70	9	T	Glomerulonephritis
*A.Ca	female	75	5	F	Glomerulonephritis
W.Ch	male	48	9	T	Diabetic Nephropathy
*R.Co	female	56	1	F	Polycystic Kidneys
R.Con	female	39	47	F	Glomerulonephritis
*W.Co	male	33	1	T	Unknown
C.Fe	male	53	2	T	Diabetic Nephropathy
A.Fu	female	42	2	F	Glomerulonephritis
*M.Ge	female	60	53	F	Hypertension
I.Jo	male	37	5	T	Glomerulonephritis
B.Go	male	33	1	T	Diabetic Nephropathy
*J.Gr	male	29	1	T	Glomerulonephritis
W.Ka	male	46	1	T	Glomerulonephritis
R.Ke	male	47	1	T	Glomerulonephritis
*R.Le	male	55	2	T	Diabetic Nephropathy
*M.McA	female	51	47	F	Hypertension
R.McA	male	64	4	T	Unknown
*M.McC	female	56	23	F	Analgesic Nephropathy
J.McF	male	48	45	T	Glomerulonephritis
*C.McG	female	62	6	T	Analgesic Nephropathy
J.McK	female	64	1	F	Hypertension
L.McL	female	55	7	F	Pyelonephritis

TABLE 20 : Cont.

PATIENTS' INITIALS	SEX	AGE (YRS)	MONTHS ON CAPD	SYSTEM[+]	ORIGINAL DIAGNOSIS
A.McL	male	51	1	T	Glomerulonephritis
*M.McV	female	54	8	T	Polycystic Kidneys
P.McV	male	61	1	T	Pyelonephritis
*A.Me	female	36	18	T	Glomerulonephritis
C.McC	male	47	18	T	Glomerulonephritis
*T.Mo	male	45	1	T	Glomerulonephritis
A.My	female	43	27	T	Polycystic Kidneys
*M.Mu	female	71	1	F	Glomerubnephritis
*C.Pa	female	68	48	F	Analgesic Nephropathy
*M.Re	female	64	6	F	Diabetic Nephropathy
R.Ro	male	39	1	T	Renal Tumour
B.Pa	female	50	9	F	Diabetic Nephropathy
I.Sc	female	49	36	T	Analgesic Nephropathy
E.Si	female	43	32	T	Glomerulonephritis
M.Sm	female	60	1	T	Polycystic Kidneys
W.St	male	75	2	T	Glomerulonephritis
*A.Wa ,	female	49	1	F	Polycystic Kidneys
J.Wi	male	51	10	T	Polycystic Kidneys
*W.Yo	male	44	27	T	Diabetic Nephropathy

[+] F = Fresenius System

T = Travenol System II

* Patients from whom a serum sample was also obtained

5.3 METHODS

5.3.1. Determination of Opsonic Activity

This was carried out by radiometric assay as described in Chapter 3. In each experiment, aliquots of *S. epidermidis* were opsonised for 15 minutes at 37°C with either NHS or the test fluid sample. A third aliquot, suspended in PBS, was used for turbidometric determination of the bacterial count. Suspensions of the total CAPD peritoneal cells (predominantly macrophages) were prepared as in section 2.4.1. The sample opsonised with NHS was included in each experiment in order to standardise conditions so that differences in cell activity would not affect the results. The opsonising capacity of the fluid was calculated as follows :-

$$\frac{\%\text{ of bacteria ingested when opsonised with sample}}{\%\text{ of bacteria ingested when opsonised with NHS}} \times 100$$

5.3.2. Determination of Bacteriostatic Activity

A suspension of 2×10^5 log-phase *S. epidermidis* in 50µl of PBS was added to 0.5ml of filter-sterilised peritoneal fluid or serum in a plastic microtube. Unresticted growth was determined in controls containing RPMI 1640 medium + 25mM Hepes (Flow Laboratories) instead of the sample. To all of the tubes 50µl of PBS was also added. In addition to evaluating the growth of bacteria in each of the peritoneal fluids and sera, the effect of

adding extra Tf to each of these was also tested in order to determine whether the level of Tf in each sample was optimal. This was achieved by addition of 50µl of 1mg/ml solution of iron-free human Tf (Behring-Hoechst, Hounslow, U.K.) in PBS instead of just PBS. Finally 20µl (2µCi) of [^{3}H]-uridine was added to each tube, and these were incubated at 37^{o}C for two hours. The samples were then harvested and counted as described in 3.3.2. Each sample was set up in triplicate. The bacteriostatic activity of the test fluid was calculated as the percentage growth in the test sample compared with the RPMI 1640 control. The effect on growth of S.epidermidis of addition of extra Tf was calculated as follows :-

Change in growth = (% growth in test sample) − (% growth in test sample + Tf)

5.3.3. Determination of Total Protein

Protein was precipitated with salicyl sulphonic acid and the turbidity of the suspension at 650nm compared to a standard curve. (This procedure was carried out by Dr. Spooner in the Biochemistry Department, Gartnavel General Hospital, Glasgow.)

5.3.4. Estimation of IgG, C3 and Transferrin Levels

This was carried out by radial immunodiffusion, using Partigen LC plates (Behring-Hoechst). Appropriate dilutions of standard normal human serum (Behring-Hoechst) were used to construct standard graphs.

5.3.5. Statistical Methods

The methods used were sum of ranks, signed rank, and Spearman rank correlation, as appropriate.

5.4 RESULTS

5.4.1. Levels of IgG, C3 and Transferrin in Peritoneal Fluids and Sera.

The levels of IgG, C3 and Tf in 38 of the 44 individual PDE samples were measured and compared with those in normal peritoneal fluid, CAPD patients' sera, and normal sera (Table 21). The levels of all three proteins in PDE were much lower than those in the sera or normal peritoneal fluid, the difference being of the order of 50 to 100 fold. Levels in normal peritoneal fluid did not differ significantly from those in normal sera, and the IgG and C3 levels in CAPD patients' sera were also similar to those in normal sera, although Tf levels were slightly lower ($p<0.05$).

When the results from individual samples were considered, a significant correlation was found between the levels of IgG, C3 and Tf ($p<0.001$) in PDE (Figure 19). No such correlation was found for normal peritoneal fluid or for sera (Figures 20,21 and 22). There was also no correlation between the levels of IgG, C3 or Tf in PDE and those in the corresponding patients' sera (Figure 23). In addition no significant difference in levels of IgG, C3 and transferrin in PDE was found when patients were grouped according to diagnosis (Table 22). In particular no difference was found between the levels of proteins in the PDE of diabetic and non-diabetic patients.

In 28 of the patients tested, the level of total

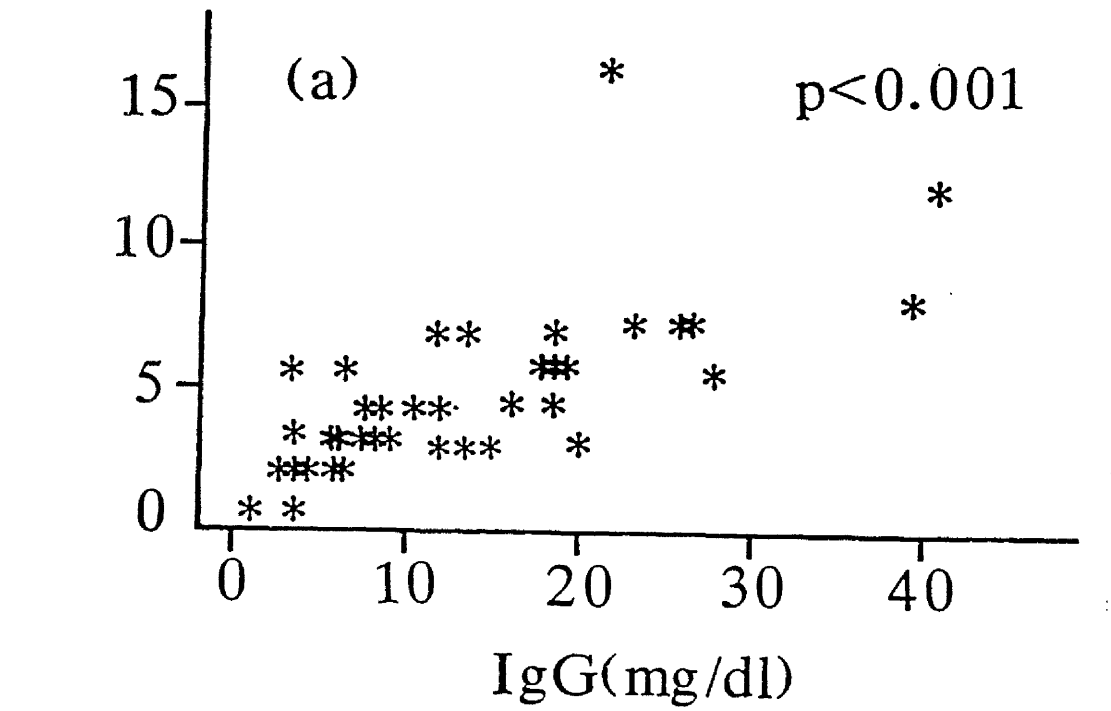
TABLE 21 : Serum Protein Levels in Peritoneal Dialysis Effluent, Normal Peritoneal Fluid and in Normal and CAPD Patients' Sera.

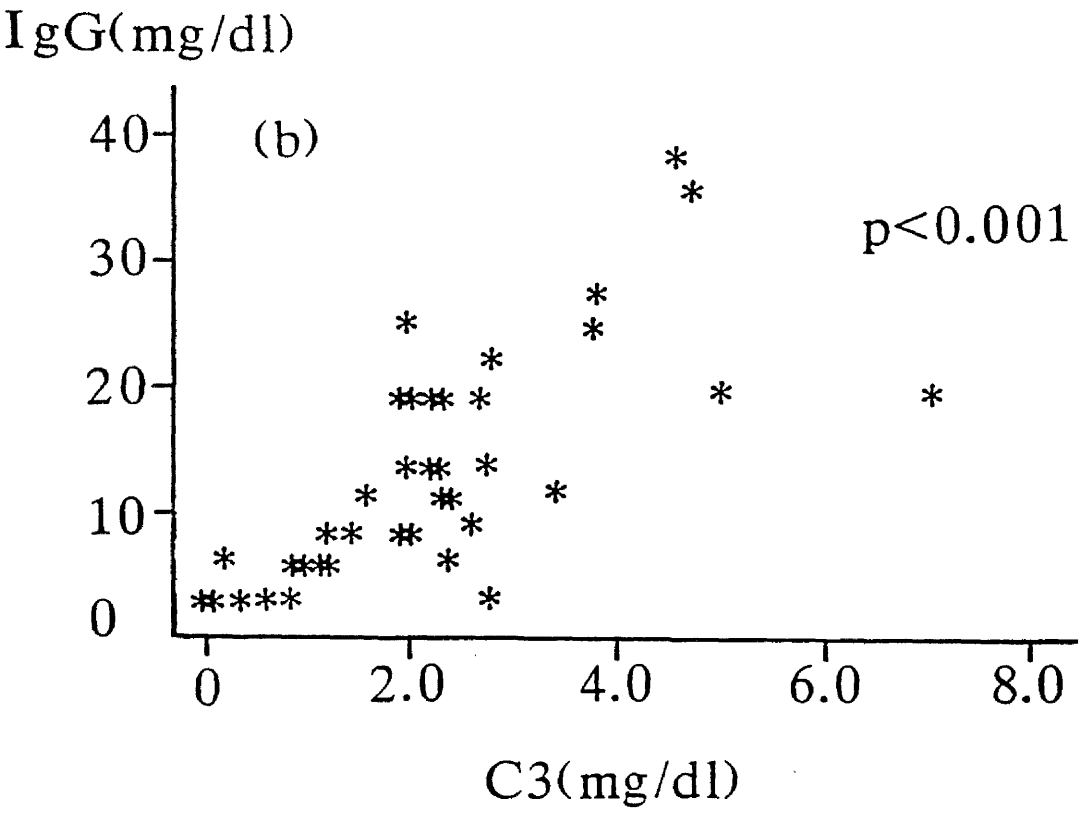
	PERITONEAL DIALYSIS EFFLUENT	NORMAL PERITONEAL FLUID	CAPD PATIENTS' SERA	NORMAL HUMAN SERA
NUMBER OF SAMPLES	38	8	17	6
IgG	12.7*+ (5.7-20.1)	1085 (958-1104)	1160 (705-1215)	1120 (1092-1185)
C3	2.2 + (1.2-2.8)	110 (100-118)	150 (122-185)	140 (130-170)
TRANSFERRIN	4.1 + (2.5-6.7)	225 (204-230)	200 # (168-242)	250 (214-305)

* Median (interquartile range). All figures are mg/dl

+ $p<0.001$ compared with normal human serum

# $p<0.05$ compared with normal human serum


TRANSFERRIN (mg/dl)
(a)
p<0.001
15
10
5
0
0
10
20
30
40
IgG(mg/dl)


IgG(mg/dl)
(b)
p<0.001
40
30
20
10
0
0
2.0
4.0
6.0
8.0
C3(mg/dl)

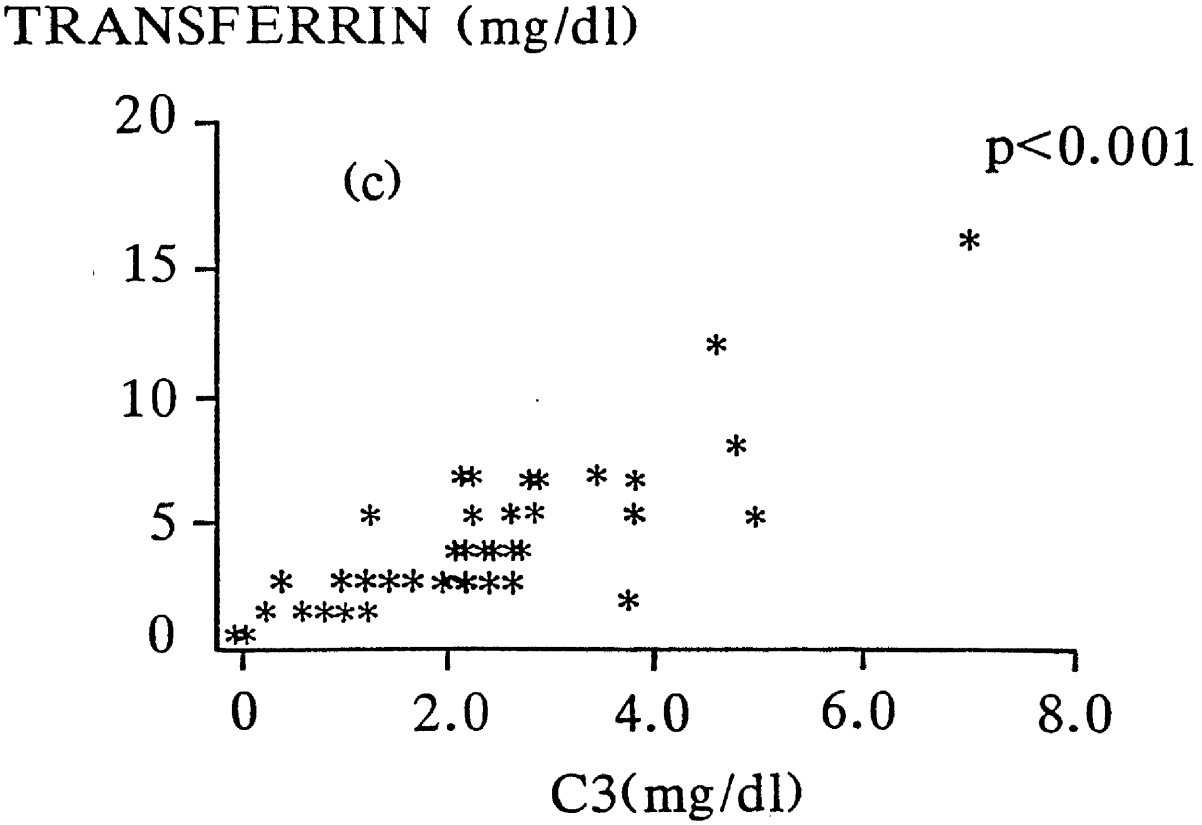
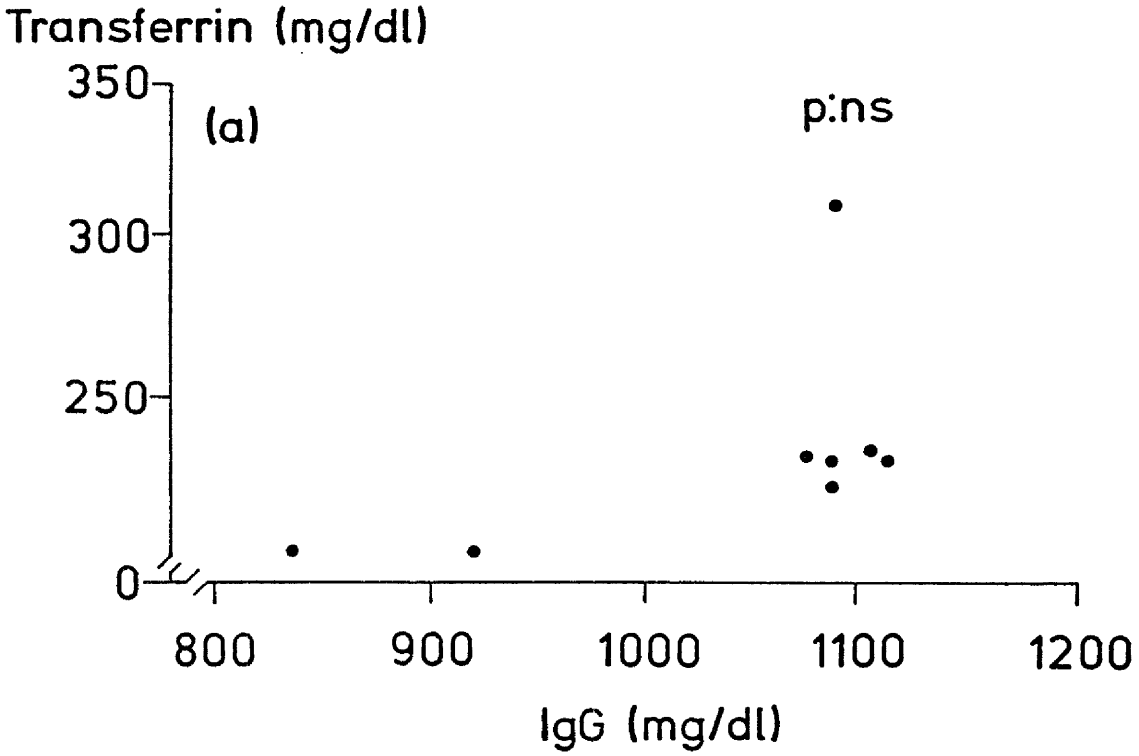


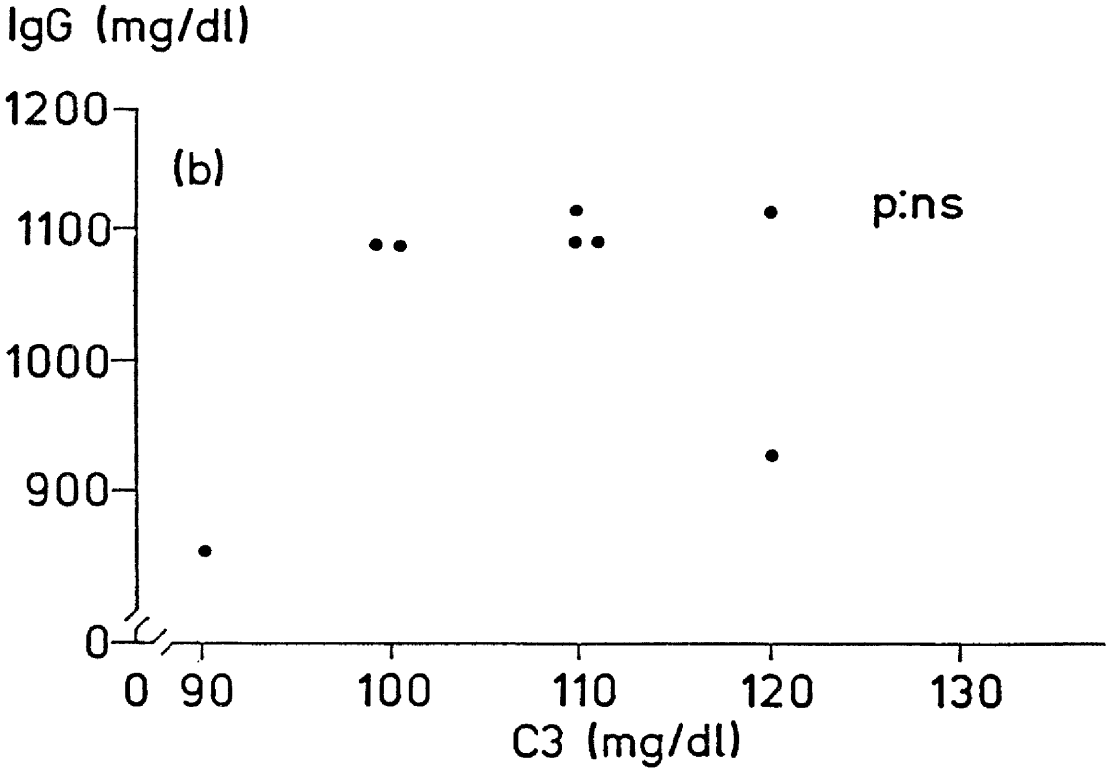
FIGURE 19 : Comparison of Levels of IgG, C3 and Transferrin in Peritoneal Dialysis Effluent from CAPD Patients.

(a) IgG compared with Transferrin

(b) C3 compared with IgG

(c) C3 compared with Transferrin


Transferrin (mg/dl)
350
300
250
0
(a)
p:ns
800
900
1000
1100
1200
IgG (mg/dl)


IgG (mg/dl)
1200
1100
1000
900
0
(b)
p:ns
0
90
100
110
120
130
C3 (mg/dl)

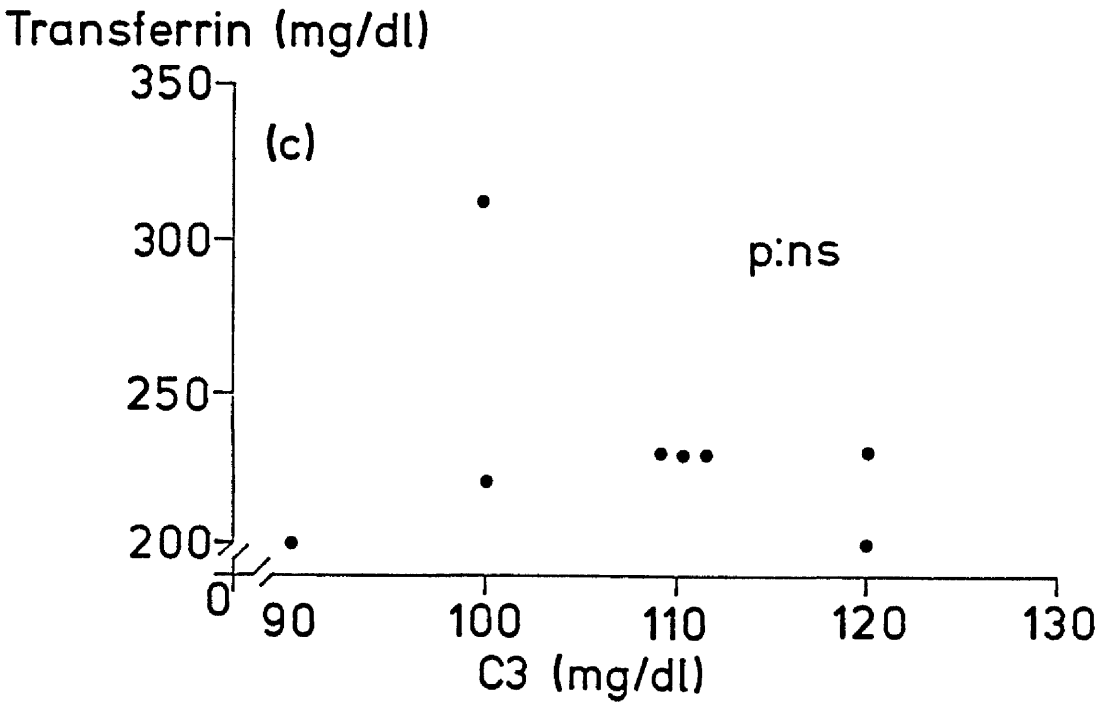
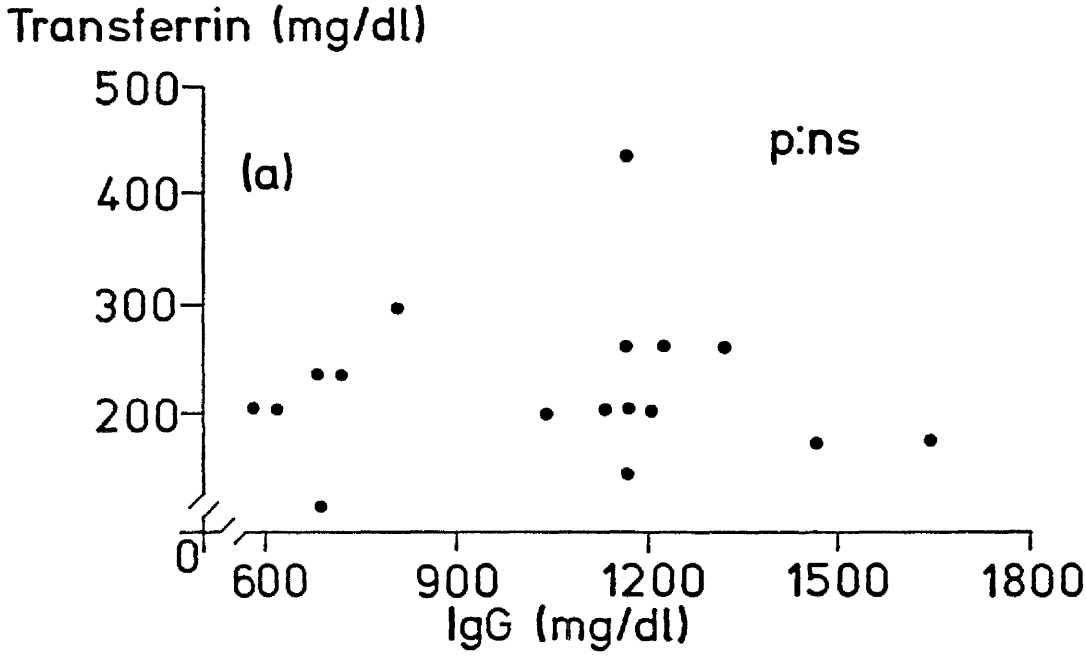


