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Effects of the aqueous extract from *Poria obliqua* Bres. on the roots of *Allium cepa* L., *Vicia faba* L. and *Tradescantia zebrina* Loud

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The various specific effects of the extracts from plants, used in popular medicine against cancer, on plant cells and especially on mitosis has been investigated in Department of Plant Anatomy and Cytology of the Warsaw University already from 1953. Since the preliminary tests indicated on the activity of some of these extracts it was assumed that the study of various fractions of these extracts on various test plants will enable to separate specially active substances.

In 1959 we started to investigate the influence of the aqueous extract from *Poria obliqua* Bres. (*Inonotus obliquus* Pers.) using as the test object roots of *Allium cepa, Vicia faba* and *Tradescantia zebrina* cultivated in tap water. Extractions from *Poria* were done in the cold or hot tap water. The most useful was the dried water extract obtained from Mgr. S. Piaskowski. Most often following solutions of the dried water extract were used: 0.2, 0.5, 0.7, 0.8, 1, 1.5 and 2 per cent. pH was that of the tap water used for solvent. To these solutions the test plants from water cultures were transferred for periods ranging from 2 hours to several days. After different periods of treatment with extract solutions the root tips were fixed or the whole plants were transferred again to water cultures and the root tips were fixed after 6, 12, 24, 48, 62 (or more) hours or they were kept for further culture and observations. The fixative used were acetoalcohole (1 : 3) or Navashin fixative modification (CrAF 0.5—1—20). The fixation time was 4 hours. Smear preparations were stained with acetoorcein or acetocarmin. The microtome sections were stained with gentianviolet. Feulgen's method was used both for smears and sections.

This is only a preliminary report of larger studies not completed.

The roots treated for 24 hours in room temperature by 1 per cent fresh solution of the extract and transferred to water show a little different colour of root cap and of the whole of root tip. After still longer period, from 24 to 36 hours, their colour changed still more (Plate I, fig. 1).

The cells of the elongation region lose their turgor and became flabby. These are symptoms of necrosis because after 3—4 days these parts of the roots usually fall off. Smaller concentrations of the extract as e.g. 0.5—0.7 per cent initially cause an inhibition and later a very strong retardation of the roots growth. With concentrations from 0.7 to 0.8 per cent after 3—4 days of very slow growth the root tips of the majority of *Allium, Vicia* and *Tradescantia* roots died out and the transfer to water is without effect. *Tradescantia* in water culture develops root hairs. However, when the roots are in 0.1—0.2 per cent extract solution for a period from 1 to 2 hours they lose the ability to develop root hairs. When after the transfer to the water the root continues its growth the root hairs are formed only on that part that was formed already in water (Plate I, fig. 2). Thus the formation and growth of root hairs was inhibited by the extract.

Morphologically the apical meristems together with root caps of roots treated by 1 per cent extract through 24 hours are badly damaged or distroyed and after the transfer to water they behave as decapitated ones. They form numerous lateral roots (usually just above the injured part) and often they regenerate the apical meristem and continue to elongate after several days. The different behaviour depens most probably on the different degrees and types of damage.

The toxic action of the fresh 1 per cent or stronger extract on roots meristematic cells was proved beyond doubt. After 24 hours of treatment with this extract no mitotic figures were found in treated root tips of *Allium, Vicia* and *Tradescantia*. Probably all mitoses were completed because newformed nuclei are observed. It may be also that a sporadic regression of newly started mitotic divisions takes place. After the transfer to water the cells from apical meristems never behave normally. In nuclei pycnotic at the beginning a chromatolysis is observed both in apical meristem and in root cap. The dageneration of the nuclei resembles that described by T i s c h l e r as karyorhexis and consists in fragmentation of the nuclei into small granules penetrating in cytoplasm or, more often, resambles ordinary karyolysis.

