"I have been able to make the dumb plant the most eloquent chronicler of its inner life and experiences by making it write down its own history. The self-made records thus made show that there is no life-reaction in even the highest animal which has not been foreshadowed in the life of the plant."

———Sir J.C. Bose
Motility is one of the fundamental properties of all living beings. In fact, it is the obvious sign of life and is expressed in a number of ways. The common forms are cytoplasmic streaming, cell shape changes, ciliary motion, and most advanced of all is muscle contraction. In all cases of motility, some features are found to be common and it is expected that these phenomena depend on the same or similar structural elements and that the motive force is the product of similar events at the macromolecular level. The motility is usually measured by the extent of the reaction elicited by an environmental change. It may not be observed when the induced change takes place at the cellular level without modification of the general behaviour, but the reaction is perceptible as and when the organism moves or changes its position. The last reaction is common to the animals and also perceptible to lower groups of plants but rarely observed in higher plants. In fact, among the various behaviours in higher plants, the peculiar one is that a leaf or a part of the leaf system or flower in certain cases show very rapid movement under certain conditions, a phenomenon analogous to animals. It is unique in the sense that the form of motility in plants occurs in vegetative or sexual organs of species classified under families which
are phylogenetically very different. That is, this phenomenon is not an usual characteristic of some specific families or genera.

Study of motility in plants confronts with the inherent complexity of a coordination of diverse processes leading to manifestation at different levels of organisation. The basic concepts of plant motility are being projected and tried for more accurate results and some progress is being made in unravelling the physico-chemical basis of certain motile functions, though some fundamental aspects of the phenomena are still remained unresolved.

The motility in the plants can be discussed under the following categories:

1) Spontaneous movement  2) Movement on stimulation

1. Spontaneous Movement

a) Circadian rhythms

The word circadian is derived from 'circa' and 'diem'; 'circa' means about and 'diem' means the day. It is the time between the repetition of a definite phase of the movement, that is, for example the time between successive peaks or troughs. All circadian systems have a period of approximately 24 hr. Persistent circadian rhythms have been observed in most of the major group
of plants. Many of the characteristics of this type of rhythms in different plants are closely similar and this is known as the Biological Clock. The basic system is thought to control the various aspects of physiological and chemical activities which are normally considered in case of endogenous rhythms. In circadian rhythms main responsible basic oscillating system is not similar in all cases.

b) **Nyctinastic movement**

Nyctinastic movement operated by pulvini frequently occur in compound leaves especially those of members of the Leguminosae. In the evening the leaflets tend to fold together and the whole leaf may droop downwards. The following morning the leaf rises and the leaflets become expanded. Although these type of movement is primarily a response to changes in illumination, an internal rhythm is often established which continues to initiate the movement at the usual times even in continuous darkness.

c) **Nutations**

This type of movement is exhibited by stems, tendrils or leaves. As the plant grows upward, the weight of the aerial part increases. The plants with soft stems cannot stand erectly, on the other hand the stems tends to hold some objects and coil around it or
2. Movement on Stimulation
   a) Geotropism
      This type of movement occurs when the plant organ moves or orient according to the direction of the gravitational pull. This type of behaviour is not fixed and can be changed by a range of circumstances.
   b) Phototropism
      The movement of the plant in which the plane of curvature is determined by the spatial relationship of the organ and the light stimulus is known as Phototropism. Actually the movement is the result of a change in the pattern of growth induced by a change in the spatial distribution of light falling on the plant.
   c) Nastica
      Nastic movement is caused as a result of the response to artificial or environmental stimulus. *Mimosa pudica* is a typical example of this category having an anisotropic pulvinus at the junction of the leaf and the stem. The typical movement exhibited by the plant *Mimosa pudica* is an example of Seismonastic movement.
Phenomenology of the movements in *Mimosa pudica* has been studied in this Institute first by Bose (1902 to 1918). This was then followed by other investigators (Das and Palit, 1934-35; Dutt and Guhathakurta, 1956-57; Datta, 1959-60). However, very few attempts have yet been made to elucidate the mechanism of the movements in *Mimosa* biochemically. The question arises as to what are the molecular mechanisms that underlie cell motility in animal and plant systems.

The purpose of the present work is to investigate the molecular mechanism of movement of leaves in *Mimosa pudica* taking into consideration the contractile proteins thus far known in different motile systems. The main advantage of *Mimosa pudica* over other plants as a model for the study of excitability in plants is the very fast rate of its reactions.

I. MOVEMENTS AS STUDIED IN *MIMOSA PUDICA* L.

The study of rapid pulvinar responses has been confined almost entirely to the leaf of *M. pudica* L. The leaf is compound with two or four pinnae, each of which bears numerous pairs of pinnules. Primary and secondary pulvini are situated at the base of the petioles and subpetioles and a tertiary pulvinus occurs
at the base of each of the pinnules. When the leaf is stimulated the various pulvini become curved in such a way that the main petiole droops, the pinnae come close together, and the pinnules turn upwards so that the members of each pair are brought in contact. Two distinct and interesting problems are presented by these movements: the nature of the mechanism involved in rapid pulvinar response, and the means by which a stimulus applied to one part of the plant can induce reactions in pulvini some distance away. Such responses are usually denoted as seismomasty.

Apart from this seismomastic movement *M. pudica* plant undergoes two other characteristic movements. One is the spasmodic evening fall which in broader sense is the nyctinastic movement and the other is the sudden spontaneous dropping of the leaflets known as autogenic response. Results of the investigation of Dutt and Guhathakurta (1959) showed that the leaf executes the characteristic evening fall even when the plant is subjected to a condition of continuous light or continuous darkness, whether it is under normal variation of temperature or under constant temperature. The studies on autogenic response gave the idea of the interaction of the down moving auxin stream with the pulvini of the leaves (Dutt and Guhathakurta, 1949).
A.  Cytological Studies

The primary pulvinus of *M. pudica*, like most other pulvini, possesses a central vascular strand surrounded by a thick layer of parenchyma. Towards the centre, the parenchymatous cells are separated by well developed intercellular spaces. These spaces diminish in size and disappear altogether just below the epidermis. In the unstimulated pulvinus the parenchyma is highly turgid but longitudinal extension is resisted by the central strand. These parenchymatous motor cells contain all the elements in an outer cytoplasmic layer: lobate nucleus, spherical mitochondria, sparse ergastoplasm, Golgi bodies and chloroplasts (Fleurat-Lessard, 1969); the centre of the cell is occupied by an expanded vacuolar system, described by Mangenot (1927) and Toriyama (1953), comprising a large colloidal vacuole and a tannin vacuole. The occurrence of fibrils is noted in the cytoplasmic layer and in the large vacuole. In the colloidal vacuole, however, the fibrillar aspect is not constant. The general structure and poor lignification of the various tissues give a great extensibility to the organ and this allows movements of large amplitude to take place. Above the main pulvinus and a transitional zone, the petiole is elongated and rigid. Within the transitional zone, the central
vascular cylinder of the pulvinus divides to give four separate bundles which become rearranged to give the typical petiolar pattern. Mangenot (1927a) confirmed this result. Kundu and Saha (1968) distinguish two types of sieve tubes, some are wide and short and the others narrow and long with persistent nuclei. The cell group capping the xylem pole is also important and the origin of this tissue has been diversely stated by different authors: according to Bose (1931), Kundu and Saha (1968) it is a strand of internal phloem; in the opinion of Sibaoka (1962), Toriyama (1962) and Esau (1970) it is a strand of protoxylem. Sen (1963), Fleurat-Lessard and Gavaudan (1973) regarded it as specialized perixylem parenchyma. The important physiological point is that cells in this tissue and others in the phloem have electrical properties which might be involved in the process of movement (Dutt and Guhathakurta, 1962).

