In recent years the effects of various chemicals on animal and plant tissues have received considerable attention, in view of the rôle of tar and certain hydrocarbons in the production of malignant growths. The mechanism of tumor formation is unknown, although the causative agents of some types of overgrowth are well recognized. The study of the effect of carcinogenic agents on the root tip of the onion is especially desirable, since the species is well known cytologically and has been considered by animal and plant cytologists as the standard material for the study of the physiology of the cell. The gross effects of some carcinogenic agents such as coal tar, dibenzanthracene, and Scharlach R on plants have been reported (Levine, 8). The present report deals with the effects of the same chemicals on the root tips of Allium cepa, the common onion.

The earlier literature is well known and has been reviewed a number of times. Here only a brief résumé will be given of the work that has developed in connection with the study of coal tar and other chemicals, which, some writers believe, are carcinogenic on roots.

Komuro, in 1930–1931 (2, 3, 4) studied the effects of short exposures of root tips of Vicia faba and Pisum sativum to coal tar suspensions and described a number of cellular changes, such as the vacuolization of nuclei, chromosome aberrations, fragmentation of the nuclei, giant cells, and hypochromatic cells. He exposed root tips of the plants to coal tar for periods of seven and a half and ten minutes, washed them in tap water, and then fixed them. In a later study (5) he increased the period of exposure to fifteen minutes, thirty minutes, and five days. In roots exposed for fifteen or thirty minutes the presence of vacuolized nuclei, which became pyknotic, was observed; multinucleate cells and nuclei with many nucleoli were common, and amitotic divisions were present. Many nuclear divisions were noticed, but all, Komuro contends, were abnormal. Great stress is laid upon the difference between root tips washed after exposure and those unwashed.

Vicia faba root tips exposed for five days were fixed in alcohol. The cells showed shrinkage, exposing large intercellular spaces; hyperchromatic and pyknotic nuclei are described as the dominant features of these cells.

In these roots Komuro describes a structure which he calls Phytotoxic-tumor, found in the periblem above the growing point. His belief is that the tumor is formed through amitotic divisions of the cells in this region. In other roots, the piliferous layer may give rise to nodules. In the tissue surrounding these nodules, especially the plerome, Komuro reports all the cytological abnormalities mentioned above.

While Komuro’s figures are not entirely convincing, the reported phe-
nominal are of interest since some of these details are suggestive of the nuclear changes reported for animal and plant tumors (Levine, 7).

Mottram (10) assumes that the difference between normal and cancer cells lies in their content of genes. Disturbance of the chromosomes, he argues, must result in changes in the genes. To effect these changes, he subjected growing bean roots to gamma radiations from radium, to water heated to 49°-50° C., to coal tar, and to gentian violet solutions. Roots so treated were fixed in Bouin's fluid and stained with iodine gentian violet or Heidenhain's iron hematoxylin. Mottram found that these treatments did no more than temporarily decrease the rate of growth of the roots. Cytological studies centered about the anaphase stages. Mottram noticed fragmentation of chromosomes together with delayed migrations of the chromosomes to the pole of the spindle. He stresses the importance of the penetrability of the agent in affecting the mitotic structure as significant in cancer production.

Lewis (9) believes that malignant cells are permanently altered cells which are capable of reproducing their kind. He contends, however, that malignancy is due to alterations of the cytoplasm rather than to changes in the chromosomes or genes. While the somatic mutation theories of Whitman (12), Bauer (1), and others, have not advanced our knowledge of the etiology of cancer, the striking phenomena observed in tar-treated roots, as reported below, seem to be essentially an effect on the cytoplasm with little change in the chromosomes except in so far as these are influenced by the state of the cytoplasm.

Ortiz Picón (11) exposed root tips of \textit{Allium sativum} to tar for five to fifteen minutes and kept them in water twenty-four to twenty-eight hours after the exposure. After washing they were fixed in "Susa." The cells in the pleome and periblem showed plasmolysis, then chromosome changes, and finally complete disintegration of the nuclear material in the cytoplasm. Meristematic cells with two to three nuclei were observed, but no giant cells nor any of the atypical proliferations reported by Komuro. Roots exposed for longer periods were destroyed. Ortiz Picón believes that the cellular changes originate in the osmotic disorders which produce the plasmolytic phenomena and that the tar does not have a specific effect upon the roots.

It has been pointed out (Levine, 8), principally in the sunflower, \textit{Ricinus}, and tobacco, that repeated painting of the organs of these plants fails to produce recognizable external tumorous masses like those produced by the crown-gall organism. An histologic study of the treated organs, which is now in preparation, shows marked proliferation of the meristematic tissue with the differentiation of the newly formed growth into parenchyma and xylem, and ultimately the aberrant overgrowth of wood. Swellings of the stem are common and appear in many instances to be more pronounced than those of control plants which were only injured.