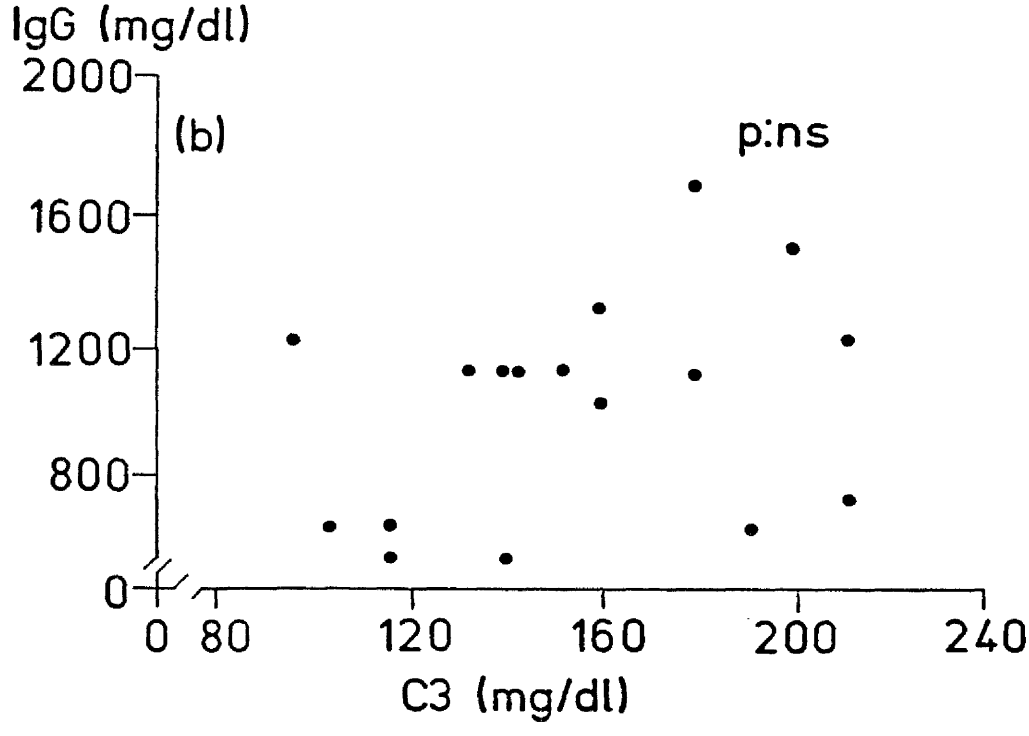
FIGURE 20 : Comparison of Levels of IgG, C3 and Transferrin in Normal Peritoneal Fluids

(a) IgG compared with Transferrin

(b) C3 compared with IgG

(c) C3 compared with Transferrin


Transferrin (mg/dl)
500
400
300
200
0
(a)
p:ns
600
900
1200
1500
1800
IgG (mg/dl)


IgG (mg/dl)
2000
1600
1200
800
0
(b)
p:ns
0
80
120
160
200
240
C3 (mg/dl)

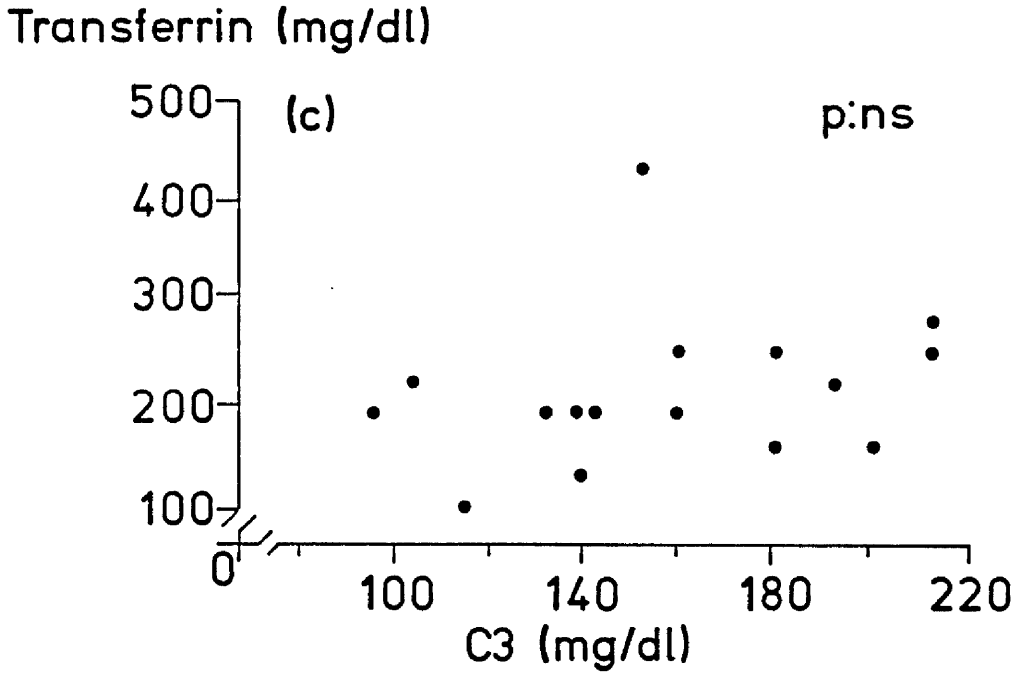
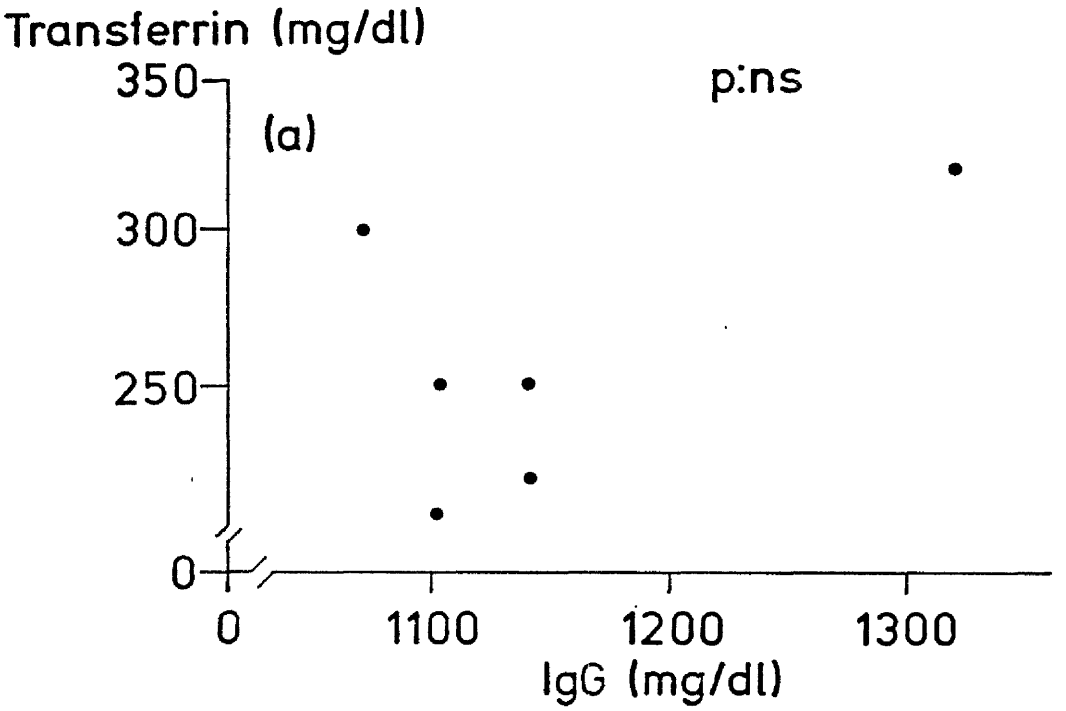


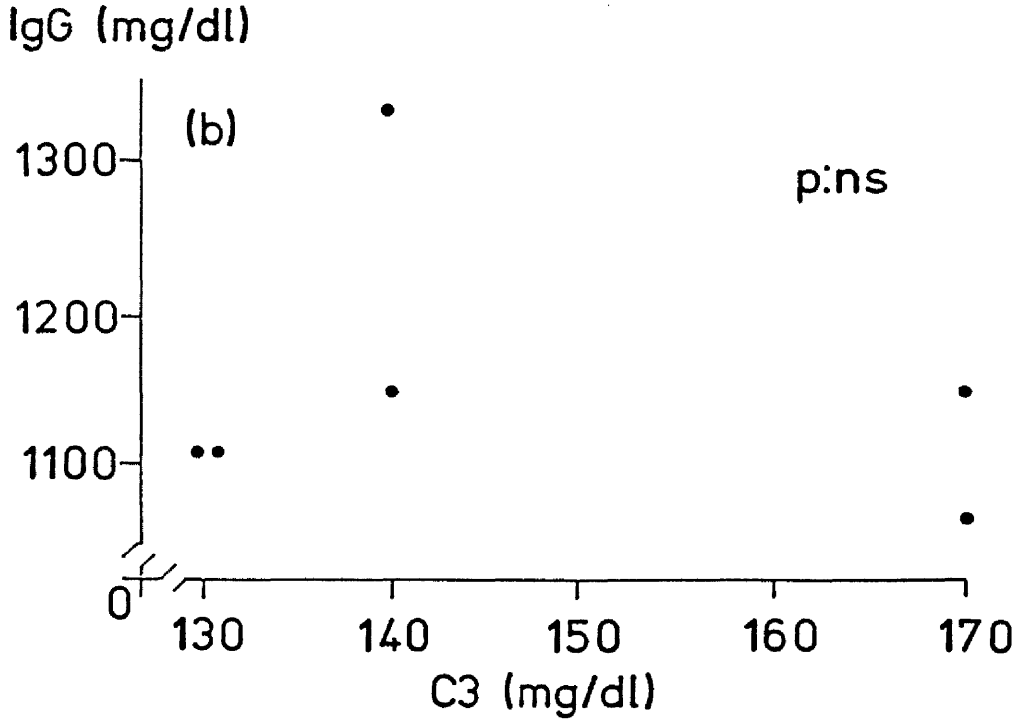
FIGURE 21 : Comparison of Levels of IgG, C3 and Transferrin in CAPD Patients' Sera

(a) IgG compared with Transferrin

(b) C3 compared with IgG

(c) C3 compared with Transferrin


Transferrin (mg/dl)
350
300
250
0
(a)
p:ns
0
1100
1200
1300
IgG (mg/dl)


IgG (mg/dl)
1300
1200
1100
0
(b)
p:ns
130
140
150
160
170
C3 (mg/dl)

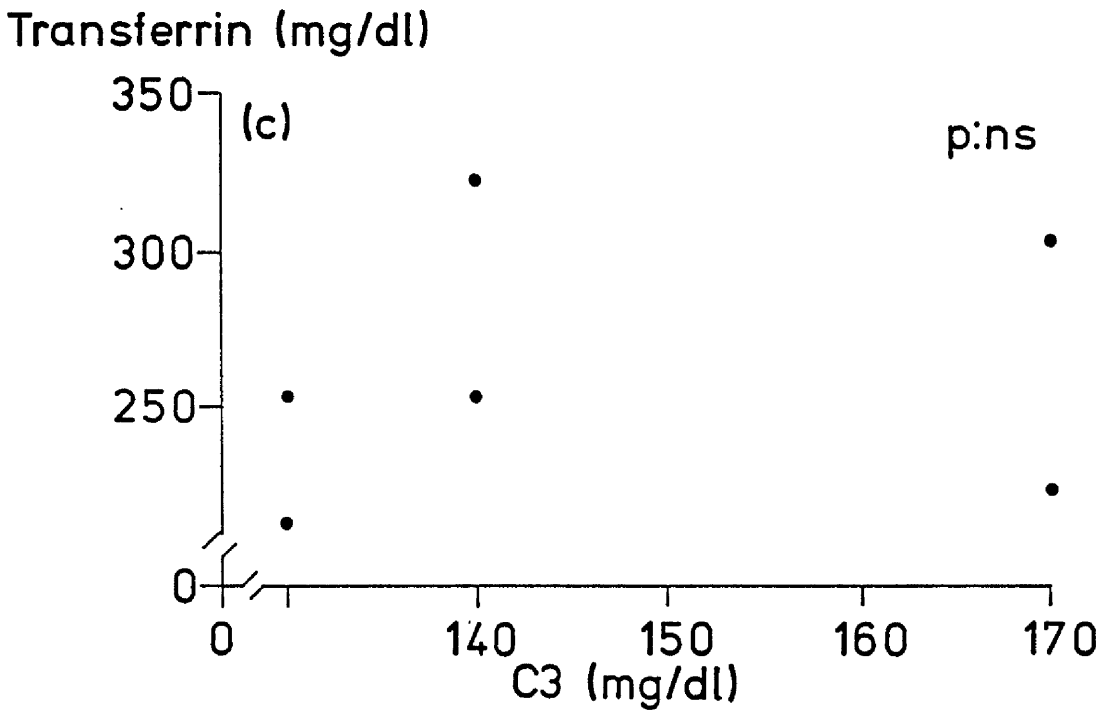
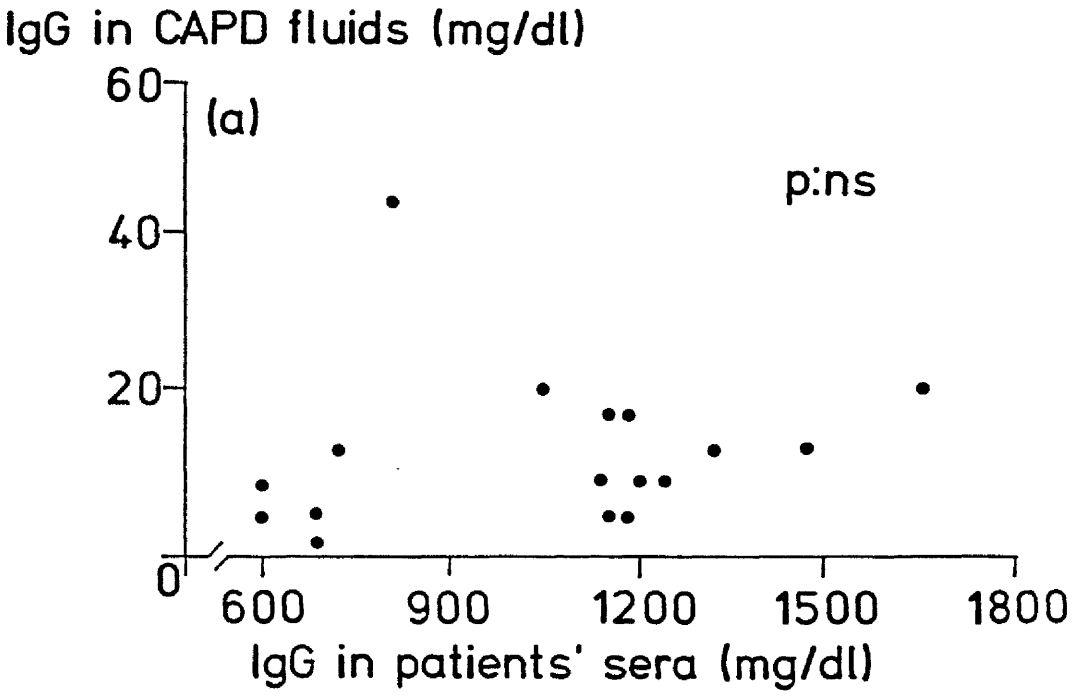


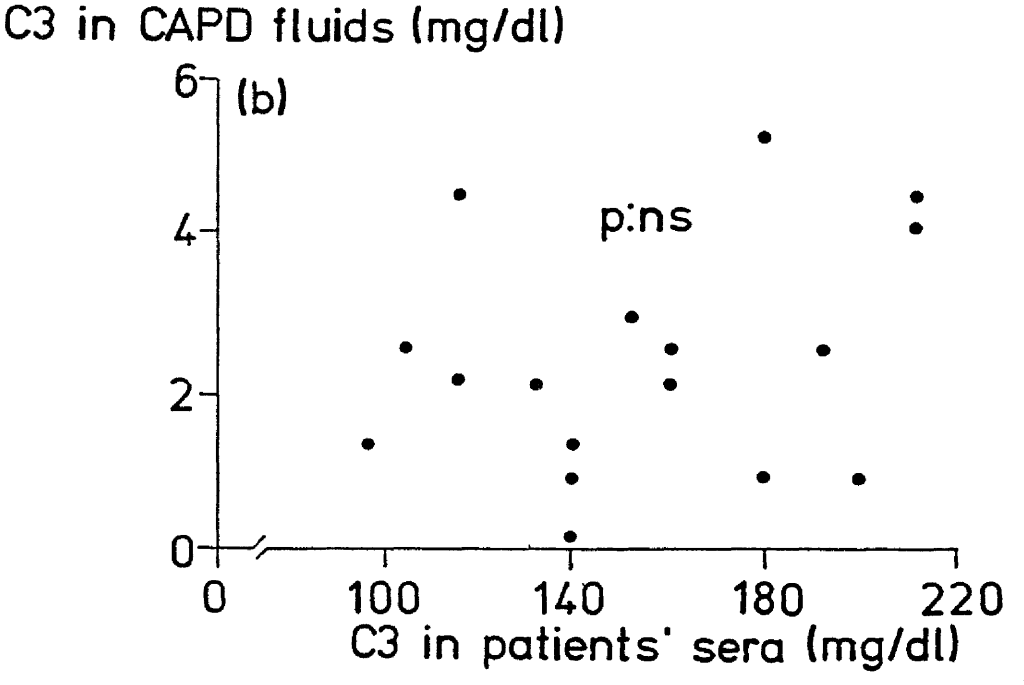
FIGURE 22 : Comparison of Levels of IgG, C3 and Transferrin in Normal Human Sera

(a) IgG compared with Transferrin

(b) C3 compared with IgG

(c) C3 compared with Transferrin


IgG in CAPD fluids (mg/dl)
60
(a)
p:ns
40
20
0
600
900
1200
1500
1800
IgG in patients' sera (mg/dl)


C3 in CAPD fluids (mg/dl)
6
(b)
p:ns
4
2
0
0
100
140
180
220
C3 in patients' sera (mg/dl)

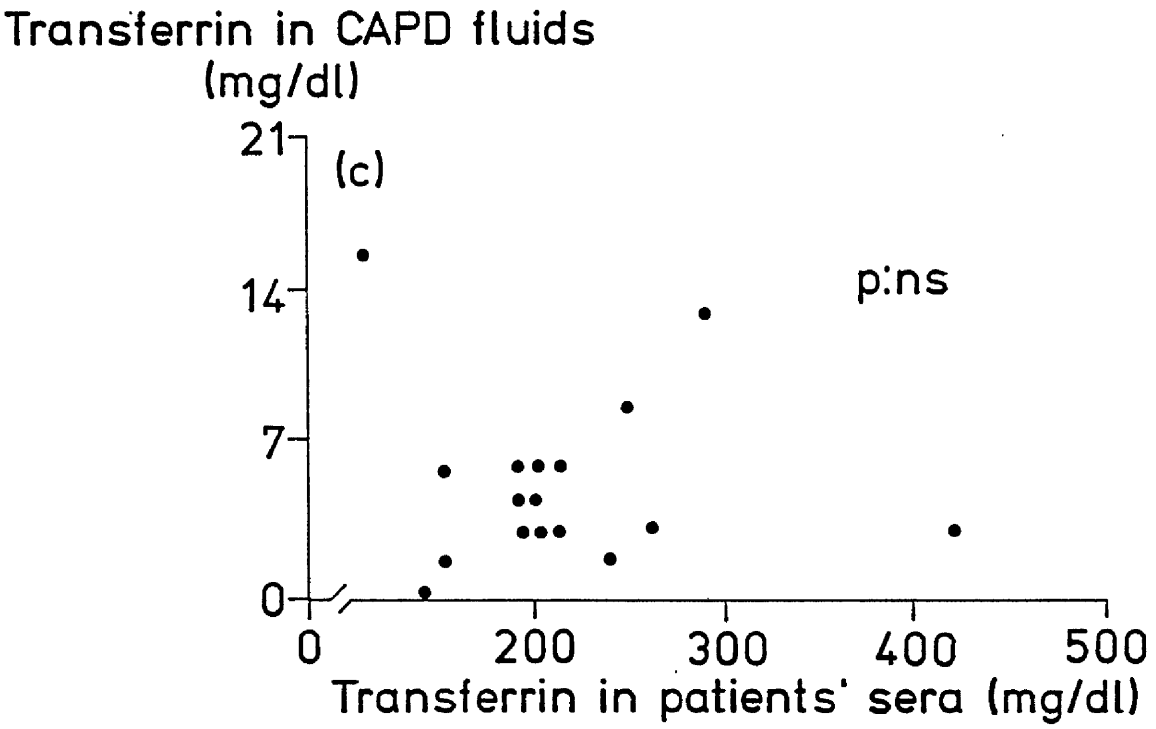


FIGURE 23 : Comparison of Levels of IgG, C3 and Transferrin in CAPD Patients' Sera with Levels in Corresponding Dialysis Effluents

(a) IgG

(b) C3

(c) Transferrin

TABLE 22: Serum Protein Levels in Peritoneal Dialysis Effluent from Patients on CAPD Grouped According to their Original Diagnosis for Renal Failure.

	ORIGINAL DIAGNOSIS			
	GLOMERULONEPHRITIS	DIABETIC NEPHROPATHY	POLYCYSTIC KIDNEYS	OTHERS+
NUMBER OF SAMPLES	12	7	7	12
IgG	13.6* (4.6-23.5)	8.1 (7.8-20.0)	14.6 (13.2-30.0)	8.4 (3.9-19.0)
C3	1.5 (0.4-2.8)	2.1 (1.3-2.4)	2.5 (1.9-3.8)	2.2 (1.0-3.2)
TRANSFERRIN	5.0 (1.2-7.4)	3.4 (2.9-4.8)	4.2 (2.9-7.3)	4.2 (1.5-5.9)

* Median (interquartile range). All figures are mg/dl

+ This group includes patients with pyelonephritis, hypertension, analgesic nephropathy and those with their diagnosis unknown.

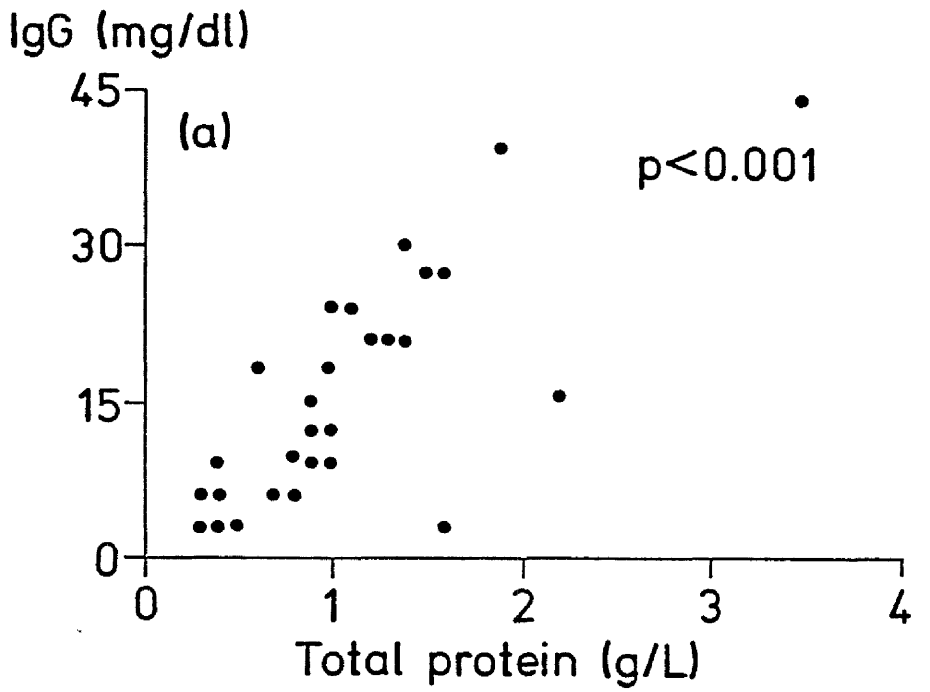
There were no significant differences between any groups.