At the distal end of the petiole, the vascularization of the secondary pulvinus is achieved by the union of elements originating from peripheral and central bundles. Histological data show that a complicated correlation systems exist between the various parts of the plant.

B. Conduction of Excitation in *M. pudica*

In the past it was thought that the excitation
of the *M. pudica* was transmitted by the pressure variation in specialized cells (Hofmeister, 1867; Sachs, 1874) and this phenomenon is analogous with that of the lower group of animals (Fee, 1849). Pfeffer (1873-74) and Bose (1907) considered the possible existence of three conduction mechanisms; the first compared plant conduction to that observed in the animal nerve system; the second one was based upon the transport of stimulating substance and the third was the theory of pressure variation mentioned above. The data of Bose (1907), Ricca (1916), Houwink (1935), Sibaoka (1954) and Umrath (1959) make it clear that in *M. pudica* three types of conduction as differentiated by their bioelectric properties do exist. These can be differentiated on the basis of the type of effective stimulus, the velocity of propagation and the anatomical pathway followed by the excitation. This excitation is dependent on the following factors:

(i) the age of the plant organ on which the measurement is made (ii) the intensity of stimulus and (iii) time of the year.

a) **The medium conduction**

This conduction is distinguished by a wave that originates when the stimulation is not injurious and it can be stopped by an area of dead tissue. Bose (1907),
Sibaoka (1950 and 1950a), Houwink (1935) and Stoeckel (1968) reported the various characteristic events of this mode of conduction in the petiole of *M. pudica* leaf both by mechanical and electrical methods. The velocity of the wave is between 16 and 110 mm/sec.

b) The slow conduction

The slow conduction originates when the stimulation is injurious. The velocity of propagation of the slow excitation has been found to vary between 2.5 and 5.0 mm/sec. Ricca, as early as 1916 showed that a stimulating substance was released by wounding and he supposed that this material was transported in the transpiration stream.

c) The rapid conduction

The velocity is 180-300 mm/sec and found usually in young leaf system and also resulting from strong stimulation. Umrrath (1959 and 1966) described it as a fast potential variation, the mechanism of which is still unknown.

C. Transmission of Stimulus

The earliest recorded attempt to elucidate the process of conduction seems to have been that of Dutrochet (1837). He removed a ring of cortex from the stem and found that the stimulus caused by burning a leaf
was able to pass through the decorticated region and therefore concluded that the passage of the stimulus was connected with movement of liquid in the wood. Haberlandt (1920) showed that drops which emerge from the stem when it is cut do not come from the xylem but from special cells, the so-called tube cells, which are situated in the phloem. These tube cells resemble large sieve tubes, but have a single large pit in the transverse walls. They also possess a nucleus. He found that the stimulus could pass through a zone of the petiole which had been killed by heat and therefore concluded that transport of the stimulus did not take place through the protoplasmic connections between the cells but was effected by means of a disturbance in the hydrostatic equilibrium in the tube cells. Linsbauer (1914), while considering the possibility that stimuli could be transmitted by means of hydrostatic changes in the tube cells of the phloem, concluded that since they could also be transmitted through stems in which all tissues external to the wood had been removed in one or more internodes, an alternative mode of transport must be by sap movement through the wood.

According to Ricca (1916) some chemical substance liberated from cells in the region where the stimulus is applied can be carried in the transpiration stream through the xylem and even across a narrow
water filled discontinuity. When this substance, which may be donated as a hormone, reaches the leaves it induces contraction of the motor cells of the pulvini. Snow (1924) observed that rapid basipetal conduction of excitation occurred in leaves of *M. pudica* which were in nearly saturated air or had even been submerged under water for three hours. Later, he (Snow, 1925) showed that when the tip of a pinna of *M. spegazzinii* was cut under water, containing a stain the rate at which the excitation moved basipetally was 2.4-10 times greater than that at which the stain was transported through the xylem. He also found that the excitation travelled much more rapidly in leaves which had been submerged under water for some hours than in others which had remained in air and were still attached to the plant.

Rapid conduction in the stem was found by Ball (1927) to be unaffected by removal of all tissues outside the xylem in the internodes, but it failed to pass through a zone of the stem where the tissues on the inner side of the wood had been removed, leaving the xylem, phloem and cortex intact. It did not pass through a discontinuity or through a zone killed by steam except when living cells in the same or an adjacent internode were stimulated by cutting the stem with a pair of scissors or by squeezing it with a forceps. The logical
Deduction from these experiments is that the rapid conduction of excitation in the stem of *M. pudica* takes place in living cells situated within the cylinder of wood including the narrow elongated cells on the inner side of the protoxylem.

D. The Stimulating Factors

a) Electric stimulation

It has long been recognized that the passage of excitation through plant tissues may be associated with changes in electric potential. Bose (1928) compared this stimulation of the petiole pulvinus unit of *M. pudica* leaflet with that of isolated nerve and muscle of animal. The excitation caused at any point on the stem or on the leaf system is conducted as an electric action potential and can be detected by electric instruments. By means of an electric probe first devised by himself, Bose (1928) discovered a particular layer of cells which preferentially carried the electric excitation. He first observed that an action current accompanies excitatory transmission through plant tissue and both travel with the same velocity. This important finding helped him to a great extent to show how the excitatory transmission in plant is analogous to the excitatory conduction in animal nerve. According to him excitation in the contractile tissue of the pulvinus is propagated from cell to cell like that occurring in the
animal heart. Dutt and Guhathakurta (1962), with the help of multiple pen recording units with amplifiers for recording the electrical changes at different points, showed that the biphasic impulse appearing with the fall of leaf is not limited only to the petiole, it can be traced in the subpetiole and in the stem near the pulvinus. The impulse appears in all such places quite simultaneously.

