DISCUSSION

From the compounds belonging to the group of amides only a few have so far been tested for their cytological potential. Notably among them are Formamide, which was shown to be strong antimitotic on amphibian cells by Brachet (1964). Hydroxyurea was reported to be strong radiomimetic agent in mammalian cells (Openheimer et al, 1965) and in plant material (Khilman et al,1966). Earlier reports attribute similar property to Urea, while Ennis and Lubin (1966) reported that cyclohexamide and aceto oxycyclomide specifically inhibit protein synthesis in L-cells growing in suspension. Brachet(1964) had attributed the effect of formamide particularly with reference to blocking of RNA and DNA synthesis to its capacity to break-S-S bondage of nucleic acids. About hydroxyurea there are conflicting views. On the one hand Openheimer et al,(1965) belive that hydroxyurea effects cells through its degredation product hydroxylamine, which in turn exerts cytotoxic effects, while Khilman et al(1966) after an elaborate experiment concludes, that hydroxyurea acts as an inhibitor of DNA synthesis. Several reports show thiourea and thioacetamide to be tumorigenic in rat liver in addition to being very potent to produce of nodular cirhosis (Fitzhugh and Nelson,1948). Crombie (1952) carried a series of investigations on a group of isobutylamides and showed them to have strong insecticidal property. A few similar isoButylamides were recently

isolated from the plant Piper peeploides and initial trial with these compounds showed positive cytological effects. Some of them were completely ineffective.

Antimitotic activity:

An antimitotic may be defined as an agent which prevents a mother cell or nucleus from giving rise to two valid daughter cells or nuclei (Bowen and Wilson, 1954). Such an agent may disrupt active mitosis or inhibit the start of mitosis and the effect should be reversible within limits. Naturally, there are many compounds which exhibit antimitotic activity when tested against suitable systems. Their mode of action is not very well understood. Even, the action of most commonly used drug colchicine is not clear (Eigsti and Dustin, 1955; Hadder and Wilson, 1958).

In the present series of experiments following compounds namely, formamide, dimethylformamide, pellitorine, NIB-veratamide, NIB-capramide, Nikathamide, Hexamine, Piperine and PPA-amide showed this property. Among the first two compounds i.e., formamide and dimethylformamide, the latter showed more activity. Formamide was used at 1M concentration. Treated with this solution for two hours, the mititoc index was reduced from 5.20 to 0.94 giving a reduction of about 85 %.

The only report about the effect of formamide on mitosis is that of Brachet (1964). He treated eggs of Pleurodeles waltlii and Rana esculanta with different concentrations of

formamide which varied between 1M and 0.01M. According to his results 1M formamide immediately stopped cleavage and gastriculation without killing the cells. But when the treatment was continued for 18-20 hours embryos were completely checked from further development and inhibition became irreversible.

Cytological effects of the treatment may be divided into irreversible and reversible reactions. The former ones are immediately killing effects or such strong toxic effects that the tissue eventually dies. Many of these are concerned with a complex of reactions, particularly the ones which effect the destruction of the spindle mechanism. At the same time toxicity of a compound is a function of its concentration, water solubility as well as duration of treatment. The effect of formamide is evidently reversible, at least within the range of concentration and duration used. The mitotic index which had been reduced to 0.94 recovered after putting it back in water at the conclusion of treatment and after 48 hrs., it gained almost the normal rate of mitosis and mitotic index was 4.93. Dimethylformamide at this concentration was completely lethal. Here only 0.1M concentration induced significant reduction in frequency of dividing cells for the same period of treatment while 0.05M treatment given for 2 hrs. gave a reduction of 50% over control (table-19), while a reduction of nearly 85% over control was recorded following treatment for 6 hours (table-20). When the recovery was allowed in 0.05M dimethyl formamide treated

bulbs, the mitotic index picked up the rate and came back to normal level after 48 hours(table-21). When resting seeds of barley were used as test material, 0.5M for 6 hours limited the germination to 67% only and reduced seedling height from 12.8 cm. to 8.4 cm., a significant reduction of nearly 34%. This brings us to the conclusion that at the same concentration and identical period of treatment dimethyl formamide is many times more potent than formamide. This reasoning is strengthened by the data on the frequency and type of chromosome aberration recorded following treatments with these agents. Pellitorine which is N-isobutyl 2 trans: 4 trans decadienamide is another strong antimitotic agent. Here also, the effect seems to be a function of both the concentration as well as the duration of treatment. A concentration of 0.02% effects a complete mitotic inhibition in three hours, while after 0.005% for 4 hours the index was only 0.58 (table-I). That the mitotic inhibition was brought by only 3 hours of treatment indicates that the compound has a strong antimetabolic activity and effects some vital functional unit of the cell. But this effect is temporary (table-2) hence reversible. Data in table-3 indicates that there is a sudden drop in the percentage of dividing cells in post metaphase with a corresponding increase in the prophases and prometaphases. This could be due to either prophase poisoning or the disruption in the normal functioning of the spindle mechanism. Prophase, inhibition is considered an artifact of the inhibition of the nuclear membrane.