\textbf{Methods and Materials}

Our experimental studies consisted in subjecting root tips of the bulbs of \textit{Allium cepa}, the common onion, to coal tar, Scharlach R, dibenzanthracene, and, as controls, petrolatum and a mixture of ether and water. The coal tar
was applied in two ways. In the first, 10 c.c. of tar were dropped in a jar containing 100 c.c. of tap water. The tar was then raised with a glass applicator and a single drop floated on the surface of the water in which the roots were to be tested, forming a thin covering or film. A modification of this method consisted in preparing an emulsion of tar in water by thoroughly shaking 5 to 10 gm. of tar in 90 or 95 c.c. of tap water. This failed to yield accurately measured suspensions, for a considerable quantity of tar adhered to the surface of the flask. The second procedure was the Mottram method: coal tar was shaken with approximately 10 times the volume of ether; this in turn was mixed with 100 times its volume of water. Here, also, a considerable amount of the tar remained in the mixing flask. This treatment made a finer emulsion and gave the water a brown coloration with a considerable amount of tar floating on the surface.

Scharlach R is readily soluble in ether. We used 0.2 gm. in 10 c.c. of ether, which was dissolved in 1 liter of tap water. For the dibenzanthracene experiments 0.1 gm. of 1:2:5:6-dibenzanthracene in 25 c.c. benzene was added to 960 c.c. of tap water. The mixture was thoroughly shaken. The dibenzanthracene was almost entirely dissolved in the benzene but some of it recrystallized on the basal portion of the bulb around the root area, and also on the surface of the water. The presence of ether and benzene was detectable for three to four days after the onions were exposed to these solutions.

Bulbs of a yellow variety of Allium cepa of a uniform weight, approximately 80 to 100 gm., were used throughout these experiments. The bulbs were examined for molds and other saprophytes. They were carefully washed and the outer layer of scales was removed. They were then weighed and placed in glass cylinders of 320 c.c. capacity, which were imbedded in sand to the mouth of the vessels or covered with black paper. The root end of the bulb fitted into the mouth of the cylinder so that this portion of the bulb was always exposed to the material in the cylinder. The plants were exposed in a moderately lighted room, the temperature of which varied between 20° and 24° C.

A long series of exposures was made, especially with the coal tar preparations. All were repeated a number of times. The short exposures were for five minutes up to twenty-four hours. During the first hour the roots were exposed for five minutes, ten minutes, fifteen minutes, twenty minutes, thirty minutes, forty-five minutes and sixty minutes; then every hour until the twenty-fourth hour. Treated roots were studied forty-eight hours up to ten days after exposure at twenty-four-hour intervals and then at longer intervals up to twenty days. Roots were also studied from one to six days, at daily intervals after exposure. Control experiments were conducted concurrently in each series. The effects of injury on untreated and treated roots were also studied. Over 115 bulbs were used in these experiments.

The root tips, after definite exposures, were fixed in Bouin's fluid, Flemming's weaker solution, or Meves fixative. The roots were imbedded in 52° to 54° paraffin. Serial sections were made 5 to 7.5 microns in thickness; these were stained by Flemming's method and with Heidenhain's iron hematoxylin.
GROWTH OF NORMAL AND TARRED ONION ROOTS

On exposure of the root end of healthy onion bulbs to water, small fibrous roots are formed within twenty-four hours. With daily changes of water the roots continue to increase in length, growing 2 cm. the first and 0.5 to 1 cm. on succeeding days. New roots are formed so that at the end of ten or eleven days, a yellow onion of 80 to 100 gm. will have produced about 60 roots with an average length of 10 to 12 cm. The roots are long, straight, glistening white in color, and the tips are just perceptibly thickened.

Viable onion bulbs set on the surface of water which is covered with a thin layer of coal tar show a decided reduction in the size and number of roots formed. In one series of experiments, 9 onions produced an average of 24 roots each, with an average root length of 4 cm. after a period of eleven days. The daily increase in growth averaged about 0.3 to 0.5 cm. The

roots were brown in color and in many instances thin layers of the tar adhered to them. The water-coal tar cultures were changed daily. The roots frequently showed hooked tips, twisting, and in a considerable number of instances bulbous tips, giving the impression of diminutive clubs. These club-shaped structures developed from the root tip or from a region slightly above it. They first made their appearance at the end of three days, progressed for a short time, and then seemed to disappear.

