MATERIALS AND METHODS

The experimental material consisted of primary roots of onion and root meristems of barley.

(1) Onion (*Allium cepa* L.) 2n=16

Healthy commercial bulbs of onion were used for the study. This is an ideal material and has been used very extensively in this type of study. This also forms a standard material of the "Allium test" (Levan, 1949).

(11) Barley (*Hordeum vulgare* L.) 2n= 14.

Six rowed, naked barley of Ladakh origin was used. This is a native of Ladakh and grows at an altitude of 9-12,000 ft. Naked barley was used in view of its disposition of the embryo in the seeds which permit uniform penetration of the chemicals treated as well as germination.

Chemicals used:

The chemicals used in this study belong to the group of compounds, known as amides. Amides are derivatives of ammonia and the amines in which one or more of hydrogen atoms are replaced by acyl group ($R-\overset{O}{\overset{\|}{C}}-$). these are basic compounds but are biologically very important (Watson, 1965). The compounds used are given in table 1 with their common name, chemical structure, chemical name and source. Some of them are available commercially and have some therapeutic value while others have been synthesized for the first time from the plant *Piper peeploids* and are therefore new compounds. All of these are either open chain compounds or closed ring ones.

METHODS:

Germination of bulbs:

Bulbs of onion were placed on clean glass vials filled with distilled water. These were germinated at 20 ± 2°C. Water in the vials was replaced every morning. After 48 hours the roots were of desired length (0.5 cm) for experimentation. Vigorously rooting bulbs were selected and these were reduced in number to a 20 to 25 of uniform appearance. These were assigned to the treatments at random. Three replications were maintained in each case.

Preparation of the test solution:

Not all these compounds are soluble in water but, all of them are soluble in alcohol. Whenever the compound was soluble in water, the desired solutions were made in distilled water. When compound was not soluble in water it was first dissolved in 2-5 ml of absolute alcohol and required suspentions were made in distilled water (details are given with each experiment).

The treatments were given for different periods of time and fixations were made either immediately or after varying periods of recovery. For recovery, the treated bulbs were washed in running water for 5 minutes and returned to distilled water for recovery. Fixations were made in freshly prepared mixture of acetic acid : ethyl alcohol (1:3). No pre-fixatives were used.

Staining:

Material was always stained according to Feulgen technique following hydrolysis in 1N-HCl for 10-12 minutes.

Mitotic index:

Mitotic index was calculated by determining the number of dividing cells in relation to total. The cells in division were grouped as prophases, metaphases while anaphases and telophases were counted togeather and rest of the cells in non dividing stage were grouped as interphase. First four hundred cells in each slide encountered while moving the mechanical stage from a starting point were scored and 4 to 8 slides were studied in each case.

Cytological technique:

Chromosomal aberrations were generally scored at anaphase and in few cases at metaphase. For this purpose treated roots were fixed in acetic alcohol (1:3) directly and left in fixative for 24 hours. They were hydrolysed in 1N-HCl at 60°C for 12 minutes in case of onion and 17 minutes in the case of barley. Following hydrolysis, these were washed with water and stained with leuco-basic fucshin. Squash preparations were made in a drop of aceto carmine from a deeply stained portion of the meristem just behind the root-cap.

All the observations were made from the temporary slides.

Making slides permanent:

Wax was removed from the temporary slides very carefully and the slide was inverted in a mixture of acetic acid, N-butyl alcohol (1:1), coverslip facing downwards, till the cover got seperated. They were then passed through one change of N-butyl alcohol for 10 minutes and mounted in euparol.

Scoring of chromosomal aberrations:

The method of scoring the chromosomal aberrations was kept constant throughout the study. The observation of slides was ~~kept~~ started through one end to another. Any type of aberration was studied and recorded. Abnormalities generally met, were chromosomal bridges, fragments, chromosomal or chromatid breaks, pyknotic masses, micronuclei, stickiness and some spindle abnormalities. For the sake of comparison of the effects, frequency of abnormal cells in each treatment was calculated.

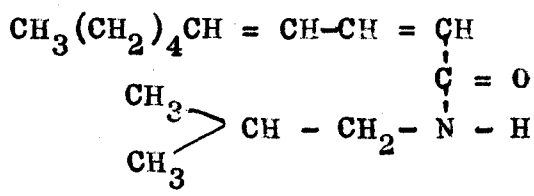
Drawings and Microphotographs:

Camera lucida drawings were made from temporary slides by Carl Zeiss camera lucida apparatus. All drawings were made at the table level using the Meiopta ocular 100 X with N.a. 1.3 oil immersion objective and 10 X eye piece. Microphotographs were taken from temporary slides by 35 mm. camera fitted to Meiopta microscope, using ocular 100X with N.a.1.3 oil immersion objective and 10X eye piece. Agfa Printon films were used exclusively for photography.