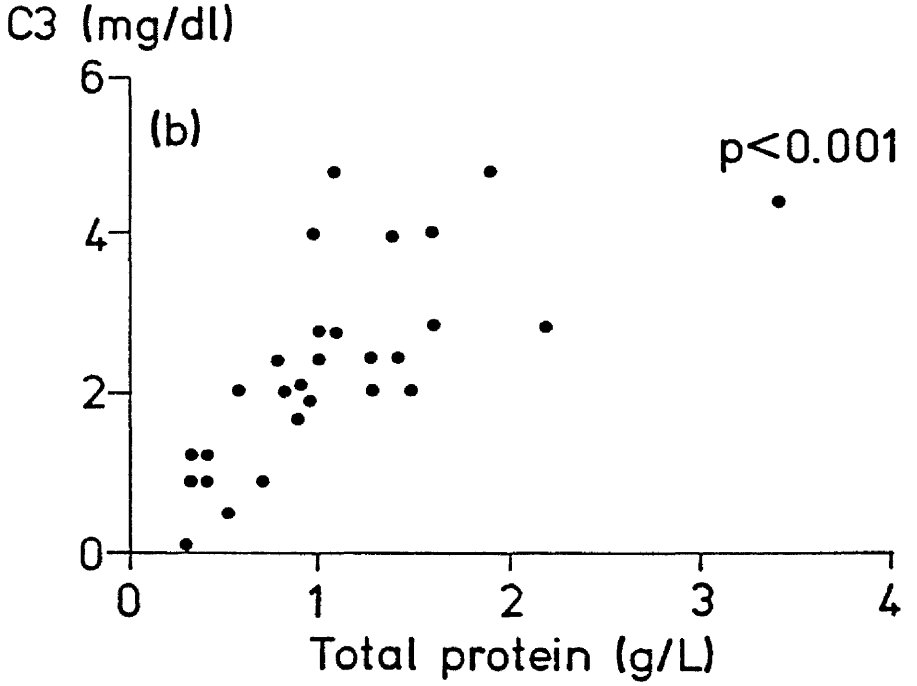
protein in the PDE was also measured and found to correlate significantly with the levels of IgG, C3 and Tf (Figure 24). There was no difference in the total protein levels in PDE between patients on the two systems (Travenol and Fresenius) the median figures being 1.3 g/ l (interquartile range 0.6-1.6) and 0.9 g/ l (0.6-1.1) respectively. A significant inverse correlation ($p<0.05$) was found between the level of total protein and the length of time a patient had been on CAPD (Figure 25). However, if patients who had been on CAPD for less than six months were excluded, the correlation became non-significant.

Although the levels of IgG, C3 and Tf in PDE varied widely between individual patients, they remained relatively constant between successive samples in each of the 13 patients from whom two samples were obtained (Table 23).

5.4.2. Opsonic Activity

The ability of PDE to opsonise S.epidermidis for ingestion by peritoneal cells was significantly lower than that of normal peritoneal fluid ($p<0.001$), the median ingestion being 59% (interquartile range 46-71) and 86% (interquatrile range 82-93) respectively, of the ingestion occurring when bacteria were opsonised with normal human serum. A correlation ($p<0.01$) was found between the opsonising capacity of the PDE and the level of IgG (Figure 26a). A less significant correlation ($p=0.02$) was also found with C3 levels (Figure 26b). No such correlation was found for normal peritoneal fluid (Figures 26c and 26d).


IgG (mg/dl)
45
30
15
0
(a)
p<0.001
0
1
2
3
4
Total protein (g/L)


C3 (mg/dl)
6
4
2
0
(b)
p<0.001
0
1
2
3
4
Total protein (g/L)

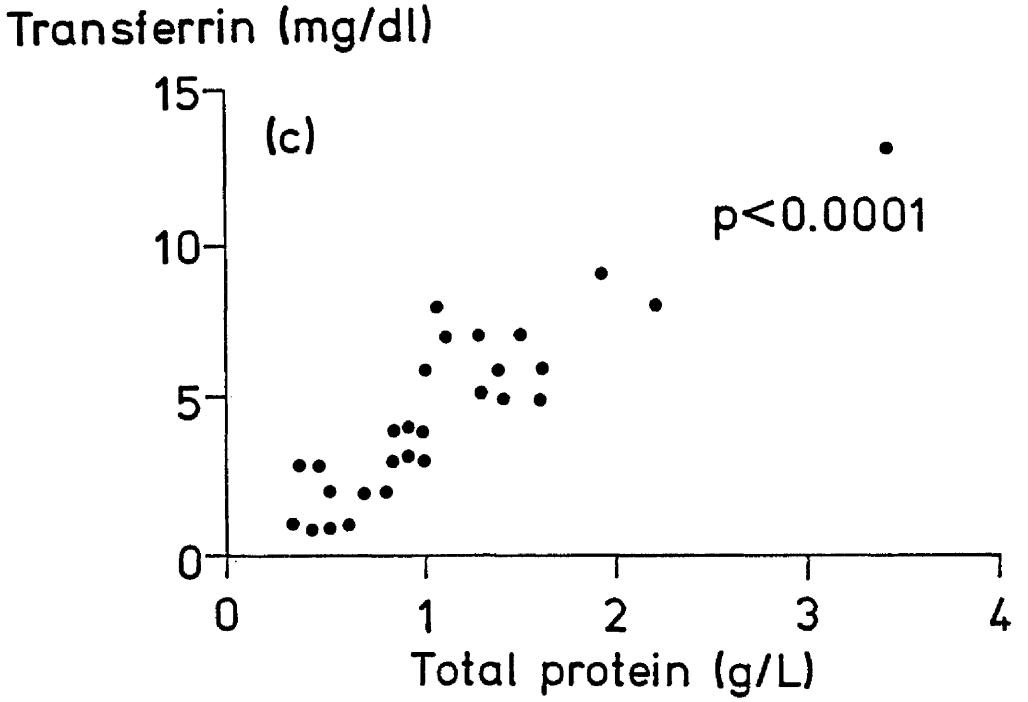


FIGURE 24 : Comparison of Levels of Total Protein in Peritoneal Dialysis Effluent with Corresponding Levels of IgG. C3 and Transferrin.

(a) IgG

(b) C3

(c) Transferrin

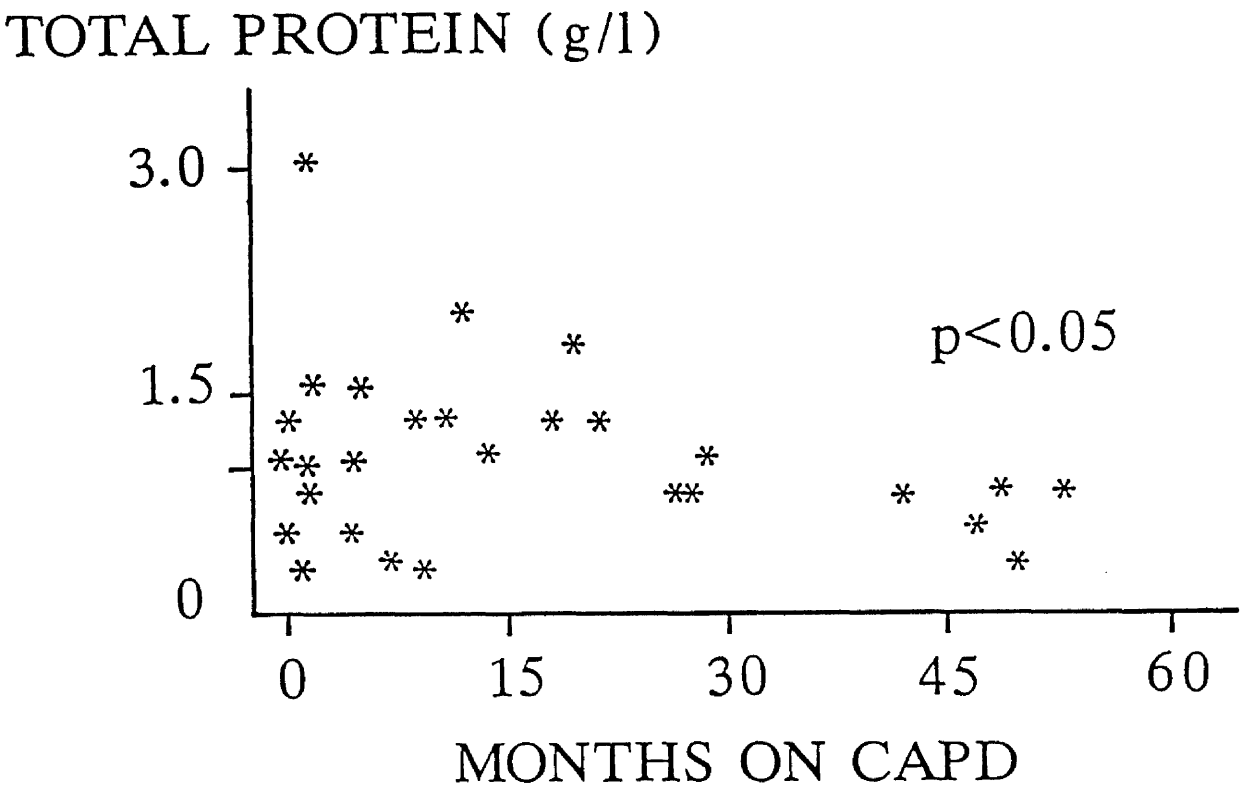


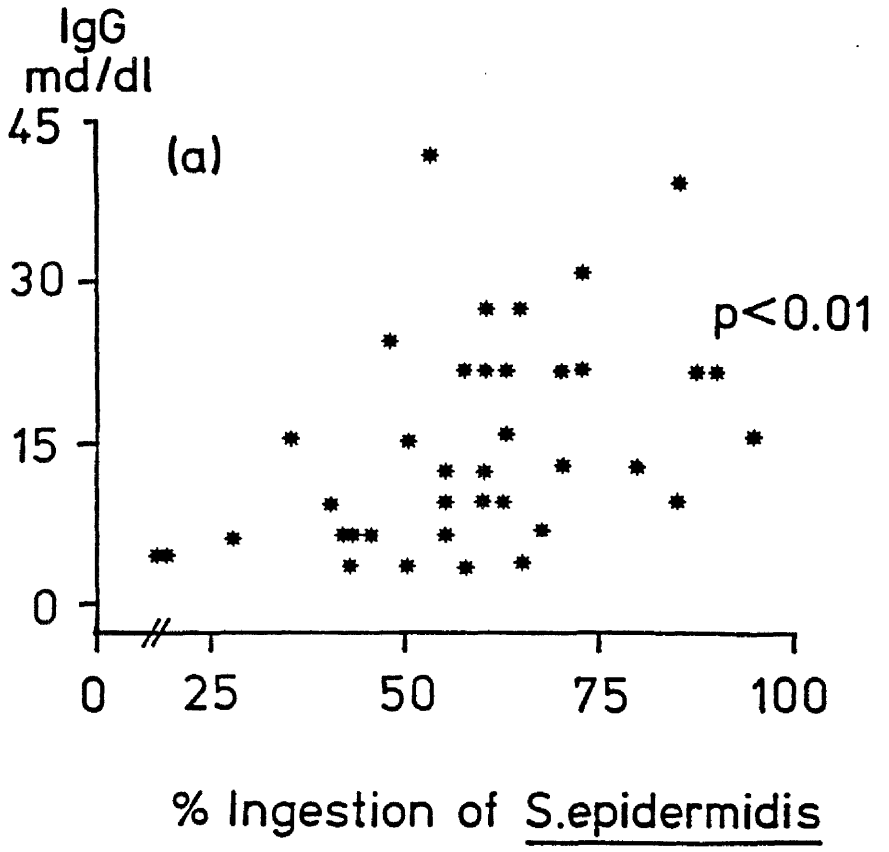
FIGURE 25 : Comparison of Length of Time on CAPD with the Level of Total Protein in Peritoneal Dialysis Effluents.

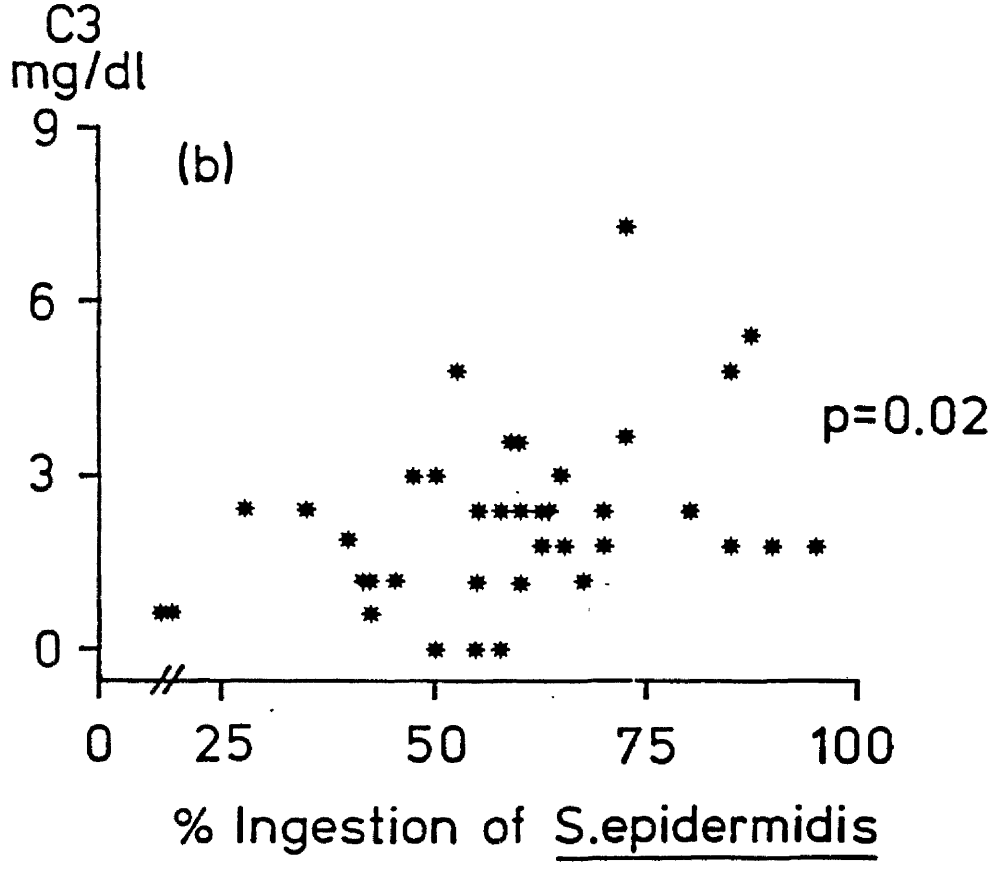
TABLE 23 : Levels of IgG, C3 and Transferrin in Peritoneal Dialysis Effluent of Individual Patients from whom Two Separate Samples were Obtained.

PATIENT NUMBER	TIME BETWEEN SAMPLES (MONTHS)	CONCENTRATION (mg/dl): IgG	C3	TRANSFERRIN
1	1	19.4 a	3.6	4.0
		18.4 b	3.2	2.6
2	1	5.0	0.9	1.7
		4.8	0.5	1.4
3	2	7.8	1.3	3.4
		6.8	2.1	3.8
4	3	6.6	0.5	1.3
		4.8	1.2	1.0
5	1	3.5	2.8	5.4
		4.8	2.8	8.1
6	0.5	12.8	3.4	6.5
		14.2	2.4	6.3
7	4	10.5	2.3	5.8
		14.2	2.8	7.3
8	3	8.6	1.1	2.4
		7.8	1.1	2.3
9	2	8.1	1.9	4.3
		7.3	2.1	3.8
10	2	12.6	2.4	4.9
		13.0	2.8	4.2
11	1	16.2	2.3	3.2
		12.5	1.7	2.0
12	0.5	15.9	2.3	3.2
		15.2	1.8	0.9
13	5	9.4	1.3	3.6
		11.4	2.5	4.2

a First Sample

b Second Sample


IgG
md/dl
45
30
15
0
(a)
p<0.01
0
25
50
75
100
% Ingestion of S.epidermidis


C3
mg/dl
9
6
3
0
(b)
p=0.02
0
25
50
75
100
% Ingestion of S.epidermidis

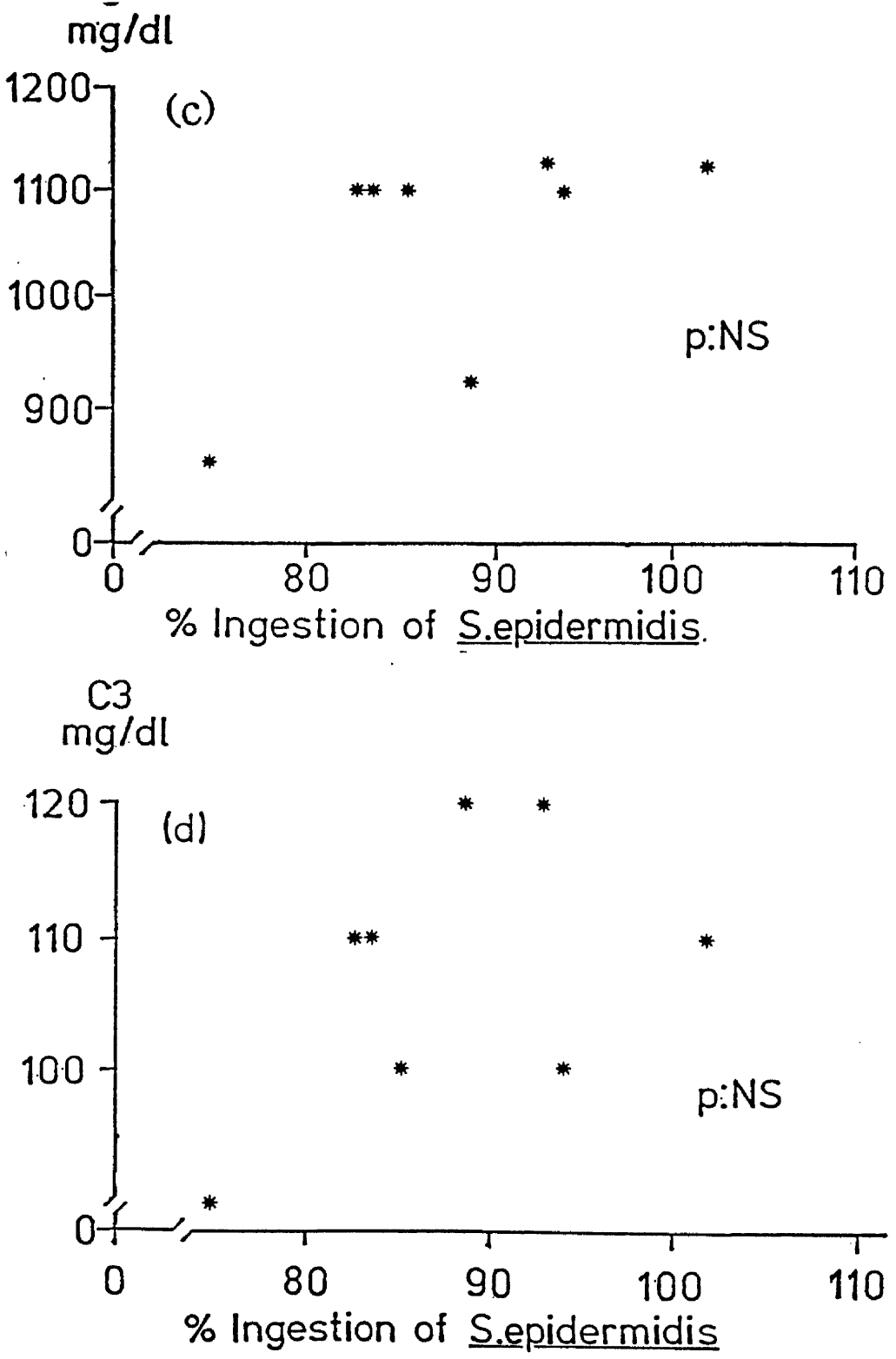


FIGURE 26 : Comparison of Opsonising Capacity of Peritoneal Dialysis Effluent from CAPD Patients with the Levels of (a) IgG and (b) C3, and Comparison of Opsonising Capacity of Normal Peritoneal Fluid with the Levels of (c) IgG and (d) C3

*Percentage ingestion of S.epidermidis is relative to ingestion of bacteria opsonised with normal human sera.

5.4.3. Bacteriostatic Activity

The growth of S.epidermidis in peritoneal fluids and sera was always less than in RPMI 1640 culture medium which was used as a control (Table 24). However, growth in PDE was significantly greater than in normal sera ($p<0.05$). There was no significant difference between the growth in normal or CAPD patients' sera, or normal peritoneal fluid. In addition no correlation was found between growth of S.epidermidis in PDE and in the corresponding patients' sera (Figure 27). A significant effect of adding extra Tf was only shown with PDE from CAPD patients ($p<0.05$) and not with normal peritoneal fluid or sera (Table 25).

A significant inverse correlation was found between the level of Tf and the growth of S.epidermidis in PDE (Figure 28a; $p=0.002$). There was no such correlation for normal peritoneal fluid, patients' sera or normal sera (Figure 28b,c,and d). A significant inverse correlation ($p<0.01$) was also found between Tf levels in PDE from CAPD patients and the effect of adding extra Tf, i.e. the lower the level of Tf found in the PDE the greater the effect of adding extra Tf (Figure 29a). Again no such correlations were was found with the controls (Figure 29b,c and d).

5.4.4. Relationship to Peritonitis

When the opsonic activity of PDE samples was compared with the frequency of episodes of peritonitis, a significant inverse correlation was found ($p<0.01$; Figure 30). Furthermore, the incidence of peritonitis was one episode per 14.8 patient months in patients with high PDE opsonic activity (>70% of control) against S.epidermidis

TABLE 24 : Growth of S.epidermidis in Peritoneal Dialysis Effluent, Normal Peritoneal Fluid, and Normal and CAPD Patients' Sera.

	PERITONEAL DIALYSIS EFFLUENT	NORMAL PERITONEAL FLUID	CAPD PATIENTS' SERA	NORMAL HUMAN SERA
NUMBER OF SAMPLES	44	8	17	6
GROWTH OF S.epidermidis*	20.0 (5.6-37.8)+o	5.8 (1.4-11.8)	10.2 (3.8-17.8)	12.8 (9.6-14.3)

* as percentage of growth in control (RPMI 1640 culture medium)

\+ median (interquartile range)

o $p<0.05$ compared with normal human serum

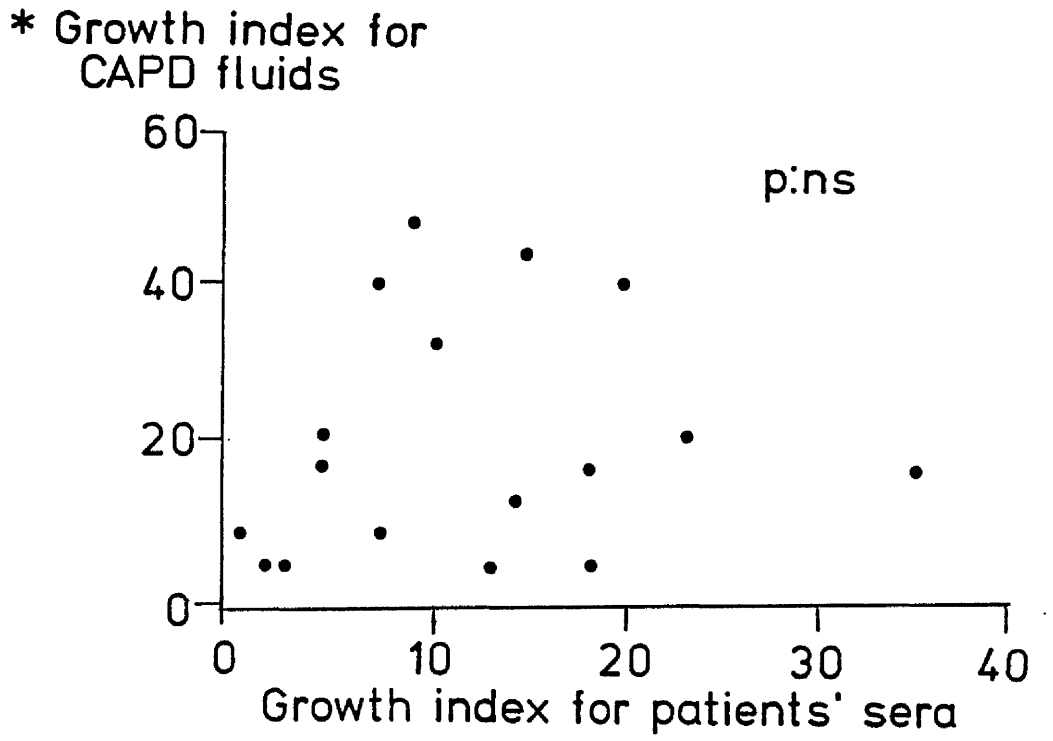


FIGURE 27 : Comparison of the Growth of S.epidermidis in Patients' Sera with Growth in the Corresponding Peritoneal Dialysis Effluents.