Secondary roots were observed on the tar treated bulbs in old cultures only, while on the untreated roots the secondary growths appeared on the seventh to the tenth day, and earlier when the distal parts of the primary roots were injured. These secondary roots generally occupied a position on the proximal end of the roots. Later the distal portion showed them also.

In another series of experiments, 24 bulbs of approximately 80 to 90 gm. were set in fresh water, at a room temperature of 20°–24° C.; the roots formed were counted and measured after three days. Sixteen bulbs with an
approximately equal number of roots, of approximately the same length, were selected. All the roots were removed at the point of emergence from the bulb. Twelve bulbs were set in water covered with a drop of coal tar, so that the root end of the bulb touched the surface of the tar and water. Fig. 1 shows 2 bulbs (A) with their second crop of roots after eight days' treatment, and one of the control bulbs (B). The roots of the control are three to four times longer than those of the treated bulbs and are more numerous. The treated bulbs show the characteristic growths described above. The tar and water mixture was changed daily and an equal quantity of tar was used every time the water was changed.

These roots survived for a period sufficiently long to enable the plant to react to the irritant. It appears that the toxic effects produced by the tar make their appearance gradually; they inhibit cell division, a fact which is supported by the observations of Ortiz Picón (11) and Mottram (10) and amply confirmed by numerous serial sections of the roots in our experiments. The club-shaped root tips which begin to appear on the third day, and which represent types of cell proliferation and hypertrophy, are apparently responses to the effects of the trauma and possibly of the tar. These structures may be considered a type of neoplasm, though no analogous growths have been observed in animals known to the writers.

The gross effect of Mottram's tar method on roots of the onion was also tested. It is quite possible that the differences in the physical make-up of our materials vitiated the beneficial effects of dissolving the tar in ether. When bulbs were placed in the cylinder containing the ether-tar-water preparation the expected evaporation of the ether did not occur readily. A chemical analysis of the mixture covered by the onion for eight days, kindly made for us by Doctor Emil J. Baumann, showed a negligible quantity of ether. Yet ether was detected in these preparations after two or three days depending upon the room temperature. It is well known that ether is soluble in water up to 10 per cent.

A series of onions of the same general weight was treated by Mottram's
The bulbs were first set out in fresh water to insure their root-producing power. After seventy-two hours those which failed to grow were discarded while those that had produced a fair number of roots were set in the ether-tar-water preparation. Twelve bulbs formed an average of 13 roots each in water. When these bulbs were placed in jars filled with an emulsion of coal tar-ether-water in proportions of 1:10:1000, the roots became thin and apparently dehydrated within forty-eight to seventy-two hours. Only the root tips appeared to remain unchanged. On the fourth day they regained their original thickness, but on the fifth day they were soft and flaccid, and collapsed when removed from the water. The roots did not increase in size but disintegrated, forming a slimy mass in which an abundance of bacteria were found. When these bulbs were left in the same mixture it was noticed that new roots (Fig. 2) began to make their appearance on about the tenth or eleventh day. From this time on the Mottram solution gave results similar to those obtained with the coal tar emulsion in water or the coal tar film on water, the ether apparently having entirely evaporated. It appears that the ether added to the coal tar had a marked toxic effect causing pronounced plasmolysis at first and then cytoplasmic changes together with the coagulation of the nuclei and, finally, death of the entire root. The new roots appeared singly or in twos, but rarely in greater number.

A cytological study of these first formed roots showed normal structures but the number of division figures appeared to be less than in water-grown roots. From a comparative cytological study it is clear that the ether exerted a marked toxic effect on the roots.

Roots were also subjected to Scharlach R in ether and water, to ether and water, and to dibenzanthracene in benzene and water. Such roots showed slightly different reactions grossly. Those exposed to Scharlach R and ether became stained after twenty-four hours; they were flaccid but by the forty-eighth hour they had recovered their turgidity and appeared to be growing; on the fifth day new roots began to develop.

The roots exposed to ether and water became flaccid, lost their turgidity, and began to accumulate slime by the forty-eighth hour. When these roots were washed in water they seemed to recover.

Roots in dibenzanthracene in benzene shaken with water showed loss of turgidity in twenty-four hours. There was a strong odor of benzene from the water for 116 hours. The roots failed to grow and no new roots were formed.

Roots of the onion three days old were set in a film of petrolatum on water, and studied for six days. Up to the fourth day they grew slowly. After that there was little growth and no new roots were formed. It seems possible that petrolatum interferes with the normal function of the roots. After the sixth day the roots appeared oil-soaked and somewhat translucent. They became woody and hard at this time.