D'Amato (1950) has shown that different compounds induce a prolongation of prophase period due to prophase poisoning. Inhibition of mitotic spindles by colchicine though not well understood, is well known (Davidson and Macloed,1966). This is also characteristic of radiations (Marquardt,1938) and after treatments with various chemicals (Ostergren, 1950; Patau and Patil, 1951).

N-isobutyl capramide is less effective than pellitorine. Here lowest effective concentration 0.02% and higher concentrations upto 0.10% were tried and the period of treatment, varied from 4-12 hrs. Inspite of the fact that such higher concentrations were used for periods upto 12 hrs. complete mitotic inhibition was not achieved. The lowest mitotic index recorded was 2.1% i.e. 25% of the control. Compared to pellitorine, NIB-capramide is a weak antimitotic. Inspite of the fact that both of these have active group present in the molecule, but the difference in the effect is only due to a specific position of these groups. This was so far regarding the open chain compounds, but with regard to the close ring compounds the efficiency of the compounds was in the following order: NIB- veratamide, Nikathamide and PPA-1 while Hexamine, NIB, cinnamide and piperine had little or no effect.

The antimitotic effect of NIB veratamide is almost comparable to pellitorine. Here treatment with 0.02% for 4 hrs. stops the division of cells almost completely. But there is some indication of prophase

poisoning, because there is not any proportional reduction in all stages of cell division. The percentage of cells in premetaphase stages is shown in table-13. It can be seen that while in controls the percentages remain generally about 60-70%, it remains almost 90% in the treated material. This sufficiently indicates prophase inhibition due to this compound. In short, all the members of this amide group which show antimitotic property have one thing in common and that is their capacity to induce prophase poisoning.

Nikathamide is another compound that shows antimitotic property, when the material was treated with 0.5%. Nikathamide treated for 8 hrs. gave complete inhibition of mitosis. In the lower concentrations i.e. 0.25% and 0.125% the division of cells continued but at a very reduced rate. However, following recovery the rate of mitosis came back to normal status after about 48 hours.

Against these, five compounds namely NIB-veratamide, hexamine, PPA-I, piperine and acetamide were completely ineffective. All these compounds even at as high a concentration as 1% and prolonged treatments of 24 hrs. had no effect on the rate of mitosis. None of these induced mitotic inhibition.

During the last twenty years quite a number of chemicals were shown to possess prophase inhibition at lower concentrations. Cornman (1947) found parascorbic acid and coumarin to suppress the initiation of mitosis

in some Allium species. The effect of coumarins and its derivatives was studied by D'Amato (1954). In lower concentrations prophase inhibition of mitosis was observed while in higher concentrations various chromosomal aberrations were found. D'Amato (1952) and D'Amato and Avanzi (1949) also showed acridines as mitotic inhibitors. Ericksson and Rosen (1949) demonstrated that protoanemonin caused inhibition at pre-prophase in Zea mays root tips. Similar effects were attributed to Benzimidazole, nitrogen mustard and nucleic acid derivatives(Duncan and Woods,1959) and base analogues (Kaul and Natarajan,1966). Patau and Patil (1951)studied the effects of sodium nucleate on the root-tips of Rhoeo didcolour; and showed that the treatment caused a steady decline of the influx into early prophase and attributed the effect to the increasing damage to resting nuclei which they termed preprophase inhibition of mitosis. This compound was earlier shown by several people(Huskin,1948; Kodani,1948; Wilson and Cheng,1949; Huskin and Cheng,1950) to induce reductional grouping. Wilson and his collaborators worked with a number of antibiotics (Wilson,1950; Wilson and Bowen, 1951; Bowen and Wilson, 1954; Hawthorne and Wilson,1952) of all these actidione was most active and caused mitotic reduction by checking transition from one stage to another also inhibited the onset of mitosis.

Harmones, like indole-acetic acid shows antimitotic activity. The supposed mode of action in this case is inhibition of glycolysis resulting from

combination of IAA with the enzyme, triose phosphate dehydrogenase (Wilson and Morrison,1958), while the mode of action of compounds such as the herbicidal chlorophenoxy types and other phenols is much more difficult to assay.