Very intersting are the effects of the treatment by 0.7—0.8 per cent extracts through 24—48 hours, or of 1.5 per cent extract in 3, 6—12 hours. In these conditions we observe a specific action of the extract on the chromatin and the chromosomes. In prophase with initially loose and distinct spiralization (Plate I, fig. 3) tape-like structures are formed connected by bridges (Plate I, fig. 4) or more or less rounded structures are formed which easily disintegrate in small granules or even disappear (Plate I, fig. 5).

In metaphase a very strong shortening of the chromosomes was stated.

Their length may by one half of the normal (Plate II, fig. 1 and 2) or they form spherical bodies (Plate II, fig. 4). At metaphase with shortened chromosomes the kinetic spindle is quite distinct and seems to be normal.

If other plants react in the same way this may be a very helpful treatment for long chromosomes counting.

In anaphase and telophase we observe also shortening of the chromosomes and often their vacuolation or even fragmentation (Plate III, fig. 3 and 4; Plate IV, fig. 1—5). In comparison with normal anaphase (Plate III, fig. 1) or telophase the chromosomes from the treated cells are shorter (Plate III, fig. 3) or even spherical (Plate IV, fig. 2), they often show constrictions (Plate III, fig. 4; Plate IV, fig. 1, 4 and 5) or fragmentate (Plate IV, fig. 1, 3, 4 and 5).

It was assumed that the vacuolation found in metaphasic, anaphasic or telophasic chromosomes (Plate II, fig. 5 and 6; Plate III, fig. 2 and 4; Plate IV, fig. 1, 4 and 5) may be due to the slight heating (up to 50—60°C) in acetoorcein smearing but control preparations with untreated plants showed no vacuolation of the chromosomes (Plate III, fig. 1).

The "vacuolation" of the anaphasic chromosomes results probably from the shrinkage of the chromosomes with simultaneous despiralization. The vacuolation is only spurious. Besides this, true vacuolation as a result of chromosomes degeneration does exist also.

Very striking is the shortening of the chromosomes up to spherical shape (Plate II, fig. 3 and 4; Plate IV, fig. 1, 2 and 4). It seems that to certain degree this phenomenon is reversible. It is possible that the shortening starts earlier for instance for the metaphase already at prophase and together with the shortening of the chromosomes also other reversible changes in the still living cell are connected. The removal of the active substance by washing out may cause the return of the chromosomes to their initial state if the whole cell is able to recovery. If the cell with shortened chromosomes died out and the extract is still acting then the chromatin destruction and chromatolysis enuses or only chromatolysis without fragmentation is observed.

Reasuming our preliminary results on the action of water extract of *Poria obliqua* on the cells of *Allium cepa, Vicia faba* and *Tradescantia zebrina* we may state that:

1. The cells from apical meristems and root caps of the roots are specially sensitive to treatment with the extract.

2. The extract inhibits cell divisions probably specially through the action on the chromatin causing its final lysis.

3. Resting nuclei after initial period of pycnosis disintegrate in small droplets (karyorhexis) which is followed by karyolysis or without previous karyorhexis, karyolysis take place.

4. The chromosomes are shortened to spherical bodies and after prolonged action of the extract chromatolysis enuses.

5. Extract from *Poria obliqua* inhibits the elongation (growth) of cells. This may be seen from the damages in the region of cell elongation in *Allium cepa, Vicia faba* and *Tradescantia zebrina* roots and from the inhibition of the root hairs formation in *Tradescantia zebrina.*

Detailed presentation and discussion of the results obtained will be published later.

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Explanation of the plates

Plate I

Fig. 1. — *Allium* roots after 24 hours in 1% *Poria* extract and after 24 hours in water. Note change in appearance and clouring of the root tips

Fig. 2. — Roots of *Tradescantia* after 2 hours in 0.2% *Poria* extract, kept for several days in water. Evident are the regions without root hairs whose growth had been inhibited. Below these regions root hairs have grown already in water

Fig. 3. — Prophase in a cell from the root of *Vicia* after 12 hours in 0.8% *Poria* extract. Note despiralization of the chromosomes

Fig. 4. — Prophase in a cell from the root of *Allium* after 48 hours in 0.8% *Poria* extract and after 24 hours in water. Tape-like bridge-connected agglomerations of vacuolated chromatin

