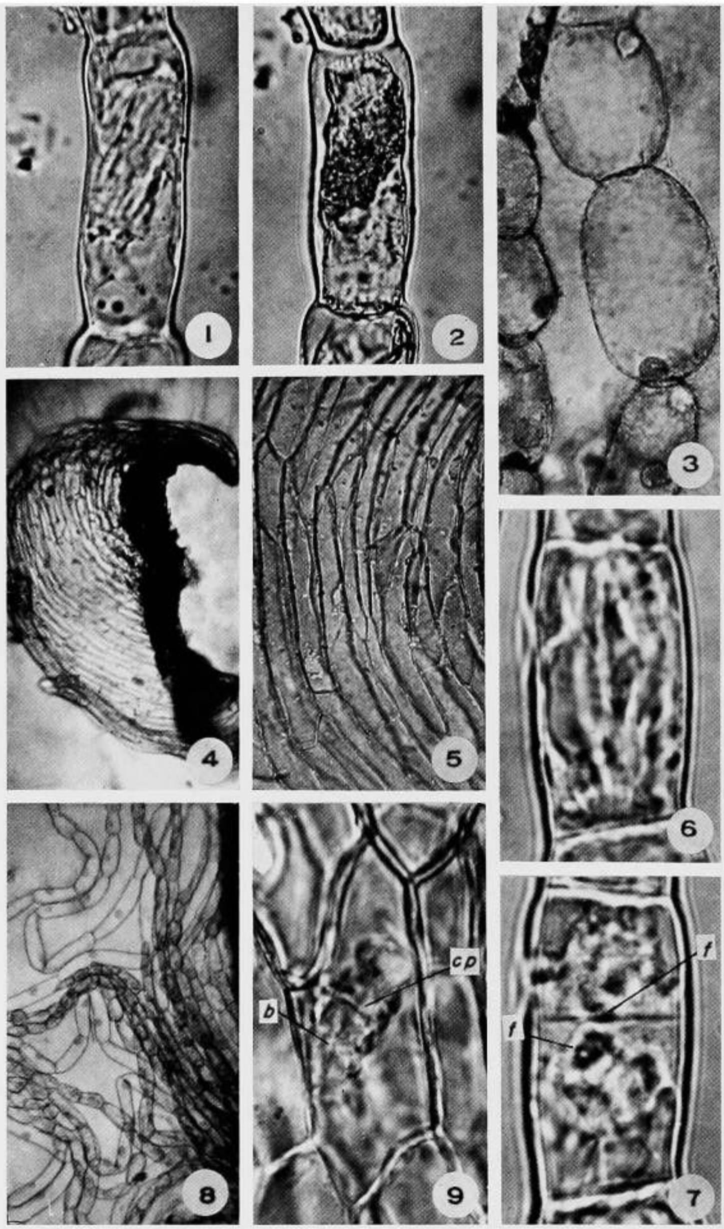
necrosis in a rather nonspecific manner in cells undergoing mitosis (Figs. 1-2). Subjecting cells to 135 ppm picloram reduced the rate of cytoplasmic streaming, but cells in early prophase at the time of treatment completed mitosis even though dicentric bridges were evident in anaphase. The mature cells survived for five days. Reducing the concentration to 100 ppm allowed the hair cells to survive more than 10 days even though cellular abnormalities were apparent within six days following treatment (Fig. 3).

