REVIEW OF LITERATURE
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The possibility of participation of leucocytes in local inflammatory response was described by Hunter in 1794 and Dutrochet in 1828 (Senn and Jungi, 1975). The haematogenous origin of inflammatory cells was not settled until the late 19th century when Hering (1867) and Cohnheim (1867) demonstrated the haematogenous origin of inflammatory cells in the mesentery of the living frog. Later Liberkuhn (1870) described peri-vascular locomotion of leucocytes on a glass surface. Phagocytosis and chemotaxis were discovered subsequently (Leber, 1888; Gabritschevsky, 1890 and Metchnikoff, 1893).

When microbes invade the skin and mucous membrane they first encounter host antagonists i.e., anti-bacterial antibodies, complement components, the properdin system, the kinin system, lysozymes .......... transferrin and vaso-active neurohormones. These factors reactivate and stimulate the phagocytic cellular response and this humoral cellular interaction usually provides a remarkable host defence against bacterial diseases (Hirsch, 1965). Polymorphonuclear
cells, called microphagocytes by Metchnikoff (1893) form first line of cellular defence of the mammals.

Phagocytic cells of the body, which are essential for host defence against infection can be divided into -

I. **Peripheral blood**

1. Polymorpho-nuclear neutrophil,
2. Bilobed eosinophil,
3. Monocyte.

II. **Reticulo-endothelial system**

1. Alveolar macrophages of lung,
2. Kupffer cells of liver,
3. Spleen and bone marrow macrophages,
4. Other tissue macrophages.

The polymorphonuclear neutrophil is the chief phagocytic cell of blood and can quickly leave the vascular tree by virtue of it's amoeboïd movements to engulf and destroy bacteria and other microbes. The bilobed eosinophil, although somewhat more limited in its phagocytic activity, can also kill pyogenic bacteria. In contrast to the neutrophil and eosinophil, which are end stage cells, the monocyte can transform in the tissues into larger macrophages with broader phagocytic and biosynthetic capacities.
**Figure 1** Schematic representation of specific immunity.

Antigenic material is recognized, phagocytized, and processed by macrophages. Information is then transferred to circulating T-lymphocytes or B-lymphocytes, which give rise either to small lymphocyte or plasma cell. Small lymphocytes produce lymphokines or chemical mediators important in host-defense against virus, fungus, and malignancy. Plasma cells give rise to immunoglobulins, which are important in host resistance to bacteria, parasites, and other allergens.
**Figure 2** The steps in the inflammation response.

Tissue injury or bacterial invasion stimulates vascular dilatation and increased vessel permeability, aiding the delivery of plasma proteins, antibodies, and circulating leukocytes to the area. Leukocytes adhere to the vessel walls, migrate between the endothelial cells and enter the interstitial space, with the aid of opsonins from the plasma proteins, bacteria and cellular debris are phagocytosed and destroyed.
5) Activation of the phagocyte
6) Microbicidal activity (Destruction of foreign material).

Random Movement -

Random movement has been defined as non-directional movement of cells. It is measured by the migration of cells through a capillary tube (Random tube migration) or through a porous filter (Random filter migration). It is evident that this non-directional movement is a separate phenomenon from directional movement (chemotaxis) by the observation that in a number of disease states like Chediak-Higashi Syndrome, Diabetes mellitus and Candidiasis with deficient cell mediated immunity, the random movement is normal whereas chemotaxis is abnormal (Miller et al, 1971; Miller, 1973 & 1975 and Clark & Kimball, 1971). Further, capillary tube migration and filter migration may measure separate events as in patients having Chediak-Higashi Syndrome showed defective random filter migration but normal random tube migration (Clark & Kimball, 1971).

Chemotaxis -

This term refers to directional movement of polymorphonuclear leucocytes or mononuclear leucocytes to the sites of microbial invasion. There are two
major steps involved in it. Activation of phagocytic cell membrane, chemotactant and the cell movement. The measurement of chemotaxis is performed with some modification of Boyden chamber (Boyden, 1962). The number of leucocytes that pores through the membrane in response to the stimulus is measured and serve as an index of the tested leucocytes intrinsic motility and tested attractants chemotactic effect. The complement system, which activates chemotaxis, includes trimolecular complex C^5, C^6, C^7 and low molecular weight fragments C^3A and C^5A. Inhibitors of chemotaxis have been found in the serum of few children with recurrent infection (Ward and Schlogel, 1969). Defective chemotaxis due to intrinsic deficiency in neutrophil has been described in patients with diabetes mellitus (Mewat and Baun, 1971). Moudgil and co-workers (1977) using in vitro exposure of polymorphonuclear leucocytes to anaesthetics including lidocaine and mepacaine demonstrated retarded chemotaxis in a dose-related fashion.

