INTRODUCTION

During the last several years a fantastic development in the field of biochemistry and medicine has been observed in which the major contributions in regard to understanding the biochemical basis of the diseased conditions in human individuals were made by biochemists. Recently a new approach in the field of medicine has been made which describes the pathological disorders and complexities in molecular terms. This approach was originally initiated in the early 1950s when Professor Pauling recognized sickle-cell anemia as a molecular disease, and he remarked "Disease is a great teacher". The eminent successes in the field of biochemical genetics during the successive years culminating with the synthesis of a gene in the laboratory by the noted scientist Professor Khorana has suggested some fanciful ideas about the possibility of direct chemical manipulations with the chromosomes for therapy and treatment of biochemical diseases at some future time.

While therapies for biochemical disorders are a logical extension of traditional medicine other directions of biochemical research are leading to understanding the molecular aspects of the physiological states. It is known that life is nothing but an expression of physical and chemical principles involving a series of reactions taken part by various macromolecules such as nucleic acids, proteins etc. These macromolecules form the real matrices of the living systems. Human
being is simply an elegant machine with elaborate macromolecular structures. Besides the structural aspects of these macromolecules they have many functional forms e.g., proteins are functionally active in the form of hormones, antibodies, enzymes etc., nucleic acids are functionally active in the form of genes regulating information transfer and (also involved in the memory effect etc., etc).

It is known that the flow of information used by the living systems occurs from DNA to RNA to proteins to cellular metabolism and finally to intracellular activities with feedback controls regulating the events at each level. During the recent years enough emphasis has been given on the critical role of control effects generated either by certain metabolites at key biochemical cross-roads in changing properties of enzymes or by changing the transcriptional and translational events at the level of synthesis of these enzymes. By doing so the biochemical events are regulated in the living systems. If by any means this regulation mechanism is affected, serious pathological disorders like cancer, leukaemia etc. might occur or severe metabolic aberrations develop leading to abnormal state of health.

Although very extensive investigations are being carried out in the field of leukaemia all over the world the identification of this disease in terms of alterations in the molecular events has not been possible.
In the human leukaemias it has been found that there is generally an increase in the leucocyte enzymes which tends to be characteristic of immature leucocytes (1) and decrease in those proteins characteristic of the mature leucocytes (2,3). It is thus possible that leukaemogenesis may be considered as a disorder of cellular differentiation. Hence the molecular mechanism for the development of leukaemia regardless of its etiology may be due to disorders in the control of protein synthesis, resulting in a block in cell differentiation. In leukaemia translational control may be of particular interest since evidence has already been presented which demonstrates that changes in amino-acyl-t-RNAs or changes in their levels could occur through alterations of amino acid pools, amino-acyl-t-RNA synthetase or t-RNA themselves (4,5).

Leucocytes represent a group of heterogeneous cell populations comprised of granulocytes, lymphocytes and monocytes and they are primarily involved in the defence mechanism in their role in phagocytosis as well as in antibody formation. Leucocytes like any other cells contain various subcellular fractions loaded with enzymes with specific metabolic characteristics. The metabolic pattern is affected under various pathological conditions and there occurs a significant alteration in relation to the activities of various enzymes associated with the metabolism of leucocytes (6-9) along with changes in the nucleic acid metabolism and protein biosynthesis in the leucocytes under conditions of leukaemia (10-15).
It is known that most of the granule associated enzymes of the leucocytes are lysosomal in nature (16) and that these enzymes have a very important role in the leucocyte function which is found to be altered under conditions of leukaemia (2,6). Information regarding the alterations of enzymes of carbohydrate metabolism in leucocytes under leukaemic condition indicates that there occurs a marked alteration in the enzyme make-up of the pathways concerned particularly in respect of the glycolytic cycle and the TCA cycle although not much is known about the activities of enzymes of the uronic acid pathway in either normal or leukaemic leucocytes with the exception of β-glucuronidase activity (17).

