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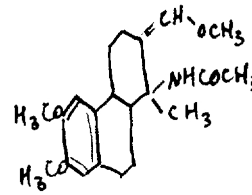
COLCHICINE



1. Colchicine is an alkaloid present in corms and roots of various Liliaceae, particularly Colchicum autumnale, the meadow saffron. (Klein & Pollauf '29) Although it has never been fully synthesized, the researches of Windaus and others indicate the formula reproduced here: (from Henry '3 '39) It will be observed that the nitrogen is on a side chain and that there is an acetanilide nucleus present. (~~Note 1~~) Not so! The ring is probably saturated

Saffron extracts have been used since Byzantine times as an empirical specific for gout, (Rushy et al., '30) Water and uric acid excretion are stimulated. However intestinal inflammation and respiratory depression frequently occur so that this drug is not widely used in modern practice. Temperature plays an important part in determining the toxicity of this drug, warm blooded animals, or frogs kept at a high temperature, having 400 times the sensitivity as cold-blooded. (Funner, ..., '33) Jacobson ('25) reports a specific paralysis of cardiac parasympathetic terminations. Lorthiori finds a [increasing] modification of the Arneth leucocyte count [due to more rapid production of young neutrophiles], a lowered renal threshold for glucose and a decrease of liver glycogen, all indicating an interference with renal mechanisms. Clark & Barnes '40 find that colchicine poisoning, iodoacetate poisoning, and adrenal-cortical deficiency are all relieved by the administration of salt-water or of cortical extract.

There are also scattered reports in the French litterature of stimulatory effects on parathyroid and testicular tissue.

It is plain that the general pharmacological effects of this drug have not as yet been reconciled in any comprehensive theory on the mechanism of its action.

2. It is not on the basis of the above-listed properties that Colchicine merits the attention that it receives from biologists today. A number of other effects of this drug have been recorded which are traceable to its effects on cell-division. The modifications, described below, which Colchicine introduces into cell-division have been called the C-Mitosis (Levan '38). The early history of its clarification has been reviewed quite adequately by Dermen '40, and Fyfe '39, and will not be repeated here. The work of Levan ('38,'39) describes the c-mitosis as follows. The material is the root-meristem of Allium cepa; the concentrations of the drug varying from .0075% to 1%, applied by merely growing the roots in the test solution:

Prophases proceed normally, and polar caps may appear in the earliest stages. The spindle however does not become organized. As a result the metaphase is never oriented, and the chromosomes do not appear on the equatorial plane. Instead they remain scattered about the cell, apparently for as long as seven or eight hours. The kinetochore is also somewhat influenced for it does not divide as it would in the normal rhythm. The length of the chromosome does however soon split, and repulsion or some other force spreads the halves out so that x shaped chromosomes appear, the point of juncture being the unsplit kinetochore. Eventually this too divides, yielding pairs in a tetraploid figure. Telophasic transformation now ensues, but the disorganization of the chromosomes results in canalized and in polymorphic, pycnotic nuclei. These late

C-telophases give the false appearance of amitosis. These reconstituted nuclei are now ~~tetraploid~~ diploid or~~, at any rate, are 2x~~ with respect to the parent haploid nuclei. Thus, similarly haploids can be made to yield homozygous diploids. This process of chromosome doubling can be repeated almost indefinitely. In the reduction-division of the sporocytes (Levan '39) a fundamentally similar pattern applies. In somatic mitosis the presence of ~~x~~ diplo-chromosomes and the absence of the spindle are ~~clear~~ diagnostic features ~~for~~ of colchicine activity in the tissue concerned.

This mechanism of polyploidy depends on the ~~non-function~~ inhibition of the spindle and is not to be confused with that induced in the resting phase by hetero-auxin (Levan '39b)nor by that resulting from the absence of new wall-formation ~~by~~ in material treated with purine alkaloids (Gosselin '40).