Adherence -

It is an important phenomenon comprising of sticking of granulocytes to the vascular endothelium near the inflammatory stimulus (Grant, 1974) and also adherence of micro organisms to phagocyte surface which enhances their engulfment. It is called
opsonisation. These processes are facilitated by humoral factors called opsonins (Weston, 1976). Granulocytic margination is the first event to occur after application of stimulus and is an antecedent step to chemotaxis, opsonisation, phagocytosis and microbial killing. The important factors involved in this process are sub-classes of immunoglobulin G (IgG), antibodies and the complement components C$_3$A, a potent chemotaxin (Ward and Newman, 1969), and C$_3$B, which is necessary for opsonisation of microbes (Alper et al, 1972). They are recognised by surface receptors on granulocytes and help in making the firm contact with microbes (Cline and Golde, 1977).

**Phagocytosis**

After the offending micro-organism is opsonized and contacts the cell surfaces, pseudopodia are extended and the organism is engulfed. The process of phagocytosis is more complex. The technique for assay of phagocytosis involves incubation of a microscopically visible particles with phagocytic leucocytes by using Nitro blue tetra-sodium test (Park et al, 1968). Radio-activity labelled bacteria, starch particles, immune complexes, or erythrocytes and polyvinyl-toluene particles have been utilized toquantitate phagocytosis. The method adopted by Stossel (1975) in which paraffin oil particles
containing the dye oil red 0 is most widely used. Investigations of the process of ingestion have uncovered many facts of this complex series of events: the importance of cell membrane receptors for IgG and C₃ in the process of opsonization, the role of micro-tubules and the contractile proteins, actin and myosin within the cell, the importance of the divalent cations, manganese, cobalt, magnesium and calcium in phagocytosis (Weston, 1976).

Activation of the Phagocyte

After phagocytosis, a series of metabolic events occur that have been called the "respiratory burst". This consists of an increase in glucose utilization through the hexose monophosphate shunt, an uptake of oxygen, and the production and release of substances, super-oxide (O₂⁻) and hydrogen peroxide, which are thought to be important in microbicidal activity (Klebanoff, 1975b). The enzymes responsible for the respiratory burst include NADH and NADPH. These enzymes reduce oxygen to peroxide and reduce colourless nitro-blue tetrazolium dye to a blue compound, formazan that can be quantitated by spectrophotometric reading (Baehner et al, 1975).
Microbicidal activity -

Following the ingestion of micro-organisms a series of distinct events occur within the polymorphonuclear leucocytes, which lead ultimately to the destruction of the invading organism. The killing and digestion of the ingested material by phagocytic leucocyte is either oxygen dependent or oxygen independent. It occurs with the help of intraphagocytic leucocytic digestive enzymes (Klebanoff, 1975a).

ASSAY METHODS FOR GRANULOCYTIC ADHERENCE -

Different methods for separating leucocytes from erythrocytes and lymphocytes from granulocytes by applying principles of differential densities centrifugation techniques, were developed by using serum albumin (Vallee et al, 1947), gum acacia (Spear, 1948), exchange resin (Tullis, 1952), two albumin solutions of differing densities (Agranoff et al, 1954).

Ottensen (1955) separated lymphocytes from granulocytes and erythrocytes by centrifuging blood in a tube which contains a specially derived perspex body in the capillary tube in it's axis and by adjusting its specific gravity.
Jago (1956) used heparinised blood to separate granulocytes from lymphocytes by using the centrifuging and sedimentation principles. Philippu (1956) used a method described by Szilard (1926) and separated different types of leucocytes by centrifugation of leucocyte suspension in Wintrobe tube.

Ventzke et al (1959) separated leucocytes by method of Skoog and Beck (1956) and then by using plastic tube, separated granulocytes from lymphocytes by centrifugation and sedimentation. Johnson and Garvin (1959) removed granulocytes from heparinised whole blood by passage through a short column of siliconised glass wool, which adhere to it, while lymphocytes were yielded in the effluent blood.

Garvin (1961) further intensively studied varieties of factors affecting leucocytes and platelet adherence to siliconised glass beads column instead of glass wool.