This discussion brings out this fact very clearly that although a large number of chemical of differing Chemical property have been tested, yet we do not have any proper and perfect understanding of the action of these compounds. Among amides reported here only a few exhibited activity while others had no effect. It is pretty clear from the structure of these compounds that all of them possess essential methylating or alkylating groups. But activity seems to have some specific correlation with the total structure. Let us, examine the causes of mitotic inhibition. The most important pre-requisite for a cell to divide is the synthesis of DNA which takes place at interphase or as commonly called resting phase. Along with the synthesis of proteins some basis bistones which also go into the organisation of chromosomes is important. Therefore, any reaction that effects the synthesis of these essential components are bound to cause inhibition. Biesele (1958) has pointed out that in addition to this, there are other pre-requisites i.e. division of centromere, which is slightly different from rest of the chromosomes as revealed by the staining tests; uncoiling of nucleoprotein strand during chromatid separation, activation of mitotic centres responsible for the organisation of the spindle and release of energy by

carbohydrate metabolism or glycolysis, the mutual interdependence and adjustment between DNA, RNA and proteins in the organisation of the new chromosomal material and perhaps the synthesis of a formate like an auxin which may act as a trigger (Mehra-1960). Interference at any of these stages is likely to lead to the blocking and inhibition of mitosis. Let us, therefore, understand how amides could bring out this effect.

According to Brachet (1964) formamide stops mitosis by bringing about a complete destruction of spindle fibres. It inhibits both DNA & RNA synthesis. In a few cases Feulgen positive inclusions were found in the cytoplasm similar to those DNA containing granules observed by Chevremont (1960) in the cytoplasm of tissue culture cells treated with acid deoxyribonuclease. If so, it is possible that in cells treated with agents which produce a partial denaturation of DNA single stranded DNA might be eliminated in the cytoplasm and serve as a primer for DNA polymerase. The results of such a process would be the synthesis of cytoplasmic DNA. In fact this hypothesis has already found support in some experiments by Prescott et al,1962 who found that the uptake of primer DNA by pinocytosis is followed with cytoplasmic DNA synthesis in amoebae. As regards the negative results with some of the compounds (table-30) inspite of the fact that all of these compounds possess essential reactive groups, it is plausible that mere presence of such groups is not sufficient but what is more

important is their specific placement with respect to the whole molecule. Thus Dustin (1947) found ethyl carbamate (urethane), ethyl phenyl carbamate, isopropyl phenyl carbamate and B-chloroethyl carbamate having antimitotic activity and remarkably methyl carbamate even in big doses did not interfere with mitosis and was free of any narcotic properties characteristic of other carbonic esters tested.

Of all the hypothesis given so far for the effect of mitotic poisons there is a reason to believe that amides act either as enzyme inhibitors preventing either the onset of prophase or the polarization of spindle. Most of the amides have a capacity to break the hydrogen bonds. In this connection, experiments of Ambrose and Ayenger (1952) are significant. They have shown that urethane at 0.56M and urea at 11M are capable of dissolving the chromosomes, just like phenols.

It is generally held that radiomimetics disturb nucleoprotein metabolism and the coating of the chromosomes with thymonucleic acid at prophase. This is possible as amides may conceivably interfere with purine metabolism. Amides, acting principally on spindle may perhaps combine with -SH proteins that have already been shown histochemically to be present in the spindle as well as in the nuclear sap. (Levan,1945).

Cytological aberrations:

Both formamide and dimethyl formamide

exhibited a strong radiomimetic effects on barley and onion somatic cells. 1M formamide solution treated for 2 hrs. induced chromosomal aberration like stickiness, fragments, binucleate cells and chromosomal erosion in addition to somatic reduction at metaphase. There were several cases of irregular separation, resulting in laggards.

Dimethyl formamide, induced all such types of abnormalities but at a lower concentration i.e. 0.5M. This included chromatid as well as chromosomal breaks. The rate of chromosomal exchange was low. In barley root tips where, scoring was done at metaphase abnormalities like chromosome breaks, breakage at the centromeric region, dicentrics and sticky bridges were observed. With the increase in period of treatment the breaks per cell increased from 0.88 in 1 hr. to 1.35 in two hrs. and 2.37 after 3 hours. The rate of abnormalities were thus time dependent. The data shows a linear ascend (table-24) and thus an additional effect. There were no exchange after 1 hr. treatment, but after 2 and 3 hrs., exchanges per cell were of the rate of 0.04 and 0.19 respectively. However, preponderance of breaks were confined to the centromeric region.

Formamides are known to break hydrogen bonding (Brachet, 1964) and denature DNA at low temperature (Brooks and Lawley, 1961). It seems, therefore, possible that young growing roots which are metabolically very active and were in a state of DNA synthesis, the treatment with