**The Normal Tissues of Allium cepa Roots**

In longitudinal sections of the normal root tip of *Allium cepa* eight days old, 4 distinct areas are recognizable as shown in Fig. 3. The growing center of the root tip is shown at 'a.' It consists of small embryonic cells which by
FIG. 3. **LONGITUDINAL SECTION OF NORMAL ROOT TIP GROWN IN TAP WATER FOR EIGHT DAYS,**
**SHOWING EMBRYONIC TISSUES OF THE ROOT**

\[a = \text{growing center; } b = \text{plerome; } c = \text{periblem; } d = \text{dermatogen; } e = \text{calyprogen.} \times 190.\]

FIG. 4. **CROSS-SECTION OF ROOT SHOWING ORIGIN OF SECONDARY ROOT. \(\times 95\)**
FIG. 5. LONGITUDINAL SECTION OF ROOT ABOVE ROOT TIP GROWN IN WATER FOR NINE DAYS
Note development of root anlage. × 95.

FIG. 6. AREA FROM SECONDARY ROOT ANLAGE, SHOWING BELATED CHROMOSOMES ON SPINDLE.
× 835
growth and division give rise to the root. The lower cells of this growing center give rise to the root cap or calyptrogen, 'e.' This tissue consists of a layer of irregularly shaped cells which protect the embryonic, growing center. As new cells are formed, the lower cells of the older, outer layer disintegrate. The cells formed from the upper layer of the growing center give rise to the tissues which form the root proper. The central cone of cells, the plerome, 'b,' forms the central cylinder with its fibrovascular bundles. The cone of tissue surrounding this central layer, the periblem, 'c,' forms the cortex of the root and frequently serves as the storage organ. Another layer

![Image](image_url)

**Fig. 7. Giant Cell with Nuclei of Different Sizes, from Root Traumatized Six Days Previously.** × 200

of cells surrounding the cone of parenchymatous cells is the epidermal layer or dermatogen, 'd.' This consists of a thin layer of tissue one to two cells in thickness.

The young root, besides showing early differentiation into these several tissue layers, presents different zones of activity. In the region of the growing center the cells are small. The cell walls generally stain faintly, and the nuclei are small and actively dividing. The cytoplasm forms a dense granular reticulate structure. Vacuoles are small and most frequently lacking. At a distance of 3 to 4 mm. back of the growing center the cells divide less frequently, and in the central cylinder the cells are larger. Here the nuclei are clearly differentiated. The zone is one of growth. Vacuoles in the cytoplasm are relatively small.
Still further back of the growing point, a distance of 1 cm. from the tip, the cells are elongated and assume the characteristic structure of the mature root. The cell is occupied by a large central vacuole with a delicate layer of cytoplasm lining the cell wall. The nucleus is peripherally placed. Cells on the periphery of the central cylinder, the endodermis or pericycle, from which secondary roots take their origin, are shorter and more densely filled with cytoplasm. The nuclei are well differentiated and the cytoplasm shows small vacuoles.

Fig. 4 is a cross section of a young root showing the structure and seat of origin of a secondary root. Fig. 5 is a photomicrograph of a longitudinal section of a root showing a secondary root anlage. An enlargement made of a small portion of this nodule is shown in Fig. 6. Special attention is drawn to these figures, since the mass of tissue which forms the secondary root develops by growth and differentiation into a secondary root only after or about the time it emerges from the mother root (Fig. 4). In older portions of slightly injured roots, multinucleate giant cells (Fig. 7) were observed. These cells were found in the cortical and stele portions of the root. We have been unable to find any mention of these cells in the literature except for those references to tar-treated roots made by Komuro (1932). We studied a large number of cross-sections of roots in which injuries induced large lacunae which became infected with spore-producing molds.

**The Effect of Injury on Roots of Allium cepa**

Normal roots two or three days old were injured by longitudinal scratches or small pricks about the root tip, or by gently squeezing the root tip. At various intervals from two to six days after injury, the roots were fixed and studied microscopically.

The injured roots grown in water change their direction of growth and increase in length is interfered with. However, after a period of four to five days they resume a normal appearance. Injured roots invariably produce secondary roots much earlier than normal roots. Frequently, evidences of these lateral roots appear on the sixth day.