Rabinowitz (1964) used glass beads column, described by Garvin (1961), to separate various types of leucocytes. He again (1965) used same method to study adherence and separation of leucaemic cells on glass beads column.
Brandt (1965), also assayed granulocytic adherence by taking siliconised glass beads 0.1 mm in diameter, which were packed to a height of 3 cm over small pieces of glass wool, in a siliconised glass syringe 1 cm in diameter, the whole, being enclosed in a water jacket at 37°C. 2 ml of heparinised whole blood (50 units/ml) was added to the column and after 10 minutes allowed to flow out from the column. The flow rate was adjusted by controlling the pressure on the plunger of the syringe to 0.1 ml/minute. An absolute neutrophil count (TLC x % of neutrophil) was performed over the influent and effluent blood and ratio was termed as "Adhesiveness index" (A.I).

Kvarstein (1969) also used glass beads column to study granulocytic adherence. Penny et al (1966) used method devised by Brandt (1965), to see the effect of various drugs and physical factors on granulocytic adherence.

Brayant and Sutcliffe (1972), prepared leucocyte rich plasma from heparinised blood by centrifuging. The granulocytic adherence to glass was determined by using capillary glass tubes and adhesion was expressed as percentage of control adhesion or as percentage of test W.B.C. count.
Percentage of control adhesion = \( \frac{\text{Test A.I.}}{\text{Control A.I.}} \times 100 \)

Percentage of W.B.C. count = \( \frac{\text{Test A.I.}}{\text{Test N.B.C. count}} \times 100 \)

This method was advantageous over previous techniques (Garvin, 1961). In contrast to procedures employing glass bead columns, only adherent cells are measured and artificial evaluation of adhesion due to sequestration of cells can be excluded. Furthermore, cells in capillary tubes can be examined directly with a microscope and differential count and characteristics such as cell aggregation can be noted.

Penn (1923), Garvin (1961) and Kvarstein (1969) noted that optimal adhesion occurs at 37°C and adhesiveness is reduced as the temperature is lowered. Brayant and Sutcliffe (1972) also observed similar findings. Brayant and Sutcliffe (1973) saw effect of cyclic AMP on granulocytic adherence by using glass beads column.

Boxer et al (1978). Klempner and Gallin (1978) used nylon wool and tuberculin syringes with 27 gauze needles in place of pasteur pipettes and leucopac nylon fibres. Schiffer et al (1977) also used nylon fibres to study the effect of local anaesthetic agents on granulocytic adherence. Present study was carried-out with Klempner and Gallin technique having tubercular syringe with nylon wool column upto two centimeter height from below and 21 gauze needle.

Granulocytic adherence and infection –

Adherence is an essential step in the physiology of phagocytosis and host defence. Granulocytes must adhere to the vascular endothelium before diapedesis into extravascular compartment, directional movements, phagocytosis and killing of bacteria at the site of inflammation. Inhibition of any of these steps may impair the host defence against bacterial invasion. Adherence is the least studied aspect of granulocytes uptil now.

Granulocytic adherence has been studied in a few diseases associated with recurrent infections and has found to be associated with impairment of other neutrophilic functions. Rabinovitz (1965) and Penny and Galton (1966 b) observed impaired granulocytic adherence in chronic myeloid leukaemia,
acute myeloid leukaemia, myelomatosis, macroglobulinaemia and paroxysmal nocturnal haemoglobinuria. Granulocytic adherence to nylon fibres was found to be depressed in 12 out of 29 patients of multiple myeloma (Mac Gregor et al., 1978). Lazy leucocyte syndrome and Chediak Higashi Syndrome are associated with decreased adherence of granulocytes (Boxer et al., 1974, 1976 and 1978).

Inflammation augmented the granulocytic adherence to nylon fibres in vitro. Mean adherence in these patients was twice normal (56.4 ± 5.6% V/s 29.4 ± 5.2%) and was inhibited by in vitro administration of anti-inflammatory agents (Lentnek et al., 1976).

The anti-inflammatory drugs interfering with the granulocytic adherence are corticosteroids, aspirin, ethanol, acetaminophen, indomethacin, phenylbutazone, colchicine, tetracycline, lidocaine, EDTA, normal saline and bradykinins (Mac Gregor et al., 1974, 1976; Penny et al., 1966a; and Schiffer et al., 1977). Ethanol, however in mild to moderate doses (0.2 gm/litre concentration) increases but in high doses (6.4 gm/litre concentration) inhibits granulocytic adherence (Hallengren and Foryrson, 1978). These drugs when mixed with adherence increasing factor of inflammatory diseases, the augmenting effect
of this factor is neutralised and normal granulocytic adherence results (Mac Gregor, 1976). O’Floherty et al (1978) suggested that activated complement induces stickness of granulocytes, which endangers their sequestration. The results help to explain the recently reported pulmonary leucostasis and dysfunctions accompanying haemodialysis (Mac Gregor, 1977). An increase in cyclic AMP level is also known to cause decrease in granulocytic adherence (Brayant and Sutcliffe, 1973).

