DISCUSSION
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It has been known for some time that a variety of anaesthetics can influence immune responses in animal and man. Speculation whether general anaesthetics decrease immunocompetence, making patients more susceptible to infections and malignancies, has been voiced (Duncan, 1976; Graham, 1911; Kanto, 1974; Lee, 1975; Logerfo, 1973; Walton, 1979).

As indicated previously, the non-specific immune response is epitomised by the inflammatory response and usually involves polymorphonuclear leukocytes as the main effector cells. The production, mobilization and functional state of these cells all appear to be affected by anaesthesia.

With regard to productivity, there is substantial evidence starting from the early observation that prolonged exposure to nitrous oxide results in leucopaenia in human and that nearly every anaesthetic depresses white cell production. Since it is a well known fact that all anaesthetics are cellular poisons, this statement should not come
as a surprise. It seems that depressed white cell production during and following anaesthesia is due to the inhibition of cellular mitosis rather than delayed cellular maturation or release from the bone marrow. In contrast to prolonged exposure to nitrous oxide, most anaesthetic experiences in today's operating rooms are short lived and there does not appear to be any significant leucopaenia associated with them - or if there is, the inhibition of granulocyte production is reversible. Thus while it is recognized that all anaesthetics depress white cell productivity, it is likely that this depression can be withstood by healthy patients undergoing brief anaesthetic exposure (Duncan, 1976).

In most immune systems a wide variety of reactions may be affected by anaesthesia and surgery, this possibility being appreciated as early as 1875.

....."Let us remember that chloroform does not act solely on the nerve tissue. Far from that, it has actions on all the tissues and attacks each one at a time which is a function of it's susceptibility..... Anaesthetic is not a special poison for the nervous system. It anaesthetizes all the cells, benumbing all the tissues, and stoping their irritability....." (Bernard, 1875).
Phagocytosis is a primary defence mechanism against infection. Abnormalities of neutrophil function remain the most important variable of immunological defence against infections (Alexander, 1972). Since there is evidence that anaesthetic agents render cells immobile, it is conceivable that they may affect phagocytic activity and granulocyte adherence. Indeed as early as 1911, Graham showed an inhibition of phagocytosis when human and rabbit leucocytes were exposed to ether. Hamburger, in 1916, reported leucocytes in vitro after exposure to chloroform. Bruce (1967) showed that halothane anaesthesia caused a substantial reduction in the number of salmonella-bacteria ingested by each peritoneal neutrophil, 4 hours after intraperitoneal injection in mice. Kosciolek (1967) reported a decrease in phagocytosis in blood obtained from surgical patients after halothane and ether anaesthesia. Leucocytes from these patients immediately after surgery and 24 hours later, exhibited a decreased ability to phagocytose staphylococcus aureus.

Cullen, Hume, Chretien (1972) reported a decrease in phagocytosis of latex particles and nitroblue-tetrazolium (N.B.T.) reduction in patients during either halothane or nitrous oxide - narcotic
anaesthesia without surgery. In a later study (Cullen, 1974), however, halothane 0.5 - 2.5% or nitrous oxide 80% produced only minimal, statistically insignificant inhibition of latex particle phagocytosis or N.B.T. reduction. Cullen (1974) has suggested that the inhibition of phagocytosis reported in vivo during anaesthesia might result from other factors, such as stress or arterial blood flow. Recent studies have demonstrated that IgG receptor sites are present on the cell surfaces of monocytes and neutrophils, but the latter cells require complement in addition to IgG in order to accomplish efficient phagocytosis (Douglas, 1970). It might be speculated that anaesthetic agents hinder opsonization or alter the cell receptor sites.

Ostergren (1944) showed that most anaesthetic agents caused a dispersion of metaphase. Lovis and Kimball (1971) observed a similar development of C-mitosis in botanical species under influence of anaesthesia.