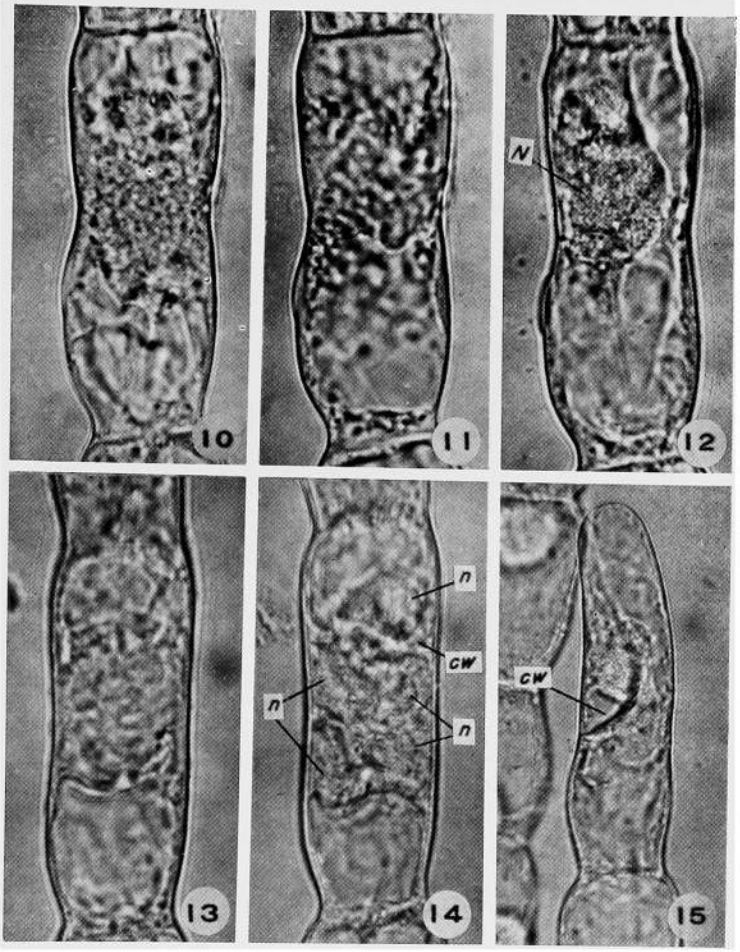
The cytoplasm of leaf epidermal cells of *Vicia* subjected to 135 ppm picloram coagulated within four hours and nuclear structure was destroyed by the second day. However, in a significant number of leaf cells subjected to 20 to 50 ppm, final cell lengths were much greater than in controls. In 10 ppm, elongation of tissue cells was striking (Figs. 4-5). Further-



Figs. 1-9. 1-2, *Tradescantia* staminal hair cell was in midprophase when treated with 200 ppm picloram at 12:57 ca. ×600. 1, cell is now in anaphase and exhibits gradual gelation (14:16). 2, by the next day a binucleate cell has developed and it appears to be dead. 3, growth has occurred in *Tradescantia* cells that have been exposed to 20 ppm picloram for 14 days. ca. ×250. 4, segment of *Vicia* leaf that has shown marked elongation during 5 day treatment with 10 ppm picloram. ca. ×50. 5, higher magnification of Fig. 4 showing marked elongation of cells. ca. ×90. 6-7, *Tradescantia* hair cell treated with 1.6 ppm trifluralin at 14:30. ca. ×1000. 6, dicentric bridges formed in anaphase (16:39). 7,

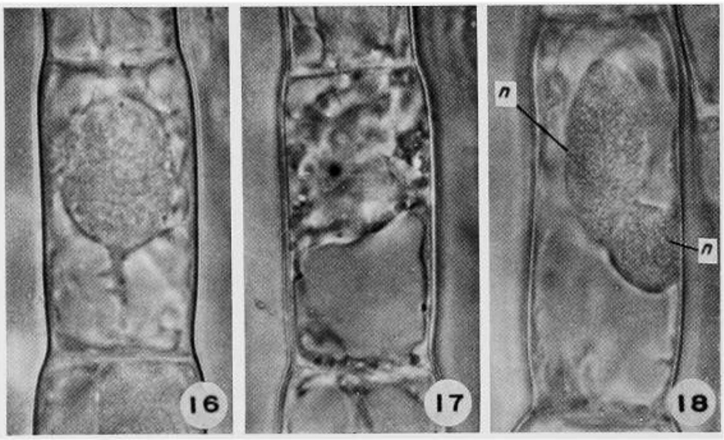
and all cells were dead by the second day. Both chemicals strongly inhibited mitosis. In fact, only cells in late anaphase could complete mitosis when subjected to only 10 ppm of either. The cells survived for five days in 10 ppm 2, 3, 6-TBA, but were dead within three days when treated with the same concentration of 2, 3, 5, 6-TBA. Mitosis could proceed in 2 ppm of either chemical, but dicentric bridges were common in anaphase (Fig. 9).

Although Zimmerman *et al.* (1952) have emphasized that substituted benzoic acids should be considered as growth regulators, the present study indicates that these compounds are capable of inhibiting mitosis and causing abnormal mitotic figures even at low concentrations. Furthermore, these results show that the effects in *Vicia* leaf cells are more pronounced than in *Tradescantia* hair cells.