Fig. 5. — Prophase — as above. Tape-like agglomerations of vacuolated and fragmented chromatin are evident

Fig. 3, 4 and 5 root tips fixed in acetoalcohol (1 : 3), smear preparations were stained with acetoorcein. Microphotographs in anoptral contrast. Magnified ca 1500 ×

Plate II

Fig. 1. — Side view of metaphase in a cell from a control root of *Allium*

Fig. 2. — Metaphase in smeared cell from a control root of *Allium*

Fig. 3. — Metaphase in a cell from the root of *Allium* after 24 hours in 0.7% *Poria* extract. Shortening of chromosomes is evident

Fig. 4. — Metaphase in a cell from the root of *Allium* after 48 hours in 0.8% *Poria* extract. Shortening of chromosomes to vacuolated, granular forms is evident

Figs. 5 and 6. — Metaphases in cells from the roots of *Allium* after 36 hours in 0.8% *Poria* extract. Diminution of size and vacuolation of chromosomes is evident

Root tips were fixed in acetoalcohol (1 : 3). Smear preparations were stained with acetoorcein. Microphotographs of Fig. 4, 5 and 6 in anoptral contrast. Magnified ca 1500 ×

Plate III

Fig. 1. — Anaphase in a cell from a control root of *Allium*.

Fig. 2. — Late anaphase in a cell from the root tip of *Allium* after 24 hours in 0.8% *Poria* extract. Note shortening of chromosomes and "vacuolation" caused probably by despiralization and contraction of chromosomes

Fig. 3. — Early anaphase — as above. Diminution of chromosomes is evident

Fig. 4. — Anaphase in a cell from the root tip of *Allium* after 24 hours in 0.8% *Poria* extract. Diminution of chromosomes as well as constrictions and deformations are evident
Root tips were fixed with acetoalcohol (1 : 3). Smear preparations were stained with acetoorcein. Microphotographs of Fig. 2, 3 and 4 in anoptral contrast. Magnified ca 1500 ×

Plate IV

Fig. 1. — Anaphase in a cell from the root of *Allium* after 48 hours in 0.8% *Poria* extract and after 24 hours in water. Note shortening of chromosomes, various types of vacuolation, agglomerations, and deformations

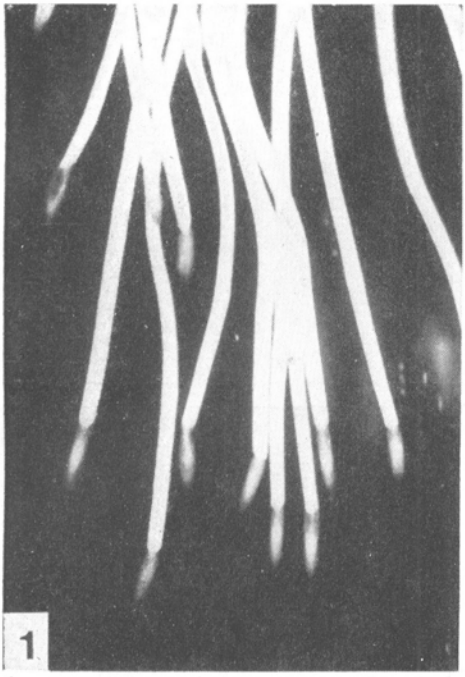
Fig. 2. — Late anaphase in a cell from the root of *Vicia faba* after 48 in 0.8% *Poria* extract and after 24 hours in water. Chromosomes have shortened to spherical forms.