Penny (1966a) suggested that chelating agent like EDTA binds calcium ions and this calcium can be replaced by other divalent cations like magnesium, manganese and nickel. The contact between the neutrophil surface and glass surface or phagocytosed particle might thus involve a divalent electrophilic bridge. Complement components are required for chemotaxis (Boyden et al, 1965 and Ward et al, 1965) and the reaction of neutrophils to both chemotactic and emigration stimuli would seem to involve at least in part a membrane alteration resulting in increased adhesiveness.

Anaesthetics enhance infection by depression of phagocytosis and inhibition of mobilization of phagocytes into the area of infection (Bruce & Wingard, 1971). Graham (1911) observed reduction in
phagocytosis by human and animal leucocytes when exposed in vitro & vivo to different bacteria during ether anaesthesia.

Goldstein et al (1971) observed that cyclopropane and methoxyflurane reduce the murine pulmonary bactericidal activity. Bruce (1976) found tenfold reduction in number of phagocytised bacteria during halothane anaesthesia, in addition number of neutrophil was also decreased in his study. Kosciolek (1967) reported depressed phagocytosis by neutrophilis during ether and halothane anaesthesia in both man and rabbits. Moudgil et al (1977), Stanley et al (1976) and Hill et al (1977) showed that inhalational anaesthetics including halothane depress the chemotaxis of neutrophil with clinically used concentration in human. All these studies suggest that anaesthesia is the prominent cause of depressed phagocytosis which gradually returned to normal some 60 hours later (Bruce & Wingard, 1971). The degree of leucocytic depression is related to duration of anaesthetic exposure (Rubin 1904; Snel, 1903; Wingard, 1971).

A number of studies (Eastwood et al, 1963; Lassen, 1956; Parbrock, 1967) indicate that exposure to an anaesthetic concentration of NaOH may produce
leucopaenia in animals and in men. It was further shown by Kripke et al (1977) that 20% N₂O is the lowest concentration required for producing such leucopaenia.

Cullen et al (1975) showed that phagocytosis is slightly depressed after induction of anaesthesia but before operative intervention and this existed for halothane, N₂O as well as for opioid N₂O anaesthesia, thereby showing that this depression was independent of any particular type of anaesthetic agent (Cascorbi, 1981).

On the other hand the effect of local anaesthetics on phagocytosis are less well studied. Moudgil and co-workers (1977) demonstrated restarted chemotaxis in a dose related fashion by using in vitro exposure of neutrophil to lidocaine and marcurine. This finding was important at the site of local anaesthetic infiltration (Duncan & Cullen, 1977). Stanley et al (1976) extended these findings to in vivo exposure and were able to show that patient receiving lidocaine epidural with epinephrine showed depressed chemotaxis, this effect was reversible after 8 hours. Stanley et al (1978), Hill & Colleagues (1977) further demonstrated this effect in the absence of surgical stress.
ANAESTHESIA AND THE CELL

The cell - the basic unit of life - has a pivotal role in body defences and is susceptible to anaesthetic actions (Moudgil et al, 1976). Heilbrunn (1920) demonstrated that cytoplasm of sea-urchin eggs becomes less viscous after exposure to ether, chloroform, paraaldehyde, chloral hydrate and urethane. (Seitriz (1941) further observed that chloroform and cyclopropane stopped cytoplasmic streaming in the slime mould physarum polycephalum by a rapid and reversible gelation of the cytoplasm. This effect was suggested to be due to rapid and reversible locking of the linear protein molecule).

Schoenborn, Watson and Kendrew (1965) and Schoenborn (1968) demonstrated that anaesthetic agents can interact with proteins. The anaesthetic agents may affect the antigen and antibody reactions by altering the configuration of various molecular receptor sites (Moudgil et al, 1976).

Anaesthesia and cell motility -

Nunn et al (1970) demonstrated reversible inhibition of lymphocyte motility on exposure to halothane. Of further interest are the observation that mobilization of phagocytes is also adversely
affected by anaesthesia. Bruce (1966) observed that halothane anaesthesia caused decreased mobilization of neutrophils in response to intra-peritoneal injection of pseudomonas endotoxin. Kimbell and Brody (1963) observed that focal accumulation of neutrophils in rabbit's ear skin windows, was markedly depressed after anaesthetising the animals with ether or halothane. Lowenberg (1934) also made similar observations of depressed locomotion in vitro.