A study of the root tips two days after injury is inflicted shows evidence of some necrosis and some disturbance of the cell layers due to regeneration. The cells are clearly differentiated and the healing effects are quite in evidence. A far more striking effect produced by delicate injuries to the root tip is shown in Figs. 8 and 9, fixed three days after trauma. Fig. 8 is a photomicrograph of a longitudinal section of a root showing a thickened, club-shaped root tip with some necrotic tissue in its center. There appears to be considerable regeneration with hypertrophy, as shown in enlargement, Fig. 9. Centers of cell proliferation seem to be present also. The root cap has entirely disappeared and is replaced by a thickened epidermal layer of cells, free of nuclei. The cells back of the necrotic areas appear under higher magnification to be active and stimulated by the injury. This preparation is suggestive of a hyperplasia. It is evident that injury to the onion root causes reactions which are significant, with the production of swellings consisting of hyperplastic and hypertrophic cells.
FIG. 8. LONGITUDINAL SECTION OF A ROOT TIP FIXED THREE DAYS AFTER INJURY
Note bulbous root tip. × 27.

FIG. 9. SECTION SHOWN IN FIG. 8, ENLARGED
Note necrotic area, hypertrophied cells and areas of hyperplasia. × 65.
Effect of Coal Tar and Scharlach R on Injured Root Tips

It is well known that the so-called plant cancer, crown gall, can be produced artificially only by introducing *Bacterium tumefaciens* into a young portion of a plant that has been previously injured. A study of the effects of the carcinogenic agents on injured roots was made to determine whether the stimulated, injured tissue would produce overgrowths. Root tips of the yellow onion, slightly injured, were subjected to coal tar and Scharlach R. The gross effects produced by coal tar and Scharlach R dissolved in ether and diluted in water were approximately the same in the injured and uninjured roots. The cytological effect seemed to be due to ether rather than to the solutes. When the coal tar film method was used or when Scharlach R was dissolved in water, the lethal effects were entirely eliminated.

Injured roots three days old were subjected to a tar film for various short periods. Roots immersed in a 10 per cent coal tar emulsion in water for fifteen minutes showed considerable proliferation about the region of injury. The nuclei were coagulated and the staining property of the cells considerably altered so that the safranin used in the Flemming's triple stain was the most conspicuous color in the preparations. There were few or no division figures. Studies of similarly treated roots were made at various periods after the injury and treatment. Such injured roots grown for eighty-three hours after

![Image: Section of Injured Root Tip Exposed to Scharlach R for Forty-six Hours]

Fig. 10. Section of Injured Root Tip Exposed to Scharlach R for Forty-six Hours

Note proliferating tissue at the injured root tip. × 27.
treatment with coal tar showed a breaking down of the root cap tissue with a destruction of the growing center. The nuclei appeared homogeneously stained. Further cytological changes will be dealt with in greater detail below.

Scharlach R dissolved in water had no marked effect on injured roots, which behaved not unlike normal ones. The injured roots were five days old and were subjected to the Scharlach R solution for forty-six hours. Fig. 10 is a low-power view of a longitudinal section of a root tip so treated and is suggestive of Komuro’s *Phytocertumor*. There is an active proliferation of cells, as shown in Fig. 11. Some of these cells are well differentiated and several prophase stages are seen. Complete serial sections of this root show this proliferating tissue to be made up of a globular mass of cells some 6 mm. to 1 cm. back of what was the normal root tip. On closer examination it is noticed that the cells about this nodular mass of tissue are mature elongated normal cells (Fig. 10). It is suggested, in view of Komuro’s (5) observations, that this abnormal tissue may be attributable to the treatment; a more plausible interpretation is that it represents a disturbed anlage of a lateral root. We have observed a number of these nodular masses in injured roots further removed from the root tip. None of these masses occurs in the piliferous or epidermal layer.

An attempt was also made to determine what part of the cytological changes induced in the roots could be attributed to the ether. Mottram (10) assumes that the ether evaporates before it exerts any biological effects on the roots.

Injured roots five days old were exposed to a 10 per cent solution of ether in water. The mouth of the glass cylinder used for exposing these roots was
completely covered by the bulb. The room temperature was 20°–24° C. After 140 hours of exposure growth seemed to have ceased. No further gross changes were observed. The necrotic areas stained deeply. The nuclei in cells about the injured parts were clearly defined, although staining homogeneously. Some of the cells were apparently hypertrophied. It would appear that the nuclear damage persists even after the ether has entirely disappeared. In some cells the cytoplasm appeared to be but slightly injured. There was no evidence of cell division. In injured roots exposed for forty-six to forty-eight hours the cytological changes were more marked. There was evidence of extreme plasmolysis and separation of cells. The nuclei were coagulated, forming deeply staining homogeneous bodies.

It appears from these studies that the evaporation of ether from a solution in water is not sufficiently rapid to eliminate the influence of this substance on the root tissue. It seems, also, that nuclear changes once induced are not overcome, although the cytoplasmic changes seem to disappear after the roots are returned to fresh water.