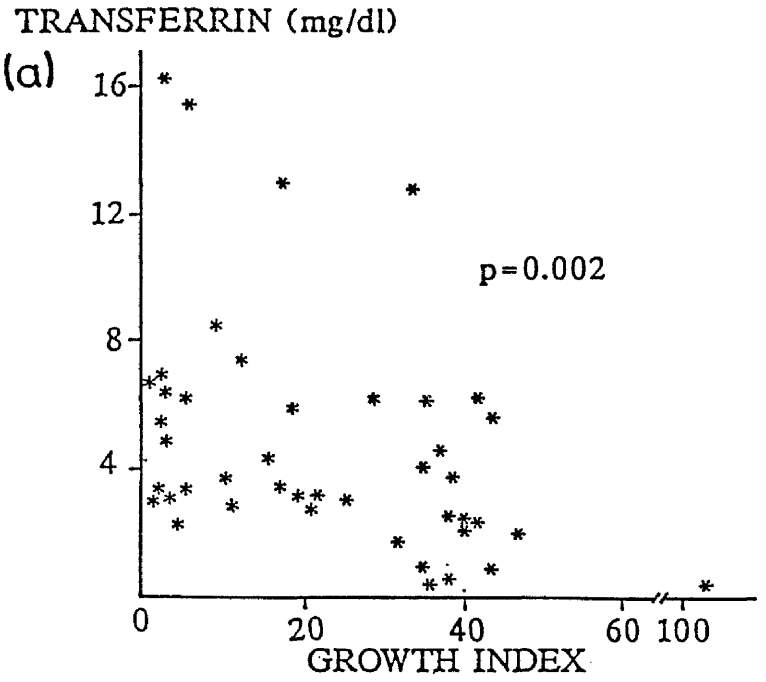
* as percentage of growth in control (RPMI 1640 culture medium)

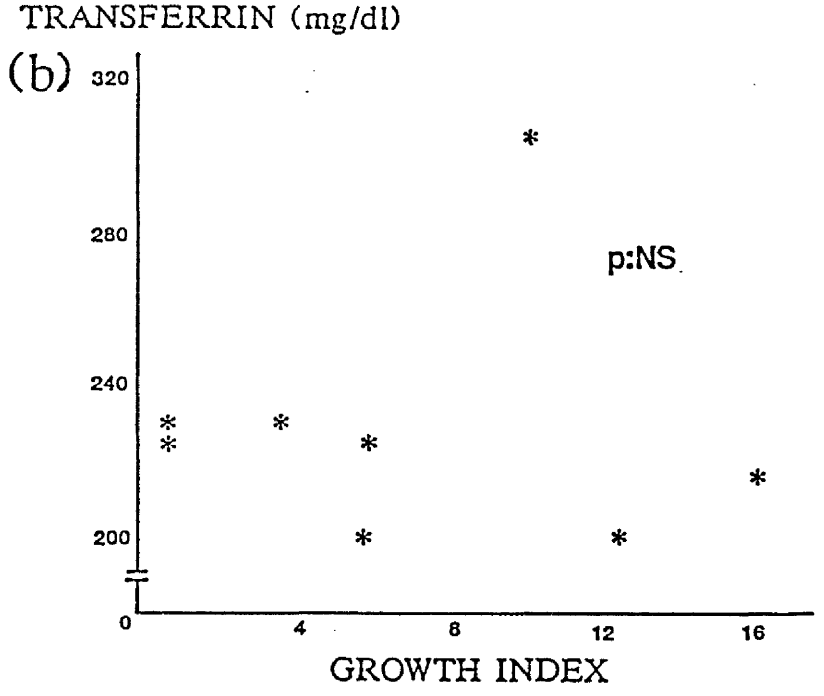
TABLE 25 : The Effect on Growth of *S.epidermidis* of Addition of Extra Transferrin to Peritoneal Fluids and Sera.

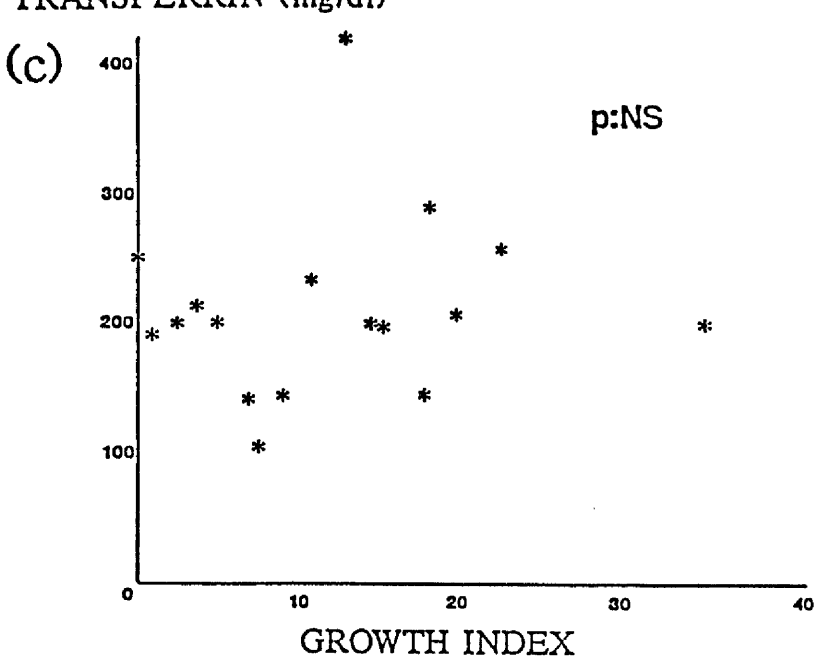
	PERITONEAL DIALYSIS EFFLUENT	NORMAL PERITONEAL FLUID	CAPD PATIENTS' SERA	NORMAL HUMAN SERA
NUMBER OF SAMPLES	44	8	17	6
PERCENTAGE REDUCTION IN GROWTH OF *S.epidermidis* ON ADDITION OF EXTRA Tf	2.0+* (0.38-8.58)	-0.1 (-0.88-0.18)	1.2 (-0.05-3.80)	0.7 (-0.30-2.48)

+ Median (interquartile range)

* $p<0.05$ compared with normal human serum


TRANSFERRIN (mg/dl)
(a)
16
12
8
4
p=0.002
0
20
40
60
100
GROWTH INDEX


TRANSFERRIN (mg/dl)
(b)
320
280
240
200
0
p:NS
4
8
12
16
GROWTH INDEX



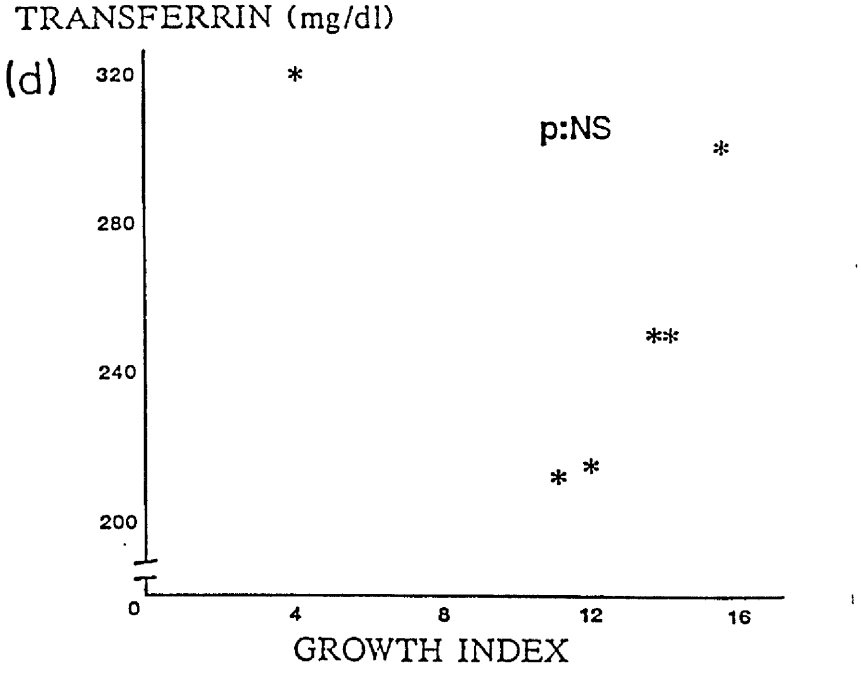


FIGURE 28 : Comparison of Growth of S.epidermidis with the Level of Transferrin

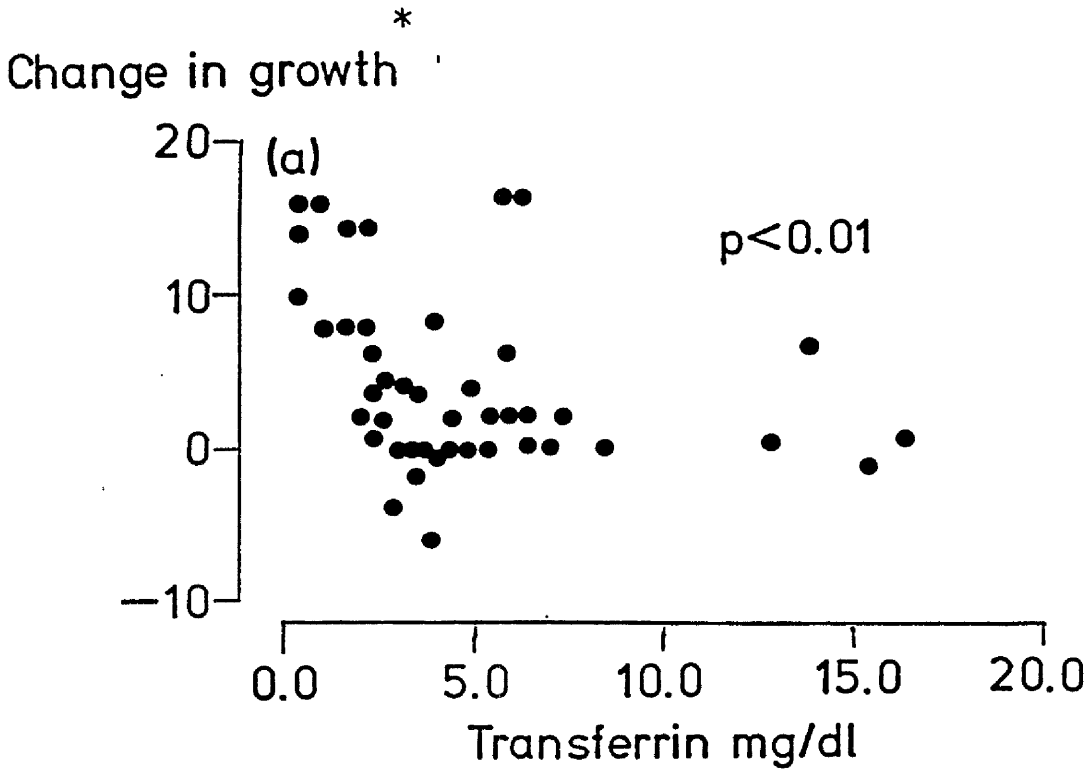
(a) in peritoneal dialysis effluent of CAPD patients

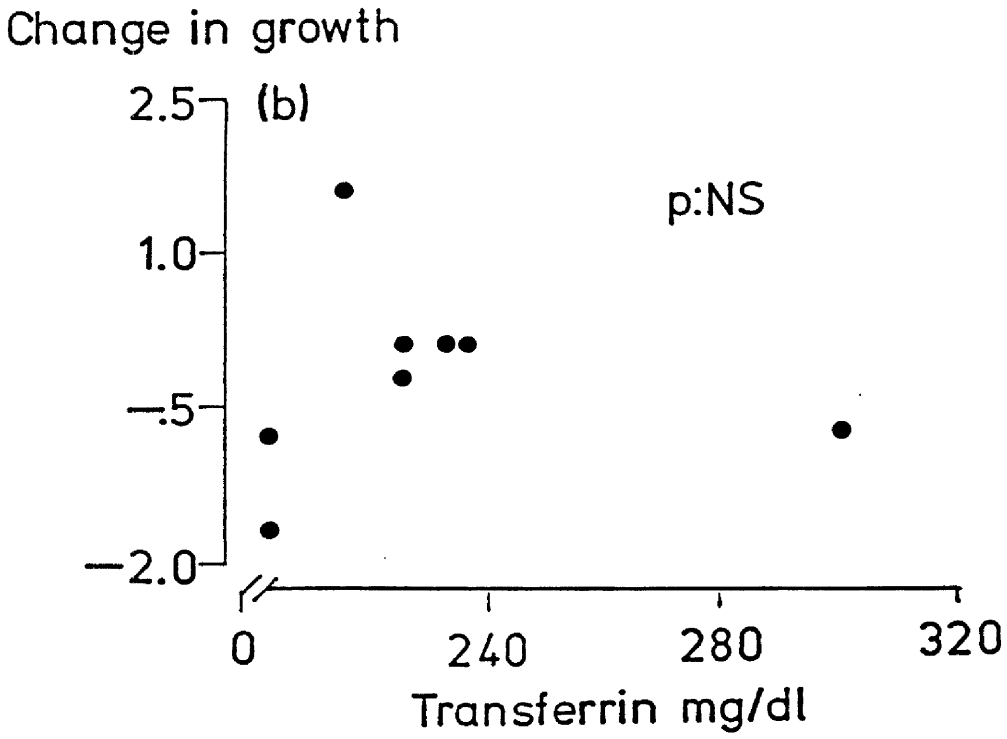
(b) in normal peritoneal fluid

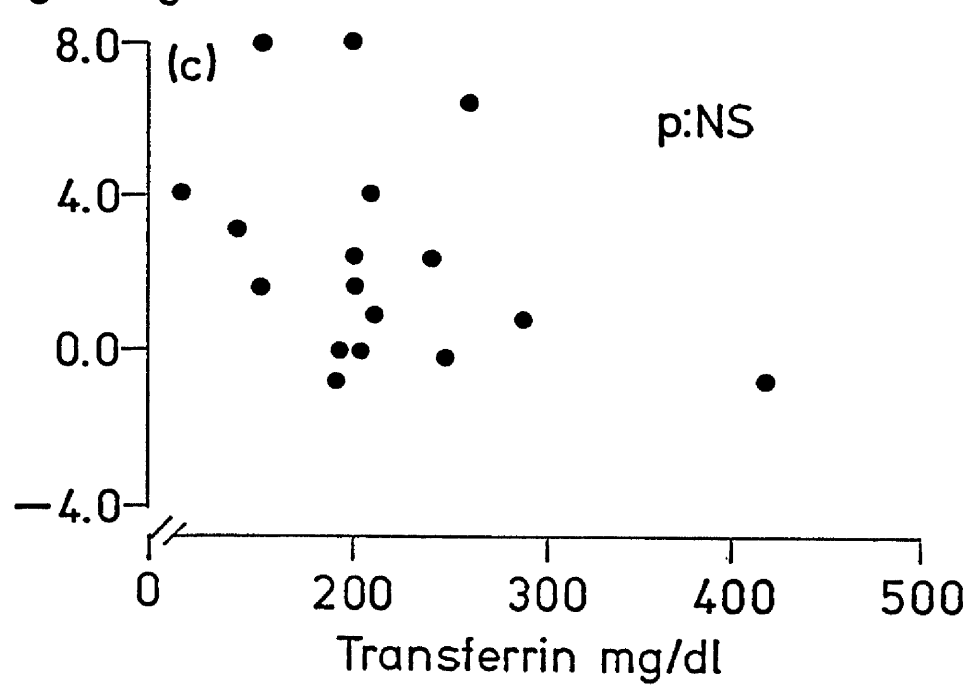
(c) in patients' sera

(d) in normal serum

*Growth Index = rate of growth as percentage of growth in uninhibited control (RPMI 1640 culture medium)


Change in growth
20
(a)
10
0
−10
p<0.01
0.0
5.0
10.0
15.0
20.0
Transferrin mg/dl


Change in growth
2.5
(b)
1.0
−.5
−2.0
p:NS
0
240
280
320
Transferrin mg/dl



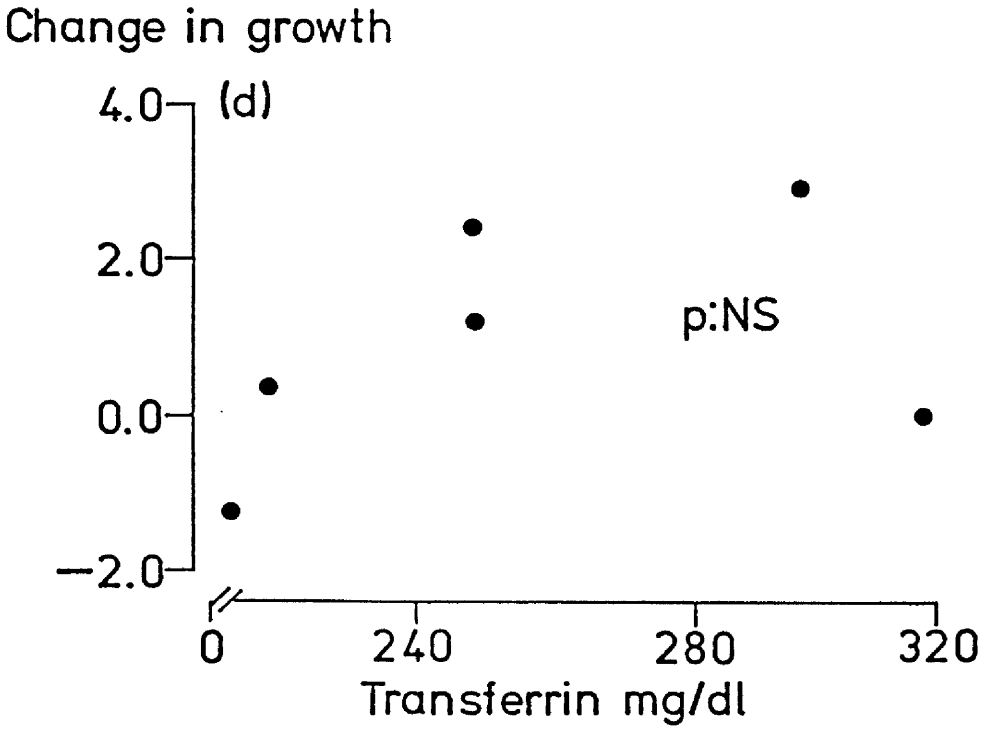


FIGURE 29 : The Effect on Growth of S.epidermidis of Addition of Extra Transferrin Compared with the Level of Transferrin

(a) in peritoneal dialysis fluid from CAPD patients

(b) in normal peritoneal fluid

(c) in CAPD patients' sera

(d) in normal sera

* see section 5.3.2. for definition

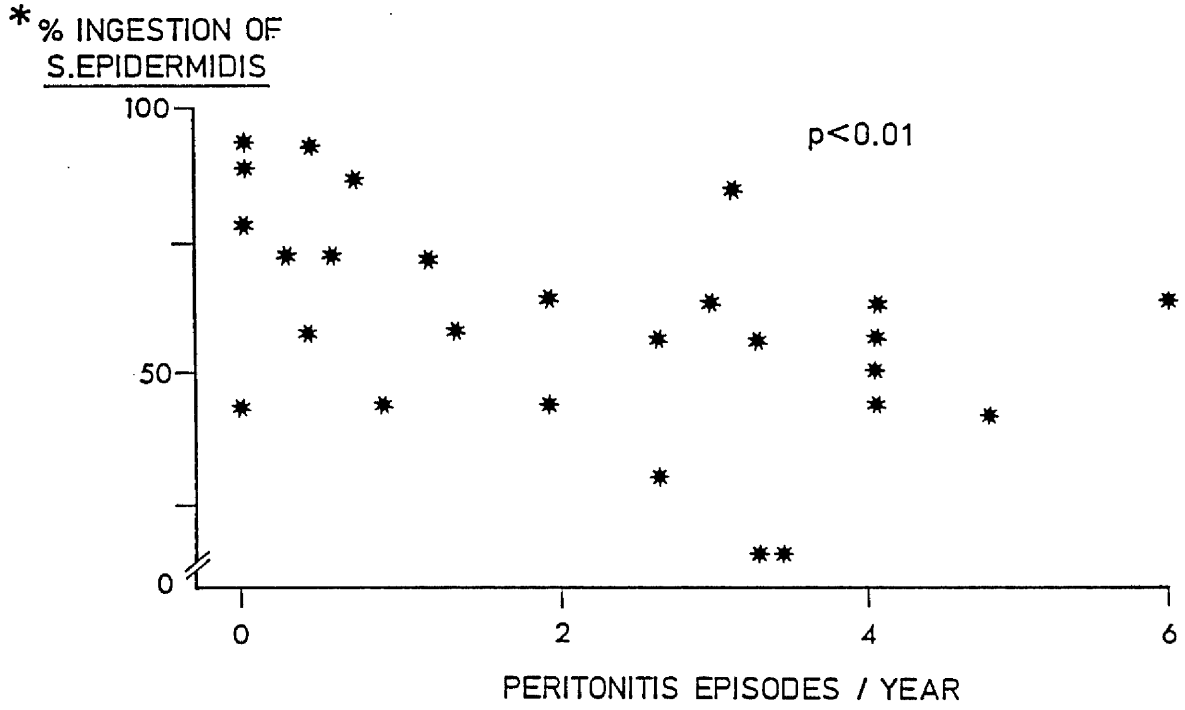


FIGURE 30 : Comparison of the Frequency of Peritonitis Episodes in CAPD Patients with the Opsonising Capacity of their Peritoneal Dialysis Effluents

* Percentage ingestion of S.epidermidis is relative to ingestion of bacteria opsonised with normal human serum.

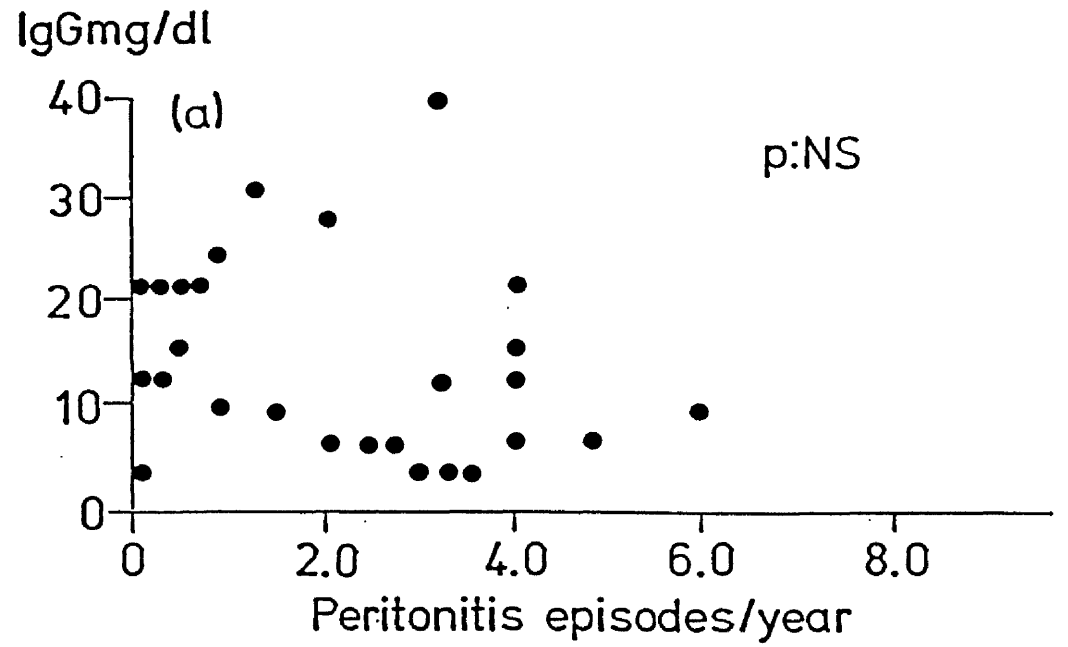
(Table 26), but was nearly 3 times greater (one episode per 5.4 patient months) in patients with low opsonic activity (<70% of control). However, no correlation was found between the levels of either IgG or C3, and peritonitis (Figure 31a and b). There was also no correlation between the frequency of peritonitis episodes with either the bacteriostatic activity of the fluid or with Tf levels (Figures 32 and 33).

TABLE 26 : Relationship Between Peritonitis and Opsonising Activity of Peritoneal Dialysate Effluent

	>70% OPSONIC ACTIVITY OF CONTROL	<70% OPSONIC ACTIVITY OF CONTROL
NUMBER OF PATIENTS	10	28
OPSONIC ACTIVITY+	84%* (72-88)	55% (43-61)
TOTAL EXPOSURE TIME (MONTHS)	177	352
TOTAL NO. OF EPISODES OF PERITONITIS	12	65
AVERAGE NO.OF PATIENT MONTHS BETWEEN EPISODES	14.8	5.4

* Median (interquartile range)

+ Compared with normal human serum - for details see text



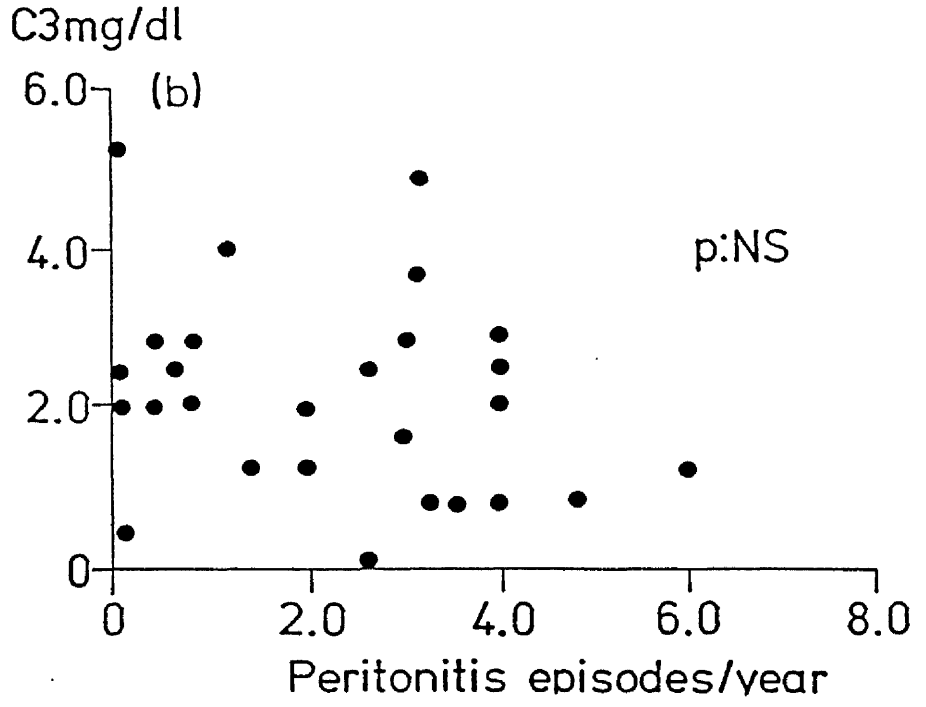


FIGURE 31 : Comparison of the Frequency of Peritonitis Episodes in CAPD Patients with (a) IgG and (b) C3 Levels in their Peritoneal Dialysis Effluents.