Anaesthesia and random cell movement -

The spontaneous motilities of mouse leucocytes (Nunn, Sharp and Kimbell, 1970; Rabinovitch and Destefana, 1974) and a variety of unicellular organisms (Bruce, 1975; Sharp et al, 1969; Wiklund et al, 1972) are decreased by halothane, methoxyflurane, cyclopropane, chloroform and ether. The effects are reversible and may be secondary to anaesthetic effects on micro-tubular systems necessary for modification of the membrane structure (Nunn et al, 1970).

Anaesthesia and chemotaxis -

In contrast to random cell motility, the attraction of leucocytes to a nidus of infection is directional and under the influence of chemotactic factors. Although chemotaxis is impaired by ethanol
and thermal injury (Seifert et al, 1973), the effects of anaesthesia and operation have not been ascertained. Bruce (1966) demonstrated reduced leucocyte mobilization to the mouse peritoneal cavity in response to salmonella or pseudomonas lipopolysaccharide when mice were anaesthetized with 1% halothane, but the influence of altered splanchnic blood flow during anaesthesia could not be excluded.

In human patients, the leucocyte mobilization viewed through a skin window is not inhibited by surgical procedures during cyclopropane, N₂O and ether anaesthesia (Brayten et al, 1970). However, ethanol, shock and diabetes are capable of inhibiting the response. The effects of anaesthetics on leucocyte mobilization and directional motility in vitro are unknown (Duncan & Cullen, 1976).

Anaesthesia and cellular adherence -

This is the least studied function in relation to anaesthetics. Leucocyte adherence to the vessel wall is inhibited by topically applied lidocaines in a dose related manner (Giddon et al, 1972). But the effects of other anaesthetics are unknown (Duncan and Cullen, 1976).
Anaesthesia and phagocytosis -

In 1911, Graham showed an inhibition of phagocytosis when human and rabbit leucocyte were exposed to ether. Hamburger (1916) reported a dose related inhibition of phagocytosis by equine leucocytes too in vitro exposure of chloroform. Bruce (1967) and Kosciolek (1967) reported similar results with ether and halothane anaesthesia. Cullen, Hume and Chretien (1972) reported decreased phagocytosis in patients during halothane or nitrous oxide - narcotic anaesthesia without surgery.

Recent studies have demonstrated the presence of IgG receptors on the cell surfaces of monocytes and neutrophils, but Douglas (1970) has suggested that neutrophils require complement in addition to IgG for efficient phagocytosis. Moudgil et al (1976) have speculated that anaesthetic agents hinder opsonization or alter the cell receptor sites, thereby reducing the phagocytosis.

More recent studies using in vitro technique with halothane and N₂O have demonstrated only minimal effects upon phagocytosis by human leucocytes (Cullen, 1974; Cullen et al, 1972). Local anaesthetic inhibition of mouse macrophages (Rabinovitch, 1974) and human leucocytes (Cullen, 1974) is demonstrable only with very high concentrations of drugs which are usually achieved only at the site of infiltration.
Narcotics in clinical use have not been evaluated, however, levorphanol the structural analogue of morphine can cause 80-90% inhibition of phagocytosis in vitro (Wurster et al, 1971, Zucker Franklin et al, 1971).

The administration of 1% halothane impairs phagocytosis of peritoneal salmonella in mice (Bruce, 1967) but human studies after halothane - \( \text{N}_2\text{O} \), Pentothal Inovar - \( \text{N}_2\text{O} \), or morphine - d tubocurarine - \( \text{N}_2\text{O} \) anaesthesia (Cullen et al, 1975; Rosenbaum et al, personal communication to Duncan & Cullen, 1976), without operation have shown only minimal impairment of latex particle phagocytosis by peripheral blood leucocytes. The N.B.T. reduction is reduced after morphine - \( \text{N}_2\text{O} \) anaesthesia (Rosenbaum et al, 1976, personal communication) but this may represent a steroid effect rather than a direct effect of the anaesthetic agents (Chretien et al, 1972). Reduced phagocytic activity by fixed macrophages of the reticulo-endothelial system has been demonstrated during anaesthesia in man (Lofstrom et al, 1974) and in animals (Goldstein et al, 1971).