The possibility that anaesthetic agents may alter the course of an infection has been under consideration for the last 70 years. In 1903 Snell observed increased mortality in guinea-pigs infected with anthrax, after exposure to ether, chloroform
and chloral hydrate. Rubin (1904) made similar observations in rabbits infected with streptococci or pneumococci and exposed to ether or chloroform. Both the depth and duration of anaesthesia were important in increasing the severity of these infections. In 1910, Opie anaesthetized dogs with chloroform, with or without concomitant bacterial infection, and studied their livers. He observed that he had "...succeeded in producing lesions of a character and intensity not obtained by simple administration of chloroform". While these early studies seem to suggest that anaesthesia may enhance an infection, other workers have observed a reverse effect. Waterhouse (1915) a surgeon from Charing Cross Hospital suggested a beneficial effect of diethyl ether in cases of pyogenic infection. Also, a bactericidal effect from ether vapour was noted by Topley (1915).

Bruce et al (1975) anaesthetized 18 patients with either halothane and nitrous oxide or nitrous oxide, thiopental-Innovar\textsuperscript{R}. During anaesthesia, 7.9 less neutrophils per 100 cells contained latex and 15.7 less monocyte per 100 cells contained latex. This represent a decrease of 17 and 28 percent reduction of phagocytosis respectively from pre-anaesthetic value.
Moudgil and co-worker (1977), using in vitro exposure of polymorphonuclear leucocytes to anaesthetics including lidocaine and marcaine, demonstrated retarded chemotaxis in a dose related fashion. Stanley and associates (1978) extended these findings to in vivo exposure and were able to show that patients receiving lidocaine epidurals with epinephrine manifested depressed white cell chemotaxis. This effect was reversible after 8 hours. Inhalational anaesthetics including halothane, have also been shown to interfere with the chemotactic mechanism in humans. Hill and colleagues (1977) further demonstrated this effect in the absence of surgical stress. The effect of local anaesthetics on white cell phagocytosis are even less well studied. Lidocaine has been shown to decrease both nitroblue tetrazolium reduction and phagocytosis of latex particles by human polymorphonuclear leucocytes in vitro. The finding was only important at the site of local anaesthetic infiltration (Duncan, 1976).

By screening the above literature, it appears that majority of leucocyte functions are disturbed during and after the anaesthesia. Much work has been done on chemotaxis and phagocytosis in relation to anaesthesia but the leucocyte adherence is neglected
function till now. As we know that adherence (attachment to material) is also an important function as chemotaxis and phagocytosis; this present study was carried on 60 patients to know the effect of different anaesthetic agents on granulocytic adherence at different time intervals.

The role of granulocytes, in combating infections is undisputable, which after being attracted at the site of infection, phagocytose the infective organisms and ultimately kill the micro organism. Based on this mechanism granulocyte adherence was studied in various diseases associated with recurrent infections. Granulocyte adherence has been found to be impaired in chronic myeloid leukaemia, acute myeloid leukaemia, myelomatosis, macroglobulinemia, paroxysmal nocturnal haemoglobinuria (Robinowitz, 1965; Penny & Galton, 1966 and Mac Gregor et al, 1978). Laxy leucocyte syndrome and Chediak Higashi Syndrome (Boxer et al, 1974, 1976 & 1978).

The present study has been undertaken in 60 patients having different surgical procedures under different anaesthetic agents. Blood sample taken before induction of anaesthesia was taken as control sample for the same patient. Although various methods have been described for assaying the adherence
(Garvin, 1961; Brandt, 1965; Brayant and Sutcliffe, 1972; Schifter et al, 1977; Mac Gregor et al, 1974) but in the present study the method described by Klempern and Gallén (1978) has been followed, which is much simpler and is more sensitive as compared to other method available.

Blood samples from the patient were studied at same time to avoid the effect of temperature and other environmental factors (Garvin, 1961 and Kvarstein, 1969).

In the present study, the mean granulocytic adherence in control sample of group I was 87.66 ± 3.41, in group II it was 89.94 ± 1.95; in group III it was 91.04 ± 2.12; in group IV it was 90.12 ± 2.78 and in last and Vth group it was 90.03 ± 1.14. The mean G.A. obtained by Klempern and Gallin (1978) was 82.6 ± 8.2 using 80 mg of nylon fibres. The difference in adherence actively can be attributed to the different environmental factors like temperature, humidity etc. and the difference in nylon fibres used.

A highly significant (P ≤0.01) decrease in granulocytic adherence was observed in II, III and IVth samples of the patients undergoing different surgical procedures under the influence of different
anaesthetics, except in sample III of group I where P value was significant (P ≤ 0.05).