Cytological investigations on the effects of colchicine, particularly interesting to geneticists for obvious reasons, have been made on a great number of organisms, plant and animal, some of which are summarized in the following table:

TABLE I

Investigations on cytological effects of Colchicine.

Material	Effect	Reference
Tradescantia, stamen hrs	c-mitosis	Nebel & Ruttle '38
Nicotiana & Datura	polyploidy	Blakeslee ..'37
Zea,Allium,etc root	c-mitosis	Eigsti '38
Allium root	c-mitosis (good illus.)	O'Mara '39
Triticum root	c-mitosis	Beams & King '38
var. roots	c-mitosis	Shimamura '39
Pinus seeds	mixed polyploidy	Mirov..'39
Pteridophyte gam.	polyploidy	Rosendahl '41
Marchantia	polyploidy	Blakeslee '39
Spirogyra	binucleate	Yamana..'41
Saccharomycetes	No Effect	Richards '38
Schizomycetes	No Effect	Walker.. '40
Review on plant material	Boas & Gistle '39.	
Tissue culture (an.)	arrested metaphase	Ludford '36 Clearkin '37
T.C. human cancer	arrested metaphase	Oughterson '37
Mouse organs after injection	arrested metaphase	Brues & Cohen '36
Frog (tadpole) tail epidermis	arrested metaphase	Lein '41
Frog egg	arrested cleavage	Keppel .. '39
Frog egg	pycnotic nuclei	Welsh unpub
Rabbit ova	arrested cleavage,tetrapldy	Pincus.. '39
Orthopteran testis	giant sperm, differentiation of cellular elements without cell division	Dooley '41

Protozoa	No Effect	Beams & Evans '38,'40

Teratological effects, no doubt resulting from the distortion of cleavage have been reported as follows:

Oryzias (teleost)	Waterman '40	various
Gallus (chick)	Ancel & Lallemond	'stropnosomic'
Rana (anuran)	Welsh unpub.	exogastrulae

We have every reason to believe that the 'arrested metaphase' of the zoologists is equivalent to the c-mitosis of the botanists. The inhibition of spindle function is the prime cause.

3. While colchicine demonstrates c-mitosis with the clearest and greatest efficacy, it is by no means the only reagent that will induce polyploidy in the manner described. The French workers, particularly the Gavaudans, Simonet, and Guinochet have described , in a series of papers in the C.R. Soc Bid a great many such compounds, including acenapthen, p-dichlorobenzene, naphthalen and chloro-naphthalene, phenyl-urethane, ether, chloral hydrate, and others. There is no resolution of this list on the basis of chemical structure. Indeed Ludford '36 finds that slight modifications (demethylation) of the colchicine molecule destroy its c-activity.

In particular, however, the anesthetics are represented in the list, and it has been suggested that colchicine acts as an anesthetic. This view is dealt with more fully below.

In concordance with the effects on spindle a great number of consistent observations have been made indicating that one physical correlative of the spindle inhibition is a modification of the viscosity rhythm of mitosis (Heilbrunn '28). The initial colchicine effect is a reduction in that viscosity. Wilbur '40 on Arbacia eggs finds that initially rise in viscosity after fertilization and the accompanying metaphase is inhibited, but that the "standard" viscosity is unaffected. Beams & King come to a similar conclusion on Triticum seedlings, as does Wada ('40) on living Tradescantia staminal hairs, and Lein '42 on Glycera eggs in hypotonic seawater. T

The causal relations of these phenomena are not revealed, however. That is to say, is the colchicine effect directly on the cytoplasmic viscosity, or is the diminution of the latter an incidental consequence to a more direct inhibition of the spindle. The supposed common behavior of anesthetics in decreasing viscosity is interesting in this connection. Further progress in this direction is probably waiting for independent progress of research on the nature of the spindle.

Lillie('14,'18) has observed changes in water-permeability and electrical polarity of the cell membrane during sea-urchin egg division. However, no reports appear in the literature with respect to colchicine effects.