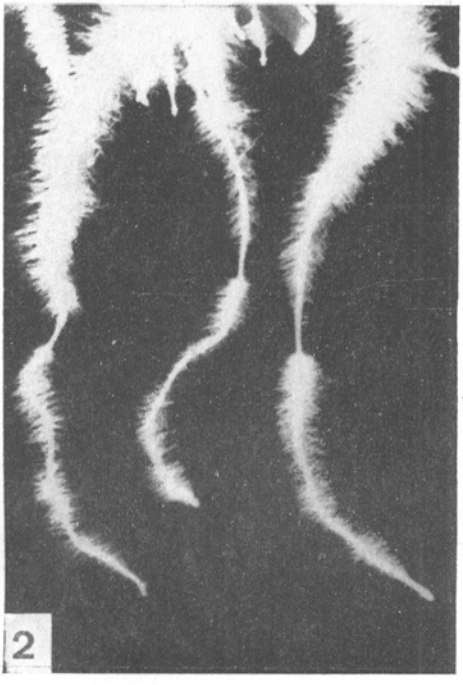
Fig. 3. — Telophase in a cell from the root of *Vicia faba* — as above. Fragmentation of vacuolated chromosomes is visible.

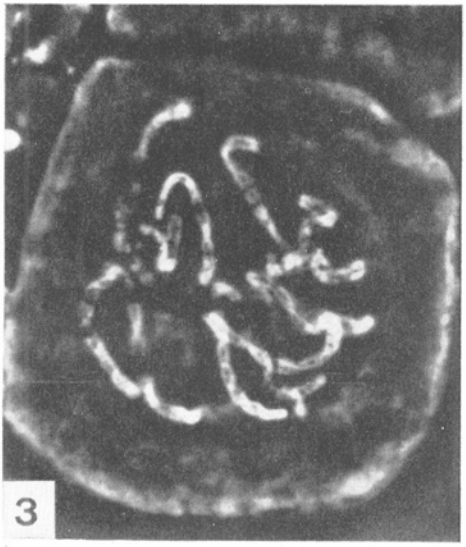
Fig. 4. — Anaphase in a cell from the root of *Vicia faba* — as above. Note diminution and vacuolation of chromosomes

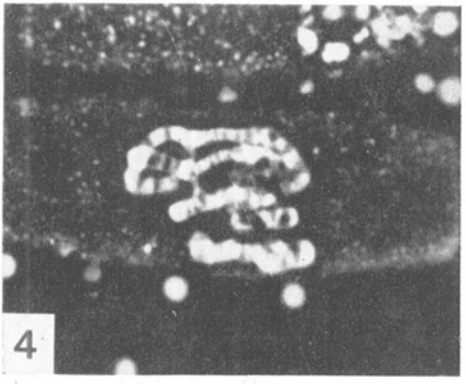
Fig. 5. — Anaphase in a cell from the root of *Allium* after 48 hours in 0.8% *Poria* extract and after 24 hours in water. Diminution, vacuolation, constrictions and fragmentation of chromosomes are evident
Root tips fixed with acetoalcohol (1 : 3). Smear preprations were stained with acetoorcein. Microphotographs in anoptral contrast. Magnified ca 1500 ×