formamide (1M concentration) was able partially to denature DNA, either by breaking the -H-H bonds or by blocking some vital enzyme system which resulted in the recovery of chromosomal breaks. Dimethyl formamide was more toxic because it contains two methyl groups with a potential of alkylating DNA and, therefore, the toxicity in terms of cytological aberrations was more in this compound than in the formamide. From open chain compounds pellitorine showed very severe cytotoxic effects, and induced a host of abnormalities, including bridges, laggards, fragments, micronuclei, polyploid cells and Pyknotic masses. Of all these aberrations the most prominent were laggards, fragments and pyknotic masses. Following a treatment with 0.02% for 3 hrs. more than 26% cells contained only pyknotic masses with a frequency of 2-6 per cell. Fragmentation of chromosomes was the main reason resulting in laggards and were recovered later on as micronuclei. The stickiness of chromosomes was associated with all the treatments in both the experiments on onion and persisted throughout. Another open chain compound i.e. N-isobutyl capramide also showed similar cytological damage but at higher concentration namely 0.06% and 0.1% i.e. nearly 3-5 times the effective concentration of pellitorine. Again, while pellitorine was effective just after a treatment of 3 hrs.,NIB-capramide needed 12 hrs. to induce the same effects. This means, if both compounds be tested at the same concentration pellitorine is far more active than NIB capramide.

Among the aromatic compounds Nikathamide and N-isobutyl-veratamide showed positive cytotoxic effects. Both these agents induced chromosomal abnormalities like fragments, sticky bridges, binucleate cells and polyploid nuclei. N-isobutyl veratamide showed same effects at 0.02% for 4 hours as Nikathamide at 0.25% for 4 hrs. That means, at the same concentration NIB-veratamide is more than ten times more effective than Nikathamide. NIB-veratamide causes a higher proportion of fragments. In contrast to these two compounds N-isobutyl-cynamide (NIB-cinamide) was completely inactive. Only in prolonged treatments few chromatid bridges were recovered and such treatments was generally lethal. The data presented show that inspite of analogous structure, compounds differ in their biological activity from each other significantly. This clearly indicates that a mere presence of reactive groups is not enough, but some other factors are responsible. According to Darlington (1942) stickiness is due to the disturbances in the nucleic acid metabolism of the cell. Amides, therefore, seems to disturb nucleic acid metabolism directly or indirectly which may also be reason for chromosome breakage. Breakage of chromosomes has been observed following treatments with radiations and a variety of chemicals. (Auerbach,1958; Natarajan,1966). A number of hypothesis have been given to explain chromosome breakage in intact tissues (Revell,1958; Wolff,1963). Taylor et al,1962, showed through experiments that such compounds as would interfere with the biosynthesis of DNA

or RNA at any stage would result in chromosome breakage but this breakage would be specific or localized depending on the type of reaction being blocked. Kihlman (1962) repeated and confirmed the results of Taylor et.al., (1962). Recently, however, Ahnström and Natarajan(1966) concluded from their experiments that chromosome breakage was brought about by reversal of DNA polymerase reaction. Presence of bridges is sufficient to indicate that there has been some amount of chromosome breakage. Bridges were recoverdd only after 36 hours of recovery period when the cells had sufficient time to synthesise new chromosomal strands. Formation of bridges and fragments without centromere are liable to be excluded from 2 daughter nuclei at telophase and become a micronuclei. The presence of micronuclei through all recovery intervals confirms that there is a substantial amount of chromosome breakage. The presence of fragments at anaphase and micronuclei ~~at~~ immediately after treatment suggests that the breakage must occur in the post synthtic period either at late G_2 or at prophase.

Presence of pyknotic masses within cell is another abnormality. These are Feulgen positive bodies in the cell and are formed from the degeneration of nuclei (Patau and Patil, 1951). According to these authors single pyknotic masses are formed from degeneration of prophase nucleus, while two or more such masses are most likely derived from either anaphase or telophase.

Pellitorine is an isobutyl amide of an

unsaturated straight chain with trans-trans configuration. Compounds of similar structure have been found to possess a considerable insecticidal property (Crombie,1952). There is considerable mitotic inhibition alongwith appreciable distortion and breakage of chromosome without killing the organism. It seems, therefore, logical to test the carcinogenetic potential of these compounds. Almost all compounds showed some cytological effects excepting acetamide, piperine, while NIB-cinamide possesses very little cytotoxic property.

False Anaphase bridges (Sticky bridge):

Anaphase bridges are common abnormalities in these cells which divide irregularly or the tumour cells (Koller,1953) and in cells which have been irradiated and fixed a few hours after irradiation for cytological analysis (Marquardt,1949). These bridges are formed by the daughter chromosomes which stick together at their ends. They may part again at telophase when the chromosome are stretched between the two poles. There are frequent instances where the bridge persist throughout telophase and resting stage. If more than one chromosome pair is involved, the bridge will be solid, thick column, which does not break apart and which is responsible for the characteristic dumb-bell shaped compound nucleus. The breakage in the sticky bridge is a mechanical disruption of the chromosome and it usually occurs at the locus where the chromosomes have stuck together. Frequently the intercalary segment which lies between the centromere

and the point of stickiness, is stretched by the force of tension without the chromosome being broken. Then at the end of telophase, the thin and attenuated chromosome fibre is attached by the nucleolytic or proteolytic enzymes of the cytoplasm, and as a result the Bridge disintegrates (Koller,1947).