If one electrode is inserted into the stem or petiole of M. pudica or even laid on the surface so as to make a moist contact and a second is connected to the lower part of the stem or inserted into the surrounding earth, a change of potential can be detected when the excitation passes the first electrode. The change consists of a pulse of negativity, the action potential amounting to 20-100 mV. It rises rapidly to a maximum and then declines. Houwink (1935), who used ice as a stimulant, found that when a petiole was stimulated, the main pulvinus responded a little later but the excitation did not pass beyond it. A second stimulus applied to the stem produced an action potential which was detected when it reached the electrode further up the stem.

Umrath (1928) found that in M. pudica and M. spegazzinii an action potential could be induced in
a region of the stem from which all the tissues outside the xylem had been removed but not in a region where half of the stem had been cut away and all the tissues on the inner side of the xylem removed by scraping. This supports the view that rapid conduction in the stem takes place through tissues on the inner side of the xylem and not through the phloem.

Sibaoka (1962) has investigated the location of the excitable cells in the petiole of *M. pudica*. By inserting microelectrodes into the cells of various tissues he found that the elongated parenchyma cells in the phloem and protoxylem regions had a resting potential of about -160 mV, while that of all the other cells was only about -50 mV. When basipetal conduction of excitation was started by stimulating the petiole electrically at its apex, a membrane action potential was always induced in the cells which had a larger resting potential. This suggests that it is these cells which probably provide the pathway for the conduction of excitation.

b) Effect of light

The excitability of the pulvinus in *Mimosa pudica* to an increase of light intensity known since Darwin’s work (1882) has been studied by Fondeville (1963) and recently by Roblin (1975). It has been shown that when
the leaves are illuminated during the dark period of the photoperiodic cycle, they are stimulated after a latent period which is a function of irradiance ranging generally from 40-120 sec. The action spectrum drawn up by Fondeville, Schneider, Borthwick and Hendricks (1967) has clearly revealed that the reaction is mediated by specific photosensitive pigment; considering the maximum near 450 nm, these authors postulated that it was a flavin or a carotenoid.

Applewhite and Gardner (1971) supposed that phytochrome is the active pigment as movements are induced by light emitted in two spectral bands (460-520 and 620-660 nm). This phytochrome action involves changes in permeability as suggested by Hendricks and Borthwick (1967) is supported by various reports (Haupt, 1964; Jaffe and Galston, 1966; Jaffe, 1968; Beevers et al., 1970; Yunghans and Jaffe, 1970; Jaffe and Thoma, 1971) and by the work with contractile vacuoles. The contraction of the upper half and the ventral ridge of the pulvinule occur along with the contraction of the contractile tannin vacuoles in the dark, either after light or irradiation with red light. Neither pinnules nor vacuoles change their conformation in the dark after irradiation with far red light. This behaviour of the vacuoles to red and far red irradiation is typical of phytochrome action and correlates with the leaf movement (Toriyama...
and Jaffe, 1972; Setty and Jaffe, 1970). 

E. Biochemical Mechanism of the Movement

a) Increase of permeability

Several authors (Bose, 1918; Dutt and Guhathakurta, 1956-57; Fondeville, 1969) have proposed that the mechanical response in the motor cells is caused by an increase in permeability of the cell membrane and also there is a sudden diminution of the turgor pressure. There is, however, no conclusive evidence that a rise in permeability actually does occur when the mechanical response starts in the motor cells. But it has long been known that the contraction of the stimulated cells is accompanied by ejection of liquid into the intercellular spaces. Dutt and Guhathakurta (1956-57) actually measured the amount of sap expelled and showed that this was reabsorbed during recovery using a micropotometer. This expulsion of the sap mainly occurs in the lower half of the pulvinus, the part known to be most sensitive. From time to time it has been surmised that this phenomena might be due to a sudden increase in the permeability of the protoplasts to solutes. This would allow some of the osmotically active substances to escape from the cells and enable contraction of the stretched cell walls to force out some of the water of the cell sap (Blackman and Paine, 1918). Since a transient
increase of membrane conductance which depends on the permeability to ions is seen to occur concomitantly with the action potential in the excitable cells of animals it may also occur in the motor cells of sensitive plants.

b) Role of contractile protein and ATPase enzyme

Recent results suggest that the rapid movement in Mimosa is caused by a reaction of the ATP-ATPase system. Poglazov (1956) found first that ATPase activity in the fresh leaf of Mimosa is markedly higher than that in the senile insensitive leaf as in the leaves of other plants which do not exhibit the rapid movement. No activation with addition of Mg$^{2+}$ and Ca$^{2+}$ but an inhibition with ethylenediaminetetraacetic acid (EDTA) are observed. The maximum activity is shown at pH 5-6. However, Lyubimova et al. (1966) also found an ATPase in homogenates of the various tissues in Mimosa which is activated by Mn$^{2+}$ with the optimum pH at 6.5. This is widely distributed in the insensitive parts, such as leaflet, pinna rachis and petiole but scarcely in the sensitive pulvini. They pointed out therefore, that the ATPase seems not to be related to the mechanism of rapid movement and this may be identical with results obtained by Poglazov (1956). Lyubimova and associates (1966) extracted another ATPase found largely in the puvini which is
activated with \( \text{Mg}^{2+} \) and \( \text{Ca}^{2+} \) with an optimum pH between 8 and 9. The precise biochemical nature and physiological function of the ATPase found in the pulvini are still unknown.

ATP appears to be involved in coiling of pea tendrils. The ATPase activity of pea tendrils decreases during coiling and increases again during the recovery phase. A cell free preparation from tendrils containing ATPase \cite{Jaffe and Galston, 1967} showed a decrease in viscosity upon the addition of ATP. This is consistent with the view that a contractile ATPase is involved. This evidence was interpreted as indicating the participation of an actomyosin like contractile ATPase in pea tendril coiling. This enzyme however can use other nucleoside triphosphates as substrates.

Indirect evidence that contractile proteins are involved in the movement has been stated by Vanden Driessche (1963). Driessche's observations were based on the following results. When the basal cut end of the petiole is immersed in the solutions of mersalyl and protamine sulfate, the pinnules do not maintain the open position. Treatments with EDTA by the same method show no effect on maintaining the open position of the pinnule, but the pulvinules of the treated leaf lose
their motor responses to shock stimulus. Driessche concluded that the maintenance of the open position in the pulvinar cells is dependent on the energy set free by ATP hydrolysis in the pulvinar cells which is blocked with mersalyl; this position corresponds to the elongated state of the contractile proteins, a state no longer possible in the presence of protamine sulfate and divalent cations such as Ca$^{2+}$ and Mg$^{2+}$ play a role in the movement of the pulvinus. Toriyama and Jaffe (1972) suggested that calcium ions can play a role; they have observed that Ca$^{2+}$ ions, mainly located on the tannin vacuole are released after stimulation and that at the same time the fibrillar content of the colloidal vacuole alters its conformation. These last data suggest that the fibrils may have contractile properties.