Figs. 10–15. The effect of nitralin on dividing *Tradescantia* staminal hair cells. ca. ×900 except 15. 10–12, dividing cell treated with 3 ppm at 11:00. 10, cell in early prophase (11:19). 11, irregular metaphase plate (17:13). 12, formation of nucleus with doubled chromosome complement (second day). 13–14, dividing cell treated with 1 ppm at 10:51. 13, chromosomes are aggregating into several clumps (16:24). 14, a five-nucleate cell with multi-septa has formed (second day). 15, a cell with incomplete cell wall photographed on second day following treatment with 3 ppm. ca. ×600.

Nitralin: Hair cells of *Tradescantia* treated when in early prophase with 0.1 to 5 ppm nitralin exhibited mitotic aberrations of various kinds. For example, cells possessing nuclei with doubled chromosome complements (Figs. 10–12) and multinucleate cells with multi-septa (Figs. 13–14) were common. The chromosomes became indistinct and mitosis was greatly prolonged. However, if cells are in late prophase at time of treatment, mitosis proceeds in a normal manner even when subjected to 5 ppm nitralin. In hairs treated with 3 ppm, an occasional cell was found that did not complete cell wall formation following mitosis (Fig. 15). As can be seen in Figs. 16-18, cells subjected to 0.09 ppm could proceed from interphase through mitosis but formed two nuclei of unequal size. At concentrations of 0.01 through 0.08 ppm the hair cells completed mitosis in a nearly normal manner. But, movement of chromosomes in anaphase was delayed and dicentric bridges were common in cells subjected to even 0.01 ppm nitralin.