The sticky bridges are difficult to distinguish from the true dicentric bridges. Discrimination is possible only if the chromosomes stick together at some distance from the end and especially if the stickiness is restricted to a small intercalary section. The configuration which results from this kind of stickiness may be termed as Pseudo-chiasma (Koller,1953 and Levan, 1949). The chromosome deficiency which results when an intercalary sticky bridge breaks has fatal consequences and the cell dies.

Certain points of anaphase chromosomes show difficulty of separation. This leads to the origin of bivalent structures which may be having one or more contact and accordingly may resemble rod or ring bivalents. The adhesion between the points in question is so strong that the pulling at the centromeres will draw out the parts of the chromosomes proximal to sticky bridge and very often effect fragmentation of the chromosomes. In acute cases the whole mass of chromosomes tends to stick together.

Chromosome stickiness was found in almost all the treatments. However, it was maximum with pellitorine and its analogue but least in NIB-cinnamide, PPA-

Amide and Piperine which induced no fragmentation though they effect a reasonable amount of stickiness through all the treatments. Darlington and Koller (1947) attributed chromosomal stickiness to excessive nucleic acid charge and failure of gene reproduction Yamama (1947) relates it to a reduction in viscosity of the matrix substance rendering the chromosome surface more fluidy. The other reasons could be that amides cause breakage of Hydrogen bonds and dissociation of nucleo protein which results in stickiness. Similar reasons have been attributed to urethane. It has been suggested that stickiness of chromosome may be brought about by cross-polymerisation of DNA. Chromosome stickiness occurs spontaneously and can be produced experimently by changes in temperature (Barber and Callen,1943) chemical agents (Darlington and Koller,1947; Ostergren,1950) and by supersonics (Selman,1952). It may also be genetically controlled phenomena, as in maize (Beadle,1932).

However, in the case of amides, it seems most probable that this stickiness is brought about by either polymerization or due to disruption of H-bonds.

Fragmentation:

Free fragments are found at metaphase as well as anaphase. They are liberated at metaphase if both chromatides are cut off at the same level. In other cases, free fragments are detected at anaphase, where they remain lagging between the separating anaphase groups. For a long time now, chromosome fragmentation has been frequently correlated with mutagenesis. Levan (1951) has listed

30 radiomimetic compounds which range from inorganic to complex organic substances and thus give little clue to biochemical basis of action. There is evidence that some radiomimetics effect chromosomes at more or less specific sites. There is reason to believe that certain chemicals have a preferential affinity for secondary constrictions (Kihlman and Levan, 1951; Swaminathan,et.al., 1963; Natarajan,1964). Again, there is a considerable discrepency between the results of different workers with regard to the radiomimetic potential of some compounds. Levan and Tjio (1948) reported that phenols produced chromosome breaks, while Loveless and Revell (1949) failed to reproduce the results. A number of chemicals like urethane (Oehlkers,1943), sulphur mustard (Darlington and Koller,1947), carbamates (Haddow,1948), nitrogen mustard (Ford,1949),phenols (Levan and Tjio,1948), maleic hydrazide (MaCleish,1953) acridines (D'Amato, 1954), coumarines (D'Amato,1950), vegetable oils and fats (Swaminathan and Natarajan,1956), phenylnitrosoamine,caffeine,nucleic acid analogues (Kihlman,1962), certain phosphates (Gallinsky,1949), base analogues (Kaul and Natarajan,1966), organophosphates (Hussein and Hussein,1963), Herbicides (Sawamura,1965) and hydroxyurea (Kihlman,et.al.1966).

Macfarlane (1954) showed that metel elements like phenylmercuric compounds in very dilute solution induced chromosome fragmentation. Similar effects were shown by heavy metel salts like Aluminium chloride and

cadmium nitrate (Oehlkers,1953). Micronutrient elements which are so vital for proper metabolism and growth of plants, produced chromosomal fragmentation and mutations almost akin to X-rays when applied in more than optimum doses (Von-Rosen,1954,57).

From a long list of compounds like this it is rather impossible to find a single explanation for the observed radiomimetic effects. However, chemical breakage of chromosomes may be ascribed to a variety of causes which could be enumerated as follows:-

a. Defective synthesis of any of the vital constituents of chromosomes, the DNA, complex residual protein or DNA.

b. It may be due to direct action of chemical which may bring about depolymerisation of DNA causing its liquification and stickiness because of which fragmentation occurs at anaphase due to polar attractive forces.

c. It may be due to denaturation and dissociation of protein base or due to rupture in the integrity of DNA with proteins.

d. It can also be postulated that some chemicals may combine with the DNA molecule rendering it incapable of further reproduction. In otherwords, it may have a biochemical pathway through the enzymatic phase and

any variation in enzyme production may cause chromosome fragmentation.

e. Any block in the biochemical pathway of the nucleotide synthesis will also lead to chromosome breakage.