From these studies it is quite clear that there are at least two distinct effects produced on roots of the onion subjected to injury and subsequently exposed to coal tar and Scharlach R. One consists of cell proliferation together with hypertrophy associated with the injury; the other, quite distinct from the former, is the reaction of the tissue in response to the interaction of the chemicals of the cell and those of the solutions used. The reaction is not specific for the chemicals used, but is an expression of the organism employed.

Cytological Studies on Untreated and Treated Root Tips

Untreated Root Tips: In view of the recent studies of the effects of coal tar and other agents on plant tissues it was found desirable to study more carefully the control material used in these experiments. The untreated root tip 5 mm. to 6 mm. long shows an abundance of nuclear and cell divisions in all stages. The cytoplasm of these cells was carefully observed.

The untreated tissues of the onion frequently show cells in the late anaphase stage, in which belated chromosomes are observed. The rate at which the chromosomes move to the poles of the spindle varies. There is an abundance of evidence that some chromosomes, 2 or more, often remain on the spindle fibers. This phenomenon has been observed in somatic cells and in the normal reduction divisions of a large number of plants and animals. It is known that chromosomes left on the spindle disintegrate or, in some pathological conditions, form small nuclei. No such structures were found in the specimens we studied. The evidence seems to point to the fact that chromosomes on the spindle in early telophase stages are belated and ultimately reach the poles.

Another commonly observed cytological change is the clumping of chromosomes in the early telophase stage. This is frequently observed in normal tissues.

We have mentioned the presence of multinucleate giant cells in injured roots (Fig. 7). No giant cells were observed in our chemically treated roots.
Nuclei with two or three nucleoli are common. Vacuolization of the cells behind the growing center of the root is a usual occurrence.

_Treated Root Tips:_ Our studies on the effects of coal tar, Scharlach R, dibenzanthracene, and ether were made on the root tips of onions two to three days old exposed to coal tar floated on or suspended in water, or to a solution of 1 gm. coal tar in 10 c.c. ether shaken in one liter of tap water. The roots were exposed for five minutes, ten minutes, fifteen minutes, twenty minutes, thirty minutes, forty-five minutes, and hourly for twenty-four hours, then daily for ten days. To study the residual effects of the tar on roots of a number of bulbs, we permitted them to remain in fresh water, changed daily, for varying periods up to twenty days. The effects of the other chemical substances were tested at periods which we considered critical.

Roots of a comparatively large number of bulbs were exposed for short periods. Solutions in which ether was used were made fresh and used immediately afterward. Using the 5 per cent to 10 per cent water emulsion of coal tar on onion roots three days old, for five minutes, we failed to discover aberrant chromosome fusions, or other abnormal behavior of these structures. Figs. 12 and 13 represent two cells from the same root in which chromosomes have divided. The chromosomes in the former have spread through the cell, apparently moving to the poles at different rates of speed. The chromosomes do not stain uniformly and unstained areas appear in them.
In Fig. 13 the chromosomes have divided, while others show longitudinal splitting. The cells with nuclei in the resting condition show considerable plasmolysis. The nucleolar material appears to be of the usual form. The vacuoles are irregular, and the cytoplasm has the appearance of a coarse granular reticulate structure. One of us has studied the question of fragmentation of chromosomes after tar treatment. A large number of serial sections show definitely that many cases of suspected fragmentation of chromosomes after this period of treatment are due to cutting of the chromosomes (Fig. 14).

![Fig. 13. Cells from Periblum Regions of Root Tip Grown in 10 Per Cent Coal Tar Emulsion in Water for Five Minutes. X 1435](image)

Root tips subjected to the Mottram coal tar method for five minutes showed coagulation of the nuclear material and marked plasmolysis. Chromosomes divided or in the process of division appeared unaffected, although some of them failed to stain uniformly, as shown in Fig. 15. Cells appropriately sectioned show a mere band of contracted cytoplasm about the coagulated nuclear mass. It appears most probable that these nuclear and cytoplasmic changes are somewhat augmented by the presence of ether (compare Figs. 14 and 15).
FIG. 14. CELLS FROM THE PERIBLEM REGION OF A ROOT TIP GROWN IN 10 PER CENT COAL TAR WATER EMULSION FOR FIVE MINUTES
Note vacuolization of cytoplasm. $\times 770$.

FIG. 15. CELLS FROM PERIBLEM OF A ROOT TIP EXPOSED FOR FIVE MINUTES TO A MIXTURE OF COAL TAR, ETHER, AND WATER IN PROPORTIONS 1:10:1000
Marked coagulation of nuclei, plasmolysis of cytoplasm, chromosomes in division unaffected. $\times 835$. 307
Roots exposed for longer periods to a coal tar film or emulsion show generalized plasmolysis. The division figures appear to be less numerous than in control roots of the same bulb or roots of the same age from other bulbs. Some epidermal cells stain heavily with safranin; others show a dense cytoplasm with large central vacuoles. Fig. 16 represents a section of a root tip exposed to a 5 per cent coal tar emulsion for fifteen minutes. The epidermal and periblem cells appear markedly vacuolate and plasmolyzed.