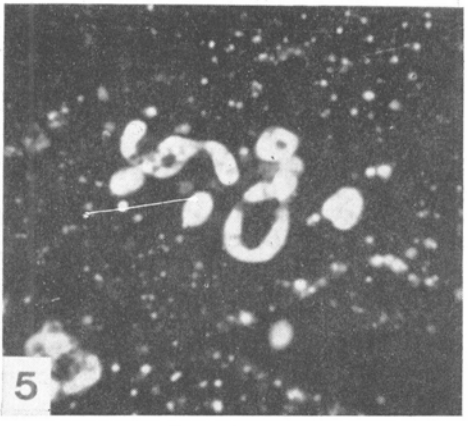
Plate I











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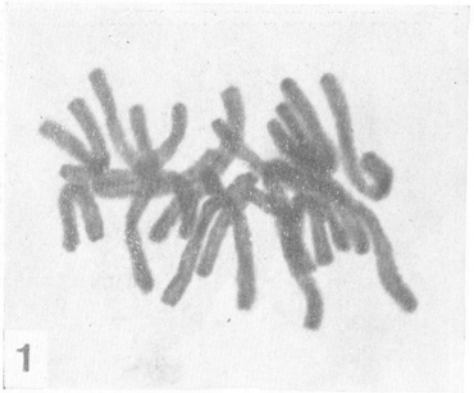
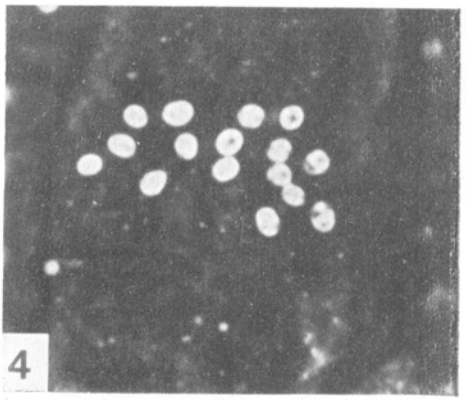
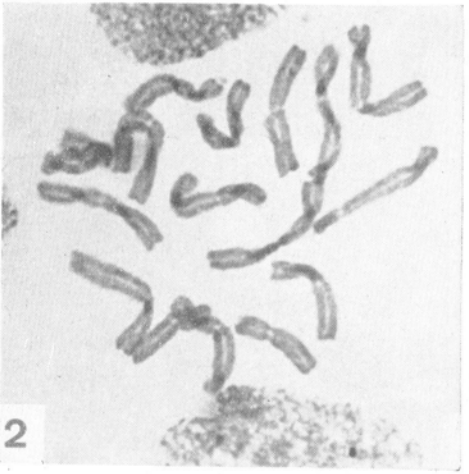
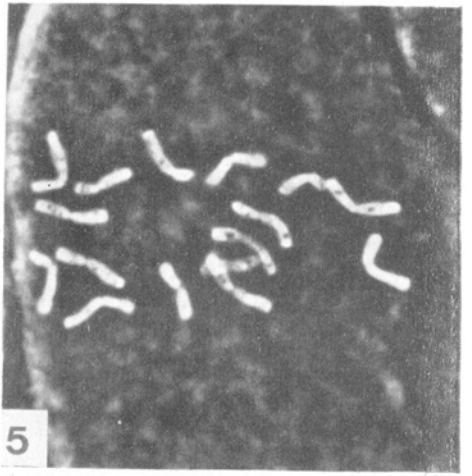
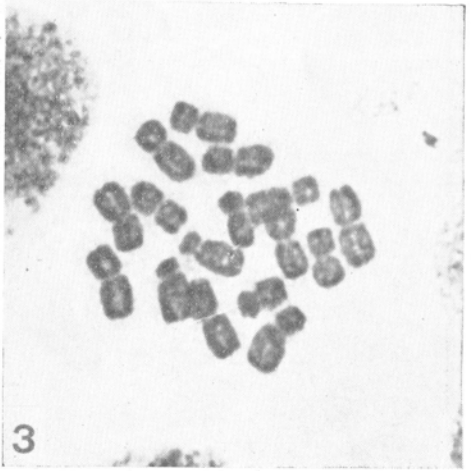