In the present series of experiments, fragments were recovered in varying frequencies in all treatments excepting with NIB-cinnamide and piperine. Maximum frequency of fragments were found in treatment with Hexamine while PPA-Amide had only sticky bridges. Here a fundamental different in the mechanism of action of these compounds is obvious. Pellitorine induced a good frequency of fragments while in other compounds a reduced frequency was noticed.

An interesting feature of chromosome breakage by amides is the general occurrance of aberrations of chromatid type even after 60-120 hrs. i.e. the time when chromosome aberrations should be present. Kihlman (1951) dealing with chromosome breaking effect of purine derivatives, suggested that some of them would not be able to penetrate the nuclear membrane for their molecular size and low solubility in lipoid solvents. As a consequence, these substances could act on chromosome only during mitosis a condition evidently leading to aberrations of chromatid type only. These compounds are able to persist in the treated roots for many days to be active on the cells while entering mitosis. Another possibility is that that breaks induced by amides in the resting

stage may remain open and fusion occurs only when chromosomes are effectively split leading to chromatid aberrations.

Spindle abnormalities and polyploidy:

Disturbances in mitotic spindle have already been observed after irradiation and several other chemicals. At anaphase the spindle abnormalities present very diverse pattern such as discontineous and incompact spindles, over-elongated spindles, belated separation of some chromosomes and wide scattering of chromosomes. Evidence of complete inhibition of spindle was, however, not found. The general effects of spindle abnormalities has been irregular separation, C-mitotic effects, laggards, and multipolar spindles and polyploidy. Polyploid cells were recovered in almost all treatments. Notable among these were isobutylamides of open chain type. These phenomena are best interpreted as caused by an interference with the invisible external spindle organizers (Darlington and Thomas,1937).

The ultimate result is the development of C-metaphase and in the absence of anaphasic separation of daughter chromosomes there is the formation of polyploid nucleus containing double the number of chromosomes.

Here again a large number of compounds are known with similar effects. There mode of action may be different. In addition to classical drug colchicine, there are carbondisulphide, naphthalene or related compounds (Steingger and Levan,1947) Hydroxy-quinoline

(Prakken and Swaminathan,1951); Coumarins and derivatives (D'Amato,1954)1; Acridines (D'Amato,1954); sulphanilamide (Mehra,1949); Gammaxene (Sharma,et.al.,1959) and potassium cynide (Levan and Wagenheim,1952).

The fragmentation of polyploid cells possibly arises out of the failure of metaphase chromosomes to go to two poles, because of the disorganization of the spindle apparatus. The disorganization is caused by the viscosity disbalance in the plasma due to chemical treatment and other irregularities noted are also mere reflection of the metabolic disruption of the metabolic equilibrium within the cell. Another interesting obsevation is the formation of the multipoar nuclei. There can be no doubt that multipolar formation is further instance of the breakdown of the normal metabolic process within the cell.

The mode of action of amides and isobutylamide on cell division and chromosomes:

With a view to finding a possible mode of action of amides on the cellular systems experiments were carried out using compounds belonging to aromatic, aliphatic as well as heterocyclic amides. In almost all the compounds important reactive ethyl and methyl groups were present with an essential difference that these groups were placed along the molecule at different positions. Hence, at the very start following questions arise regarding the mode of action:-

i. Whether a mere presence of reactive group in the structure of a compound bestowes it with physiological properties.

ii. The presence of the reactive group at a specific position of the molecule that makes it biologically reactive ?

iii. Total structural geometry of the compound makes a compound biologically active?

After the discovery of sulphur mustards as a chemical mutagen, many compounds of similar structure were synthesized and tested. It soon became established that the mutagenic activity might be connected with a special molecular configuration. Thus in the nitrogen mustard and mustard like compounds the presence of two alkylating groups was believed to be essential to

produce chromosomal damage. D'Amato (1950) found that when CH_3O and Cl are substituted for the amino groups in the acridine molecule, it became inactive. Kihlman (1952) compared the potency of 25 purine derivatives. He found that 8-substituted caffeine derivatives are the most effective compounds. It was also observed that the potency of oxypurines as chromosome breakers increases with increasing N-methylation, while that of the methylated oxypurines decreases or is lost, If methyl groups are substituted with groups possessing larger carbon chains. Kihlman concluded that as regards the purines, chromosome breaking ability does not depend upon any particular atom or groups of atoms, but on the molecule as a whole.

In almost every report claiming the discovery of a new chemical mutagen, the author attempts to explain the mechanism by which the biological effect is brought about. There is general tendency to find one common mechanism by which the chromosomes respond to various chemical agents.

Inspite of the similarity shown by the end effects of chemical mutagens, it can be assumed that the mechanism by which these effects are produced, is not the same.

TABLE XXX

Relative biological efficiency of different amides and isobutyl-amides as revealed by Allium test.