In some root tips exposed for twenty minutes to 5 per cent coal tar emulsions in water we have seen chromosomes showing irregular fusions similar to those described and figured by Komuro. Fig. 17 is a photomicrograph of a portion of the periblem in which the chromosomes are clumped. The cytoplasm in these cells is markedly vacuolate. The resting nuclei appear to be normal, although some of them stain densely.

Vacuolization of the nuclei is not a common phenomenon following short exposures to coal tar-water emulsion. Fig. 18 is a photograph of a longitudinal section of a root, showing plerome and periblem regions, which was exposed for forty-five minutes to a 10 per cent coal tar emulsion. The nuclei are in resting and early prophase stages and show irregularly shaped vacuolar areas. This effect is progressive, for after an exposure of an hour the irregular vacuoles become more numerous, with complete disintegration of the nucleus; this occurs simultaneously with the complete vacuolization of the cytoplasm, as shown in Fig. 19.

After treatment with the water-ether-tar mixture, onion roots showed destroyed and plasmolyzed cells. Nuclei were frequently ruptured; this was at first believed to be due to mechanical disturbances in sectioning, but the frequent appearance of these injured nuclei indicates that this is one of the results of treatment.

Root tips treated for six hours show cells in spireme stages (Fig. 20) in which the chromosome band appears contracted and vacuolate. Dividing
Fig. 17. Root Tip Exposed to 10 Per Cent Coal Tar Emulsion for Twenty Minutes
Perihem cells show effect; slight massing of chromosomes; large vacuoles. × 835.

Fig. 18. Root Tip in 10 Per Cent Coal Tar Emulsion for Forty-five Minutes
Vacuolization of cytoplasm, vacuolate nuclei giving spireme effects. × 835.
chromosomes appear much shorter and irregular in shape. In these prepara-
tions it was possible to follow the cells from one section to another. The
evidence points strongly to the contraction of the chromosomes in these stages.

In preparations of root tips subjected for twelve hours to the Mottram
mixture the cell cytoplasm shows evidence of disintegration. The granular
reticulum is coarse and the vacuoles are irregular and tend to fuse. The
resting nuclei in some roots at this stage are clearly differentiated, but the
dividing chromosomes are irregular in shape, varying from short stubby rods
to small globular masses, as shown in Fig. 21. Following twenty-four-hour

![Fig. 19. Root Tip Exposed for One Hour to 10 Per Cent Coal Tar Emulsion](image)

Partial destruction of cells due to intense vacuolization produced in some areas of the root
tip. × 835.

exposure to the same treatment few division figures were observed; the cyto-
plasm became coagulated, forming coarse granular bodies in which some well
differentiated resting nuclei were still visible although most of the nuclei were
coagulated.

In the roots exposed to tar emulsion or tar film the injury induced was
not so marked twenty-four hours after exposure. In these preparations there
are still evidences of dividing nuclei. The resting nuclei are well differenti-
tiated and only an occasional nucleus is homogeneously stained.

In roots exposed to the coal tar film method for three days the epidermal
cells were plasmolyzed and vacuolate. Some nuclei are homogeneously
stained while others are well differentiated. The periblem cells show vacuoli-
FIG. 20. Root Tip Exposed to Tar-Ether-Water for Six Hours, Showing Nuclear Destruction. × 835

FIG. 21. Root Tip Exposed to Tar-Ether-Water for Twelve Hours, Showing Contraction of Chromosomes. × 1920
zation with separation of cells. The cytoplasm and nuclei appear injured and densely stained. After six days’ exposure to coal tar and water emulsion, the epidermal cells became densely stained and failed to differentiate. The cells in the periblem and plerome show deeply stained cell walls, and the nuclei have one or two nucleoli. The cytoplasm is a coarse granular structure and appears aberrant.

We also studied the structure of root tips that were exposed for various periods to coal tar and then permitted to grow in fresh water for periods varying from one to six days. Roots exposed to a film of coal tar on water for thirty minutes were washed carefully to free them, so far as possible, of all tar and were then placed in fresh water changed daily for three days. The majority of the roots showed little growth. Very few division figures were observed in these roots. The nuclei were well differentiated and 2 to 3 nucleoli were found in each nucleus. There were no further changes. A root of a bulb exposed for one hour and set in fresh water for twenty-four hours showed separation of cells, plasmolysis, and vacuolization with few or no dividing nuclei or cells.