Kuo and Lou (1966) have compared by histochemical methods the ATPase content in various motor organs of graded excitability chosen in different plant species, namely Mimosa pudica, Sesbania cannabina and Robinia pseudoacacia. It can be seen that the higher the excitability of the organ, the greater its enzymic activity. In a single motor organ, they have also shown a physiological gradient as demonstrated by the uneven distribution of ATPase activity; more intense in the
more irritable side. Inside a motor cell ATPase activity has been detected histochemically on the plasmalemma when Mg\(^{2+}\) and Ca\(^{2+}\) ions are added (Toriyama, 1963) and on the tonoplast (Watanabe, 1971). Biswas and Bose (1972) isolated and purified an Mg\(^{2+}\) K\(^{+}\)-activated ATPase from *Mimosa pudica* that has not the same biochemical properties as that extracted from muscle; however, it exhibits viscosity decrease on addition of ATP which is a property known to be characteristic of the contractile proteins constituting the animal muscle.

In considering the whole mechanism, it must be mentioned that ouabain and other cardiac glycosides inhibit the pulvinar movement (Jonas, 1976) and the ATPase histochemical reaction (Watanabe, 1971). Now, it is known that cardiac glycosides inhibit the active flux of Na\(^{+}\) and K\(^{+}\) in various animal cells (Glynn, 1964) and also in plants (MacRobbie, 1962) and the associated hydrolysis of ATP by inhibiting a transport ATPase (Skou, 1965).

What emerges from the studies on the movement of *Mimosa pudica* is the following: (i) The sensitivity finds its expression in rapid leaf movements and conduction of the excitation through the different parts of plant from the starting point of stimulus elicited by
various agents. (ii) The motor responses take place in particular basal foliar structures, called pulvini and involve at least turgor variation, migration of ions, contractile proteins and utilization of ATP. The present knowledge of the biochemical mechanism involved in plant motility is not perfect but indicative that there might be some similarities with that of motility in animal systems involving microfilaments, microtubules and membrane components.

II. MOVEMENTS AS STUDIED WITH ANIMAL AND OTHER SYSTEMS

In animal the motility is expressed by the interactions of several kinds of filaments, microtubules and cytoplasmic ground substances in which they are embedded. The most elaborately evolved of these systems is the myofibril present in the muscle cells and its action can be explained in terms of the properties of the four different proteins, known as myosin, actin, tropomyosin and troponin. In the interactions of these four proteins chemical energy is released by the hydrolysis of ATP by one of the major contractile protein myosin and converted into mechanical energy. Myosin contains ATPase or in general nucleoside triphosphatase activity (Bendal, 1969).
A. Role of Actin, Myosin, Tropomyosin and Troponin in Cell Motility

Motility in cells is expressed in diverse activities such as cytoplasmic streaming, phagocytosis, morphogenetic movements, amoeboid motion, cytokinesis, and mitosis. These movements have now been related to four general classes. (i) Bacteria are propelled by the mysterious beating of their simple flagella which are composed of a single type of protein lacking any enzymatic activity (Iino, 1969). (ii) Certain peritrichous ciliates possess a contractile stalk containing a rubber-like organelle, the spasmoneme, which contracts when exposed to Ca\(^{2+}\) (Weis-Fogh and Amos, 1972). (iii) The beat of cilia and sperm tails (and perhaps some cytoplasmic movements as well) is apparently due to the interaction of microtubules and the ATPase dynein (Mohri, 1976; Olmsted and Borisy, 1973). (iv) In muscle contractile force is generated by the sliding interaction of actin and myosin filaments, with the energy provided by the hydrolysis of ATP (Huxley, 1969).

Until recently, only little was known about the molecular mechanism of these diverse group of biological phenomena exhibiting movements which lack the highly organized contractile apparatus present in
muscle but possess the contractile proteins actin and myosin. Important aspects of contractile proteins prevalent and operative in non-muscle cells have been the subject of reviews (Pollard and Weininger, 1974; Clarke and Spudich, 1977 and Hitchcock, 1977) and published symposia (Inoue and Stephens, 1975; Goldman et al., 1976). The contractile proteins from cells other than muscle are referred to as non-muscle actin and myosin. In order to differentiate these proteins from their myofibrillar counterparts, they are also called as cytoplasmic actin and myosin as first suggested by Bray (1973). The cytoplasmic actins and myosins are specifically named by using the name of the cell of origin, e.g. Acanthameoba actin and Physarum myosin etc.

a) **Non-muscle actin**

Numerous studies clearly show that actin is a major component of various cells, mostly eukaryotic which lack a highly organized contractile apparatus and closely resembles actin from muscle. Since 1974 actin has been purified from vertebrate blood cells including erythrocytes (Tilney, 1976; Sheetz et al., 1976), macrophages (Hartwig and Stossel, 1975), leukocytes (Clarke and Spudich, 1977) and platelets (Elzinga et al., 1976; Gallagher et al., 1976) and from brain tissue (Storti et al., 1976). It has been examined in other
Vertebrate tissues (Grovmdan and Wieland, 1975) and cultured cells (Bray and Thomas, 1976; Whalen et al., 1976). Actin has also been purified from invertebrate eggs (Kane, 1976) and sperm (Tilney, 1976) and from the green alga Nitella (Palevitz, 1976), Physarum (Hatano and Owaribe, 1976; Jacobson et al., 1976), Acanthamoeba (Pollard, 1976; Gordon et al., 1976) and Dictyostelium (Cooke et al., 1976). These cytoplasmic actins share with muscle actin the ability to form double helical filaments from globular monomers to form periodic arrowhead-shaped complexes with heavy meromyosin (HMM) to activate the Mg$^{2+}$ ATPase of myosin or HMM, and to interact with the regulatory proteins troponin-tropomyosin.

Physico-chemical parameters of cytoplasmic actins so far investigated have been found to be almost identical with the same parameters for muscle actin. Actins consist of a single polypeptide, the native molecular weight of actin from muscle (Tsuboi, 1968; Rees and Young, 1967) and platelets (Booyse et al., 1973) are approximately the same as the molecular weights (45,000 daltons) in denaturing solvents. Amino acid compositions of actin from five different cells (viz. Acanthamoeba, Dictyostelium, Physarum, sea urchin eggs and human platelets) have been determined. The
compositions of these actins are remarkably similar to one another and to muscle actin containing some unusual amino acid, e.g. \( N^\alpha \)-methylhistidine and \( N^\varepsilon \)-methyllysine (Abramowitz et al., 1975; Gordon et al., 1976; Adelstein and Kuehl, 1970; Weihing and Korn, 1972). Actin from Physarum (Hatano and Oosawa, 1966) and brain (Puszkin and Berl, 1972) has been found to contain bound nucleotide. Human platelets' actin also seems to contain bound nucleotide because phosphate is released (presumably from bound ATP) during polymerization (Booyse et al., 1973). Cytoplasmic actins which contain less than 1 mol of nucleotide/45,000 daltons of protein seems to be incompletely purified or partially denatured. Among cytoplasmic actins, only Physarum actin has been investigated for the presence of divalent cation which contain about 1 mole of \( \text{Ca}^{2+}/ \text{mole of monomer} \); the bound \( \text{Ca}^{2+} \) could be exchanged for \( \text{Mg}^{2+} \) (Pollard and Weihing, 1974).