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Name of the compound.	Antimitotic	Chromosome breakage.
Pellitorine	+++	++
NIB-Capramide	-	+
NIB-Veratamide	+++	++
N-isobutyl Cinnamide	--	-
Formamide	+	+
Dimethyl formamide	+	)++
Nikathamide	+++	++
Hexamine	-	++
PPA-Amide	--	+++
Piperine	-	-
Acetamide	-	-
P.O.amide	-	-

Legends:

+++	Very active.
++	fairly active.
+	active.
-	Non-active.

Mechanism of action:

Possible mode of action of these compounds is not very clear, however, one or more of the following chemical reactions may be operating and can be responsible for the biological activity of amides and isobutylamides:

i. Alkylation of genetic material.

ii. Effect on H-bonds.

iii. Cross linking.

iv. Steriochemical factors.

Mechanism of alkylation:

According to Price (1958) alkylation may be defined as "the conversion of H - x ⇝ R - x. The source of the alkyl group R, may be a simple alkylating agent, R-Y from certain cyclic compounds $\overset{}{C} - \overset{}{C}$ (Y) or from an unsaturated compound C=C". Price has further explained the mechanism of alkylation when unsaturated compounds with Carbon Carbon double bond are involved in the process. Two types of mechanisms have been put forth, depending upon the nature of group attached to double bond.

$$C = C - R_a \xrightarrow{H^+} HC - \overset{+}{C} - R_a$$

$$\longrightarrow H - C - \overset{+}{C} - R_a$$

If the group R_a is an electron donor then the reaction can proceed in only acidic medium. Such type of reactions are acid catalysed because of the

initial attack by a proton and involves a carbonium ion (C^+) intermediate.

If the group R_b attached to double bond is electron attracting then reverse type of mechanism is involved i.e. the reaction should move either in alkaline or neutral medium because it is initiated by a nucleophilic reagent X^- and as such proceeds through a carbonion (C^-) intermediate. Such reactions are reversible:

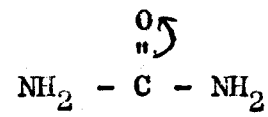
$$X^- + C \overset{\curvearrowright}{=} C - R_b \text{ ---- } X - C - \overset{-}{C} \overset{\curvearrowright}{} - R_b$$

$$\underset{OH^-}{\overset{H^+}{\rightleftharpoons}} X - C - \overset{\text{"}}{C} - R_b$$

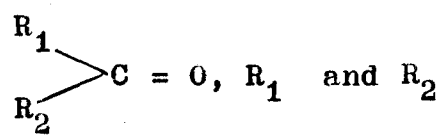
In case of test compounds used in these experiments e.g. amides and isobutylamides, only second type of mechanism can be put-forth because of the $\overset{\curvearrowright}{C} = O$ grouping attached to a double bond. Further the reactions were studied in neutral medium. All these compounds were either neutral in character or very weakly basic in nature (Amides are very weak bases).

Effect on Hydrogen bonding:

Urea which is known to be responsible for breaking the H-bondage may be shown to act as follows:-

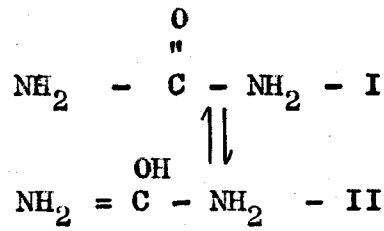


The carboxyl of urea is the seat of negative charge, with O being more electronegative than C and so this carbon, oxygen double bond may get polarised. Degree of polarisation being dependent on the substituents of carbon.

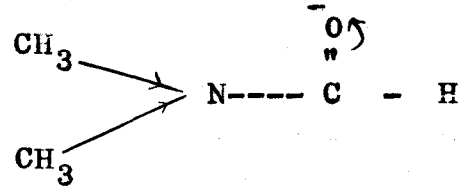


Normally O of the carbonyl is sufficiently electronegative to form effective H-bondage, so it attracts the H-of the normal bond (H-bond) with the result that H-bond breakage takes place. In fact, this is breaking of bond at one place and forming at the other place.

If it is so, a substituent at R_1 or R_2 having more electron repelling properties than NH_2 should enhance the intensity of negative charge on oxygen. That might be one of the causes responsible for more efficient H-bond breaking property of formamides than that of urea. Though it is an established fact that in case of urea tautomerision of the following type takes place:-