Since the isolation and characterization of rabbit PMN leucocytes as particles analogous to the lysosomal fractions of other cells (16), series of investigations have been carried out on the importance of these granules in the intracellular events like phagocytosis which seems to be controlled by several acid hydrolases present in them (16,18). The process of phagocytosis is found to be regulated by several drugs and metabolic regulators (19-22) and it has been found that the extent of phagocytosis is altered under conditions of leukaemia (23). It has also been found out that the granulocyte enzymes are good mediators of the inflammatory processes (24). Anti-inflammatory drugs are known to suppress the release of lysosomal enzymes from guineapig and rabbit PMN granulocytes thereby altering the phagocytic action (25,26). Besides these, lysosomal enzymes of these granulocytes play the active role in
the phagocytic process (27). Certain basic proteins are found to be present in the granules of these cells and they are found to have antimicrobial activity (28-30). Under leukaemic conditions they are altered in respect of certain properties (31).

Investigations have also been carried out on the importance of granules present in the platelets about which not much informations are available although \( \beta \) -granules have been found to be involved in platelet aggregation (32) and clotting system (33) and are associated with the lysosomal fractions (34). \( \beta \) -granules of the platelets contain mitochondria and are responsible for energy metabolism and so many diverse platelet functions such as clot retraction, adhesiveness, aggregation and phagocytosis (35,36).

Although some perfunctory informations are available about changes in the concentration of enzymes like acid phosphatase, alkaline phosphatase, \( \beta \) -glucuronidase, acid cathepsin, peroxidase, catalase and succinate dehydrogenase of the heterogeneous populations of leucocytes and platelets under conditions of leukaemia (6,37,38), very few attempts have so far been made either to determine the activities of these enzymes in different cell populations or to estimate the nature of distribution pattern of these enzymes in the various subcellular particles of these isolated granulocytes and platelets under leukaemic conditions (39). Normal granulocytes are mature cells and are called PMN leucocytes whereas leukaemic granulocytes contain
both mature PMN leucocytes and also immature cells which are not polymorphonuclear. In order to assess the biochemical nature of the granules of these granulocytes and platelets in regard to the distribution of these lysosomal, mitochondrial and supernatant enzymes under conditions of leukaemia, investigations have been carried out with a view to identify this pathological condition in the molecular terms.

In the following few paragraphs studies concerning the nature of leucocytes, their pathological characteristics, metabolic characteristics as well as investigations on the activities of the enzymes and nature of the leucocyte and platelet granules with respect to their enzyme contents and also their functional role etc. under normal and leukaemic conditions are presented.

**Leukaemia**

Leukaemia is characterized as a group of neoplastic disorders of blood cell forming tissues (40,41). On the onset of the disease, leucocytosis occurs due to perpetual proliferation and exfoliation of mature and immature leucocytes into the peripheral blood and such character terminates only with the death of the individual. Leukaemia is often associated with anaemia and thrombocytopenia. The leukaemic process is one of the maturation arrest in which the primitive cells cannot mature beyond a certain point, perhaps because of some metabolic defects. In leukaemia there may be involvement of any one of the white
cell forming tissues like bone marrow, lymphoid tissues, the reticuloendothelial system or system of plasmocytes.

The broad subdivisions of leukemia may be made on the basis of two findings: (i) the site of origin of the proliferating leukocytes and (ii) the predominance of cells of different maturity. The myelogenous or myeloid leukemias are those involving leukocytes normally produced from the bone marrow, while lymphogenous or lymphoid leukemias involve leukocytes formed chiefly in lymphoid tissues. The characteristic feature of chronic leukemias is the presence of more mature cells in the peripheral blood whereas more primitive, less differentiated cells occur in clinically acute leukemias. Polymorphonuclear leukocytes, myelocytes and metamyelocytes are chiefly found in chronic granulocytic (myeloid) leukemia (CML). When the neutrophil series is replaced by eosinophil or basophil series the leukemia formed is termed as chronic eosinophilic and chronic basophilic leukemias respectively. In acute myeloid leukemias (AML) myeloblasts, promyelocytes, myelocytes and polymorphonuclear leukocytes are the chief varieties of the leukocytes present. The rarest variety of leukemia is monocytic leukemia where monocytosis of considerable degree consisting of many immature cells is present in the blood (42). The leukemia of the monocyte series with monoblast and monocytes in the blood and bone marrow has been subdivided into two categories, (i) myelomonocytic leukemia or Neugeli type of acute leukemia where monocytes may be derived from myeloblasts
or early myelocytes and (ii) Schilling type of acute leukaemia where monocytes originate from the reticuloendothelial system. When the cells of acute leukaemia show no evidence of differentiation beyond the myeloblast, monoblast or lymphoblast stage, the type of acute leukaemia is called 'stem cell leukaemia'.