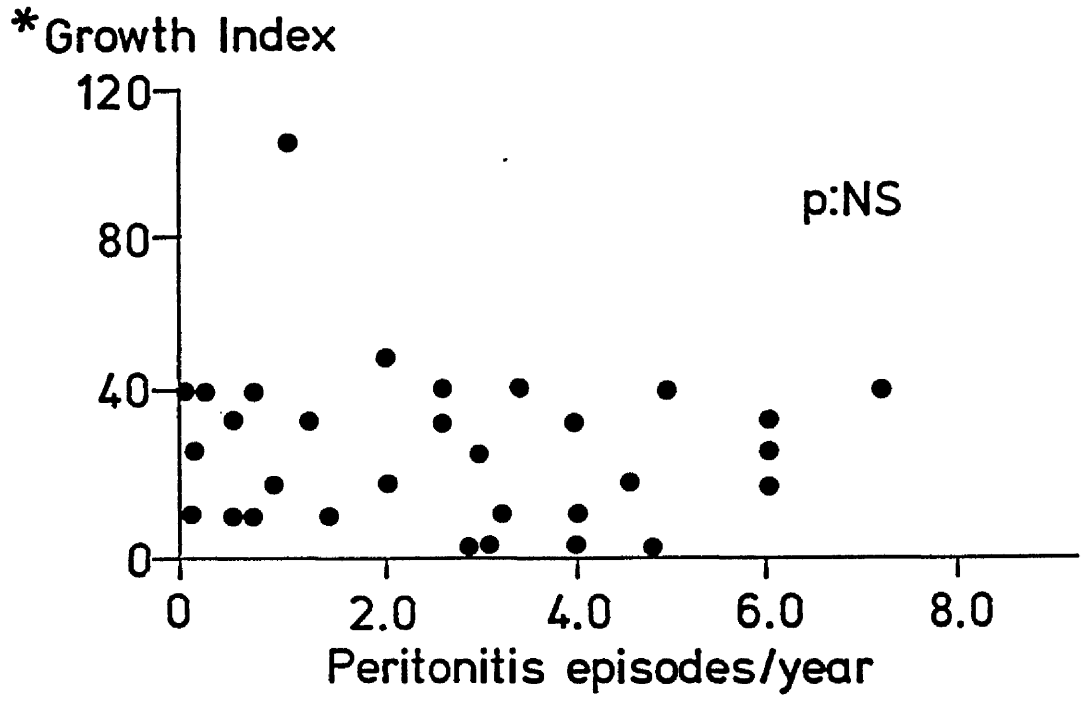


FIGURE 32 : Comparison of the Frequency of Peritonitis Episodes in CAPD Patients with Growth of S.epidermidis in Peritoneal Dialysis Effluent.

* Growth Index = rate of growth as percentage of growth in uninhibited control (RPMI 1640 culture medium)

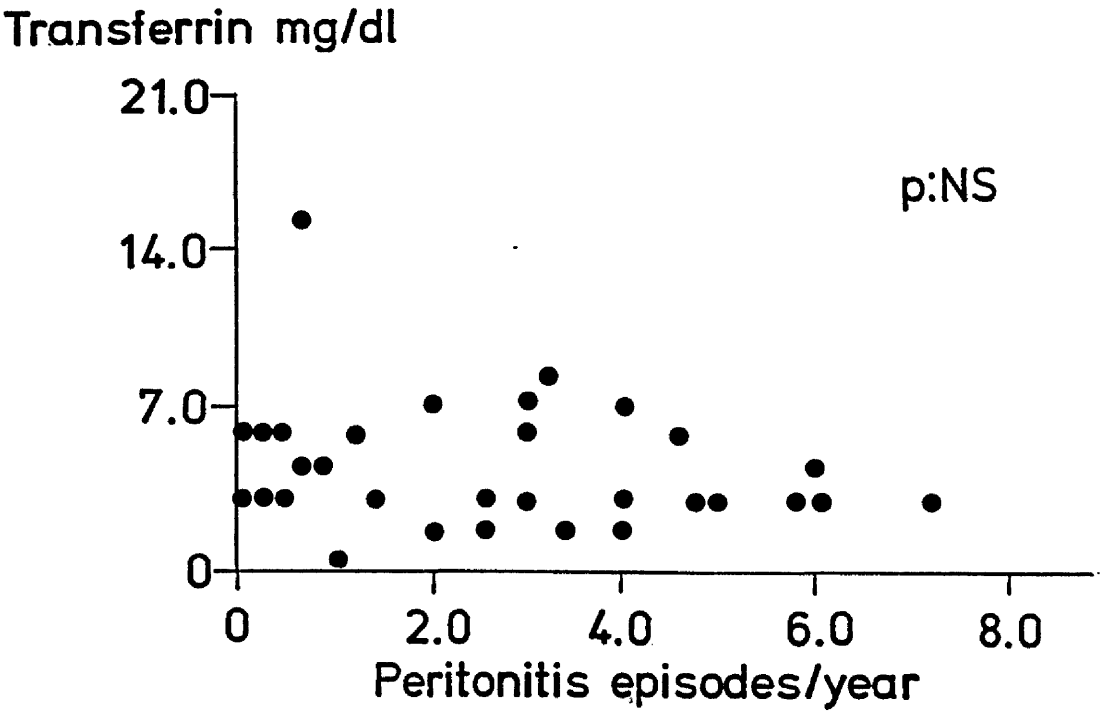


FIGURE 33 : Comparison of the Frequency of Peritonitis Episodes in CAPD Patients with the Levels of Transferrin in Peritoneal Dialysis Effluents.

5.5 DISCUSSION

During CAPD, the frequent drainage of fluid and replacement by fresh dialysate results in the removal of proteins such as opsonins and Tf. It is therefore not surprising that the levels of these proteins in PDE are only 1-2% of those found in serum or normal peritoneal fluid. These findings are similar to those reported by others (Steen et al,1986; Keane et al,1984; Verbrugh et al,1983). Furthermore, in this study the level of these proteins was measured in PDE following the long overnight dwell time when the levels would be at their highest. During the day, with shorter dwell times, the levels of these proteins in the peritoneal cavity will be even lower.

When levels of IgG, C3 and Tf in PDE and the corresponding patients' sera were compared no correlation could be found, i.e. the level of proteins in sera had no effect on the levels found in the peritoneum. This is in agreement with the conclusion that permeability of the peritoneal membrane is the major factor in determining the quantity of serum proteins that gain entry to the peritoneal cavity rather than the actual serum levels (Krediet et al, 1986; Dulaney and Hatch, 1984). Furthermore a significant correlation was found between levels of IgG, C3, Tf and total protein which suggests that the entry of serum proteins into the peritoneal cavity does not involve a specific transport process but depends on the filtering efficiency of the membrane itself. Yewdall et al (1986), have recently also shown that plasma proteins, including

opsonically active proteins, enter peritoneal fluid in relatively non-selective proportions with the effect that CAPD fluid resembles a very dilute plasma.

When levels of total protein in PDE and length of time on CAPD were compared, a significant inverse correlation was found, suggesting a decrease in the permeability of the peritoneal membrane with the passage of time. As the correlation was not seen if only patients who had been on CAPD for more than 6 months were examined, the main change in membrane permeability may occur within the first 6 months of dialysis.

Similar losses of serum proteins in the PDE were found in both diabetic and non-diabetic patients, which is in disagreement with the finding of Krediet et al (1986). A possible explanation for this difference could simply be due to the way in which the protein levels were measured. The work here was based on the levels of proteins in the overnight dwell PDE. However, Krediet et al (1986) worked on the average daily protein loss in the PDE over 3 consecutive days, which therefore included protein losses in all the daily exchanges. If there were indeed even small differences in the permeability of the peritoneal membrane between diabetic and non-diabetic patients, and hence differences in the rate of protein loss, this could be emphasised and possibly become significant over the four hour dwell times, but not overnight. The overnight eight hour dwell time may allow sufficient time for the levels of proteins to equilibrate in the two groups and hence no difference would be found. Protein loss in the PDE has also been shown to be substantially increased during peritonitis

(Krediet et al,1986; Blumenkrantz et al,1981; Rubin et al,1981) due to possible alterations in the peritoneal blood flow affecting membrane surface area or permeability.

Although the levels of IgG and C3 were similar in CAPD patients' sera and normal sera, as also found by Keane et al (1984), a significant difference between the Tf levels was found. Depressed Tf levels have been reported in patients with chronic renal failure (Milman et al,1984) and is a common feature of inflammatory disease (Bothwell et al,1979). Protein depletion has been suggested as an alternative explanation for low Tf levels (Bothwell et al, 1979), but no difference was seen in serum IgG or C3 levels between CAPD patients and normal controls which might have been expected if protein depletion were involved.

It is well established that transferrin provides an important antimicrobial defence mechanism by rendering iron unavailable to microorganisms in vivo (Brock, 1986). The finding of a significant inverse correlation between the growth of S.epidermidis in PDE and the level of Tf, which was not found with normal sera or peritoneal fluid, and the difference in growth rates in PDE and controls suggests that the Tf levels are not optimal in PDE. This is further supported by the finding that in PDE the degree of depression of growth on addition of extra Tf correlated inversely with the level of Tf in the PDE. No such effect was seen in normal peritoneal fluid or sera, in which Tf levels were much higher. It must also be remembered that although PDE did allow growth of S. epidermidis, it was still considerably less than that observed in the RPMI control. Hence, although PDE was significantly less

bacteriostatic than control peritoneal fluid or sera, it was by no means an ideal culture medium for bacteria. Also, even when extra Tf was added to control sera, some growth of S.epidermidis still occured, which suggests that other factors (possibly nutritional or immunological) in addition to inadequate Tf levels may determine the bacteriostatic activity of PDE.

Although no correlation was found between bacteriostatic activity of individual PDE samples and susceptibility to peritonitis, the greater growth of bacteria in PDE in comparison with sera or normal peritoneal fluid may in part be responsible for the predisposition to peritonitis. It has been suggested that the entry of iron into PDE as a result of haemolysis would further reduce the antimicrobial activity of PDE (Bloodworth and Harber, 1986).

Levels of IgG and C3 in PDE were found to correlate with opsonic activity, in agreement with the findings of Lamperi and Carozzi (1986b), Steen et al (1986), and Verbrugh et al, (1983). No such correlation was found in normal peritoneal fluid which suggests that the level of opsonins are sub-optimal in PDE. The ability of PDE to efficiently opsonise S.epidermidis is believed to depend primarily on the antibody or heat stable components of PDE rather than the complement or heat labile ones. This was shown by Keane and Peterson (1984) when they opsonised bacteria with decomplemented NHS (heat inactivated) and showed that phagocytosis of S.epidermidis was still efficient. However when the opsonic source was IgG-deficient serum which contained normal levels of

complement, phagocytosis of S.epidermidis by peritoneal macrophages was relatively inefficient. Escherichia coli and S.aureus, on the other hand, require to be opsonised by heat-labile components, i.e. complement, for effective phagocytosis (Keane and Peterson,1984; Peterson et al,1977). Without complement, phagocytosis of these organisms was found to be minimal. These reports also show that phagocytosis of S.epidermidis proceeds predominantly through an Fc receptor mechanism, while that of E. coli and S. aureus occurs predominantly via C3b receptors. In the present work the opsonising activity of PDE was also found to be less than that of normal peritoneal fluid, showing that the levels of opsonins are indeed suboptimal in PDE. This may severely inhibit optimal phagocytic activity as it is well known that opsonins are required for efficient ingestion of micro-organisms by phagocytes (Johnston and Stroud,1977; Stossel,1974; Winkelstein,1973).

An additional effect of a quantitative deficiency of complement could be possible interference with the recruitment of cells into the peritoneum in the immune response, due to lack of chemoattractants C3a and C5a. Inadequate antibody levels may produce the same effects, as in agammaglobulinaemia depressed production of chemotactic activity was noted by Steerman et al,(1971) due possibly to insufficient immune-complexes being formed to stimulate C3a and C5a production by the classical pathway of complement activation.

Recent work by Verbrugh et al (1986), suggests that complement, even when present, may not be functionally active. Inactivation of complement by CAPD solutions was

demonstrated to be largely due to the low pH of these solutions. This may mean that although complement was detected here in the PDE of patients it may be functionally inactive. Hence it is possible that both qualitative and quantitative defects may exist which may severely hamper the defences of the peritoneum.

When opsonic activity of PDE samples were compared with the frequency of episodes of peritonitis a significant inverse correlation was found, in agreement with the findings of Lamperi and Carozzi (1986b) and Steen et al (1986). This finding may allow possible early identification of patients at high risk. Such a correlation was not seen between the levels of IgG or C3 and peritonitis despite the fact that the concentration of these proteins correlated with opsonic activity. Here the specificity of the IgG for S. epidermidis, rather than just the level of IgG, may be important. Also, as far as S.epidermidis is concerned, complement is not thought to be as important as IgG in its opsonisation (Keane and Peterson,1984). In any case, the causes of peritonitis are undoubtedly multifactorial, and the inability to obtain a significant correlation between peritonitis rates and any single parameter is not unexpected. Inadequate fibronectin secretion by CAPD peritoneal macrophages has, for instance, been suggested by Polin and Douglas (1984), to play a part in the increased peritonitis rate seen in some CAPD patients. This may be related to the ability of fibronectin to act as an opsonin, thereby enhancing the attachment and phagocytosis of micro-organisms. The addition of purified IgG to PDE has been proposed as a method of enhancing the

host's immune defences against peritonitis (Keane and Peterson,1984) and there is some clinical evidence in favour of this (Lamperi and Carozzi,1986b). In conclusion, it therefore appears that the normal humoral defence mechanisms of the peritoneum are weakened in patients on CAPD when compared with those of normal controls, and this may be one of the causes of the susceptibility of these patients to peritonitis.

CHAPTER 6

A LONGITUDINAL STUDY OF PERITONEAL DEFENCE MECHANISMS OF PATIENTS ON CAPD

6.1 INTRODUCTION

All the work presented in this thesis up to this point has been related to CAPD patients as a whole, and the results obtained compared directly with samples obtained from normal controls. On no occasion was any systematic attempt made to examine what changes in peritoneal immunity may occur the first few months on CAPD.

This area has also been neglected by other workers as there appears to be no data currently available giving a complete overview of changes that may occur in the first few months to the peritoneal defences of patients on CAPD. Young et al (1986) did examine changes in protein levels in CAPD patients' plasma as dialysis proceeded (but not in the PDE itself) and found a decrease in plasma IgG during the early months on CAPD. However, this gives no indication as to the situation in the peritoneum over the same initial period. Steen et al (1986), on the other hand, did examine IgG and C3 levels and the opsonising capacity of PDE and found no correlation with time on CAPD. However, their study was not performed with new patients followed through for a period of time, but with a random selection of patients who had been on CAPD for times varying between 1 and 37 months. No emphasis was therefore placed on examining the possibly important changes during the initial few months. In view of this, and in order to provide more information about the immune status of these uraemic patients this chapter reports the results of a study on 16 patients who were monitored for a 9 month period from the

day of commencement of peritoneal dialysis. During this time periodic samples of PDE were analysed for levels and activity of IgG, C3 and Tf, and for the number and antimicrobial activity of the peritoneal cells.

6.2 MATERIALS AND METHODS

All the materials and methods used in this chapter have been described previously. The levels of IgG, C3 and Tf in the PDE were measured as in 5.3.4. The percentage opsonising activity and percentage growth of S.epidermidis in the PDE were measured as in sections 5.3.1 and 5.3.2 respectively. Finally the bactericidal activity of the peritoneal cells from the PDE was assayed by the technique whose development was described in chapter 3.

6.3 PATIENT DETAILS

Sixteen patients commencing CAPD were monitored for up to 9 months. Details of these patients are shown in Table 27.

TABLE 27 : Details of Patients whose Cells and Serum Protein Levels and Activities in PDE were Monitored in a 9 Month Longitudinal Study.

PATIENTS' INITIALS	SEX	AGE (YEARS)	ORIGINAL DIAGNOSIS	NUMBER OF MONTHS ON STUDY	REASON FOR ABANDONING CAPD
*C.Ba	Male	39	Glomerulonephritis	9	N.A.+
A.Bl	Male	70	Glomerulonephritis	6	Died
W.Co	Male	32	Hypertension	9	N.A.
J.Ha	Female	53	Polycystic Kidneys	9	N.A.
R.Ha	Male	75	Hypertension	4	Moved to Edinburgh
C.He	Female	47	Diabetic Nephropathy	1	Catheter removed
R.Ho	Female	69	Renal artery thrombosis	1	Died
R.Lo	Male	46	Pyelonephritis	9	N.A.
H.McC	Female	21	Reflux Nephropathy	9	N.A.
W.McI	Male	56	Hypertension	6	Died
A.McL	Male	51	Glomerulonephritis	6	Catheter removed
S.McL	Female	48	Hypertension	9	N.A.
J.McN	Male	54	Diabetic Nephropathy	1	Transplanted
J.Ra	Male	74	Glomerulonephritis	2	Catheter removed
M.Wh	Female	68	Hypertension	4	Died
J.Sh	Male	46	Glomerulonephritis	1	Transplanted

* all patients were on the Travenol Disconnect System

+ N.A. = not applicable

6.4 RESULTS

Although it was originally intended to monitor all 16 patients for 9 months, only 6 of these remained continuously on CAPD for the duration of the study. The other patients ceased CAPD for a number of reasons (Table 27). It was therefore not thought appropriate to look at the individual results from each patient but instead to examine the overall differences between PDE samples obtained on day 1 and at 2,4,6,and 9 months, on CAPD.

6.4.1. IgG and C3 Levels

When IgG and C3 levels in the PDE of CAPD patients were measured over the 9 months on dialysis their concentration was found to significantly decrease with time on CAPD (Figure 34a and 34b; $p<0.05$). The drop in the concentration of these proteins was particularly noticeable up to 4 months on dialysis after which time they appeared to level out. Tf levels also showed a tendency to decrease from the starting level but this was not significant (Figure 34c).

6.4.2. Opsonic Activity

Since it has already been shown that opsonic activity is directly related to the levels of IgG and C3 (5.4.2) a corresponding decrease in the opsonic activity would be expected and this was indeed found (Figure 34d; $p<0.02$).

6.4.3. Bacteriostatic Activity

The bacteriostatic activity of the PDE, as measured by the growth of S.epidermidis in the fluid, was shown to increase significantly with time on CAPD (Figure 34e; $p<0.05$). The increase in growth was particularly large over the first 6 months on CAPD and, like the IgG and C3 graphs, tended to level off thereafter. It also corresponded with the tendency of Tf levels to fall (Figure 34c).

6.4.4. Total Cell Numbers in PDE

The total number of cells isolated from the PDE of CAPD patients over 9 months decreased significantly (Figure 34f; $p<0.05$). This reduction was greatest in the first 4 months after which the cell numbers tended to stabilise.

6.4.5. Antimicrobial Activity of the Peritoneal Cells

The ability of the CAPD peritoneal cells from these CAPD patients to ingest S.epidermidis did not decrease significantly with time on CAPD (Figure 34g). However a small but significant decrease in intracellular killing ability was observed over the same 9 month period (Figure 34h; $p<0.01$).

FIGURE 34 : Change With Time on CAPD of:

Levels of-

(a) IgG in PDE

(b) C3 in PDE

(c) Transferrin in PDE

(d) Opsonising Capacity of PDE

(e) Bacteriostatic Activity of PDE

(f) Number of cells in PDE

(g) Ingestion of S.epidermidis

(h) Intracellular killing of S.epidermidis

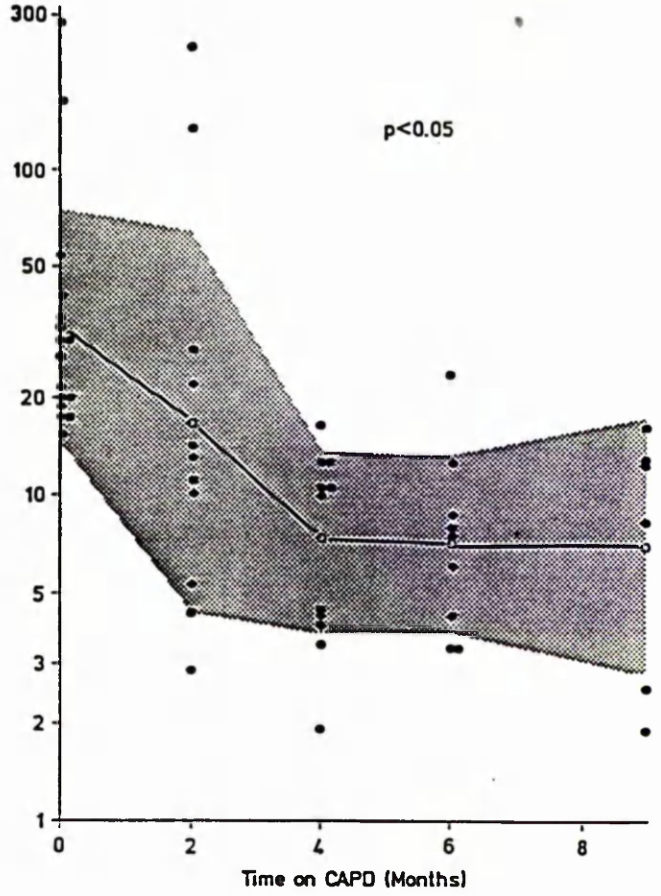
(Figures are on Pages 187-190)

● shows individual results from patients

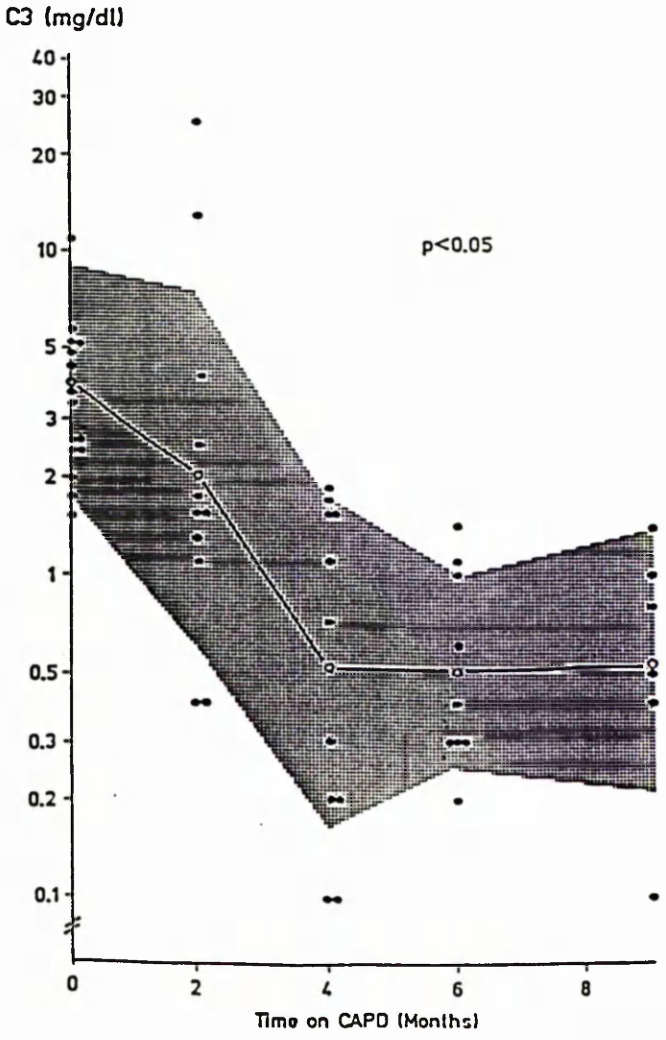
o shows the mean

▒▒ shows two standard deviations about the mean

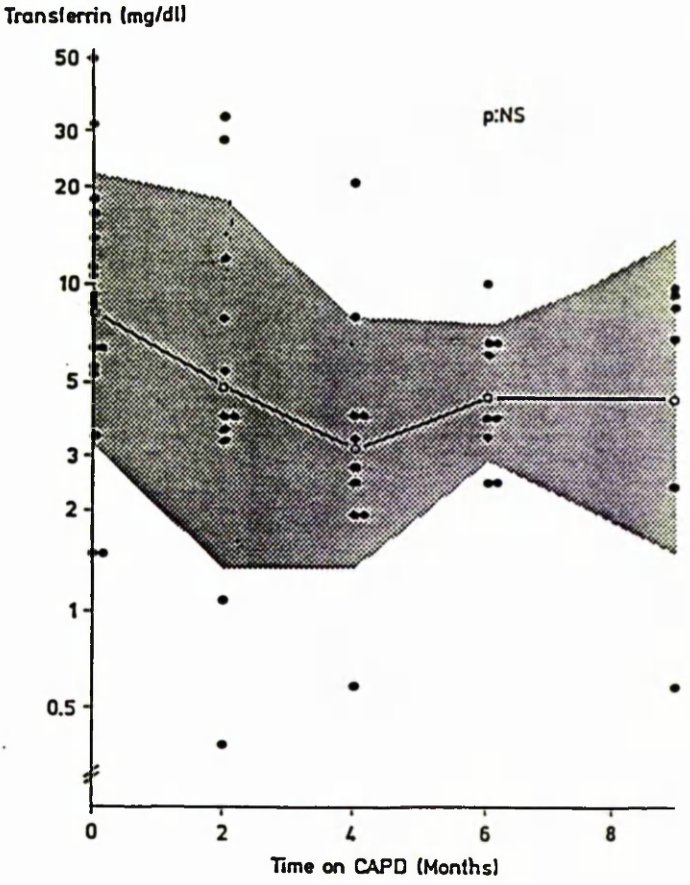
(a)