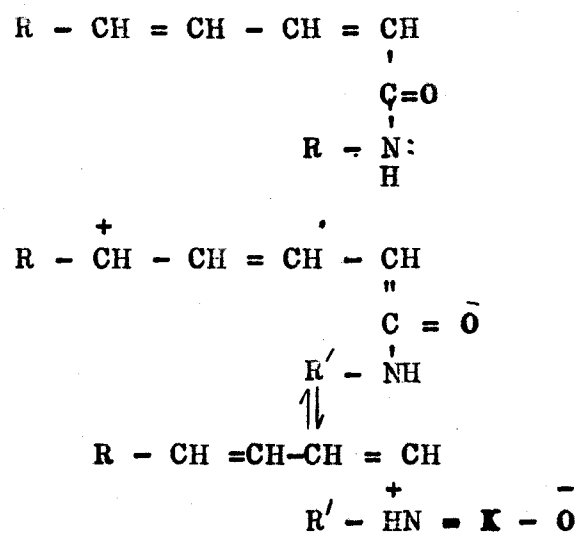


The enol formation (II) naturally weakens the H-bond forming capacity of oxygen, while in case of formamide the possibility of similar conversion is remote. So in order to test the above hypothesis dimethyl formamide was tested, because - CH_3 groups are known to be electron repelling.



Now a question may arise as to why the lone pair of electron on the nitrogen atom is not responsible for breaking H-bonds. The fact is that carbonyl always weakens the electronegative character of nitrogen (Oxygen is in the sixth group while nitrogen is in the fifth group of periodic table) and that is the reason why amides are less basic than amines. However, if N is not attached to carbonyl it can be a seat for high electro-negative charge which is not the case here. From this, it is clear that dimethyl formamide should be more active-H bond breaker than urea or formamide. This contention is well supported by the experimental data. It is possible that pellitorine can also function through its ability to break hydrogen bonds. In this case, the carbonyl group is attached to a

system of conjugated double bonds and also a nitrogen atom carrying a lone pair of electrons. Hence, the electron density at the carbonyl carbon is highly enhanced and can operate as an efficient hydrogen bond breaker.



Whereas in the case of hydrogenated pellitorine (N-isobutyl capramide) the carbonyl carbon is attached to only one source of electron rich centre, namely the nitrogen atom and its decreased activity in hydrogen bond breaking. In the case of N-isobutyl veratamide also electron density at the carbonyl oxygen is high due to the presence of the electron releasing methoxy group in the para position to the amide grouping. This will facilitate the hydrogen bond breakage, whereas in the case of N-isobutyl cinnamide the presence of

 -CH = CH - grouping depliates the electron density at the carbonyl oxygen and hence the inactivity.

Cross linking hypothesis:

Goldarce, Loveless and Ross (1949) have put-forth the hypothesis that the alkylating agents join different chromosome threads together by a covalent bond (i.e. cross linked) prior to mitosis. This was further confirmed by Alexander et al (1958) by providing some experimental evidence. Formaldehyde and hexamide are known to rubber industry for the introduction of cross links in some linear polymers, so it should not be surprising if it is assumed that both formaldehyde and hexamine play the same role in chromosome threads which are so to say linear type of polymers.

The cytological damage is not very likely to be due to cross linking of protein chains because the damage is temporary. Secondly such a long chain and bulky molecule as pellitorine cross linking a macromolecule like nucleoprotein is unlikely because of steric factors. One may ask if hexamine can, why not pellitorine. A reasonable explanation is that hexamine is a fused rigid ring structure.

Sterio-chemical factors:

Pellitorine, in which the hydrogens are trans: trans is active in causing cytological damage. Similarly in the case NIB-veralamide is active, whereas in Cis:trans, isomer of PO amide was not active. This suggests that the geometry of the molecule is also likely to be an important factor in causing cytological damage. This observation is in agreement with Crombie (1952). He states that isobutyl amide with trans:trans arrangement are effective insecticides. The insecticidal activity of amides have been attributed to acetyl choline estrase inhibition. Thus it could be suggested that the cytological damage caused by these amides could also be due to some enzyme inhibition and this suggestion is no way unreasonable. In the enzyme inhibition activity both electronic and sterio-chemical factors of the inhibitor are important.

This observation also contradicts the hypothesis put forth by Walpole(1958) when he mentions that the chain length alongwith its nature does not effect carcinogenic activity of a compound. Isobutyl amides have similar, if not identical functional groups, yet the geometry of the double bonds in the side plays a vital role. Moutschen Dahmen (1963) working with l(+) and d(-) diepoxybutane, showed that two isomers had different activity and possessed differential activity for chromosomal region.

Yet another mechanism of action could be

attributed to the molecular jumbling in two compounds i.e. NIB-veratamide and NIB-cinnamide. The former may have CONH group free to react either in complex formation or for breaking hydrogen bondage and the latter may have CONH blocked by the stearic hinderances of the adjusoant double bonds which is in configuration with the aromatic ring.

The mechanism of cytological damage caused by these compounds is not established with full certainty. At present only different hypothesis can be drawn. They are:

i. the commonly accepted alkylation of genetic material.

ii. Breakage of hydrogen bonds and

iii. Enzymatic inhibition.

The activity of amides and isobutyl-amides may be through one or all of above processes.

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