The three major varieties of acute leukaemia, myeloblastic, lymphoblastic and acute monocytic, have many striking similarities in their clinical and pathological manifestations (43).

In acute leukaemia the red cells are usually normocytic and normochromic even when their numbers are greatly reduced, variations in size and shape are not uncommon and may occasionally be striking. There may be polychromasia and reticulocytosis when red cell regeneration is active and a few normoblasts are usually present. During phases of thrombocytopenia, the morphology of the platelets is often abnormal with irregularities in size, shape and staining. In all forms of acute leukaemia the total leucocyte count in the peripheral blood may vary between very wide extremes. In chronic granulocytic leukaemia the number of circulating leucocytes is enormous. The majority of the circulating leucocytes are granulocytes or granulocyte precursors, neutrophil polymorphs, metamyelocytes and myelocytes and a variable numbers of promyelocytes and myeloblasts. Variability in size and granularity is often prominent among both the mature and immature granulocytes. There may also be an increase in total number of basophils, eosinophils,
lymphocytes and monocytes. The platelets are usually numerous in the early stages and platelet counts much above normal are not uncommon. In some cases the platelets are markedly irregular in size and shape and megakaryocyte fragments may be seen in the circulating blood. Subnormal platelet levels are often found in the later stages of the disease when thrombocytopenic bleeding may develop. In the great majority of cases, however, moderate anaemia is developed from the time of first diagnosis and such anaemic conditions tend to be more severe as the leukaemic process deteriorates. In chronic lymphocytic leukaemias the leucocytes are increased in numbers with the predominance of lymphocytes. At the initial stage of the disease the red cells appear normochromic and normocytic but as the disease progresses, hypochromia and anisocytosis develop and hemoglobin level falls. Thrombocytopenia is not prominent in chronic lymphocytic leukaemia although it develops terminally.

**Metabolism of leucocytes under normal and leukaemic conditions**

Systematic biochemical and metabolic studies of normal and leukaemic leucocytes were begun only after 1930 onwards. In many respects leukaemic leucocytes metabolically differ from normal variety (6,39). The immature leucocytes contain low DNA and with increasing maturity the DNA content is increased (6,39). No differences in the DNA composition of normal and leukaemic leucocytes have been observed but differences in the physico-chemical properties of DNA isolated from these two sources have been reported (39,44). Three satellite DNAs and a repeated DNA
fraction: have been isolated from human leukaemic leucocytes (45). The rate of DNA synthesis is low in leukaemic leucocytes as compared to their normal counterpart (6). In immature cells the RNA content is high but these changes are not specifically leukaemic (39). Gallo et al. reported that the contents of t-RNAs of several amino acids alter in leukaemic leucocytes (5). Activities of many of the nucleic acid synthesizing enzymes like dihydrofolate reductase, thymidine kinase, thymidylate synthetase, DNA polymerase are altered in leukaemic leucocytes (40,46).

The low content and high turnover rate of glycogen are characteristic for practically all the classes of acute leukaemias (37). The chief pathway of glucose metabolism in leucocytes is the formation of lactate via pyruvate and the rates of glucose utilization and lactate production are less in the white cells of CML and CLL than in the normal leucocytes (6,37).

Several enzymes of the pathways of carbohydrate metabolism like Embden-Meyerhof glycolytic pathway, Pentose phosphate pathway, pathways of glycogen synthesis, TCA cycle, uronic acid pathway etc. are altered in the leucocytes under leukaemic conditions (37,40,47,50). The rate of lipid synthesis is increased in acute and chronic myelocytic leukaemia as compared to normal but is decreased in the lymphocytic leukaemias (51).