**Group I**

The mean granulocytic adherence in this group of 10 patients, who received 5% Xylocaine heavy as subarachnoid block was 87.66 ± 3.41 in control sample. 20 minutes after subarachnoid block mean granulocytic adherence was 86.65 ± 3.86. When sample I was compared to sample II, it shows highly significant (P ≤ 0.005) decrease in granulocytic adherence. In the same way, mean granulocytic adherence in III and IV sample was 85.32 ± 6.02 and 86.74 ± 3.69 respectively. Again when we compared sample I to III and sample I to IV, it leads significant (P ≤ 0.05) in I and III sample and highly significant (P ≤ 0.01) decrease in granulocytic adherence in I and IV samples comparison. By seeing these values we can say that after induction of anaesthesia there is significant decrease in granulocytic adherence at 30 minutes, after recovery from anaesthesia and even after 24 hours of surgical procedure. We can't exclude the other factors involving at this stage such as surgical stress.
Group II -

Mean granulocytic adherence of 10 patients in this group was 89.94 ± 1.95 in control sample. In II, III and IVth samples it was 88.72 ± 2.24, 88.34 ± 1.87 and 88.37 ± 1.71 respectively. When we compared sample I to sample II, sample I to sample III and sample I to sample IV, we found significant (P ≤ 0.05) in I and II sample and highly significant in I and III as well as in I and IVth sample. Again this group shows continuous decrease in granulocytic adherence at different times.

Group III -

Patients of this group were given general anaesthesia having induction with thiopentone + scoline and maintained with N₂O/O₂/Flaxedil. Mean granulocytic adherence of 15 patients in control sample was 91.04 ± 2.12. Mean granulocytic adherence in sample II, III and IV was 84.76 ± 4.54, 82.14 ± 3.98 and 86.37 ± 2.66. Again control sample was compared to sample II, III and IV separately. Comparison in all samples shows highly significant (P ≤ 0.001) decrease in granulocytic adherence.
**Group IV** -

In this group patients received general anaesthesia having induction with thiopentone sodium + scoline and maintained with $N_2O/O_2$/Ether. Mean granulocytic adherence of 12 patients was $90.12 \pm 2.76$ in control sample. It was $84.85 \pm 3.33$, $84.88 \pm 3.37$ and $87.76 \pm 3.32$ in II, III and IV sample respectively. Comparison of control sample to sample II, III and IV separately shows highly significant ($P \leq 0.01$) decrease in granulocytic adherence at different times.

**Group V** -

These patients were induced with thiopentone sodium + scoline and maintenance was done with $N_2O/O_2$/Halothane. Mean granulocytic adherence of 13 patients in control sample was $90.03 \pm 1.14$. Mean granulocytic adherence in sample II, III and IV was $84.40 \pm 2.25$, $82.40 \pm 2.53$ and $85.86 \pm 1.30$ respectively. Comparison of control sample with II, III and IV sample leads significant ($P \leq 0.001$) decrease in granulocytic adherence at different times.

So in all the groups it was constant and significant decrease in granulocytic adherence value 30 minutes after induction of anaesthesia, at the time
Graph showing mean percentage of adherence with different anaesthetics at different times.

- Ether
- Flaxedil
- Halothane
- Marcaine 1%
- Xylocaine 5%

Samples taken at different times.
of recovery from anaesthesia and after 24 hours of surgical procedure. This decrease in granulocytic adherence solely may not be due to anaesthetic agents but surgical stress may also be a contributing factor. Cullen, Hume Chretien (1972) reported a decrease in phagocytosis of latex particles and nitroblue tetrazolium (N.B.T.) reduction in patients during either halothane or nitrous oxide-narcotic anaesthesia without surgery. Decrease of granulocytic adherence in sample II may be combined effects of anaesthesia and surgery, but in sample III and IV the surgical stress is almost over, so the effect may be of residual concentration of anaesthetic agents. Although hypotension is also an another factor which may affect neutrophil functions but during our study no hypotension was allowed during surgical procedure as well as after surgery.