1) Polymerization

Cytoplasmic actins like muscle actin polymerize into filaments identical in structure to those formed by muscle. This is a key functional property of actin because only actin organized as filaments can transmit tension and only actin filaments efficiently activate the myosin ATPase during the conversion of the chemical
energy of ATP into mechanical work. Many investigators have suggested (Taylor et al., 1976; Tilney et al., 1973) that in non-muscle cells a large percentage of the actin may exist as monomers (G-actin) or small oligomers, and undergo polymerization into filaments when needed for some contractile events.

Polymerization of actin into actin filaments (F-actin) is induced in vitro by adding KCl to a final concentration of 0.1 M or MgCl₂ to a final concentration on of 1-2 mM (Straub and Feuer, 1950). Polymerization of actin has been studied using the techniques of analytical ultracentrifugation, flow birefringence, electron microscopy and viscometry. Actin monomer from Acanthamoeba (Weihing and Korn, 1971), Dicystostelium (Woolley, 1972), Physarum (Hatano et al., 1967) and sea urchin eggs (Hatano et al., 1969; Miki-Noumura and Kondo, 1970), which is a 3S component is converted to a component of higher sedimentation coefficient.

The most suitable technique of studying polymerization is by measuring the viscosity change. The reduced viscosity of polymers formed from purified actin is well defined for a given set of conditions. The reduced viscosity reported for purified cytoplasmic actins polymerized with 0.1 M KCl is generally somewhat lower than that for muscle actin (Totsuka and Hatano, 1970).
ii) Interaction with myosin

Muscle myosin or its proteolytic fragments heavy meromyosin (HMM), subfragment 1 (Sl) binds to muscle or cytoplasmic actin filaments giving the same distinctive appearance i.e. arrowheads in electron micrographs of negatively stained specimens. Formation of arrowheads illustrates that the arrowhead shape is the consequence of very specific interactions between actin and myosin head (Moore et al., 1970). The specificity of binding of HMM with actin filaments, not to membranes or filamentous structures such as microtubules (Ishikawa et al., 1969; Pollard et al., 1970; Bettex-Galland et al., 1972), neurofilaments (Ishikawa et al., 1969; Pollard et al., 1970), tonofilaments (Ishikawa et al., 1969), or bacterial flagella (Pollard et al., 1970) can be confirmed. Binding of myosin to actin is blocked by ATP or pyrophosphate.

Although ATP appears to dissociate actin and myosin in skeletal muscle, actin undergoes a cyclical interaction with myosin in the presence of ATP which activates the myosin ATPase and simultaneously generates the mechanical forces which cause movement. All the cytoplasmic actins so far tested activate rabbit myosin or HMM like muscle actin.

Using same amount of actin from Acanthamoeba, Dictyostelium and human platelets as was used from rabbit
muscle, it was found that activation of rabbit myosin or HMM was less efficient whereas actins from chick embryo fibroblasts and rabbit muscle are equally effective activators of rabbit myosin ATPase. Eisenberg and Weihing (1970) found that when troponin-tropomyosin or tropomyosin alone is added to a mixture of Acanthamoeba actin and rabbit HMM, the activation more nearly reaches the activation caused by rabbit actin alone.

b) **Non-muscle myosin**

Myosin has been found in most types of eukaryotic cells. But the cytoplasmic myosins are studied less extensively than cytoplasmic actins. Myosin is a class of enzyme which has ATPase activity, stimulated by actin under physiological conditions, binds reversibly with actin filaments which could be demonstrated by electron microscopy. Other properties common among most myosins are a similar subunit structure (two heavy chains of identical molecular weight plus two sizes of light chains), an asymmetric shape, the ability to form bipolar thick filaments and an ATPase activity stimulated by EDTA in the presence of 0.6 M KCl. Because of the wealth of information of myosin from vertebrate striated muscles (especially rabbit and chicken) (Lowey, 1971) these myosins are
generally thought of as typical myosins with which all other myosins are inevitably compared.

Among ATPase, myosin has the property apparently unique, its enzyme activity is strongly inhibited by Mg$^{2+}$ and activated either by Ca$^{2+}$ or by K$^+$ in the presence of EDTA (Seidel, 1969). As a consequence of the Mg$^{2+}$ content of muscle ($>8$ nmole/Kg) (Bendall, 1969), myosin ATPase activity would be too low to account for the transduction of the chemical energy of ATP into force for movement; the unique ability of actin filaments is to activate the Mg$^{2+}$ ATPase activity of myosin (Eisenberg and Moos, 1968). This effect of actin seems to be due to its ability to facilitate the dissociation of the products of ATP hydrolysis from the myosin, this step is thought to be rate limiting in the ATPase reaction (Taylor, 1972; Lym and Taylor, 1971). Consequently ATP hydrolysis is tightly coupled to actin myosin interaction, which in turn utilizes, in some unknown way, the energy from ATP hydrolysis to drive the force generating mechanism causing the actin filaments to slide past the myosin. In this complicated reaction ATP plays dual role: it is the source of energy which drives the reaction but it also dissociates actin from myosin (Eisenberg et al., 1968) to start each new cycle of interaction consisting
of (1) ATP hydrolysis by myosin, (2) dissociation of products of ATP hydrolysis upon binding of actin to myosin, (3) sliding movement, and (4) dissociation of actin from myosin by the rebinding of ATP to the myosin (Lymn and Taylor, 1971).

Recently myosin has been purified and characterized from vertebrate brain (Burridge and Bray, 1975; Burridge, 1976) and liver (Brandon, 1976), macrophages (Hartwig and Stossel, 1975; Stossel and Hartwig, 1976), leukocytes (Shibata et al., 1975), platelets (Pollard, 1975) and several types of cultured cells (Ash, 1975; Ostuland et al., 1974). Invertebrate myosin has been isolated from echinoderm eggs (Mabuchi, 1976) and sperm and from squid brain (See and Metuzals, 1976). Physarum (Jacobson et al., 1976; Kessler et al., 1976; Nachmias and Asch, 1976), Acanthamoeba (Pollard and Weihing, 1974; Pollard et al., 1976; Pollard and Korn, 1973) and Dictyostelium (Mockrin and Spudich, 1976) myosins have also been characterized.