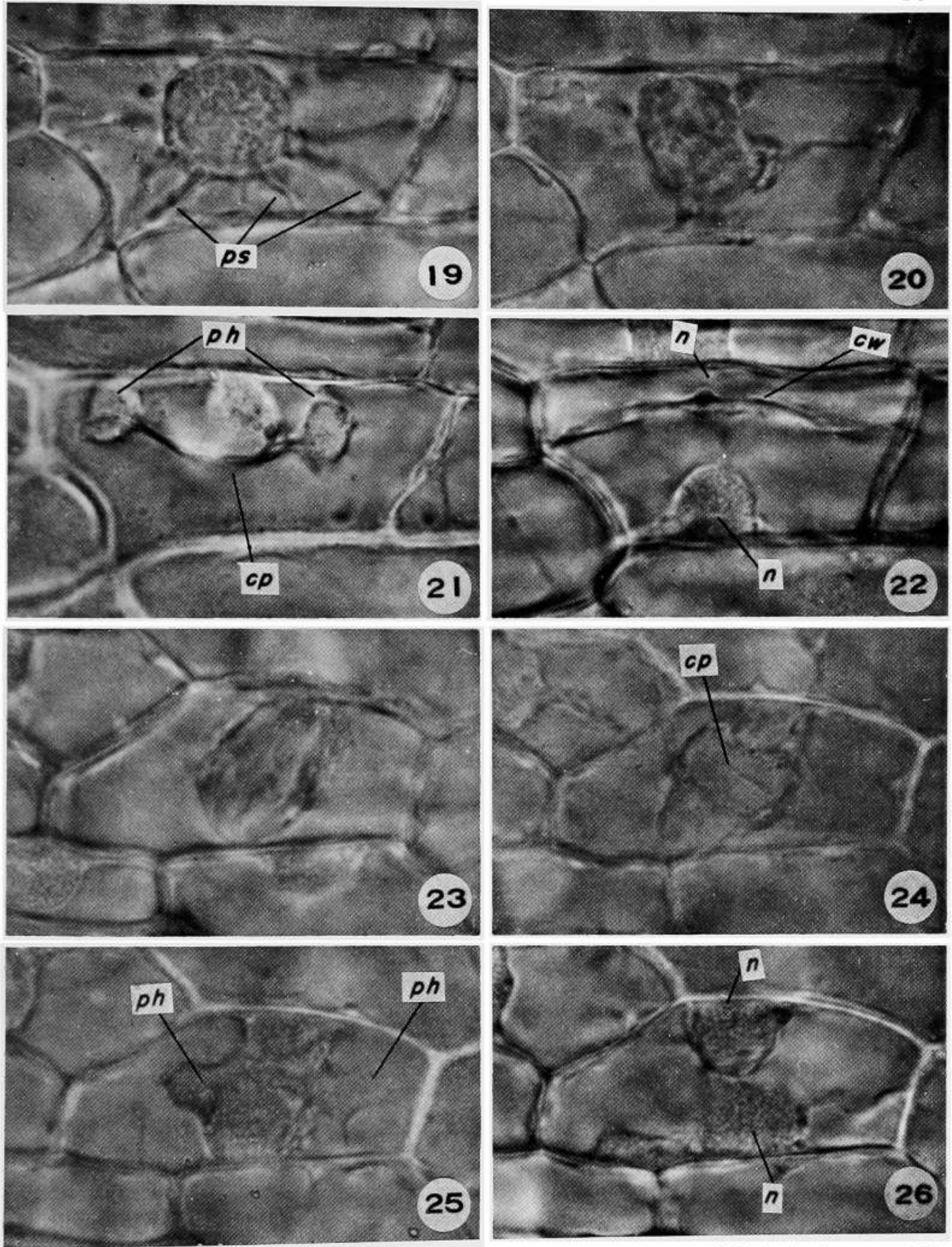


Figs. 16-18. Cell treated with 0.9 ppm nitralin at 9:55. ca. ×750. 16, cell is in early prophase (10:10). 17, cell is now in anaphase (15:34). 18, the cell is now binucleate and the nuclei are of unequal size (third day).

A segment of *Vicia* leaf was sealed in a control agar plate, examined, and a cell in early prophase photographed ten minutes after the preparation was made (Fig. 19). Fifteen minutes later, the agar layer was replaced by an agar layer containing 5 ppm nitralin. Mitosis was able to proceed (Fig. 20). The phragmoplast and cell plate gradually formed, separating the cell into two daughter cells (Figs. 21-22). A cell in anaphase at the time of treatment formed both a phragmoplast and a cell plate (Figs. 23-24), but these then gradually disintegrated (Fig. 25) and eventually a binucleate cell was formed (Fig. 26). Furthermore, when a cell that was already in the process of forming a cell plate was subjected to 5 ppm nitralin, completion of the plate was prevented (Figs. 27-28). Leaf cells in early prophase subjected to 0.3 to 3.0 ppm regressed to interphase. Cells that were in midprophase at time of treatment eventually completed mitosis. Cells in early prophase treated with less than 0.2 ppm were not affected.