Guinochet finds changes in the pH and osmotic pressure of colchicinized Triticum seedlings.(G.,'40) :

pH	vacuole	from 4.8-5.0 to 4.4-4.8	.3 decrease
pH	cytoplasm	from 5.4 to 5.2	.2 decrease
Osm Pr	'cell'	7.1 to 11.1 Atmos.	55% increase.

The increase in osmotic pressure is a reflection of the very swollen appearance that meristematic cells have after effective treatment with colchicine. The pH change is considered further below.

There is as yet no rationale for colchicine effects here. The problem may however be attacked from a chemical viewpoint. If only in a negative way, the work there has been more fruitful.

~~General considerations~~ on the chemistry of cell-division are discussed in ~~the report 'The Biochemistry of~~ Cell-Division' by the present auther, to which ~~reference should be made. If~~ the relationships between activity and metabolism ~~were there indicated to be complex~~ and obscure, the colchicine situation can only emphasize this.

Gal ('38) working on the anesthetic hypothesis tested the effects of colchicine on the activities of dehydrogenases, and checked the results against those with sodium cacodylate, which has similar cytological effects. The cacodylate had no effect on any of the Kreb's - substrates. Colchicine had no effect on succinic, glucose or glycogen dehydrogenases, but partially inhibited lactic and citric dehydrogenases. The source, unfortunately, was beef muscle and liver, in neither of which c-activity can be demonstrated. However an interesting hypothesis to test would be that the lowering of pH in Guinochet's Triticum is a consequence of the accumulation of lactic and citric acid. This hypothesis is especially interesting in view of Ruhland and Ramshorn's ('38) determinations of the RQ in meristems, and of the presence of acetic dehydrogenases, indicating anaerobic fermentation. This is discussed more completely in the succeeding report.

At any rate, colchicine does not work by blocking oxygen supply at any early stage. The effects of oxygen block are very much different. Brock, Druckrey, and Herkin ('39) find that colchicine concentrations 500x those required for complete division block in Stongylocentrotus have no effect on the oxygen consumption of these eggs. Patton & Nebel ('39) find that the drug does not affect respiration at all at .0002M, and halves it at .0004M. Both of these concentrations probably are considerably in excess of that required for c-mitosis in their material, excised Zea roots, but no figures are given. The answer to this mechanism, like that for the anesthetic inhibition of other cell activities is not to be sought in the total respiration or metabolism but to some specific moiety thereof. In spite of these negative conclusions, an enzymatic hypothesis for c-activity remains a promising one, if not the only one that is susceptible to any form of attack.

Other observations on the enzymatic activity of colchicine are mostly negatives. However, Patton & Nebel, op cit, find a depression of proteolysis at a concentration of .0001M which may be at or not too much above the threshold for c-activity in their Zea roots.

Krugelis finds no inhibition of the acid-phosphatase activity of young rat testis by colchicine. (unpub.)

Smith,'41, finds an acceleration of malt diastase, and no direct inhibition of invertase by colchicine.

Once again we can only say that no final conclusion is possible.

4. Although the main body of this paper contains all of the previous principal work on colchicine, the author is in the midst of some investigations which may yield another approach. That investigation is by no means complete: indeed it has barely started, and this paper is not to be regarded in any sense as a publication. It is intended rather as a systematization of the present situation, and as a contribution for the interest of those who have shown some solicitude for my efforts in this direction. Consequently the remainder of this paper will be on a more speculative, hypothetical basis.