Most non-muscle myosins closely resemble muscle myosin in structure. Like muscle myosin they contain two heavy chains of molecular weight 194,000-212,000 (Gazith et al., 1970; Gershman, et al., 1969) i.e. identical to that of muscle myosin. Non-muscle myosin slightly differ in size from muscle myosin. However, Burridge and
Bray (1975 and 1976) compared the sizes of the fragments of various muscle and non-muscle myosins produced by chemical cleavage at cysteine residues and found that myosins from brain, platelets and smooth muscle yielded quite different patterns. These findings suggest that similar but nonidentical forms of myosin exist in non-muscle cells and that the variation is greater than that found in actin.

Non-muscle myosins resemble muscle myosin with their enzymatic properties. Dictyostelium (Clarke and Spudich, 1974) and Physarum (Jacobson et al., 1976) myosins are activated by EDTA unlike muscle myosin. Physarum (Adelman and Taylor, 1969; Hatano and Ohnuma, 1970) and Acanthamoeba myosin (Pollard and Korn, 1973 and 1973a) hydrolyzes GTP and ITP in addition to ATP which is comparable with striated muscle where ITP, GTP, UTP and CTP are hydrolyzed in addition to ATP (Bendall, 1969).

The actin activated ATPase activity obtained at low ionic strength in presence of Mg$^{2+}$ is physiologically significant. Under these conditions, myosin activity is quite low but is stimulated by addition of actin. This stimulation is not great for most non-muscle myosins, less than 10 fold activation is reported for vertebrate and invertebrate myosins (Weihing, 1976). Myosins from lower eukaryotes Physarum and Dictyostelium can be
activated 20-40 fold (Weiking, 1976; Jacobson et al., 1976) approaching that of muscle. A crude cofactor fraction is required for actin activation of Acanthamoeba (Pollard and Weiking, 1974; Pollard, 1975) and macrophage myosin (Stossel and Hartwig, 1976). The cofactor of Acanthamoeba is a globular protein of molecular weight 100,000 daltons which has no ATPase activity and does not resemble any of the known components of the control system found in muscle. Acanthamoeba myosin itself, cannot form filaments but it does bind to actin filaments and cause them to aggregate.

c) Regulation of motility in non-muscle cells

Regulation of these processes like cytoplasmic streaming, cytokinesis, blood clot retraction and phagocytosis is complicated and probably involves more than one mechanism. In vitro and in vivo studies reveal that Ca\(^{2+}\) may regulate some of these motile systems in a similar way to Ca\(^{2+}\) regulation in muscle; functional actin myosin interaction (contraction) takes places in presence of MgATP and 10\(^{-6}\)M free Ca\(^{2+}\).

The ATPase activity of crude actomyosin preparations from brain (Fine et al., 1973), leukocytes (Shibata et al., 1972) and Physarum polycephalum (Nachmis, 1975) have been found to be Ca\(^{2+}\)-sensitive. There are indications in several of these cell types that Ca\(^{2+}\)
sensitivity is due to components associated with the
actin (Nachmias and Asch, 1974; Puszkin and Kochwa,
1974). Cohen et al., (1973) were the first who provided
evidence that there is actin linked regulation in
platelet actomyosin.

Stimulation of Acanthamoeba myosin Mg$^{2+}$-ATPase
activity by actin is unaffected by Ca$^{2+}$ (Pollard and
Korn, 1973). But when muscle troponin-tropomyosin is
added to the assay mixture, it inhibits ATPase activity
both in presence (unexpected) or in absence (expected)
of Ca$^{2+}$. Inhibition of actomyosin ATPase by troponin-
tropomyosin in the presence of Ca$^{2+}$ is unique among
myosins and suggests that troponin-tropomyosin of the
muscle type must not exist in the amoeba or it would
block actin myosin activity permanently.

Movement of the slime mold Physarum, which
contains actin, myosin and factors resembling troponin-
tropomyosin was also controlled by Ca$^{2+}$. Hatano (1970)
prepared spheres limited by plasma membrane treating the
plasmodium with caffeine which renders the plasma membrane
permeable to Ca$^{2+}$; when the external Ca$^{2+}$ concentration
was greater than $10^{-7}$M fragments exhibited cytoplasmic
streaming. Recently, Ridgway and Durham (1976) have
correlated the local intracellular Ca$^{2+}$ concentration
with streaming in Physarum. Taylor and coworker have
shown that streaming in cell free extracts of the giant
amoeba, Chaos carolinensis is dependent on traces of Ca$^{2+}$. That release of Ca$^{2+}$ precedes the onset of contraction in the ciliate Spirostomum has been shown by Ettienne (1970). For in vitro demonstration of Ca$^{2+}$ accumulation in membranes of non-muscle cells few informations are available. The fibroblast preparation inhibited ATP induced contraction of glycerinated cells which could be withdrawn by addition of Ca$^{2+}$ suggesting that the inhibition was due to removal of free Ca$^{2+}$.

Ca$^{2+}$-sensitive movement is not enough to conclude that the movement is caused by actomyosin. Ca$^{2+}$-sensitive movement is observed in the spasmoneme of certain peritrichous ciliates (Weis-Fogh and Amos, 1972; Amos, 1971) which possess a contractile stalk containing a rubber-like organelle. The organelle contracts in $10^{-6}$ M Ca$^{2+}$ and relaxes in $10^{-8}$ M Ca$^{2+}$. These changes can be repeated by simple changes of Ca$^{2+}$ concentration without any source of energy such as ATP, although addition of Ca$^{2+}$ & ATP causes repetitive cycles of contraction and relaxation.

Apart from actin linked regulation via Ca$^{2+}$ together with troponin-tropomyosin there are other types of regulation. In some cells proteins different from troponin-tropomyosin may affect interaction of actin and myosin. Actin activation of the Acanthamoeba myosin Mg$^{2+}$ ATPase requires the presence of the cofactor protein.
Since this ATPase activity is most likely to be related to the cell motility, the cofactor is in a position to turn on or off but how, is not known. At least Ca\textsuperscript{2+} does not seem to play a role (Pollard et al., 1973). For control of cell motility besides Ca\textsuperscript{2+} sensitive proteins some other factors appear to be involved. Though non-muscle cells contain tropomyosin, the evidence for the existence of troponin is however, still inconclusive. The Ca\textsuperscript{2+} binding subunit of muscle troponin known as troponin C has been reported in non-muscle cells (Fine et al., 1975; Kuo and Coffee, 1976). It is indicative that Ca\textsuperscript{2+} ions are important regulators of motility in non-muscle cells as is the case with muscle cells. It appears therefore that Ca\textsuperscript{2+} regulates the on-off state of many molecules involved in one way or another with cell motility. The observations thus far discussed indicate how diverse the regulatory pathways of actins are. Proteins have been identified that regulate (i) the transformation of monomeric actin into polymeric filaments, (ii) the aggregation of these filaments into bundles or into a mesh work gel, (iii) the transformation of bundles into gel, (iv) the association of actin filaments with the cell membrane through actinin though heterogeneity of actins might exist and (v) different ways by which actins and myosins interact.
An outline of the different molecular events involved in regulation of non-muscle cell movement is given in Flow sheet. Some motile activities are obviously mediated by an actin myosin interaction whereas others may be mediated by rapid polymerization of actin and depolymerization of actin filaments or interconversion of filaments into mesh work and many of these reactions might also be operative simultaneously in the regulation of cell motility.