Increased levels of glutamic acid and proline and decreased level of ornithine have been observed as the
characteristic feature of leukaemic leucocytes regardless of the type of leukaemia (5). Higher rate of labelled L-valine, L-leucine, L-alanine, L-cystine and L-methionine incorporation was observed in leukaemic leucocytes than that of normal variety (5).

Studies on the metabolism of normal and leukaemic leucocytes indicate that the changes in the nature and rate of growth of leukaemic leucocytes are associated with the alteration in the mechanism of protein metabolism.

Importance of PMN granules

Electron microscopic study shows that leucocytes contain intracellular components as that of the other systems (16). The presence of specific heterogeneous granules was definitely established and they are found to be of different forms, depending on their nature and maturity (52-54). Bessis showed that leukaemic leucocytes contain huge number of immature and non-specific granules which show various morphological appearance under electron microscope (55).

Cohn and Hirsch (16) first isolated and characterised the typical intracellular granules of the rabbit polymorphonuclear leucocytes as the particles analogous to the lysosomes of liver (56). They also demonstrated the critical role of these granules in the intracellular events of phagocytosis. Slosses et al. showed that the specific granules of
polymorphonuclear leucocytes are closely attached to the phagosomes (57). Cohn and Hirsch (16), Douglas (18) and Spicer et al. (58) clearly showed that the heterogeneous PMN leucocyte granules contain various hydrolyses which play the main function of the digestive process in phagocytosis. Hirsch (59) and Hirsch and Cohn (60) observed that the degranulation of PMN leucocytes occurs following phagocytosis of microorganisms. Many of the regulators and drugs like cyclic AMP, prostaglandins, caffeine etc. have direct enhancing effect on the degranulation process during phagocytosis (19-21). The phagocytic activity of leucocytes under leukaemic conditions has been found to be very low (23).

Wetzel et al. (54) and others (52, 53, 61) established that the PMN leucocyte granules are broadly divided into primary, secondary and tertiary granules. The enzymatic heterogeneity of PMN leucocyte granules isolated from both exudate and peripheral blood was also established (18, 62). It was noted that some of their enzyme contents differ under leukaemic conditions (5, 6, 40).

Zeya and Spitznagel found that several enzymatic and non-enzymatic arginine rich basic proteins are present in the lysosomal granules of polymorphonuclear leucocytes obtained from various sources (28-30). They separated and isolated such basic proteins by zone electrophoresis and found that the basic proteins are antimicrobial in nature (29). Recent investigation of Inge Olsson and Per Venge gives the information
that the leukaemic leucocyte granules contain several basic proteins; the electrophoretic mobilities exceeded that of lysozyme and differ from normal variety. He also showed that the proteins of both sources have antigenic determinants and are antimicrobial in character (31).

Polymorphonuclear leucocytes have great role in the inflammatory process Ianoff and Scherer (24) and others (26) found that several enzymatic and non-enzymatic substances of leucocyte lysosomal granules are good mediators of inflammatory process and several steroidal and non-steroidal anti-inflammatory drugs can suppress the lysosomal enzyme release from guineapig and rabbit PMN leucocytes (24,63).

Leucocytes under certain diseased conditions like Chediak Higashi Syndrome (CHS), granulomatous disease etc. contain defective granules in the PMN leucocytes and thereby their phagocytic activities are hampered under these conditions (64,65).

Study of enzymes of granulocytes

Leucocytes contain several enzymes which have clinical significance in leukaemia. Most characteristic enzyme is alkaline phosphatase (40). This is the widely studied enzyme in leukaemia and other related myeloproliferative diseases. It is well known that leucocyte alkaline phosphatase activity is extremely low in chronic myeloid leukaemia (6,37,40,41) and
such activity alters in many of the pathological conditions like granulomatous disease (65), Down's Syndrome (66), Chediak-Higashi Syndrome (67), Myelofibrosis (68), Polycythemia Rubra Vera (69) etc. It was also found that the isoenzyme pattern of the alkaline phosphatase in leucocytes of CML and other pathological conditions differ from that of normal (70,71). Robinson and Pierce (70) and others (66) suggested that the enzyme is developed by the control of the structural gene of 21 chromosome (PH trisomy) in leukaemia, Down's Syndrome and other pathological states. The enzyme is restricted to specific granules in mature PMN leucocytes (62) and intracellular distribution study of guineapig PMN leucocytes showed that the enzyme content of the granule is intermediate between the granules which contain peroxidase and $\beta$-glucuronidase (62).