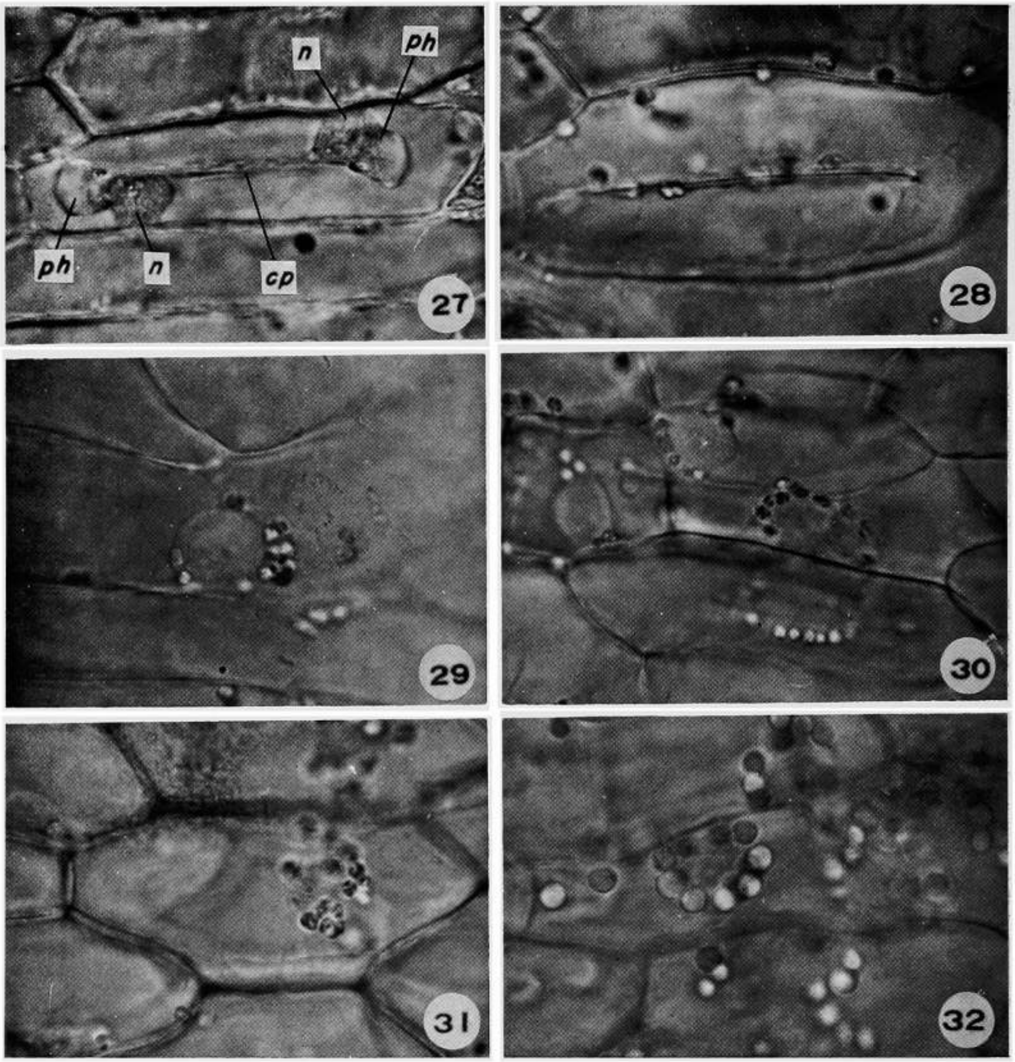
These results, as well as those of Gentner and Burk (1968) demonstrate that effects of nitralin bear a strong resemblance to the effects of colchicine (Derman 1938), napthalene acetic acid (Derman 1941) and IPC and CIPC (Ennis 1948, Sawamura 1965). Our *in vivo* studies further demonstrate that mitosis in staminal hair cells of *Tradescantia*, a monocotyledonous plant, is more severely disrupted than leaf cells of *Vicia faba*, a dicotyledonous plant, by treatment with nitralin. These findings may have a bearing on the selectiv nature of this herbicide.

Specific effects of herbicides upon chloroplast development: Leaf cells of *Vicia* were subjected to concentrations of herbicides that permit cells in early prophase to complete mitosis. That is, they were treated with 20 ppm



Figs. 19-26. The effect of nitralin on dividing cells in segments of *Vicia* leaf. ca. ×900. 19-22. 19, cell in early prophase covered with control agar plate at 13:50 (13:59). 20, control agar plate replaced with agar plate containing 5 ppm at 14:14 (14:22). 21, mid-telophase (15:49). 22, daughter cells are evident (17:01). 23-26, cell in early anaphase observed in same tissue segment as cell described above. 23, early anaphase (14:11). 24, cell in telophase following treatment with 5 ppm nitralin at 14:14 (14:26). 25, cell plate and phragmoplast gradually disintegrating (14:54). 26, binucleate cell appears (17:09).

picloram, 12 ppm pyriclor, and 2 ppm 2, 3, 5, 6-TBA. Similar cells were placed on control medium on the same days, The preparations were examined seven days after beginning of treatment. Cells grown under control conditions possessed numerous large chloroplasts (Fig. 23), whereas chloroplasts in the picloram- and pyriclor-treated tissues were small, even though cells had elongated considerably (Figs. 29–30). Cells treated with 2, 3, 5, 6-TBA grew very little,



Figs. 27–32. ca. ×600. 27, *Vicia* cell in mid-telophase when segment of leaf tissue covered with control agar plate at 14:12 (14:26). Control agar plate replaced with agar plate containing 5 ppm nitralin at 14:28. Phragmoplast material parallel to long axis of the cell had almost disappeared by 15:35. 28, the same cell with malformed cell wall five days after beginning of treatment with nitralin (fifth day). 29–32, chloroplast development in *Vicia* leaf cells observed on the seventh day after treatment with indicated concentration of herbicide. The treatments are such that cells can proceed from early prophase through mitosis during the first day. 29, 20 ppm picloram. 30, 12 ppm pyriclor. 31, 2ppm 2, 3, 5, 6-TBA. 32, control.