B. Tubulin-dynein Interaction in Motility

In comparison to muscle contraction which is mediated by actin myosin interactions and also by other factors, interactions between tubulin and dynein appear to be the basic mechanism of flagellar and ciliary movement and probably of some other type of cell motility.

Presence or absence of ciliary response is correlated with the presence or absence of arms in the outer fibre. Gibbons (1963) demonstrated biochemically and electron microscopically that once solubilized dynein could recombine at the same position of A tubule of the outer doublet as in the intact cilia on adding Mg$^{2+}$ at an alkaline pH. 14S subunit of axoneme dynein exhibited the recombining activity. Recombination is inhibited by dynein, as in the case of actomyosin.
Flow sheet diagram of regulation of cell motility
Treatment of dynein with trypsin produces Fragment A, which possesses ATPase activity but does not combine with the outer doublets (Ogawa, 1973). The subunit responsible for the binding of dynein has not yet been identified. Several investigators have observed the presence of two high molecular weight polypeptides, polypeptide A and polypeptide B on SDS-gel electrophoresis of extracts containing dynein from flagella and cilia (Linck, 1973; Burns and Pollard, 1974; Gaskin et al., 1974; Hoshino, 1975). According to Linck (1973), polypeptide A is an ATPase and polypeptide B is a structural component of dynein.

To understand interactions between tubulin and dynein, differences in the properties of dynein bound or not to the flagellar or ciliary axonemes have been analyzed. Brokaw and Benedict (1971) reported that the dephosphorylation of ATP by non-motile flagella of sea urchin spermatozoa is much slower than that of actively motile spermatozoa. Whereas ATPase uncoupled by thiourea treatment has a markedly increased activity. Considering this property and some other properties (Hayashi and Higashi Fujime, 1972; Gibbons and Fronk, 1972; Blum, 1973) it is clear that bound and isolated dynein do not behave alike.
Gibbons and Gibbons (1972) showed that motile sea urchin spermatozoa extracted with Triton X-100 and reactivated by ATP, dephosphorylated ATP at a rate (0.16 μmole Pi/min/mg protein) higher than that of non-motile ones (0.045 μmole Pi/min/mg protein) corresponding to the rate for isolated non-motile flagella. The observed difference is considered to be the activity coupled to movement.

More direct evidence showed by Otokawa (1972) is that the ATPase activity of 30S dynein is stimulated by tubulin fraction. Furthermore, Ogawa (1973) demonstrated that ATPase activity of Fragment A was stimulated by tubulin dimers derived from B tubule and also by A tubule. Tubulins originating from the central tubules and neurotubules exerted only a feeble effect on the enzyme activity (Mohri and Ogawa, 1975). This result is very interesting since the dynein arms face the adjacent B tubules and should interact with them during active flagellar and ciliary movement if the sliding theory is correct.

Very little is known about the biochemical role of other components which are responsible for the interaction of tubulin but it seems that other components might be involved. Kinoshita (1959 and 1965) reported that in glycerinated sea urchin sperm models for inducing motility
a relaxing factor is necessary. The factor was considered to be polysaccharide but no further work has been done on this.

a) Sliding mechanism and conformational change of tubulin in flagella and cilia

Attachment of the arms on the A tubule of each of the outer doublets in sea urchin sperm led Afzelius (1959) to postulate the sliding between adjacent doublets with the help of these arms and is the basic mechanism of flagellar movement. It has been experimentally proved that the active elements which convert chemical energy of ATP into movement are distributed along the length of flagella and cilia (Brokaw and Gibbons, 1973; Machin, 1958) and by simple diffusion from a source within the cells ATP can be supplied to active elements (Nevo and Rikmenspoel, 1970).

Satir (1965 and 1968) by electron microscopic examination observed that during bending of the cilia no change in the length of outer doublets occur. Briefly digested sea urchin sperm axonemes with trypsin actively disintegrate into individual doublet or groups of doublets on the addition of ATP (Summers and Gibbons, 1971). This disintegration is caused by an active sliding between the outer doublets together with a tendency of each doublet to coil into helix. This disintegration is
equivalent to the ATP induced decrease in turbidity. Recently, by electron microscopic observation, the presence of cross bridging of the doublets with the arms has been detected (Summers and Gibbons, 1973; Gibbons and Gibbons, 1973 and 1974; Gibbons, 1975).

Repeated experiments suggest that relatively tight coupling exist between the flagellar and ciliary movement and ATP dephosphorylation. The beat frequency of glycerinated or tritonated sperm flagella (Gibbons and Gibbons, 1972; Brokaw, 1967; Brokaw and Benedict, 1968) is proportional to the ATPase activity coupled to movement. Removal of outer arms keeping the inner arms intact leads to reduction of beat frequency to about half that of control spermatozoa (Gibbons and Gibbons, 1973). It has been recently found that the immotile spermatozoa lack dynein arms (Afzelins et al., 1975).

b) **Tubulin microtubule system and its characteristics**

Microtubules constitute a class of structurally similar organelles that occur in virtually all eukaryotic cells, both plant and animal and they are now recognized as important cellular elements in motility and morphogenesis. Since their discovery as the prominent components of the axoneme of cilia and flagella where they are constituted in the familiar 9+2 pattern, microtubules
have received special attention as important structural and motile elements in the mitotic spindle apparatus and as cytoskeletal structures, particularly in animal cells. Microtubules have been found to be involved in many other instances such as, movement of particles in suctorian feeding tentacle (Tucker, 1974; Rudzinska, 1965), the movement of melanin granules in the melanophore or melanocyte (Malawista, 1975; Murphy, 1975) release of insulin (Malaisse et al., 1975), axonal flow in the nerve axons etc. and in some cases arm like structures are attached to microtubules (Murphy and Borisy, 1975; Dentler et al., 1975; Burns and Pollard, 1974; Gaskin et al., 1974).

In aqueous solutions, tubulin exists as a dimeric unit of about 6S and a molecular weight of 100,000-120,000 (Shelanski and Taylor, 1968 and 1967; Renaud et al., 1968; Weisenberg et al., 1968). In presence of denaturing agents like 8 M urea or 6 M guanidine-HCl, the molecular weight reduced to 55,000-60,000 daltons, suggesting that the protein is composed of two subunits of similar size. Molecular weight, sedimentation coefficient, dimeric nature and amino acid composition of cytoplasmic tubulin resembled those of the material isolated from the outer doublets of cilia and flagella.
Bryan and Wilson (1971) demonstrated that purified tubulin from chick embryo brain could be resolved electrophoretically into two closely located components on 8 M urea polyacrylamide gels after reduction and acetylation suggesting two components differed by charge. These two subunits were termed α and β tubulin, the β subunit has greater electrophoretic mobility. The two tubulins were found in equimolar quantities in nearly all cells studied (Dustin, 1978) and were shown to have molecular weight of 54,000-58,000 daltons for α tubulin and 46,000-54,000 daltons for β tubulin (Feit et al., 1971; Olmsted et al., 1971; Raff and Kaumeyer, 1973).