Leucocytes contain both peroxidase and catalase (72). The leucocyte peroxidase is known as myeloperoxidase and has a great role in phagocytic process (73). Almost all of its activity is present in the granules of granulocytes (62) and immature granulocytes have much lower activity (60). Some of the leucocyte diseases also have much lower peroxidase activity (67). Catovsky et al. (74) and others (75) showed that neutrophil is deficient of myeloperoxidase under leukaemic conditions. Zgliczynski et al. isolated and purified the myeloperoxidase both from normal and leukaemic leucocytes and studied their physicochemical properties (76). The ultrastructural localization of myeloperoxidase in relation to the primary and secondary granules of polymorphonuclear leucocytes is recently shown by
Dunn et al. (77). Olsson et al. (76) noted that purified myeloperoxidase from normal and leukemic myeloid cells form spectrally characteristic complexes with \( \text{H}_2\text{O}_2 \) and iodine and it catalyses the iodination of the phagocytised cells when a continuous supply of \( \text{H}_2\text{O}_2 \) is formed by the addition of glucose oxidase. The defect of the phagocytic process can be detected by studying the complex.

It is known that almost all of the leucocyte catalase activity is present in the soluble supernatant fractions (62) and its activity is raised in the cells of neoplastic diseases (79). Reports on the occurrence of catalase in isolated nuclei of neoplastic origin are somewhat contradictory (80-82). Several workers noted that such activity of the catalase is high (80), or is moderate (81) or is absent (82) in the cell nuclei of neoplastic origin. Chev Kidson (83) studied the catalase activity of leucocytes under normal, leukemic and infective conditions. In acute and chronic myeloid leukemia catalase levels were found to be higher than the range for normal granulocytes whereas in acute and chronic lymphatic leukemia, the levels were of the same order as those for the normal lymphocytes. Low catalase values were found in infective leucocytes.

Leucocytes contain acid phosphatase, the activity of which is increased under leukemic conditions (6,62). Various conflicting data is observed on the localization of acid phosphatase in the leucocytes. Vercaunteren and others (64,66) showed that the acid phosphatase in PMN leucocytes present in at least two, and
probably three particle populations and considerable variation was observed in different species. Biochemical and cytological studies of unstimulated normal and CLL were performed by various workers (67). It was observed that acid phosphatase activity decreases in CLL lymphocytes as compared to the normal cells. At electron microscopic level the number of membrane bound acid phosphatase positive organelles are diminished in CLL lymphocytes. It was noted that the diminution of the enzyme activity in CLL lymphocytes is most likely due to a reduced number of lysosomes, rather than to a diminished enzyme content of these organelles. Li et al. (88) demonstrated that such decrease of the enzyme activity is the cause of drastic decrease of enzyme rich monocytes, and neutrophils. Leucocytes in CLL contain only one of the five acid phosphatase isoenzymes found in normal leucocytes, although total acid phosphatase activity is not much changed under CML, AML and leukemic reliculo-endotheliosis.

From the earlier literatures it is known that leucocytes contain β-glucuronidase (89,90). Rossiter and Wong (91) observed that both PMN leucocytes and lymphocytes contain β-glucuronidase. In 1950 Anlyan et al. reported that the activity of the enzyme varies under different types of leukemic conditions (92). An increase in the activity of the enzyme has been observed under many non-leukemic conditions (40). However, lymphocytes possesses less enzyme than granulocytes, whether leukemic or not. β-glucuronidase is the enzyme of the granule
fractions of the granulocytes (62) Nilius et al. (93) and others (67,94) demonstrated by cytochemical observation of the leucocytes that the activity varies in different diseases including leukaemia. Recently Michell et al. (62) studied the distribution pattern of the enzyme in different subcellular fractions of guinea pig polymorphonuclear leucocytes under normal conditions.