300
100
50
20
10
5
3
2
1
p<0.05
0
2
4
6
8
Time on CAPD (Months)

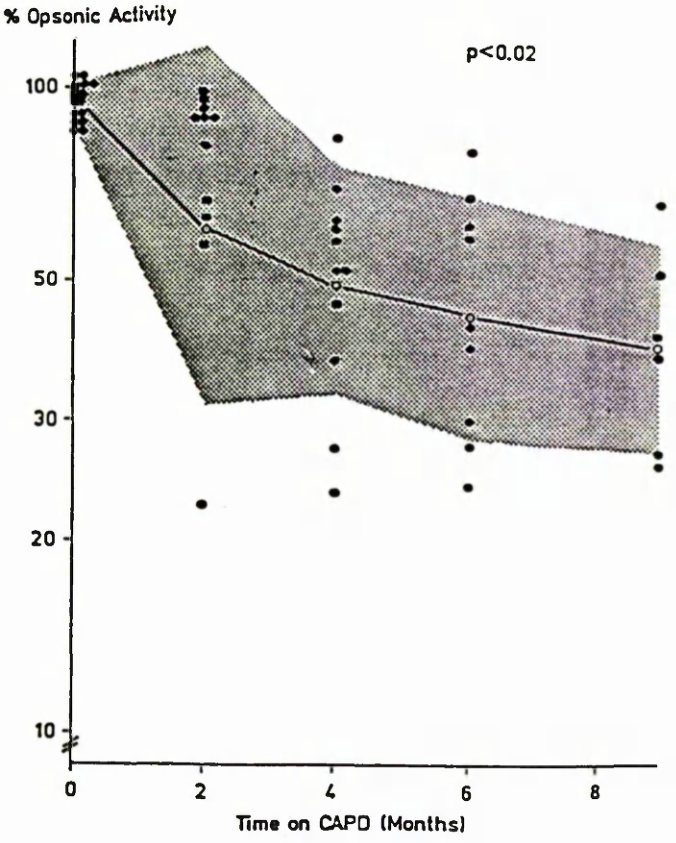
(b)


C3 (mg/dl)
40
30
20
10
5
3
2
1
0.5
0.3
0.2
0.1
p<0.05
0
2
4
6
8
Time on CAPD (Months)

(c)



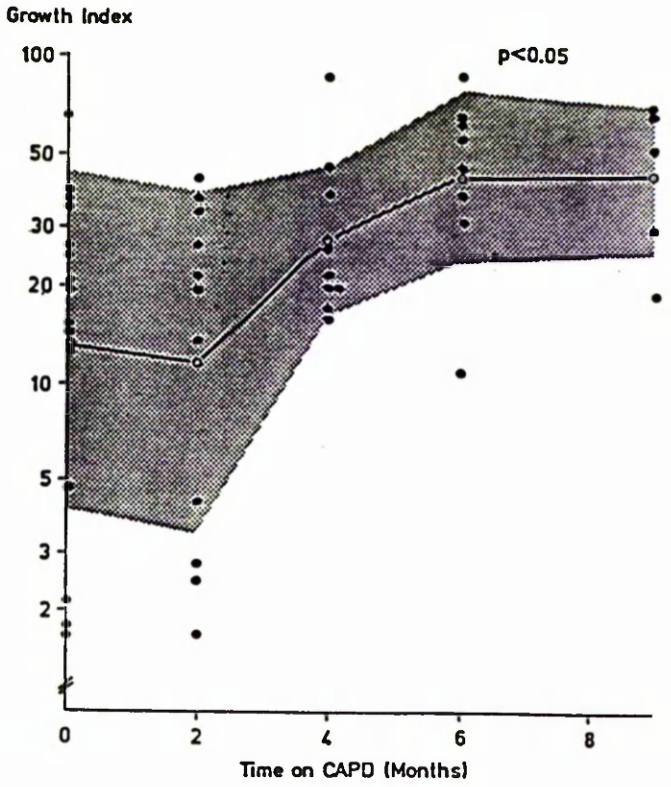
(d)



Opsonic Activity:

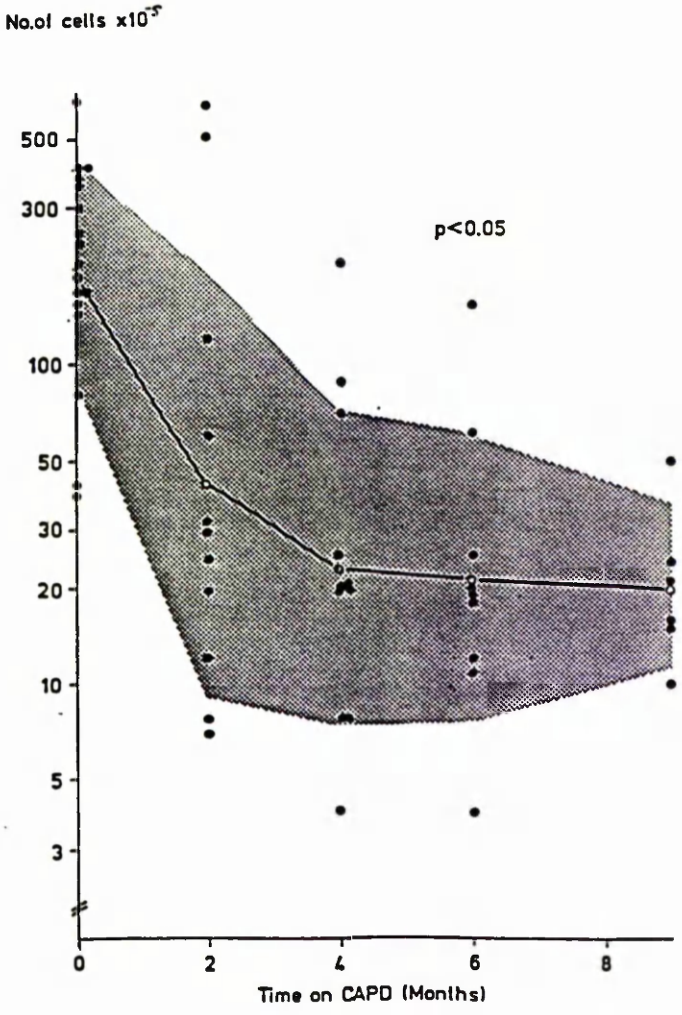
Percentage of S.epidermidis ingested when opsonised with PDE relative to ingestion of bacteria opsonised with NHS.

(e)

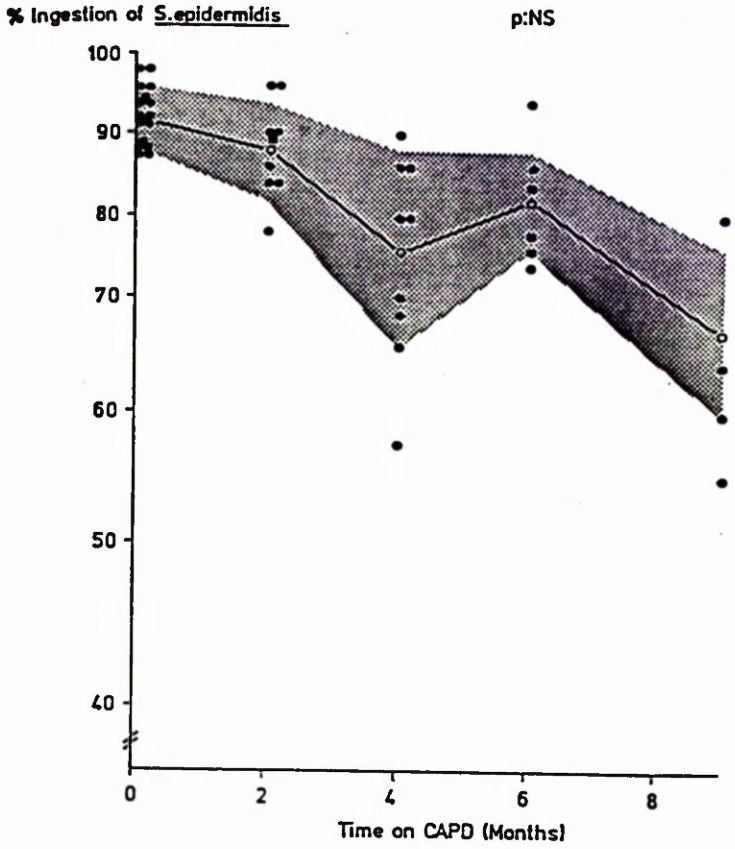


Growth Index = growth of S.epidermidis as a percentage of growth in uninhibited control (RPMI 1640 culture medium)

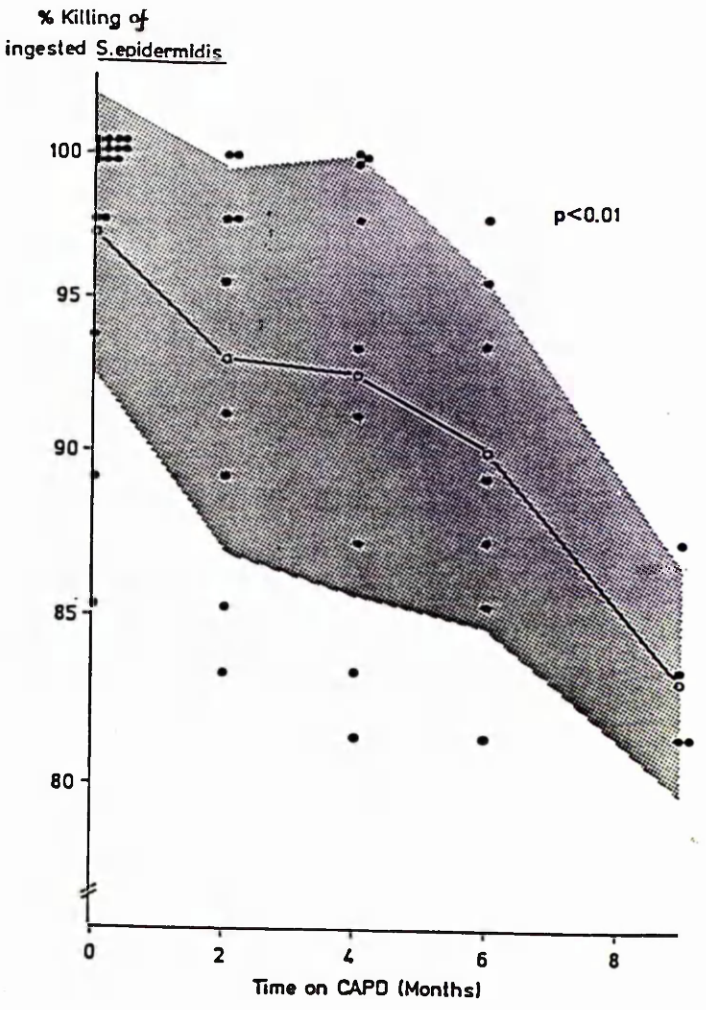
(f)



(g)


% Ingestion of S.epidermidis
p:NS
100
90
80
70
60
50
40
0
2
4
6
8
Time on CAPD (Months)

(h)


% Killing of
ingested S.epidermidis
p<0.01
100
95
90
85
80
0
2
4
6
8
Time on CAPD (Months)

6.5 DISCUSSION

The decrease in the levels of IgG and C3 in PDE and the corresponding decrease in related opsonic activity with length of time on CAPD suggests that the CAPD process reduces the immunity of the peritoneal cavity in patients that are believed to be already immunosuppressed by the uraemic state (Mezzano et al,1984; Funck-Brentano et al,1975; Touraine et al,1975). Reduction in the opsonic capacity of PDE will hinder the defences of the peritoneum in particular that of elimination of pathogens by phagocytes. Changes in the permeability of the peritoneal membrane, discussed previously in Chapter 5, may be responsible for the tendency for the levels of IgG and C3 in patients' PDE to level off after 4-6 months on CAPD. By this stage the peritoneal membrane may be altered by the CAPD process so that its permeability becomes the major factor in determining the quantity of serum proteins that gain entry (Krediet et al,1986; Dulaney and Hatch, 1984).

The decreasing bacteriostatic activity of PDE with time on CAPD adds further emphasis to the finding in Chapter 5 that CAPD fluid was significantly less bacteriostatic than normal peritoneal fluid. The tendency for Tf levels to decrease also implies that the growth of bacteria in the fluid may depend, at least to some extent, on Tf levels. However, the decrease in Tf levels did not reach statistical significance, possibly because Tf, being a smaller molecule than IgG or C3, may be less affected by any decrease in permeability of the peritoneal membrane.

When the activity of the cells was examined over the 9 month period, no significant change was found in their ability to ingest S.epidermidis. In contrast, there was a significant decrease in intracellular killing of S.epidermidis over the same time period. These findings are supported by the previous evidence in Chapter 4 showing that peritoneal cells from patients on CAPD had a significantly lower intracellular killing capacity than those from normal controls, whereas there was no difference in their ability to ingest S.epidermidis. It therefore appears that not only is the activity of the peritoneal cells reduced in CAPD patients, but the total number of cells isolated over the 9 months from the PDE significantly declines. This again agrees with the finding in Chapter 2 of a correlation between the number of cells isolated from different CAPD patients and the length of time they had been on CAPD (2.5.4) and may also be due to thickening of the peritoneal membrane.

Overall it seems that after the initial few months when the concentration and activity of the serum proteins in the PDE level off, no correlation would be found between the levels of these proteins and length of time on CAPD. The failure by Steen et al (1986), to find a significant correlation, after 9 months, between protein levels in PDE and time on CAPD is thus in agreement with the results reported here.

It was originally intended that the parameters examined above should be related to the incidence of peritonitis in each patient. However due to the low frequency of peritonitis in the patients examined here, and

the fact that only 6 patients were monitored for the full 9 months, it was not possible to relate the above findings to susceptibility to infection.

The gradual decrease in the immune defences of the peritoneum shown here in the initial few months after commencement of dialysis may mean that patients are less able to protect the peritoneum from infections after 6-9 months than they were at the start of CAPD. The clinical implications of such a decrease are provided by the findings of Smith et al (1986) and Young et al (1986), both of whom found that the incidence of peritonitis increased with time on CAPD, suggesting that a reduction in cellular and serum proteins levels and activities does indeed have a detrimental effect on the overall peritoneal defences of patients on CAPD. However, in addition to this Young et al (1986) also found a corresponding improvement in CMI during CAPD which was obviously not reflected by a reduction in the incidence of peritonitis. This suggests that CMI may therefore be of little importance in the long term in protecting the peritoneum from bacterial infection, which probably depends instead on humoral and phagocytic mechanisms. It is possible that although initially CMI responses to DNCB, for example, are indicative of the immune status of patients commencing CAPD (Tsakiris et al,1986) it may bear little relevence to patients established on CAPD.

CHAPTER 7

GENERAL DISCUSSION

GENERAL DISCUSSION

Although CAPD has been extensively used since 1978 little information has been available regarding the immune status of the peritoneum in these uraemic patients, and exactly how the technique may affect its defences. In addition it must be remembered that these patients are already believed to be immunosuppressed, so that any further reduction in the immune defences of dialysis patients may have far reaching effects, which in normal individuals would not be significant.

The two most important arms of the defence system of the peritoneum are the peritoneal cells and serum proteins and it was these factors that were examined in this thesis.

Overall a weakening of the defences of the peritoneum was found, probably as a consequence of the dialysis regime. In particular the CAPD peritoneal cells were found to be defective in their ability to cope with intracellular killing of bacteria.

The immaturity of the cells, thought to be the cause of this reduced activity, may also affect the antigen presenting function of macrophages, a suggestion supported by the reduced expression of HLA-DR antigen by peritoneal macrophages and this may hamper initiation of specific immune responses. Another factor related to the activity of these peritoneal cells was their capacity to release H_2O_2 which again was found to be deficient in patients on CAPD. The activity of these peritoneal cells therefore

appears to be sub-normal in several different ways, each of which could affect the immune status of these patients. However, the studies of H_2O_2 production and HLA-DR expression suggested that patients commencing CAPD may have an initial inflammatory reaction perhaps due to the implantation of the catheter which causes a temporary increase in macrophage activity.

The reduced levels of IgG, C3 and related opsonic activity in the PDE will further decrease the efficiency of the peritoneal phagocytic cells as opsonisation is essential for effective elimination of most pathogens. The similar reduction in Tf levels and in the corresponding bacteriostatic effect may impair the restriction of growth of bacteria in the peritoneum on initial infection, therefore allowing inadequate time for the cellular defences to be activated. The thickening of the peritoneal membrane which may be responsible for the reduction in the levels of the above proteins is probably an inevitable consequence of instilling 2 litres of an unphysiological electrolyte solution continuously into an environment which normally contains very little fluid.

When the activities and levels of the cells and proteins in PDE were related to the frequency of peritonitis a significant correlation was not found in every case, in spite of finding that most individual parameters indicated reduced activity compared with normal controls. This, however, may be due to the fact that the susceptibility to peritonitis of patients on CAPD is not related solely to a deficiency in one arm of the immune response, but is multifactorial. Individually, therefore,

a particular defect may have no effect on the overall peritonitis rate, but collectively they could be clinically significant.

Since CAPD was first introduced there have been vast improvements in reducing the possible sources of contamination when using the technique. Within the last three years, the introduction of the Travenol "O" Disconnect system in the Western Infirmary has greatly reduced the incidence of peritonitis (J.D. Briggs personal communication). More recently still, the Travenol "Y" set has been brought out which may reduce even further the likelihood of infection. The prevention of infection in the first place by good sterile technique is obviously of prime importance in controlling the peritonitis rate. All these improvements are essential as it is important to reduce the chances of exposing these patients with suboptimal defences to pathogens which to normal individuals would present no risk. In any one individual therefore, the two factors of sterile technique and peritoneal immune defences will determine the infection rate.

Many areas of research in this field still need to be examined, including the actual antigen presenting function of the peritoneal macrophages. Measurement of the expression of HLA-DR on patients' blood monocytes may determine whether they are analogous to the peritoneal cells or normal blood monocytes. It would also be of interest to measure levels of specific antibodies to certain bacteria as well as total IgG levels, as this might be more closely related to the peritonitis rate. In

addition serum levels of specific antibodies may provide more information as to the immune competence of patients. The levels of these specific antibodies might correspond more closely to levels in PDE than was found when total IgG levels were examined. Levels of IgA in PDE could also be measured as serum IgA, which unlike secretory IgA can act as an opsonin (M. Kerr personal communication) and may also be relevant to the defences of the peritoneum.

Although the results reported in this thesis may have no immediate clinical implications they have given a more detailed picture of the immune defences within the peritoneum of CAPD patients than was previously available. Possible direct improvements in the future might involve the addition of human Tf to the dialysate. Addition of IgG has already been found by Lamperi and Carozzi (1986b) to have beneficial effects. It is also possible that leaving the peritoneum empty overnight may give time for the peritoneum membrane to recover and allow it to "rest".

Although rigid adherence to strict aseptic technique is essential in preventing peritonitis, other factors play a part such as those relating to the reliability of connectors and lines, and other less well defined factors such as the quality of patient training, medical and nursing competence and experience. As mentioned before, the introduction of the disconnect system has greatly reduced the frequency of peritonitis but even this has not totally eradicated the incidence of peritonitis. The risk of developing peritonitis therefore rests on a delicate balance between colonisation of micro-organisms within the CAPD system, the dose of bacteria invading the peritoneal

cavity and the local peritoneal host defence mechanisms at the time of the insult. Improvements in catheter design, especially the use of material resistant to colonisation could be particularly important in controlling S. epidermidis infections. Stimulation of local host defence, possibly through the introduction of a vaccine might further reduce the incidence of peritonitis to more acceptable levels. Total eradication of peritonitis may not be a realistic goal and should not be a condition for continued use of CAPD.

Another major problem of CAPD which must be faced is the possible loss of ultrafiltration capacity and development of sclerosing encapsulating peritonitis, both of which are worrying complications of long term use of CAPD. The technique has only been extensively used for about 10 years, and it is not yet known what the long term effects of such a regime might be. Glucose, which is used in the dialysis fluid as an osmotic agent is not ideal as it is believed to cause loss of ultrafiltration, hyperlipidaemia and obesity in addition to a possible deleterious effect on the membrane. A suitable alternative has not yet been found.

In spite of these problems it must be remembered that CAPD has vastly increased the availability of dialysis to groups which otherwise might not have been given the opportunity; it has overcome the shortage of haemodialysis machines and offers a patient a more flexible approach to dialysis. But the results presented in this thesis emphasise the need for clinicians to consider the peritoneum of the CAPD patients as an immunocompromised site.

BIBLIOGRAPHY

ARCHER, G.L. (1985) Staphylococcus epidermidis and other coagulase negative staphylococci. In Principles and Practice of Infectious Diseases, ed. Mandell, G.L., Douglas, R.G., Bennett, J.E. Ch. 157, pp 1117-1123. New York: Wiley Medical.

BAILIE, G.R., MORTON, R., GANGULI, L., KEANEY, M. & WALDEK, S. (1987) Intravenous or intraperitoneal vancomycin for the treatment of continuous ambulatory peritoneal dialysis associated Gram-positive peritonitis. Nephron, 46, 316-318.

ACCOLLA, R.S., MORETTA, A. & CARREL, S. (1984) The human Ia system: an overview. Seminars in Haematology, 21, 287-295.

ADAMS, D.O. & NATHAN, C.F. (1983) Molecular mechanisms in tumour-cell killing by activated macrophages. Immunology Today, 4, 166-170.

ALBRECHTSEN, D. (1977) HLA-D-associated "Ia-like" antigens on human macrophages. Scandinavian Journal of Immunology, 6, 907-912.

ALOBAIDI, H.M., COLES, G.A., DAVIES, M. & LLOYD, D. (1986) Host defence in continuous ambulatory peritoneal dialysis: the efect of the dialysate on phagocytic function. Nephrology Dialysis Transplantation, 1, 16-21.

AMAIR, P., KHANNA, R., LEIBEL, B., PIERRATOS, A., VAS, S., MEEMA, E., BLAIR, G., CHISOLM, L., VAS, M., ZINGG, W., DIGENIS, G. & OREOPOULOS, D. (1982) Continuous ambulatory peritoneal dialysis in diabetics with end-stage renal disease. New England Journal of Medicine, 306, 625-630.

ARCHIBALD, F. (1983) Lactobacillus plantarum, an organism not requiring iron. FEMS Microbiology Letters, 19, 29-32.

BABIOR, B.M. (1984) The respiratory burst of phagocytes. Journal of Clinical Investigations, 73, 599-601.

BARR-ELI, M., TERRITO, M.C. & CLINE, M.J. (1981) The progeny of a single progenitor cell can develop characteristics of either a tissue or an alveolar macrophage. Blood, 57, 95-98.

BASHAM, T.Y. & MERIGAN, T.C. (1983) Recombinant gamma interferon increases HLA-DR synthesis and expression. Journal of Immunology, 130, 1492-1494.

BEELEN, R.H.J. & FLUITSMA, D.M. (1982) What is the relevance of exudate-resident macrophages? Immunology, 161, 266-273.

BEELEN, R.H.J., VON DER MEULEN, J., VERBRUGH, H., HOEFSMIT, E.C.M., OE, P.L. & VERHOEF, J. (1986) CAPD, a permanent state of peritonitis: a study on peroxidase activity. In Frontiers in Peritoneal Dialysis, ed. Maher, J.E. & Winchester, J.F. Ch.100, pp524-530. New York: Field Richards Associates.

BELLER, D.I. & HO, K. (1982) Regulation of macrophage populations V. Evaluation of the control of macrophage Ia expression in vitro. Journal of Immunology, 129, 971-976.

BELLER, D.I. & UNANUE, E.R. (1981) Regulation of macrophage populations II. Synthesis and expression of Ia antigens by peritoneal exudate macrophages is a transient event. Journal of Immunology, 126, 263-269.

BELLER, D.I., KEILY, J.M. & UNANUE, E.R. (1980) Regulation of macrophage populations I. Preferential induction of Ia immunologic stimuli. Journal of Immunology, 124, 1426-1432.

BENTLEY, C., ZIMMER, B. & HADDING, U. (1981) The macrophage as a source of complement components. In Lymphokines: A Forum for Immunoregulatory Cell Products, ed. Pick, E. Vol. 4, pp197-231. London: Academic Press.

BERGSTRöM, J., FüRST, P. & ZIMMERMAN, L. (1979) Uremic middle molecules exist and are biologically active. Clinical Nephrology, 11, 229-238.

BIANCO, C., GRIFFIN, F.M. & SILVERSTEIN, S.C. (1975) Studies of the macrophage complement receptor. Alteration of receptor function upon macrophage activation. Journal of Experimental Medicine, 141, 1278-1290.

BIRD, J., SHENG, Y.J. & GIROUD, J.P. (1984) Effects of supernatants and lysates of polymorphonuclear leucocytes; macrophage stimulatory factors. British Journal of Experimental Pathology, 65, 243-250.

BLOODWORTH, L.L. & HARBER, M.J. (1986) The importance of iron for bacterial growth in peritoneal dialysis fluid. In Frontiers in Peritoneal Dialysis, ed. Maher, J.E. and Winchester, J.F. Ch. 103, pp542-545. New York: Field Richards Associates.

BLUMENKRANTZ, M.J., GAHL, G.M., KOPPLE, J.D., KAMDAR, A.V., JONES, M.R., KESSEL, M. & COBURN, J.W. (1981) Protein losses during peritoneal dialysis. Kidney International, 19, 593-602.