In the course of some routine cytological studies on c-mitosis in the Allium cepa root-tip, the author in March 1941 came across some indications of a gradient-of-susceptibility to colchicine in the onion root tip. This was followed up by some work in the Spring of 1942 which confirmed this conception as follows:

Onion bulbs were grown in the dark over tap-water for 3-4 days until roots 1-2 cm long appeared. Then a stated colchicine solution was substituted for the water, replaced every few hours. The root-tips were then cut off at stated times, fixed, and stained for cytological study. Counts were made of 80 longitudinal sections of the types of division figures present, and segregated into the groups: prophases, normal metaphases, anaphases, telophases and c-mitoses. Camera lucida sketches were made under low power, division figures picked out, and then all of these were examined under high power, and the type and relative locality of each sketch marked. Charts II and III are graphic representations of the numbers of each type of figure in 5 sections with respect to time, at .0006% and .006% aqueous colchicine respectively. The sketches indicated very definitely that at these concentrations the effect was localized, and that furthermore, normal mitoses were always found when present, apically to c-mitoses. Furthermore the line of demarcation was shifted basipttally at the higher concentration, although this line shifted very much more with time than in response to concentration difference. No very satisfactory analysis of the charts is yet forthcoming, and thecdata are still too incomplete to be entirely significant so thaey are presented without comment.

There are at least two possibilities in the interpretation of the gradient, requiring experimental test. Because of differential absorption in the region og differentiation or elongationg there may exist an artificial concentration gradient down the root tip. Or the gradient may be intrinsic in the cells. If the first interpretation survives test by cutting and culturing experiments, it will not be possible to use this approach. If the gradient is intrinsic, the results of attempts to extinguish or accentuate it would be most interesting. Such preliminary experiments with Pb, cyanide, & methylene blue indicate only that the balance of figure-types is upset by the accumulation of prophases or of vegetative cells, in accordance with the independent properties of these reagents. At this point one can only say that experiments are being planned and will shortly proceed.

The authors attempts to measure dehydrogenase activity on whole tips was vitiated by the absorption of methylene blue by the cap and elongation regions, first, and then the meristem. When the entire tip was colored, it was the first to decolorize in the Thunberg vacuum, and this decolorization see seemed unaffected by the presence of colchicine. The conditions of the experiment demand repitition.

The attempt then to correlate collapse of spindle function with inhibition of some sort of metabolic activity then continues, but as yet is quite unsuccessful. One last important consideration ought to be kept in mind: the importance of distinguishing the intrinsic effects of colchicine and those which occur through the mediation of the cessation of cell division

The substance of this report was presented orally at a Botany 124 Seminar, Columbia University, on April 24,1942, and in written form in a report to the instructor for that course, Dr. W.G.Whaley.

The companion-report to this : 'The Biochemistry of Cell-Division' was prepared only as a background for the study of colchicine effects, and is incomplete to the extent that the colchicine problem does not join on it.

July 28,1942. Joshua Lederberg
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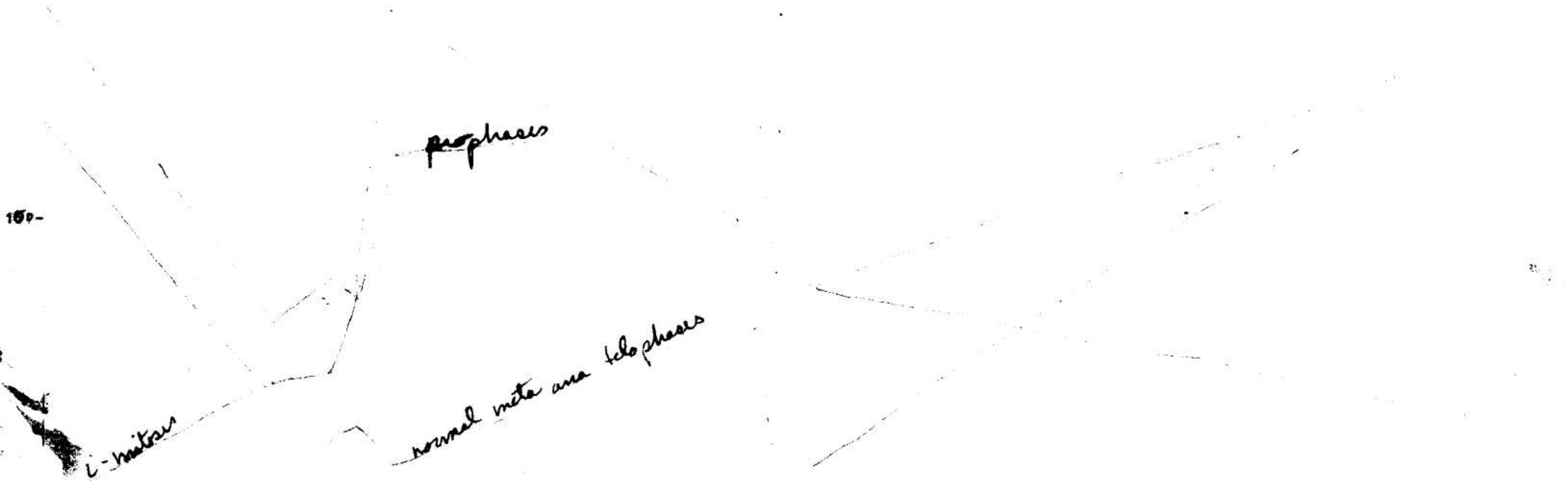