Leucocytes contain proteolytic enzymes (95,96). Such enzymes have subsequently been described and characterized in detail in several laboratories (39,62). Increased activities of the proteases are found under chronic myeloid leukaemia and acute myeloid leukaemia and such activities are decreased in the leucocytes obtained from chronic and acute lymphocytic leukaemias(6). Circulating leucocytes obtained from peripheral blood contain some di- and tri-peptidases which are activated by metal ions (95). The distribution of various peptidases in normal and leukaemic leucocytes was noted by Hascham and Krug (95). Mounter and Atiych (96) have described that the alkaline protease from neutrophilic leucocytes is similar to chymotrypsin and also two acid active proteases are present in lymphocytes. Ianoff and Scherer (24) found that the elastolytic activity is present in the leucocyte lysosomes. Stiles and Fraenkel-Conrat definitely showed that not only the cathepsin activities are changed in the leukaemic leucocytes but also their distribution patterns among the specific granules of leukaemic granulocytes are altered (39).
Ultrastructural study of intracellular components of platelets

In 1958 onwards the ultrastructural localization of platelet subcellular components was known. Feissly et al. (97) first studied the entity of the polymorph elements of platelets. They possess hyalomere and a complex granulomere components. Marcus et al. (98) showed that the thromboplastic activity is high in dense granules and hyalomere has been identified as glycogen. In 1955 Bernhard and Leplus described that there are three types of granules in megakaryocytes and platelets (99). A classification generally agreed upon is that of Schultz and Heiper in 1958 (35). Mitochondria, lysosomes, ribosomes are all present in the different types of granules. \( \alpha \)-granules are rich in lysosomes (34) and \( \beta \)-granules are rich in mitochondria (35). Although platelet mitochondria are present in small numbers in \( \beta \)-granules, they contribute much of the energy metabolism of platelets to carry out platelet functions as clot retraction, adhesiveness, aggregation and phagocytosis (36). Lysosomes are most abundant in the \( \alpha \)-granules (34). \( \alpha \)-granules are said to contain serotonin (100), ATPase activity (99), acid hydrolytic enzymes (98), fibrinogen (101), platelet factor 3 (33). Many authors believe that \( \alpha \)-granules and lysosomes are identical. Siegal and Luscher (34) did not hold this view. They separated the granules into several distinct subfractions including membranes, vesicles, lysosomes, mitochondria and dense granules and concluded that the lysosomes were a distinct...
fraction which were not identical with the d-granules. Platelets also contain other granules like \( p \) and \( s \)-granules (101, 102). Several workers isolated the different granules by differential centrifugations (103, 104).

Biochemical and metabolic studies of platelets under normal and leukaemic conditions

Surprisingly scanty informations comparing the biochemical composition and metabolism of normal platelets with those obtained from patients with leukaemia are available. Notario et al. (105) observed no abnormalities of enzyme activities in the entire cycle of respiration and glycolysis in platelets from patients with acute and chronic leukaemias. These cells exhibited increased respiration, a lower content of ATP, normal RNA but a lower turnover rate of RNA phosphorous and decreased glycogen content when compared with that of normal (37). Glucose-6-phosphate dehydrogenase activity is lower in acute leukaemia whereas the activity under chronic leukaemia is normal (106). Auer and Castens noted that fatty acid synthetase is abnormally low in chronic leukaemic platelets (107). Dehydroorotic dehydrogenase activity in platelets from both acute and chronic leukaemias was found to be elevated (108). Morphologic studies of platelets from patients with leukaemia using light, phase contrast and electron microscopy generally have been disappointing. No specific
abnormalities were detected but some correlation between plate­
let dysfunction and altered morphology especially in the chronic
leukaemia has been revealed (99).