BOTHWELL, T.H., CHARLTON, R.W., COOK,J.D. & FINCH, C.A. (1979) In Iron Metabolism in Man, Ch. 13, pp284-308. London: Blackwell Scientific Publications.

BRAMWELL, S.P., TSAKIRIS, D.J., BRIGGS, J.D., FOLLETT, E.A.C., STEWART, J., MCWHINNIE, D.L., WATSON, M.A., HAMILTON, D.N.H. & JUNOR, B.R.J. (1985) Dinitrochlorobenzene skin testing predicts response to hepatitis B vaccine in dialysis patients. The Lancet, i, 1412-1415.

BRIDGES, C.G., DASILVA, G.L., YAMAMURA, M. & VALDIMARSSON, H. (1980) A radiometric assay for the combined measurement of phagocytosis and intracellular killing of Candida albicans. Clinical and Experimental Immunology, 42, 226-233.

BROCK, J.H. (1985) Transferrins. In Metalloproteins, ed. Harrison, P.M. Vol. 2, pp 183-262. London:Macmillan.

BROCK, J.H. (1986) Iron and the outcome of infection. British Medical Journal, 293, 518-520.

BROCK, J.H. & MAINOU-FOWLER, T. (1983) The role of iron and transferrin in lymphocyte transformation. Immunology Today, 4, 347-351.

BROCK, J.H. & MAINOU-FOWLER, T. (1986) Iron and immunity. Proceedings of the Nutrition Society, 45, 305-315.

BUGGY, B.P., SCHABERG, D.R. & SWARTZ, R.D. (1984) Intraleukocytic sequestration as a cause of persistent Staphylococcus aureus peritonitis in continuous ambulatory peritoneal dialysis. American Journal of Medicine, 76, 1035-1040.

BULLEN, J.J. (1981) The significance of iron in infection. Reviews of Infectious Diseases, 3, 1127-1138.

BULLEN, J.J., CUSHNIE, G.H. & ROGERS, H.J. (1967) The abolition of the protective effect of Clostridium welchii type A antiserum by ferric iron. Immunology, 12, 303-312.

BULLEN, J.J., LEIGH, L.C. & ROGERS, H.J. (1968) The effect of iron compounds on the virulence of Escherichia coli for guinea-pigs. Immunology, 15, 581-588.

CALAMAI, E.G., BELLER, D.I. & UNANUE, E.R. (1982) Regulation of macrophage populations IV. Modulation of Ia expression in bone marrow-derived macrophages. Journal of Immunology, 128, 1692-1694.

CESARONE, C.F., BOLOGNESI, C. & SANTI, L. (1979) Improved microfluorometric DNA determination in biological material using 33258 hoechst. Analytical Biochemistry, 100, 188-197.

CHANG, Y.T. (1969) Suppressive Activity of streptomycin on the growth of Mycobacterium lepraemurium in macrophage cultures. Applied Microbiology, 17, 750-754.

CLERICI, N., REBOIRAS, S., FIERRO, C. & LEYVA-COBIAN, F. (1984) Expression of Ia like (HLA-DR) antigens on human alveolar macrophages. Clinical and Experimental Immunology, 58, 388-394.

COLES, G.A. (1985) Is peritoneal dialysis a good long term treatment? British Medical Journal, 290, 1164-1166.

CONKLING, P., KLASSEN, D.K. & SAGONE, A.L. (1982) Comparison of antibody-dependent cytotoxicity mediated by human polymorphonuclear cells, monocytes and alveolar macrophages. Blood, 60, 1290-1297.

COSTABEL, U., BROSS, K.J., ANDREESEN, R. & MATTHYS, H. (1986) HLA-DR antigens on human macrophages from bronchoalveolar lavage fluid. Thorax, 41, 261-265.

CRAPO, J.D. (1977) The role of the superoxide dismutases in pulmonary oxygen toxicity. In Superoxide and Superoxide Dismutases, ed. Michelson A.M., McCord, J.M. & Fridovich, I. pp231-238, London: Academic Press.

DAEMS, W.T. (1980) Peritoneal macrophages. In The Reticuloendothelial System: A Comprehensive Treatise, ed. Carr, I. & Daems, W.T. Vol.I, pp57-127. New York: Plenum Press.

DAEMS, W.T. & DE BAKKER, J.M. (1982) Do resident macrophages proliferate? Immunobiology, 161, 204-211.

DANA, N., TODD, R.F., PITT, J., SPRINGER, T.A. & ARNAOUT, M.A. (1984) Deficiency of a surface membrane glycoprotein (Mol) in man. Journal of Clinical Investigation, 73, 153-159.

DE MAEYER-GUIGNARD, J. & DE MAEYER, E. (1985) Immunomodulation by interferons: recent developments. In Interferon, ed. Gresser, I. Vol.6, pp69-91, New York: Academic Press.

DIGENIS, G.E., KHANNA, R. & PANTALONY, D. (1982) Eosinophilia after implantation of the peritoneal catheter. Peritoneal Dialysis Bulletin, 2, 98-99.

DI PAOLO, N., SACCHI, G., DE MIA, M., GAGGIOTTI, E., CAPOTONDO, L., ROSSI, P., BERNINI, M., PUCCI, A.M., IBBA, L., SABATELLI, P. & ALESSANDRINI, C. (1986) Morphology of the peritoneal membrane during continuous ambulatory peritoneal dialysis. Nephron, 44, 204-211.

DISKIN, C.J., COPLON, N., FELDMAN, C. & VOSTI, K. (1983) Antimicrobial activity in continuous ambulatory peritoneal dialysis. Peritoneal Dialysis Bulletin,3, 150-153.

DULANEY, J.T. & HATCH, F.E. (1984) Peritoneal dialysis and loss of proteins: a review. Kidney International, 26, 253-262.

DUNN, D.L. & SIMMONS, R.L. (1982) Fibrin in peritonitis III. The mechanism of bacterial trapping by polymerizing fibrin. Surgery, 92, 513-519.

DUNN, D.L., BARKE, R.A., KNIGHT, N.B., HUMPHREY, E.W. & SIMMONS, R.L. (1985) Role of resident macrophages, peripheral neutrophils and translymphatic absorption in bacterial clearance from the peritoneal cavity. Infection and Immunity, 49, 257-264.

DUWE, A.K., VAS, S.I. & WEATHERHEAD, J.W. (1981) Effects of the composition of peritoneal dialysis fluid on chemiluminescence, phagocytosis and bactericidal activity in vitro. Infection and Immunity, 33, 130-135.

EASMON, C.S.F., COLE, P.J., WILLIAMS, A.J. & HASTINGS, M. (1980) The measurement of opsonic and phagocytic function by luminol-dependent chemiluminescence. Immunology, 41, 67-74.

EDELSON, P.J. (1981) Macrophage plasma membrane enzymes as differentiation markers of macrophage activation. In Lymphokines: A Forum For Immunoregulatory Cell Products, ed. Pick, E. Vol. 3, pp57-83. London: Academic Press.

EHLENBERGER, A.G. & NUSSENZWEIG, V. (1977) The role of membrane receptors for C3b and C3d in phagocytosis. Journal of Experimental Medicine, 145, 357-371.

EZEKOWITZ, R.A.B. & GORDON, S. (1986) Interaction and regulation of macrophage receptors. In Biochemistry of Macrophages. Ciba Foundation Symposium 118, ed. Evered, D., Nugent, J. & O'Connor, M. pp127-136, London: Pitman.

FANGER, M.W., SHEN, L., PUGH, J. & BERNIER, G.M. (1980) Subpopulations of human peripheral granulocytes and monocytes express receptors for IgA. Proceedings of the National Academy of Science of the U.S.A., 77, 3640-3644.

FERNANDEZ-CRUZ, E., ULICH, T. & SCHREIBER, R.D. (1985) In vivo activity of lymphokine-activated macrophages in host defense against neoplasia. Journal of Immunology, 134, 3489-3496.

FINKELSTEIN, R.A., SCIORTINO, C.V. & MCINTOSH, M.A. (1983) Role of iron in microbe-host interactions. Reviews of Infectious Diseases, 5, S759-S777.

FLYNN, A. (1986) Expression of Ia and the production of interleukin-1 by peritoneal exudate macrophages activated in vivo by steroids. Life Sciences, 38, 2455-2460.

FROMKES, J.J., THOMAS, F.B., MEKHJIAN, H.S. & EVANS, M. (1977) Antimicrobial activity of human ascitic fluid. Gastroenterology, 73, 668-672.

FUNCK-BRENTANO, J.L., MAN, N.K., SAUSSE, A., CUEILLE, G., ZINFRAFF, J., DRUEKE, T., JUNGERS, P. & BILLON, J.P. (1975) Neuropathy and "middle" molecule toxins. Kidney International, 7, S352-S356.

GALLIN, J.I., BUESCHER, E.S., SELIGMANN, B.E., NATH, J., GAITHER, T. & KATZ, P. (1983) Recent advances in chronic granulomatous disease. Annals of Internal Medicine, 99, 657-674.

GEMMELL, C.G. (1984) The staphylococcus, its virulence factors and polymorphonuclear leukocyte function. In The Staphylococci Proceedings of the Vth International Symposium on staphylococci and staphylococcal infections, ed. Jeljaszewicz, J. New York: Gustav Fischer Verlag.

GANDHI, V.C., HUMAYUN, H.M., ING, T.S., DAUGIRDAS, J.T., JABLOKOW, V.R., IWATSUKI, S., GEIS, W.P. & HANO, J.E. (1980) Sclerotic thickening of the peritoneal membrane in maintenance peritoneal dialysis patients. Archives of Internal Medicine, 140, 1201-1203.

GEMSA, D. (1981) Stimulation of prostaglandin E release from macrophages and possible role in the immune response. In Lymphokines: A Forum For Immunoregulatory Cell Products, ed. Pick, E. Vol. 4, pp335-375. London: Academic Press.

GERSHON, H.E., KUANG, Y.D., SCALA, G. & OPPENHEIM, J.J. (1985) Effects of recombinant gamma interferon on HLA-DR shedding by human peripheral blood adherent mononuclear cells. Journal of Leukocyte Biology, 38, 279-291.

GIACCHINO, F., QUARELLO, F., PELLEREY, M. & PICCOLI, G. (1983) Continuous ambulatory peritoneal dialysis improves immunodeficiency in uremic patients. Nephron, 35, 209-210.

GIANGRANDE, A., CANTU, P., DE FRANCESCO, D. & LIMIDO, A. (1984) Inhibitors of lymphocyte activity in peritoneal fluid of continuous ambulatory peritoneal dialysis (CAPD). International Journal of Artificial Organs, 7, 63-66.

GIBBS, D.L. & ROBERTS, R.B. (1975) The interaction in vitro between human polymorphonuclear leukocytes and Neisseria gonorrhoeae cultivated in the chick embryo. Journal of Experimental Medicine, 141, 155-171.

GLADSTONE, G.P. & WALTON, E. (1971) The effect of iron and haematin on the killing of Staphylococci by rabbit polymorphs. British Journal of Experimental Pathology, 52, 452-464.

GLASS, E., STEWART, J. & WEIR, D.M. (1981) Presence of bacterial binding "lectin-like" receptors on phagocytes. Immunology, 44, 529-534.

GOLDE, D.W. & CLINE, M.J. (1972) Identification of the colony-stimulating cell in human peripheral blood. Journal of Clinical Investigation, 51, 2981-2983.

GOLDER, J.P. & DOE, W.F. (1983) Isolation and preliminary characterisation of human intestinal macrophages. Gastroenterology, 84, 795-802.

GOLDSTEIN, C.S., BOMALASKI, J.S., ZURIER, R.B., NEILSON, E.G. & DOUGLAS, S.D. (1984) Analysis of peritoneal macrophages in continuous ambulatory peritoneal dialysis patients. Kidney International, 26, 733-740.

GOODWIN, J.S. & WEBB, D.R. (1980) Regulation of the immune response by prostaglandins. Clinical Immunology and Immunopathology, 15, 106-122.

GRAY, E.D., PETERS, G., VERSTEGEN, M. & REGELMANN, W.E. (1984) Effect of extracellular slime substance from Staphylococcus epidermidis on the human cellular immune responses. Lancet, i, 365-367.

GORDON, S. (1980) Lysozyme and plasminogen activator: constitutive and induced secretory products of mononuclear phagocytes. In Mononuclear Phagocytes, Functional Aspects, ed. van Furth, R. Part II, Ch. 49, pp1273-1298. The Hague: Martinus Nijhoff Publishers.

GORDON, S. & RUBINI, M.E. (1967) Protein losses during peritoneal dialysis. American Journal of the Medical Sciences, 253, 283-292.

GORDON, S., TODD, J. & COHN, Z.A. (1974) In vitro synthesis and secretion of lysozyme by mononuclear phagocytes. Journal of Experimental Medicine, 139, 1228-1248.

GREEN, S.A., PLUTNER, H. & MELLMAN, I. (1985) Biosynthesis and intracellular transport of the mouse macrophage Fc receptor. Journal of Biological Chemistry, 260, 9867-9874.

GRIFFIN, F.M. & MULLINAX, P.J. (1981) Augmentation of macrophage complement receptor function in vitro. III C3b receptors that promote phagocytosis migrate within the plane of the macrophage plasma membrane. Journal of Experimental Medicine, 154, 291-305.

GRIFFIN, F.M. & MULLINAX, P.J. (1985) In vivo activation of macrophage C3 receptors for phagocytosis. Journal of Experimental Medicine, 162, 352-357.

GRIFFIN, F.M., BIANCO, C. & SILVERSTEIN, S.C. (1975) Characterization of the macrophage receptor for complement and demonstration of its functional independence from the receptor for the Fc portion of immunoglobulin G. Journal of Experimental Medicine, 141, 1269-1277.

GRIFFITHS, E. (1983) Availability of iron and survival of bacteria in infection. In Medical Microbiology, ed. Easmon, C.S.F., Brown, M. and Lambert, P.A. Vol.3, pp153-177. London: Academic Press.

GRUNER, S., VOLK, H.D., FALCK, P. & VON BAEHR, R. (1986) The influence of phagocytic stimuli on the expression of HLA-DR antigens; role of reactive oxygen intermediates. European Journal of Immunology, 16, 212-215.

GUILBERT, L.J. & STANLEY, E.R. (1980) Specific interaction of murine colony-stimulating factor with mononuclear phagocytic cells. Journal of Cell Biology, 85, 153-159.

HAEFFNER-CAVAILLON, N., CHABY, R., CAVAILLON, J.M. & SZABO, L. (1982) Lipopolysaccharide receptor on rabbit peritoneal macrophages I. Binding Characteristics. Journal of Immunology, 128, 1950-1954.

HASKINS, K., KAPPLER, J. & MARRACK, P. (1984) The major histocompatibility complex-restricted antigen receptor on T cells. Annual Reviews of Immunology, 2, 51-66.

HAU, T. & SIMMONS, R.L. (1978) Heparin in the treatment of experimental peritonitis. Annuals of Surgery, 187, 294-298.

HAU, T., AHRENHOLZ, D.H. & SIMMONS, R.L. (1979) Secondary bacterial peritonitis: the biologic basis of treatment. In Current Problems in Surgery, ed. Ravitch, M.M. Vol. XVI, No. 10, London, Year Book Medical Publishers Inc.

HOLMBERG, C.G. & LAURELL, C.B. (1945) Studies on the capacity of serum to bind iron. A contribution to our knowledge of the regulation mechanism of serum iron. Acta Physiologica Scandinavica, 10, 307-319.

HOLMES, B., QUIE, P.G., WINDHORST, D.B. & GOOD, R.A. (1966) Fatal granulomatous disease of childhood. The Lancet, i, 1225-1228.

HOLMES, B., PAGE, A.R. & GOOD, R.A. (1967) Studies of the metabolic activity of leucocytes from patients with a genetic abnormality of phagocytic function. Journal of Clinical Investigation, 46, 1422-1432.

HORWITZ, M.A., (1982) Phagocytosis of micro-organisms. Reviews of Infectious Diseases, 4, 104-123.

HUMAYUN, H.M., ING, T.S., DAUGIRDAS, J.T., GANDHI, V.C., POPLI, S., ROBINSON, J.A., HANO, J.E. & ZAYAS, I. (1981) Peritoneal fluid eosinophilia in patients undergoing maintenance peritoneal dialysis. Archives of Internal Medicine, 141, 1172-1173.

HUMES, J.L., BONNEY, R.J., PELUS, L., DAHLGREN, M.E., SADOWSKI, S.J., KUEHL, F.A., & DAVIES, P. (1977) Macrophages synthesise and release prostaglandins in response to inflammatory stimuli. Nature, 269, 149-151.

HUMES, J.L., BURGER, S., GALAVAGE, M., KUEHL, F.A., WIGHTMAN, P.D., DAHLGREN, M.E., DAVIES, P. & BONNEY, R.J. (1980) The diminished production of arachidonic acid oxygenation products by elicited mouse peritoneal macrophages: possible mechanisms. Journal of Immunology, 124, 2110-2116.

HURLEY, R.M., MUOGBO, D., WILSON, G.W. & ALI, M.A.M. (1977) Cellular composition of peritoneal effluent: response to bacterial peritonitis. Canadian Medical Association Journal, 117, 1061-1062.

INADA, S., BROWN, E.J., GAITHER, T.A., HAMMER, C.H., TAKAHASHI, T. & FRANK, M.M. (1983) C3d receptors are expressed on human monocytes after in vitro cultivation. Immunology, 80, 2351-2355.

ISERSKY, C., RIVERA, J., SEGAL, D.M. & TRICHE, T.(1983) The fate of IgE bound to rat basophilic leukemia cells. II Endocytosis of IgE oligomers and effect on receptor turnover. Journal of Immunology, 131, 388-396.

ISHIZAKA, T., ISHIZAKA, K., BORSOS, T. & RAPP, H. (1966) C' fixation by human isoagglutinins: fixation of C1 by IgG and IgM but not by IgA antibody. Journal of Immunology, 97, 716-726.

JACQUES, Y.V. & BAINTON, D.F. (1978) Changes in pH within the phagocytic vacuoles of human neutrophils and monocytes. Laboratory Investigation, 39, 179-185.

JOHNSTON, R.B. (1981) Enhancement of phagocytosis-associated oxidative metabolism as a manifestation of macrophage activation. In Lymphokines: A Forum For Immunoregulatory Cell Products, ed. Pick, E. Vol.3, pp33-56. London: Academic Press.

JOHNSTON, R.B. & STROUD, R.M. (1977) Complement and host defense against infection. Journal of Pediatrics, 90, 169-179.

JOINER, K.A., BROWN, E.J. & FRANK, M.M. (1984) Complement and bacteria: chemistry and biology in host defense. Annual Reviews of Immunology, 2, 461-491.

JOSEPH, M., CAPRON, A., BUTTERWORTH, A.E., STURROCK, R.F. & HOUBA, V. (1978) Cytotoxicity of human and baboon mononuclear phagocytes against schistosomula in vitro: induction by immune complexes containing IgE and Schistosoma mansoni antigens. Clinical and Experimental Immunology, 33, 48-56.

KAMPSCHMIDT, R.F. (1984) The numerous postulated biological manifestations of interleukin-1. Journal of Leukocyte Biology, 36, 341-355.

KAPLAN, G. & GAUDERNACK, G. (1982) In vitro differentiation of human monocytes. Differences in monocyte phenotypes induced by cultivation on glass or on collagen. Journal of Experimental Medicine, 156, 1101-1114.

KAPLAN, G., ESKELAND, T. & SELJELID, R. (1978) Difference in the effect of immobilized ligands on the Fc and C3 receptors of mouse peritoneal macrophages in vitro. Scandinavian Journal of Immunology, 7, 19-24.

KEANE, W.F. & PETERSON, P.K. (1984) Host defense mechanisms of the peritoneal cavity and continuous ambulatory peritoneal dialysis. Peritoneal Dialysis Bulletin, 4, 122-127.

KEANE, W.F., COMTY, C.M., VERBRUGH, H.A. & PETERSON, P.K. (1984) Opsonic deficiency of peritoneal dialysis effluent in continuous ambulatory peritoneal dialysis. Kidney International, 25, 539-543.

KEUSCH, G.T., AMBINDER, E.P., KOVACS, I., GOLDBERG, J.D., PHILLIPS, D.M. & HOLLAND, J.F. (1982) Role of opsonins in clinical response to granulocyte transfusion in granulocytopenic patients. American Journal of Medicine, 73, 552-563.

KOWALEWSKI, J., TOMASZEWSKI, J., HANZLIK, J., ZAWISLAK, H. & ZBIKOWSKA, A. (1971) The elimination of free, peptide-bound and protein-bound hydroxyproline into dialysate during peritoneal dialysis in patients with renal failure. Clinica Chimica Acta 34, 123-126.

KREDIET, R.T., ZUYDERHOUDT, F.M.J., BOESCHOTEN, E.W. & ARISZ, L. (1986) Peritoneal permeability to proteins in diabetic and non-diabetic continuous ambulatory peritoneal dialysis patients. Nephron, 42, 133-140.

KRONENBERG, M., SIU, G., HOOD, L.E. & SHASTRI, N. (1986) The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. Annual Reviews of Immunology, 4, 529-591.

KUNKEL, S.L., CAMPBELL, D.A., CHENSUE, S.W. & HIGASHI, G.I. (1986) Species-dependent regulation of monocyte/macrophage Ia antigen expression and antigen presentation by prostaglandin E. Cellular Immunology, 97, 140-145.

KURLAND, J.I., BROXMEYER, H.E., PELUS, L.M., BOCKMAN, R.S. & MOORE, M.A.S. (1978) Role for monocyte-macrophage-derived colony-stimulating factor and prostaglandin E in the positive and negative feedback control of myeloid stem cell proliferation. Blood, 52, 338-407.

KURLANDER, R.J., HANEY, A.F. & GARTRELL, J. (1984) Human peritoneal macrophages possess two populations of IgG Fc receptors. Cellular Immunology, 86, 479-490.

LAFUSE, W.P. & DAVID, C.S. (1984) Ia antigens. Genes, molecules, and functon. Transplantation, 38, 443-453.

LAM, C. & MATHISON, G.E. (1979) Phagocytosis measured as inhibition of uridine uptake: a method that distinguishes between surface adherence and ingestion. Journal of Medical Microbiology, 12, 459-466.

LAMPERI, S. & CAROZZI, S. (1986a) Suppressor resident peritoneal macrophages and peritonitis incidence in continuous ambulatory peritoneal dialysis. Nephron, 44, 219-225.

LAMPERI, S. & CAROZZI, S. (1986b) Defective opsonic activity of peritoneal effluent during continuous ambulatory peritoneal dialysis (CAPD): importance and prevention. Peritoneal Dialysis Bulletin, 6, 87-92.

LASKER, N., BURKE, J.F., PATCHEFSKY, A. & HAUGHEY, E. (1975) Peritoneal reactions to particulate matter in peritoneal dialysis solutions. Transactions American Society for Artificial Internal Organs, XXI, 342-344.

LEE, S. & SCHOEN, I. (1967) Eosinophilia of peritoneal fluid and peripheral blood associated with chronic peritoneal dialysis. American Journal of Clinical Pathology, 47, 638-640.

LEW, P.D., ZUBLER, R., VAUDAUX, P., FARQUET, J.J., WALDVOGEL, F.A. & LAMBERT, P.H. (1979) Decreased heat-labile opsonic activity and complement levels associated with evidence of C3 breakdown products in infected pleural effusions. Journal of Clinical Investigation, 63, 326-334.

MACGOWAN, A.P., PETERSON, P.K., KEANE, W. & QUIE, P.G. (1983) Human peritoneal macrophage phagocytic, killing, and chemiluminescent responses to opsonized Listeria monocytogenes. Infection and Immunity, 40, 440-443.

MCKEEVER, P.E. & SPICER, S.S. (1980) Surface receptors of mononuclear phagocytes. In The Reticuloendothelial System. A Complete Treatise, ed. Carr, I. & Daems, W.T. Vol. 1, 161-258. New York: Plenum Press.

MCWHINNIE, D.L., BRADLEY, J.A., BRAMWELL, S.P., HAMILTON, D.N.H., MACPHERSON, S.G., CRAM,L.P., MORE, I.A.R., FORWELL, M.A., SMITH, W.G.J., BRIGGS, J.D. & JUNOR, B.J.R. (1986) Sclerosing peritonitis- a further complication of CAPD. In Frontiers in Peritoneal Dialysis, ed. Maher, J.E. & Winchester, J.F. Ch. 124, pp638-642. New York: Field Richards Associates.

MANTOVANI, B., RABINOVITCH, M. & NUSSENZWEIG, V. (1972) Phagocytosis of immune complexes by macrophages. Journal of Experimental Medicine, 135, 780-792.

MARUTA, H. & MIZUNO, D. (1971) Selective recognition of various erythrocytes in endocytosis by mouse peritoneal macrophages. Nature New Biology, 234, 246-248.

MELEWICZ, F.M., ZEIGER, R.S., MELLON, M.H., O'CONNOR, R.D. & SPIEGELBERG, H.L. (1981) Increased IgE-dependent cytotoxicity by blood mononuclear cells of allergic patients. Clinical and Experimental Immunology, 43, 526-533.

MELEWICZ, F.M., PLUMMER, J.M. & SPIEGELBERG, H.L. (1982) Comparison of the Fc receptors for IgE on human lymphocytes and monocytes. Journal of Immunology, 129, 563-569.

MELLMAN, I. & PLUTNER, H. (1984) Internalization and degradation of macrophage Fc receptors bound to polyvalent immune complexes. Journal of Cell Biology, 98, 1170-1177.

MELLMAN, I.S., PLUTNER, H., STEINMAN, R.M., UNKELESS, J.C. & COHN, Z.A. (1983) Internalization and degradation of macrophage Fc receptors during receptor-mediated phagocytosis. Journal of Cell Biology, 96, 887-895.