These two subunits are distinct but closely related proteins. The antiserum against β tubulin does not react with α tubulin (Piperano and Luck, 1975). Each subunit has been highly conserved in the course of evolution as indicated by the similarities of tubulins from two widely separated species like chick and sea urchin. The two subunits differ by several biochemical properties e.g. α tubulin is the substrate of a unique enzyme tubulin tyrosine ligase (Raybin and Flavin, 1977) and β tubulin is phosphorylated at a serine residue (Bipper, 1972). It has been ascertained that tubulin dimers are at least largely heterodimers of αβ, rather than mixture of αα and ββ dimers (Luduena et al., 1975).
Tubulin (110,000 daltons) is capable of binding two moles of guanosine nucleotide (GDP or GTP) in two different sites exchangeable (E) and nonexchangeable (N) sites (Weisenberg, 1975; Weisenberg et al., 1976; Berry and Shelanski, 1972; Jacobs and Caplow, 1976). Nucleotides bound to E site are readily exchangeable while GTP and GDP at N site are firmly bound. During microtubule assembly GTP bound to tubulin is hydrolyzed to GDP; hydrolysis takes place at E site. Binding of GTP requires Mg$^{2+}$ and free -SH groups.

The nontubulin protein which copurify with tubulin through several cycles of assembly and disassembly at approximately constant stoichiometry are collectively known as microtubule associated proteins or MAPs. A set of two high molecular weight proteins (MW, 300,000 daltons), appearing in some preparations of microtubule protein were termed HMW (for high molecular weight) by Borisy and coworkers (Borisy et al., 1975; Murphy and Borisy, 1975) and MAPs by Rosenbaum and coworkers (Dentler et al., 1975). A second class of proteins having molecular weight between 55,000 and 70,000 daltons has been isolated and termed as tau protein by Kirschner and coworkers (Weingarten et al., 1975). However, these MAPs can be separated from the 6S tubulin and are thought to be involved in microtubule formation (Snyder and McIntosh, 1976).
c) **Tubulin-microtubule system in plants**

Their occurrence throughout the plant system is also wide spread. In addition to the already described associations, microtubules also form the fibrous component of the cytokinetic apparatus, the phragmoplast and they frequently reside in the cortical cytoplasm where they appear to participate in controlling cell wall pattern and the orientation of the cellulose microfibrils. Their wide spread occurrence in plants, especially their deposition within the cortical cytoplasm, only became apparent following the introduction of glutaraldehyde as a superior fixative for electron microscopy. Each dimer of tubulin (αβ) binds the antimitotic agents colchicine, vinblastine and podophyllotoxin (Wilson and Bray, 1974). The binding of colchicine prevents the dimers from polymerizing and has been used widely in biochemical and cytological studies to disrupt microtubule structure and hence their functions.

With the exception of studies on the flagellar tubules of the green alga Chlamydomonas (Olmsted et al., 1971; Witman et al., 1972 and 1972a) the biochemistry of plant microtubule has received only little attention. A colchicine binding protein of molecular weight 120,000 has been isolated from vascular tissue of *Heracleum mantegazzianum* (Hart and Sabnis, 1973). Tubular and
filamentous structures, collectively termed P-protein have been observed in the sieve elements of a wide range of higher plants. Protein components from *Heracleum mantegazzianum* capable of forming filaments *in vitro* have been isolated from phloem exudate and have been shown to react with vinblastine (Kleinig et al., 1971 and 1971a) in a manner characteristic of several kinds of protein including tubulin (Wilson et al., 1970). The similarity in vinblastine sensitivity together suggest the possibility of homology between P-protein and tubulin.

Colchicine binding proteins from higher plant material differs from animal preparations in atleast three probably related aspects. (1) It is present in smaller amount. (2) Its colchicine binding activity is much less stable *in vitro*. (3) The presence of an inhibitor which prevents binding with colchicine.

Electron microscopic evidence also indicated that protein structures are unaffected by colchicine or vinblastine (Sabnis and Hart). If P-protein is related to tubulin, it must be stabilised *in vivo* to resist the effects of colchicine and must remain either *in vitro* in a form unable to bind colchicine or there are some inhibitors which inhibit the binding phenomenon. Bryan (1972) has noted that the techniques used for preparing
the tubulin and assaying the binding have not been constant.

The use of [3H]colchicine binding for the detection of tubulin may have some severe limitations since those organisms in which it has not been possible to demonstrate colchicine binding in vitro are also those which require high concentrations of colchicine to block their cell divisions. The recent successful binding of [3H]colcemid to *Saccharomyces cerevisiae* tubulin by Haber et al. (1972) indicates that colcemid is probably a more promising tool for studies of tubulin from lower organisms. In addition to the difficulties of colchicine binding using lower organisms, there is no reason for the affinity for binding to be the same for all species of tubulin derived from the same cell. For example, the cell division of the sea urchin *Tripneutes gratilla* can be inhibited by 10 μM colchicine whereas 1 mM concentration is necessary to block cilia regeneration (Burns, 1973). A recent study on the colchicine binding protein has been reported from another higher plant *Phaseolus aureus* (Rubin and Cousins, 1976). The results thus far obtained are not convincing when compared with the colchicine binding capability of tubulin from animal sources.
An interesting observation is the location and orientation of microtubule in the cortex of plant cells. Parallel orientation of microtubule with cellulose fibrils in wall formation in different types of plants has been discernible (Ledbetter and Porter, 1963). A correlation between microtubule and cellulose fibres and thickening of cell wall has also been established in several cases (Heath, 1974; Hepler and Posket, 1971; Heyn, 1972). Though question has not yet been settled as to how microtubules act in the orientation of cellulose microfibrils, it is interesting to speculate that plasmalemma might have a role in the arrangement of microtubule to impart a directional signal not only for morphogenesis but also for movement. It may be worthwhile to mention in this connection that association of microtubules and microfilaments with the membrane structure in relation to cell motility and division has already been asserted from studies of animal cells in culture (Edelman et al., 1976 and 1977).

From this review though not all inclusive, it is abundantly clear that the molecular mechanism of motility either in animals or plants is very complex and variety of protein molecules are implicated in this process. A host of other factors might also be involved in regulation of this phenomenon in vivo. The common
A feature which has emerged is that a variety of contractile proteins coupled with an ATPase system generate the energy for motility in general.
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