Effect of different anaesthetic agents on granulocytic adherence

By seeing the above data, it appears that all the anaesthetic agents studied, alter the granulocytic adherence significantly. But all the anaesthetic agents are not having similar effect. Whelam et al (1982) showed significant reduction in lymphocyte number and in the response of mitogen PPD & PWM and
to histocompatibility under general but minimal changes after spinal anaesthesia. Hole (1982) further showed the same effect that depression of various lymphocyte and monocyte functions under general anaesthesia but they were absent under epidural anaesthesia. According to Hole (1982) serum cortisol increased in both pre and post operative period in general anaesthesia. Minor changes were noticed in epidural. Differences in spreading index and cytolytic activity were not seen when monocytes were cultured in medium with pooled A.B. serum, thus indicating a serum factor responsible for the monocyte depression with the patients operated under general anaesthesia.

To verify the above findings, different groups comparison were made in our study. I and II groups having spinal subarachnoid block and last 3 groups having general anaesthesia. Group I was compared to group II in different samples. The results in all the 4 samples were not statistically significant. In the same manner group I was compared to group III, IV and V but in all cases the results were statistically insignificant. Group III was compared to group IV and V to know the difference between inhalational and relevant anaesthetic agents,
but again the results in all the samples were not significant statistically.

But when group II (spinal subarachnoid block with 1% Marcaine) was compared to group III, IV and V separately, it shows statistically highly significant result with group III in all the samples except in control sample. Comparison with group IV shows significant changes in sample II and III while not significant in control sample as well as in last sample taken 24 hours after surgical procedure. Again when group II was compared to group V (Halothane) it shows statistically highly significant value in all the samples except in control samples.

The basis of adhesion is not well understood which is probably the interaction of powerful electrostatic repulsive forces between cells and substrates. There are certain divalent cations (Mn$^{+2}$, Mg$^{+2}$ and Ca$^{+2}$) which merely neutralize the negative charges (Weiss, 1971). The other factors affecting the granulocytic adherence are interaction of chemotactic factor with phospholipid bilayer of cell membrane (Wilkinson, 1974), cyclic nucleofide (Brayant and Sutcliffe, 1973), ethanol (Brayton et al, 1970), aspirin and glucocorticoids (Dale et al, 1974 and Mac Gregor et al, 1974). Certain bacterial
products (Wilkinson, 1975) and hypophosphatemia associated with hyper alimentation (Craddock et al, 1974). The complement acts as opsonins and the interaction of C$_5$A and other chemotaxins with granulocytes results in a diminished negative change at the cell surface (Gallin et al, 1975). Firm contact may be mediated by surface receptors on the leucocytes that recognise sub-class IgG or C$_3$ complement coating the particle (Cline and Colle, 1977).

The chemotaxis, adherence and phagocytosis have all been shown to be energy dependent processes during which the glycogen content of neutrophils decreases, while oxygen consumption, lactate production, glucose utilization and hexose monophosphate shunt actively increase (Klebanoff, 1975 a). Most of this energy required is derived in the form of A.T.P. from the metabolism of glucose via glycolysis pathway. The neutrophilic membrane is permeable to glucose. Most of the enzymatic steps in glycolytic pathway are reversible. However, hexokinase, phosphofructokinase and pyruvatekinase are irreversible and of these the latter two are regulated by insulin availability (Weber et al, 1966).

From the findings of above study and the granulocytic functions observed during anaesthesia
in the past by other workers (Graham, 1911, Bruce, 1967, Koscioltek, 1967, Hume, Cullen & Chrton, 1972, Cullen, 1974; Douglas, 1970; Moudgil and co-workers, 1977), it seems that a number of vital steps in granulocytic functions are impaired in patients having anaesthetic exposure. Impairment in granulocytic functions specially the adherence activity can be held responsible for the increased frequency and susceptibility of the operated patients to various type of infections.

A careful analysis of the above data and discussion shows that all the anaesthetic agents (Lignocaine, Marcaine, Thiopentone sodium, Scoline, N₂O/O₂/Flaxedil, Ether and Halothane) leads to a decrease granulocytic adherence - 20 minutes after induction of anaesthesia; at the time of recovery from anaesthesia and even 24 hours after surgical procedure.

Comparatively Marcaine given as subarachnoid block leads the less decrease in granulocytic adherence in comparison to Xylocaine given as subarachnoid block and general anaesthesia with Thiopentone sodium + Scoline + N₂O/O₂/Flaxedil or N₂O/O₂/Ether or N₂O/O₂/Halothane.