Chart II

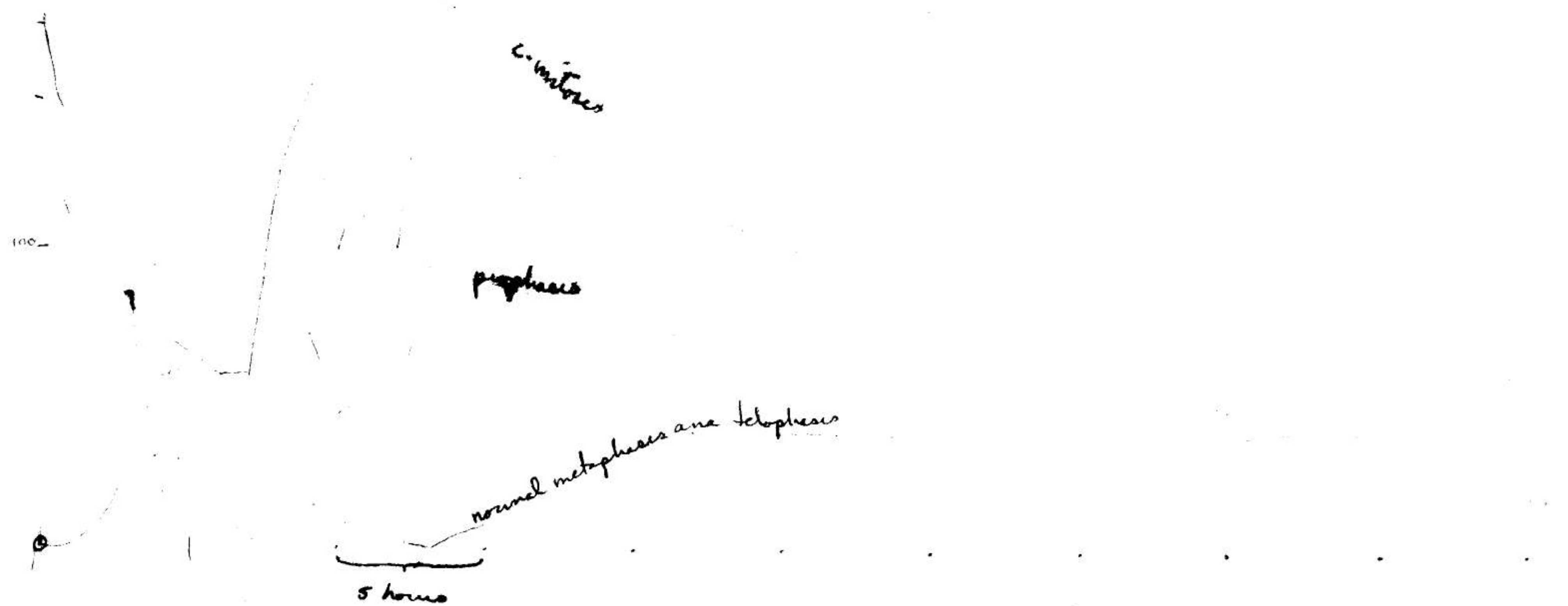

Chart III

Figs. 1 and 2.