Other roots tips exposed for one hour and afterward permitted to grow in fresh water for eighty-three hours showed complete recovery. Dividing nuclei were abundant and there was little cytoplasmic or nuclear disturbance. After eighty-three hours in fresh water, roots previously exposed for twelve hours in a coal tar suspension showed contracted and separated cells, especially the central tier of cells in the plerome; the nuclei in these cells were well differentiated. The epidermal cells, however, showed vacuoles filled with stain; homogeneous nuclei and coagulated chromosomes appeared in some of the dividing cells. These division figures appeared to have been arrested by the treatment.
Fig. 22 is a photomicrograph of a portion of a root tip exposed to coal tar for twenty-two hours and fixed 140 hours later. All stages of disintegration are recognizable. A common type of nuclear disintegration is the signet ring effect frequently observed in radium-treated tissues. These effects have been shown in the cells of the lily anther after exposure to measured quantities of radium emanation (Levine, 6).

Root tips subjected to dibenzanthracene dissolved in benzene and sus-

![Fig. 23. Root Tip Exposed to Scharlach R in Ether for 116 Hours
Coagulation of nuclei, cytoplasmic vacuolization. × 220.](image)

pended in water for seventy hours showed early necrosis. The cells are separated in the plerome layer and the cytoplasm is markedly contracted. The coagulation of nuclear material is most pronounced in the plerome and outer periblum and epidermal layers. Division figures are obscured by the marked cytoplasmic changes.

Scharlach R suspended in ether produced marked effects on healthy root tips after exposure for 116 hours. Here the principal abnormality is the coagulation of the nuclei (Fig. 23), an effect observed in roots subjected to suspensions of ether in water. Root tips after exposure to a 10 per cent
solution of ether in water for twenty-one hours showed similar effects. It appears that the ether exerts a coagulating effect upon the nuclei. Onion roots grown in petrolatum for twenty-five hours showed no cytoplasmic or nuclear aberrations. The nuclei were well differentiated and many stages in nuclear divisions were observed.

**SUMMARY AND CONCLUSIONS**

1. A study was made of the effects of carcinogenic agents, especially coal tar, on the growth and development of roots of *Allium cepa* in relation to tumor formation. Dibenzanthracene, Scharlach R, ether, and petrolatum were also studied. The effect of trauma as an agent in the production of overgrowths was studied on normally developing roots and those subjected to the various agents mentioned above.

2. Roots were exposed to coal tar preparations for periods of five, ten, fifteen, twenty, thirty, forty-five, and sixty minutes, also hourly for the first twenty-four hours and daily for twenty days. The roots were studied macroscopically and microscopically.

3. Roots subjected to emulsions or films of coal tar showed reactions in twenty-four hours. The root tips began to bend and became hook-shaped. The daily increment of growth was reduced. The number of roots formed was only about half that observed in normal bulbs grown in water.

4. Roots exposed to a tar-ether-water preparation for twenty-four hours lost their turgidity and death followed within seventy-two to ninety-six hours. At about ten or twelve days new roots began to appear. They were few in number, and their increase in length took place slowly.

5. Injured roots grown in fresh tap water showed club-shaped structures and swellings. These may represent the only type of overgrowth that these fibrous roots are capable of producing. Histologic study of these growths showed evidences of hyperplasia and hypertrophy.

6. Injured roots exposed to Scharlach R showed overgrowths which were considered to be disturbed secondary root anlagen.

7. Cytological studies of roots exposed to tar films or emulsions for short periods showed from the first hour cytoplasmic changes, plasmolysis, and marked vacuolization. Toward the end of the first hour the nuclei became vacuolate. In these stages chromosomal changes such as contraction and clumping were observed. In later stages (three, six, twelve, and twenty-four hours) there was marked cytoplasmic change, indicating a dehydrating effect. Chromosomal fusions, disintegrations and fragmentation were commonly found.

8. Root tips exposed to coal tar for three, six, and twelve days showed normal cytological structures. Few division stages were present. Many of these roots survived the early effects of the tar. The most marked cytological changes occurred within the first twenty-four hours.

9. Roots exposed to coal tar for approximately one day show marked cytoplasmic and nuclear changes when grown in water for six days after the exposure. Roots subjected to shorter exposures, studied after six days in water, showed apparent recovery. Where ether was used as the solvent the
principal changes seemed to be due to the ether. The nuclear damage persisted.

10. It is suggested that the reactions of the plant are not specific results of the chemicals used but are due to the nature of the organism itself.

BIBLIOGRAPHY

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