MELLMAN, I., PLUTNER, H. & UKKONEN, P. (1984) Internalization and rapid recycling of macrophage Fc receptors tagged with monovalent antireceptor antibody: possible role of a prelysosomal compartment. Journal of Cell Biology, 98, 1163-1169.

METCALF, D. (1982) Regulation of macrophage production. Advances in Experimental Medicine and Biology, 155, 33-48.

METZGER, H. (1983) The receptor on mast cells and related cells with high affinity for IgE. Contemporary Topics: Molecular Immunology, 9, 115-145.

METZGER, H., ALCARAZ, G., HOHMAN, R., KINET, J.P., PRIBLUDA, V. & QUARTO, R. (1986) The receptor with high affinity for immunoglobulin E. Annual Reviews of Immunology, 4, 419-470.

MEZZANO, S., PESCE, A.J., POLLAK, V.E. & MICHAEL, J.G. (1984) Analysis of humoral and cellular factors that contribute to impaired immune responsiveness in experimental uremia. Nephron, 36, 15-19.

MICHELSON, A.M. (1977) Toxicity of superoxide radical anions . In Superoxide and Superoxide Dismutases, ed. Michelson, A.M., McCord, J.M. & Fridovich, I. pp245-255. London: Academic Press.

MICHL, J., PIECZONKA, M.M., UNKELESS, J.C. & SILVERSTEIN, S.C. (1979) Effects of immobilized immune complexes on Fc-and complement-receptor function in resident and thioglycollate-elicited mouse peritoneal macrophages. Journal of Experimental Medicine, 150, 607-621.

MILES, A.A. & MISRA, S.S. (1938) The estimation of bactericidal power of the blood. Journal of Hygiene, 38, 732-749.

MILES, A.A., KHIMJI, P.L. & MASKELL, J. (1979) The variable response of bacteria to excess ferric iron in host tissues. Journal of Medical Microbiology, 12, 17-28.

MILMAN, N., STRANDBERG PEDERSEN, N. & VISFELDT, J. (1984) Serum ferritin in patients on regular peritoneal and haemodialysis treatment: relation to marrow haemosiderin iron stores. Dannish Medical Bulletin, 31, 245-248.

MORGAN, E.H. (1981) Transferrin, biochemistry, physiology and clinical significance. In Molecular Aspects of Medicine, ed. Baum, H., Gergely, J. & Fanburg, B.L. Vol. 4, pp3-123. New York: Pergamon.

NATHAN, C., NOGUEIRA, N., JUANGBHANICH, C., ELLIS, J. & COHN, Z. (1979) Activation of macrophages in vivo and in vitro. Correlation between hydrogen peroxide release and killing of Trypanosoma cruzi. Journal of Experimental Medicine, 149, 1056-1068.

NATHAN, C.F., MURRAY, H.W., WIEBE, M.E. & RUBIN, B.Y. (1983) Identification of gamma interferon as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. Journal of Experimental Medicine, 158, 670-689.

NEAUPORT-SAUTES, C., RABOURDIN-COMBE, C. & FRIDMAN, W.H. (1979) T-cell hybrids bear Fc receptors and secrete suppressor immunoglobulin binding factor. Nature, 277, 656-659.

NEILANDS, J.B. (1981) Microbial iron compounds. Annual Reviews of Biochemistry, 50, 715-731.

NEUMANN, C. (1982) Mononuclear phagocytes as producers of interferon. In Lymphokines: A Forum for Immunoregulatory Cell Products, ed. Pick, E. Vol. 7, pp165-202. London: Academic Press

NEWMAN, S.L., BECKER, S. & HALME, J. (1985) Phagocytosis by receptors for C3b(CR_1), iC3b(CR_3), and IgG(Fc) on human peritoneal macrophages. Journal of Leucocyte Biology, 38, 267-278.

NG, A.K., ZHANG, Y.H., RUSSO, C. & FERRONE, S. (1982) Immunochemical and functional analysis of Ia-like antigens on human monocyte-macrophages with monoclonal antibodies. Cellular Immunology, 66, 78-87.

OREOPOULOS, D.G., KHANNA, R., MCCREADY, W., KATIRTZOGLOU, A. & VAS, S. (1980) Continuous ambulatory peritoneal dialysis in Canada. Dialysis and Transplantation, 9, 224-226.

PAGE, R.C., DAVIES, P. & ALLISON, A.C. (1978) The macrophage as a secretory cell. International Review of Cytology, 52, 119-157.

PARAWRESCH, M.R., RADZUN, H.J. & DOMMES, M. (1981) The homogeneity and monocytic origin of human peritoneal macrophages evidenced by comparison of esterase polymorphism. American Journal of Pathology, 102, 209-218.

PASSWELL, J.H., COLTEN, H.R., SCHNEEBERGER, E.L., MAROM, Z. & MERLER, E. (1980) Modulation of human monocyte functions by Fc fragments of IgG: a comparison to other monocyte "activators". Immunology, 41, 217-225.

PATTERSON, R.J. & YOUMANS, G.P. (1970) Multiplication of Mycobacterium tuberculosis within normal and immune mouse macrophages cultivated with and without streptomycin. Infection and Immunity, 1, 30-40.

PAULI, H.G., BUTTIKOFER, E. & VORBURGER, C.H. (1966) Clinical experience with peritoneal dialysis. Helvetica Medica Acta, 33, 51-58.

PAWLOWSKI, N.A., KAPLAN, G., HAMILL, A.L., COHN, Z.A. & SCOTT, W.A. (1983) Arachidonic acid metabolism by human monocytes. Studies with platelet-depleted cultures. Journal of Experimental Medicine, 158, 393-412.

PETERSON, P.K., VERHOEF, J., SCHMELING, D. & QUIE, P.G. (1977) Kinetics of phagocytosis and bacterial killing by human polymorphonuclear leucocytes and monocytes. Journal of Infectious Diseases, 136, 502-509.

PETERSON, P.K., WILKINSON, B.J., KIM, Y., SCHMELING, D., DOUGLAS, S.D., QUIE, P.G. & VERHOEF, J. (1978) The key role of peptidoglycan in the opsonization of Staphylococcus aureus. Journal of Clinical Investigation, 61, 597-609.

PETERSON, P.K., GAZIANO, E., SUH, H.J., DEVALON, M., PETERSON, L.A. & KEANE, W. F. (1985) Antimicrobial activities of dialysate-elicited and resident human peritoneal macrophages. Infection and Immunity, 49, 212-218.

POLIN, R.A. & DOUGLAS, S.D. (1984) Fibronectin secretion by peripheral blood and peritoneal monocyte-macrophages. Journal of Leucocyte Biology, 36, 664-665.

POMMIER, C.G., INADA, S., FRIES, L.F., TAKAHASHI, T., FRANK, M.M. & BROWN, E.J. (1983) Plasma fibronectin enhances phagocytosis of opsonized particles by human peripheral blood monocytes. Journal of Experimental Medicine, 157, 1844-1854.

POPOVICH, R.P., MONCRIEF, J.W., NOLPH, K.D., GHODS, A.J., TWARDOWSKI, Z.J. & PYLE, W.K. (1978) Continuous ambulatory peritoneal dialysis. Annals of Internal Medicine, 88, 449-456.

RAFF, H.V., PICKER, L.J. & STOBO, J.D. (1980) Macrophage heterogeneity in man. A subpopulation of HLA-DR bearing macrophages required for antigen-induced T-cell activation also contains stimulators for autologous-reactive T-cells. Journal of Experimental Medicine, 152, 581-593.

RAGSDALE, C.G. & AREND, W.P. (1979) Neutral protease secretion by human monocytes. Effect of surface-bound immune complexes. Journal of Experimental Medicine, 149, 954-968.

RAZMA, A.G., LYNCH, J.P., WILSON, B.S., WARD, P.A. & KUNKEL, S.L. (1984) Expression of Ia-like (DR) antigen on human alveolar macrophages isolated by bronchoalveolar lavage. American Review of Respiratory Disease, 129, 419-424.

RHODES, J., JONES, D.H. & BLEEHEN, N.M. (1983) Increased expression of human monocyte HLA-DR antigens and Fc receptors in response to human interferon in vivo. Clinical and Experimental Immunology, 53, 739-743.

ROESER, H.P. (1980) Iron metabolism in inflammatory disease. In Iron in Biochemistry and Medicine, ed. Jacobs, A. & Worwood, M. Vol. II, pp605-640. London: Academic Press.

ROSS, G.D. & LAMBRIS, J.D. (1982) Identification of a C3bi-specific membrane complememt receptor that is expressed on lymphocytes, monocytes, neutrophils and erythrocytes. Journal of Experimental Medicine, 155, 96-110.

ROSS, G.D. & NEWMAN, S.L. (1980) Regulation of macrophage functions by complement, complement receptors, and IgG-Fc receptors. In The Reticuloendothelial System: A Comprehensive Treatise, ed. Carr, I. & Daems, W.T. Vol. 1, pp173-200. New York: Plenum Press.

ROSSI, F., BELLAVITE, P. & PAPINI, E. (1986) Respiratory response of phagocytes: terminal NAPDH oxidase and the mechanisms of its activation. In Biochemistry of Macrophages Ciba Foundation Symposium 118, ed. Evered, D., Nugent, J. & O'Connor, M. pp172-189. London: Pitman.

RUBIN, J., ROGERS, W.A., TAYLOR, H.M., EVERETT, E.D., PROWANT, B.F., FRUTO, L.V. & NOLPH, K.D. (1980) Peritonitis during continuous ambulatory peritoneal dialysis. Annals of Internal Medicine, 92, 7-13.

RUBIN, J., MCFARLAND, S., HELLEMS, E.W. & BOWER, J.D. (1981) Peritoneal dialysis during peritonitis. Kidney International, 19, 460-464.

RUBIN, J., RAY, R., BARNES, T., TEAL, N., HELLEMS, E., HUMPHRIES, J. & BOWER, J.D. (1983) Peritonitis in continuous ambulatory peritoneal dialysis patients. American Journal of Kidney Disease, 2, 602-609.

RUCH, W., COOPER, P.H. & BAGGIOLINI, M. (1983) Assay of H_2O_2 production by macrophages and neutrophils with homovanillic acid and horse-radish peroxidase. Journal of Immunological Methods, 63, 347-357.

SCHADE, A.L. (1963) Significance of serum iron for the growth, biological characteristics, and metabolism of Staphylococcus aureus. Biochemische Zeitschrift, 338, 140-148.

SCHADE, A.L. & CAROLINE, L. (1944) Raw hen egg white and the role of iron in growth inhibition of Shigella dysenteriae, Staphylococcus aureus, Escherichia coli and Saccharomyces cerevisiae. Science, 100, 14-15.

SCHADE, A.L. & CAROLINE, L. (1946) An iron-binding component in human blood plasma. Science, 104, 340-341.

SCHER, M.G., UNANUE, E.R. & BELLER, D.I. (1982) Regulation of macrophage populations III. The immunologic induction of exudates rich in Ia-bearing macrophages is a radiosensitive process. Journal of Immunology, 128, 447-450.

SCHNYDER, J. & BAGGIOLINI, M. (1980) Secretion of lysosomal enzymes by macrophages. In Mononuclear Phagocytes Functional Aspects, ed. van Furth, R. Part II, Ch. 53, pp1369-1381. The Hague: Martinus Nijhoff Publishers.

SCHREIBER, R.D., PACE, J.L., RUSSELL, S.W., ALTMAN, A. & KATZ, D.H. (1983) Macrophage-activating factor produced by a T-cell hybridoma: physiochemical and biosynthetic resemblance to gamma interferon. Journal of Immunology, 131, 826-832.

SCHWARTZ, R.H., YANO, A. & PAUL, W.E. (1978) Interaction between antigen-presenting cells and primed T-lymphocytes: an assessment of Ir gene expression in the antigen presenting cell. Immunological Reviews, 40, 153-180.

SCOTT, W.A., ZRIKE, J.M., HAMILL, A.L., KEMPE, J. & COHN, Z.A. (1980) Regulation of arachidonic acid metabolism in macrophages. Journal of Experimental Medicine, 152, 324-335.

SHALDON, S., KOCH, K.M., QUELLHORST, E. & DINARELLO, C.A. (1986) Hazards of CAPD: interleukin-1 production. In Frontiers in Peritoneal Dialysis, ed. Maher, J.E. & Winchester, J.E. Ch. 122, pp630-633. New York: Field Richards Associates.

SILVERSTEIN, S.C. & LOIKE, J.D. (1980) Phagocytosis. In Mononuclear Phagocytes. Functional Aspects, ed. van Furth, R. Part II, Ch. 35, pp895-919. The Hague: Martinus Nijhoff Publishers.

SIMBERKOFF, M.S., MOLDOVER, N.H. & WEISS, G. (1978) Bactericidal and opsonic activity of cirrhotic ascites and nonascitic peritoneal fluid. Journal of Laboratory Clinical Medicine, 91, 831-839.

SIMON, L.M., ROBIN, E.D., PHILLIPS, J.R., ACEVEDO, J., AXLINE, S.G. & THEODORE, J. (1977) Enzymatic basis for bioenergetic differences of alveolar versus peritoneal macrophages and enzyme regulation by molecular O_2. Journal of Clinical Investigation, 59, 443-448.

SMITH, B.R. & AULT, K.A. (1981) Increase of surface Ia-like antigen expression on human monocytes independent of antigenic stimuli. Journal of Immunology, 127, 2020-2027.

SMITH, K.A., LACHMAN, L.B., OPPENHEIM, J.J. & FAVATA, M.F. (1980) The functional relationship of the interleukins. Journal of Experimental Medicine, 151, 1551-1556.

SMITH, W.G.J., TSAKIRIS, D.J., JUNOR, B.J.R., BRIGGS, J.D., ALCOCK, S.R., READING, P. & HALLAM, S. (1986) Peritonitis in continuous ambulatory peritoneal dialysis. Scottish Medical Journal, 31, 85-89.

SNYDER, D.S., BELLER, D.I. & UNANUE, E.R. (1982) Prostaglandins modulate macrophage Ia expression. Nature, 299, 163-165.

SOBERMAN, R.J. & KARNOVSKY, M.L. (1981) Biochemical properties of activated macrophages. In Lymphokines: A Forum For Immunoregulatory Cell Products, ed. Pick, E. Vol. 3, pp11-31. London: Academic Press.

SPINOWITZ, B.S., GOLDEN, R.A., RASCOFF, J.H. & CHARYTAN, C. (1982) Eosinophilic peritonitis. Clinical Experimental Dialysis and Apheresis, 6, 187-191.

STARUCH, M.J. & WOOD, D.D. (1983) The adjuvanticity of interleukin-1 in vivo. Journal of Immunology, 130, 2191-2194.

STASTNY, P. & NUNEZ, G. (1984) Monocyte surface antigens. In Advances in Immunobiology: Blood Cell Antigens and Bone Marrow Transplantation, ed. McCullough, J. & Sandler, G.S. pp189-203. New York: Liss, A.R. Inc.

STEEG, P.S., MOORE, R.N., JOHNSON, H.M. & OPPENHEIM, J.J. (1982) Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. Journal of Experimental Medicine, 156, 1780-1793.

STEEN, S., BRENCHLEY, P., MANOS, J., PUMPHREY, R. & GOKAL, R. (1986) Opsonizing capacity of peritoneal fluid and relationship to peritonitis in CAPD patients. In Frontiers in Peritoneal Dialysis, ed. Maher, J.E. & Winchester, J.F. Ch. 107, pp565-568. New York: Field Richards Associates.

STEERMAN, R.L., SNYDERMAN, R., LEIKIN, S.L. & COLTEN, H.R. (1971) Intrinsic defect of the polymorphonuclear leucocyte resulting in impaired chemotaxis and phagocytosis. Clinical and Experimental Immunology, 9, 939-946.

STEINMAN, R.M., NOGUEIRA, N., WITMER, M.D., TYDINGS, J.D. & MELLMAN, I.S. (1980) Lymphokine enhances the expression and synthesis of Ia antigens on cultured mouse peritoneal macrophages. Journal of Experimental Medicine, 152, 1248-1261.

STOSSEL, T.P. (1974) Phagocytosis (Third of Three Parts). New England Journal of Medicine, 290, 833-839.

SWANSON, J., SPARKS, E., ZELIGS, B., SIAM, M.A. & PARROTT, C. (1974) Studies on gonococcus infection V. Observations on in vitro interactions of gonococci and human neutrophils. Infection and Immunity, 10, 633-644.

SZTEIN, M.B., STEEG, P.S., JOHNSON, H.M. & OPPENHEIM, J.J. (1984) Regulation of human peripheral blood monocyte DR antigen expression in vitro by lymphokines and recombinant interferons. Journal of Clinical Investigation, 73, 556-565.

TAKEMURA, R. & WERB, Z. (1984) Secretory products of macrophages and their physiological functions. The American Journal of Physiology, 246, C1-C9.

TAN, J.S., WATANAKUNAKORN, C. & PHAIR, J.P. (1971) A modified assay of neutrophil function: Use of lysostaphin to differentiate defective phagocytosis from impaired intracellular killing. Journal of Laboratory Clinical Medicine, 78, 316-322.

TANNER, A.R., ARTHUR, M.J.P. & WRIGHT, R. (1984) Macrophage activation, chronic inflammation and gastrointestinal disease. Gut, 25, 760-783.

THOMPSON, H.L., STEVENSON, J. & BROCK, J.H. (1986) The effect of iron and agar on production of hydrogen peroxide by stimulated and activated mouse peritoneal macrophages. FEBS, 200, 283-286.

TOURAINE, J.L., NAVARRO, J., CORRE, C. & TRAEGER, J. (1975) Inhibitory effect of medium sized molecules from patients with renal failure on lymphocyte stimulation by phytohemagglutinin. Biomedicine, 23, 180-184.

TRINCHIERI, G. & PERUSSIA, B. (1985) Immune interferon: a pleiotropic lymphokine with multiple effects. Immunology Today, 6, 131-136.

TSAKIRIS, D.J., AITCHISON, T.C., BRIGGS, J.D., JUNOR, B.J.R., SMITH, W.G.J. & WATSON, M.A. (1986) Incidence of peritonitis and delayed-type hypersensitivity in patients on CAPD. In Frontiers in Peritoneal Dialysis, ed. Maher,J.E. & Winchester, J.F. Ch. 102, pp536-541. New York: Field Richards Associates.

UHER, F., DOBRONYI, I. & GERGEL, J. (1981) IgM-Fc receptor-mediated phagocytosis of rat macrophages. Immunology, 42, 419-425.

UNANUE, E.R. (1981) The regulatory role of macrophages in antigenic stimulation. Part two: Symbiotic relationship between lymphocytes and macrophages. Advances in Immunology, 31,1-136.

UNANUE, E.R., BELLER, D.I., LU, C.Y. & ALLEN, P.M. (1984) Antigen presentation: comments on its regulation and mechanism. Journal of Immunology, 132, 1-5.

UNKELESS, J.C. (1986) Heterogeneity of human and murine Fc receptors. In Biochemistry of Macrophages. Ciba Foundation Symposium 118, ed. Evered, D., Nugent, J. & O'Connor, M. pp89-101. London: Pitman.

VAN FURTH, R. (1980) Cells of the mononuclear phagocyte system. Nomenclature in terms of sites and conditions. In Mononuclear Phagocytes. Functional Aspects, ed. Van Furth, R. Part 1, Ch. 1, pp1-40. The Hague: Martinus Nijhoff Publishers.

VAN FURTH, R. & COHN, Z.A. (1968) The origin and kinetics of mononuclear phagocytes. Journal of Experimental Medicine, 128, 415-433.

VAN FURTH, R., RAEBURN, J.A. & VAN ZWET, T.L. (1979) Characteristics of human mononuclear phagocytes. Blood, 54, 485-500.

VAN SNICK, J.L. & MASSON, P.L. (1976) The binding of human lactoferrin to mouse peritoneal cells. Journal of Experimental Medicine, 144, 1568-1580.

VAS, S. (1981) Microbiological aspects of peritonitis. Peritoneal Dialysis Bulletin, 1, S11-S14.

VEALE, D.R., FINCH, H. & SMITH, H. (1976) Penetration of Penicillin into human phagocytes containing Neisseria gonorrhoeae: Intracellular survival and growth at optimum concentrations of antibiotic. Journal of General Microbiology, 95, 353-363.

VERBRUGH, H.A., PETERS, S.R., PETERSON, P.K. & VERHOEF, J. (1978) Phagocytosis and killing of staphylococci by human polymorphonuclear and mononuclear leucocytes. Journal of Clinical Pathology, 31, 539-545.

VERBRUGH, H.A., VAN DIJK, W.C., PETERS, R., VAN ERNE, M.E., DAHA, M.R., PETERSON, P.K. & VERHOEF, J. (1980) Opsonic recognition of staphylococci mediated by cell wall peptidoglycan: antibody-independent activation of human complement and opsonic activity of peptidoglycan antibodies. Journal of Immunology, 124, 1167-1173.

VERBRUGH, H.A., KEANE, W.F., HOIDAL, J.R., FREIBERG, M.R., ELLIOTT, G.R. & PETERSON, P.K. (1983) Peritoneal macrophages and opsonins: antibacterial defense in patients undergoing chronic peritoneal dialysis. Journal of Infectious Diseases, 147, 1018-1029.

VERBRUGH, H.A., KEANE, W.F., CONROY, W.E. & PETERSON, P.K. (1984) Bacterial growth and killing in chronic ambulatory peritoneal dialysis fluids. Journal of Clinical Microbiology, 20, 199-203.

VERBRUGH, H.A., VERKOOYEN, R.P., VERHOEF, J., OE, P.L. & VAN DER MEULEN, J. (1986) Defective complement-mediated opsonization and lysis of bacteria in commercial peritoneal dialysis solutions. In Frontiers in Peritoneal Dialysis, ed. Maher, J.E. & Winchester, J.F. Ch. 106, pp559-564. New York: Field Richards Associates.

VERHOEF, J., PETERSON, P.K. & QUIE, P.G. (1977) Kinetics of staphylococcal opsonization attachment, ingestion and killing by human polymorphonuclear leukocytes: a quantitative assay using [^{3}H]-thymidine labeled bacteria. Journal of Immunological Methods, 14, 303-311.

VIRELIZIER, J.L. & ARENZANA-SEISDEDOS, F. (1985) Immunological functions of macrophages and their regulation by interferons. Medical Biology, 63, 149-159.

VOGEL, S.N., FINBLOOM, D.S., ENGLISH, K.E., ROSENSTREICH, D.L. & LANGRETH, S.G. (1983) Interferon-induced enhancement of macrophage Fc receptor expression: β-interferon treatment of C3H/HeJ macrophages results in increased numbers and density of Fc receptors. Journal of Immunology, 130, 1210-1214.

VOLKMAN, A. (1976) Disparity in origin of mononuclear phagocyte populations. Journal of the Reticuloendothelial Society, 19, 249-267.

WATSON, M.A., BRIGGS, J.D., DIAMANDOPOULOS, A.A., HAMILTON D.N.H. & DICK, H.M. (1979) Endogenous cell-mediated immunity, blood transfusion, and outcome of renal transplantation. The Lancet, ii, 1323-1326.

WEINBERG, E.D. (1984) Iron withholding: a defense against infection and neoplasia. Physiological Reviews, 64, 65-102.

WEIR, D.M. (1980) Surface carbohydrates and lectins in cellular recognition. Immunology Today, 1, 45-51.

WEIR, D.M. & öGMUNDSDÓTTIR, H. (1977) Non-specific recognition mechanisms by mononuclear phagocytes. Clinical and Experimental Immunology, 30, 323-329.

WHITE, C.J. & GALLIN, J.I. (1986) Phagocyte defects. Clinical Immunology and Immunopathology, 40, 50-61.

WILKINSON, P.C. (1982) Visual observations of chemotaxis and chemotropism in mouse macrophages. Immunobiology, 161, 376-384.

WILSON, C.B. & WESTALL, J. (1985) Activation of neonatal and adult human macrophages by alpha, beta, and gamma interferon. Infection and Immunity, 49, 351-356.

WINKELSTEIN, J.A. (1973) Opsonins: their function, identity and clinical significance. Journal of Pediatrics, 82, 747-753.

WRIGHT, S.D. & MEYER, B.C. (1985) Fibronectin receptor of human macrophages recognises the sequence Arg-Gly-Asp-Ser. Journal of Experimental Medicine, 162, 762-767.

WRIGHT, S.D., LICHT, M.R., CRAIGMYLE, L.S. & SILVERSTEIN, S.C. (1984) Communication between receptors for different ligands on a single cell: ligation of fibronectin receptors induces a reversible alteration in the function of complement receptors on cultured human monocytes. Journal of Cell Biology, 99, 336-339.

YAMAMURA, M., BOLER, J. & VALDIMARSSON, H. (1977) Phagocytosis measured as inhibition of uridine uptake by Candida albicans. Journal of Immunological Methods, 14, 19-24.

YEWDALL, V.M.A., BENNETT-JONES, D.N., CAMERON, J.S., OGG, C.S. & WILLIAMS, D.G. (1986) Opsonically-active proteins in CAPD fluid. In Frontiers in Peritoneal Dialysis, ed. Maher, J.E. & Winchester, J.F. Ch. 109, pp573-578. New York: Field Richards Associates.

YOUNG, G.A., YOUNG, J.B., YOUNG, S.M., HOBSON, S.M., HILDRETH, B., BROWNJOHN, A,M, & PARSONS, F.M. (1986) Nutrition and delayed hypersensitivity during continuous ambulatory peritoneal dialysis in relation to peritonitis. Nephron, 43, 177-186.

ZIEGLER, K. & UNANUE, E.R. (1981) Identification of a macrophage antigen-processing event required for I-region-restricted antigen presentation to T-lymphocytes. Journal of Immunology, 127, 1869-1875.