Outline drawings of representative 8μ sections of onion root-tips treated with threshold concentrations of colchicine.

The root apex is toward the top of the page. The broken lines are the root axis, and the plane of demarcation between mitoses and c-mitoses respectively. The small arrows are directed at the point of apparent convergence of cell layers.

- • indicates normal appearing prophase
- □ indicates normal appearing metaphase, anaphase,
- x indicates c-mitosis

Drawn at table level at 50X with the aid of a camera lucida.

J. Ledeboer, 1945.

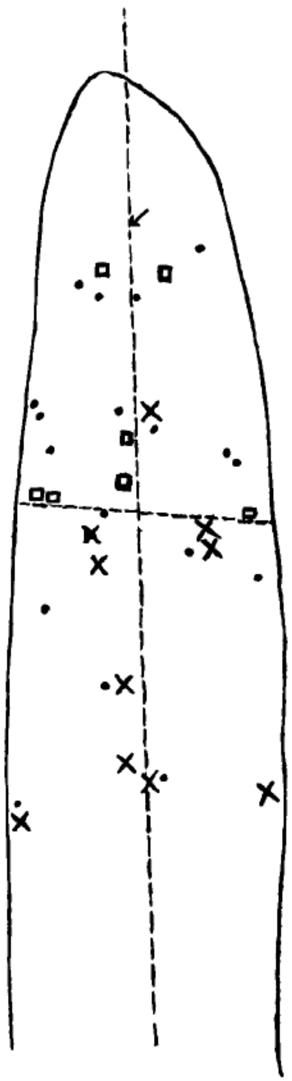

Fig. 1 Allium cepa
.006% colchicine
3 hours

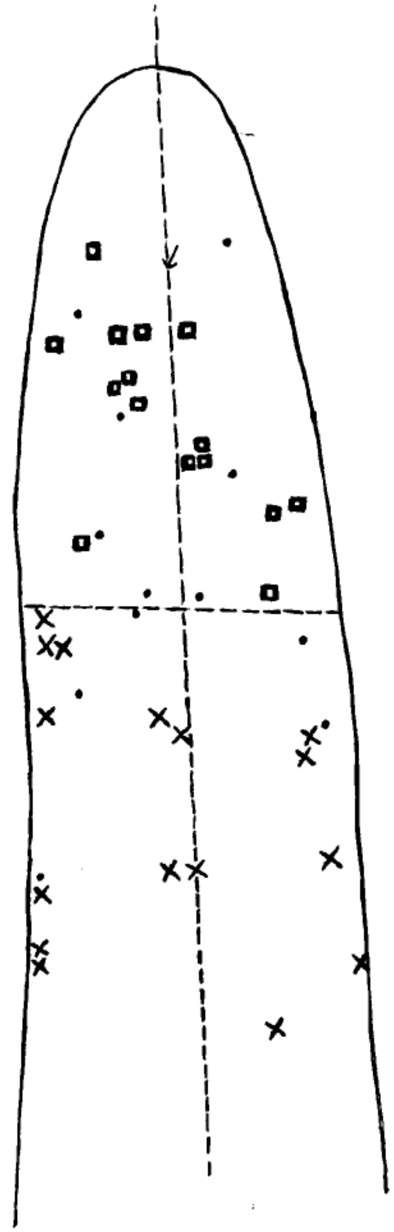

Fig. 2 Allium cepa
.005% colchicine
15 h.

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