Platelets contain several hydrolases and dehydroge­
nases (37,99,109-111) but very few informations are available
on these enzymes under leukaemic conditions. Leukaemic plate­
lets contain low lactic dehydrogenase activity (111) with
abnormal isoenzyme pattern. Several contradictory results
were, however, obtained about the presence of platelet peroxi­
dase (112,113). Recently Breton et al. (114) observed with the
help of ultrastructural study that normal human platelets and
megakaryocytes possess peroxidase activity. Catalase is known
to contain very low activity in both normal and leukaemic plate­
lets (99). Platelets contain several lysosomal hydrolases like
acid phosphatase (115), alkaline phosphatase β-glucuronidase,
cathepsin, arylsulphatase etc. (109,110). Very meagre informa­
tions are available about the activities of these enzymes under
leukaemic conditions (109). Ansell and Scott (109) noted that
platelet alkaline phosphatase activities are not altered under
leukaemic conditions. It is also noted from the work of
Tangheroni et al. that the platelet alkaline phosphatase activi­
ties also are not changed under several myeloproliferative
disorders (116).

Inflammation and leucocytes lysosomes

de Duve (56) first noted that the release of lysosomal
material has a close relationship with the inflammatory process.
Such inflammation occurs by increasing vascular permeability of the lysosomal membrane (117). Several workers (118-121) noted that the activities of acid hydrolases are elevated significantly in inflamed tissues, including rheumatoid synovial membrane. The hydrolytic degradation of cartilage mucopolysaccharide, which underlies the inflammatory process of the rheumatoid joints, occurs by the lysosomal enzyme release from cells within the tissues (122,123). It was earlier noted that the soluble extract of leucocyte lysosomal materials elicit cutaneous and joint inflammation and degrade the protein mucopolysaccharide matrix of cartilage (124,125). Lysosomes, specially of leucocytes, contain several basic proteins which appear to induce inflammation by disrupting mast cells (126) and such inflammatory process does not require histamine or serotonin (127). Leucocyte lysosomes also contain a permeability factor (a protease) which has a chemotactic influence on other leucocytes to produce inflammation (128,129).

Relation between the anti-inflammatory drugs and leucocyte lysosomes

The therapeutic effect of several steroidal and non-steroidal anti-rheumatic as well as anti-inflammatory drugs was reviewed in several literatures (117,130). The biochemical activities and mechanistic actions have been attributed to account for the therapeutic effects of the anti-inflammatory drugs (130). Chloroquine phosphate (an anti-malarial substance), phenylbutazone, oxyphenbutazone, acetylsalicylic acid,
hydrocortisone, flufenamic acid etc. were shown to inhibit
the *in vitro* and *in vivo* release of acid hydrolases from rat
and rabbit liver lysosomes (130-132). Such steroidal and non­
steroidal anti-inflammatory drugs inhibit the release of liver
lysosomal enzymes *in vivo* not only from normal rats but also
from adjuvant arthritic rats (133,134). The main function of
polymorphonuclear leucocytes is the phagocytosis and the phago­
cytic digestive process occurs leading to the degranulation of
the PMN leucocytes (59,60). It was noted that such anti­
inflammatory drugs inhibit the phagocytic process (25). Ignarro
first definitely established that the non-steroidal anti­
inflammatory drugs have direct effect on the stabilization of
the PMN leucocyte lysosomes obtained from rabbit peritoneal
exudate (63). He also found a dissimilar effect of these drugs
on stability of lysosomes from peritoneal and circulating leuco­
cytes. The acidic drugs can labilise the lysosomes obtained
from rabbit PMN leucocytes (135). Such stabilisation of guinea­
pig PMN leucocyte lysosomes also occurs by both steroidal and
non-steroidal anti-inflammatory drugs (63).