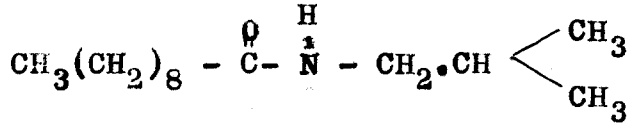
pH:

The pH of all the test solutions was maintained at 7. The citric acid buffer was used for the purpose whenever necessary.

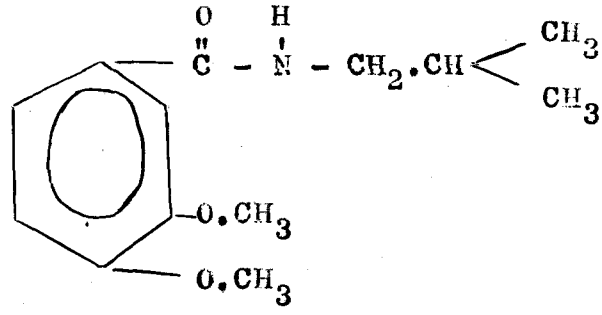
Common Name.	Chemical Name.
1.	2.
Pellitorine	N-isobutyl 2:trans:4:trans decadienamide.



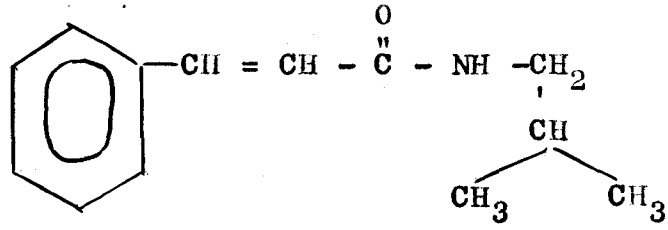
Pellitorine hydrogenated.	N-isobutyl capramide.



Synthetic amide	N-isobutyl veratamide.



ICB-amide	N-isobutyl Cinnamide.

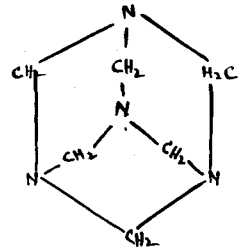
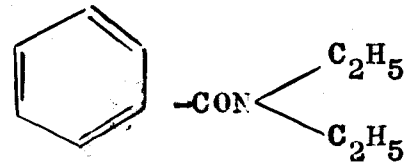
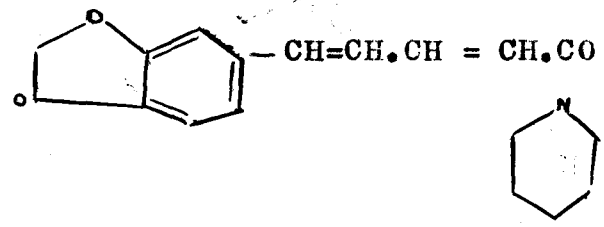
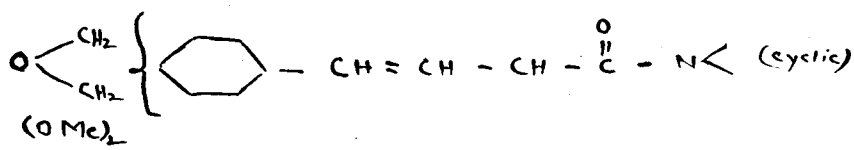


Formamide	Formamide.

$H.CO.NH_2$

Dimethyl formamide	Dimethyl formamide.

$H.Co.N(CH_3)_2$

1.	2.
Hexamine	Hexamine.
	
Coramine	Nikathamide
	
Piperine	i-piperonyl piperidine.
	
P.O.- amide	H on double bond. 1:2 not trans:trans.
	$CH_3-(CH_2)_{n_1}-CH=^{d_4}CH-CH\overset{d_3}{=}CH-(CH_2)_{n_2}$ $-CH\overset{d_2}{=}CH-CH\overset{d_1}{=}CH-\overset{O}{\overset{\|}{C}}-NH.CH_2-CH\begin{matrix}CH_3\\CH_3\end{matrix}$
Acetamide	Acetamide.
	$CH_3-\overset{O}{\overset{\|}{C}}-NH_2$
PPA-amide	PPA-amide
	

Abbreviations:

The following abbreviations have been used in the test:-

NIB-capramide	= N-isobutyl capramide.
NIB-Veratamide	= N-isobutyl veratamide.
NIB-cinnamide	= N-isobutyl cinnamide.
DMF	= Dimethyl formamide.
DNA	= Deoxy ribose nucleic acid.
RNA	= Ribose nucleic acid.
B'	= Chromatid break.
B"	= Chromosome break.
C.B.	= Centric break.
D.C.	= Dicentric.
Exch.	= Chromatid exchange.
Frag	= Fragment.
Brid.	= Bridge.
Tetra.	= Tetraploid.

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