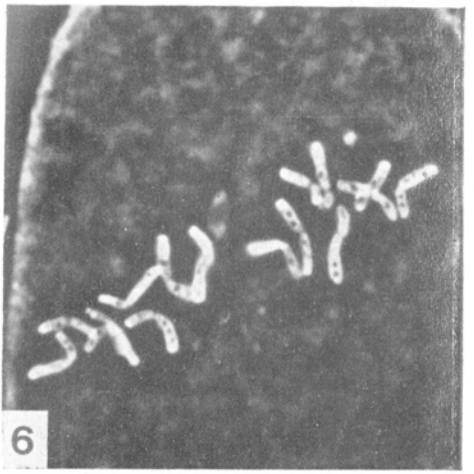
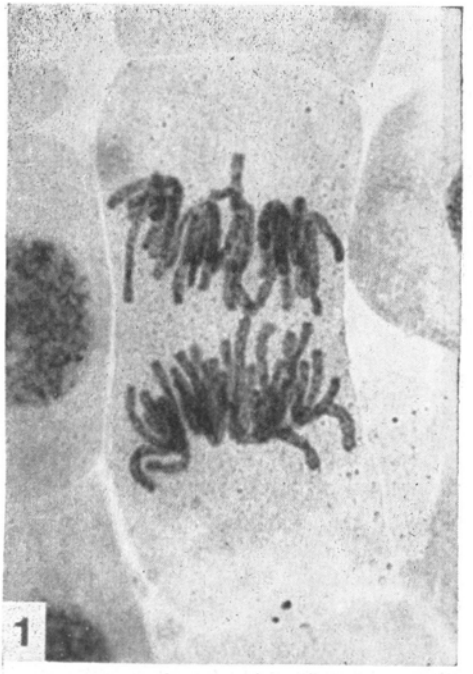
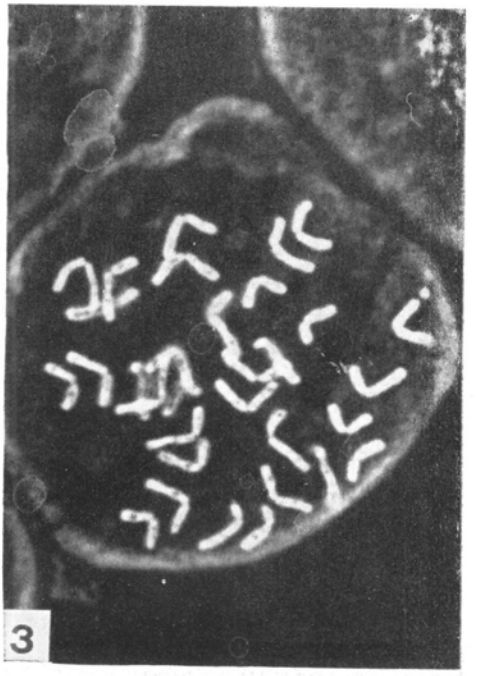
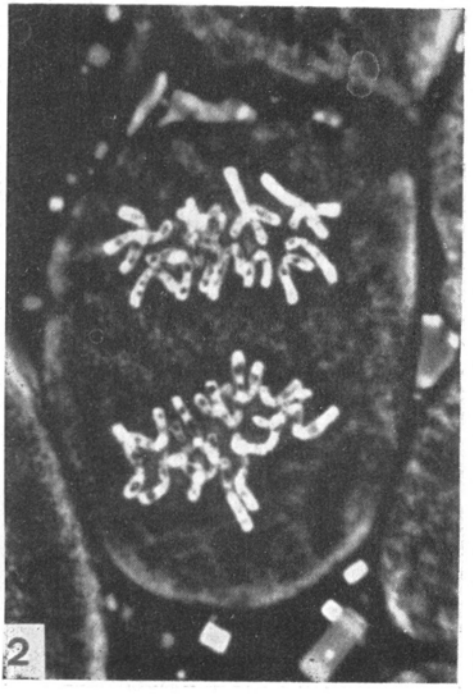


Plate III







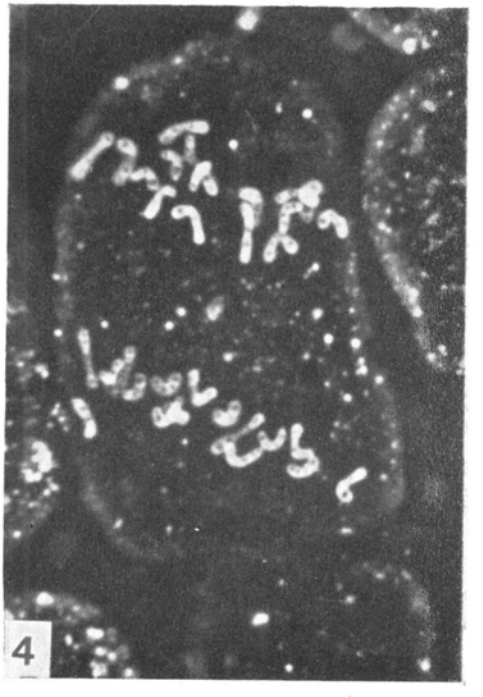


Plate IV