**Anti-inflammatory drugs and platelet function**

Aspirin has been utilized for many years as a thera­
pneutic agent because of its well known analgesic, antipyretic
and anti-inflammatory activity. In recent years unwanted side
effects particularly haemorrhage both under normal and patho­
logical conditions has been the subject of considerable concern
Recently it is noted that aspirin has a direct effect on the coagulation mechanism by inhibiting the platelet aggregation (137-140). Abnormalities in platelet function similar to those produced by aspirin under both normal and pathological conditions may be found with sulphinpyrazone (141), phenylbutazone (142), anti-histamines (143), adrenergic blocking agents (144) and phenothiazines (145) etc. O'Brien studied the effect of several anti-inflammatory, analgesic and antipyretic drugs on platelet functions and suggested that the inflammation and platelet functions are interconnected (146, 147). He found that almost all the compounds having effect on platelets also have anti-inflammatory properties and the compounds inactive in the platelet tests have no anti-inflammatory activity. He concluded that either these drugs can inhibit both the inflammatory changes and platelet response in a similar manner by operating the same mechanism as on different cells or the effect of these compounds on the inflammation is at least partially mediated through the effects on the platelets (146).

On the basis of the above described informations on the biochemical studies of leucocytes and thrombocytes under normal and leukaemic conditions attempts have been made to investigate the distribution pattern of several enzymes viz. acid phosphatase, alkaline phosphatase, $\beta$-glucuronidase, acid cathepsin, peroxidase, succinate dehydrogenase and
catalase in the leucocytes and thrombocytes both under normal and leukaemic conditions according the plan mentioned below:

Plan of work

Work embodied in this thesis consists of studies on the activities of several granule-associated and supernatant enzymes of PMN leucocytes from normal and leukaemic individuals, immature granulocytes of leukaemic (chronic myeloid leukaemia, CML) individuals and also of platelets under normal and leukaemic conditions. The intracellular distribution of these enzymes in these different types of cells have been carried out under normal and leukaemic conditions. The effects of several non-steroidal anti-inflammatory and analgesic drugs on the release of lysosomal enzymes from the granules of mature and immature granulocytes and also of platelets under normal and leukaemic conditions have also been studied.

Details about the work presented in different chapters in this dissertation are given below:

Chapter 1: General Methodology

In this chapter, case histories of the patients and description of various methods followed for the experiments are given. These methods include collection of blood samples,
isolation of heterogeneous leucocytes, isolation of polymorphonuclear (PMN) leucocytes from normal and leukaemic leucocyte preparations and immature granulocytes from leukaemic leucocyte preparations by high density bovine serum albumin gradient centrifugation, isolation of platelets, estimation of proteins, fractionation of leucocytes and platelets into various subcellular fractions. Besides these, various analytical methods such as determination of activities of enzymes like acid phosphatase, alkaline phosphatase, $\beta$-glucuronidase, acid cathepsin, succinate dehydrogenase, peroxidase and catalase in those cells are given.

Chapter 2 : Distribution study of several enzymes in the subcellular fractions of the granulocytes under normal and leukaemic conditions

This chapter deals with the assay of several enzymes in different granulocytes and also in different subcellular fractions of these cells under normal and leukaemic conditions.

Chapter 3 : Effect of several non-steroidal anti-inflammatory and analgesic drugs on the release of lysosomal enzymes from leucocyte granules under normal and leukaemic conditions

This chapter presents the effects of several non-steroidal anti-inflammatory and analgesic drugs like chloroquine phosphate, phenylbutazone, oxyphenbutazone, acetylsalicylic acid, paracetamol and phenacetin on the release of acid phosphatase and $\beta$-glucuronidase from the granules of PMN leucocytes
from normal and leukemic individuals and of immature granulocytes from leukemic individuals.

Chapter 4 : Distribution study of several enzymes in subcellular fractions of platelets under normal and leukemic conditions

This chapter presents the assay of enzymes viz. acid phosphatase, alkaline phosphatase, β-glucuronidase and succinate dehydrogenase in the homogenates and in different subcellular fractions of platelets under normal and leukemic conditions.

Chapter 5 : Effect of several non-steroidal anti-inflammatory and analgesic drugs on the release of lysosomal enzymes from platelet granules under normal and leukemic conditions

This chapter deals with the effects of several non-steroidal anti-inflammatory and analgesic drugs like chloroquine phosphate, phenylbutazone, oxyphenbutazone, acetylsalicylic acid, paracetamol and phenacetin on the release of acid phosphatase and β-glucuronidase from the granules of platelets under normal and leukemic conditions.
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