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Effect of Reserpine Phosphate on Dividing Onion Root-tip Cells. (24007)

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Goldin *et al.*(1) and Belkin and Hardy(2) have shown that large doses of reserpine, administered to mice carrying lymphoid tumor L 1210 or solid tumor Sarcoma 37, increase survival time and cause tumor regression. Induction of a deeply tranquilized state in animals reported by these authors and noted in our experiments with reserpine, inferred that antitumor activity may be the consequence of prolonged depression rather than an intrinsical and specific antitumor action of the drug. To determine more closely the mode of antitumor action of reserpine, the effects of this alkaloid in various biological systems were investigated. Initial microbiological experiments on *in vitro* activity of reserpine phosphate indicated that it does not act directly on bacteria; no influence on cell multiplication rates was recognized. However, certain colonial modifications and morphological distortions of various gram positive and gram negative cells were observed. Swelling, chain formation, and pigment inhibition were in large measure dependent upon stage of development of the cells concerned. As with many other agents, dividing cells were more vulnerable than resting stages. It was interesting, therefore, to investigate the effect of reserpine on mitosis in higher plants. This was done using root-tip cells of *Allium cepa* var. Silver Skin.

Methods. "Silver Skin" resting bulbs were germinated by placing bulb on top of shell vials of 10-20 ml capacity filled daily with freshly distilled water. The vials were coated with thin layer of paraffin to exclude light. After 3-4 days, bulbs with 15 or more roots were selected for testing. These bulbs were then transferred to similar vials containing 0.125 to 10 mg% reserpine phosphate. The solutions were changed daily for duration of experiment. To obtain as much variation in cell counts as possible, roots from 4 different bulbs were used for each dilution of compounds tested and for controls. Root tips, carefully removed at 24, 48, and 72 hours, were fixed in acetic-alcohol, squashed, and stained with aceto-carmine according to method described by Belling(3). Control bulbs were germinated in water with and without sodium phosphate, smeared, and stained in the same manner as test roots. To reduce deviation and inconsistency, roots were collected at same time each day (1 p.m.)—a time reported by Kellicott(4) to be a period of maximum mitotic division in *Allium* root-tips showing diurnal variation. Totals of at least 1000 cells in the region of cell multiplication were counted for each sample, and the number of cells in mitosis from late prophase to telophase recorded. Countings were made after one, 2, and 3 days in reserpine and after second, third, and fourth day replacement in water. Conclusions reported below are based on determinations of mitotic index and analyses of dividing cell populations in terms of phase percentage. The mitotic index is a ration of dividing to resting cells.

Results. Table I shows mitotic index values from roots exposed 24 hours to various dilutions of reserpine. At concentration of 4 mg%, the compound exerted an irreversible

toxic effect. Within the 24 hour period, at this and higher concentrations, mitotic division was absent; roots presented a flaccid condition from which no recovery was possible even though the roots were placed in fresh water for 4 or more days following reserpine treatment. Arrest was obvious in the late prophase or early metaphase stages. At 2 mg%, the compound lacked toxic properties and at lower concentrations an increase in metaphase as well as total mitotic division was apparent. Increase in mitotic index was greatest at concentration of 0.5 mg%, (Table I). Variation in number of cells in metaphase was correspondingly lower and at 0.25 mg% reached a maximum of 38% more than the controls. It would seem, therefore, that reserpine does not affect the initiating mechanisms of mitosis, nor of the mechanisms of mitosis itself.

For controls, approximately 19,000 cells of 14 untreated roots were counted. The majority (96.44%) were in the resting stage, 1.72% in metaphase, 0.74% in anaphase, 0.61% in prophase, and 0.56% in telophase. Similar values were recorded during daily examination periods throughout the experiments and with sodium phosphate used in comparative studies.

When the counts for different periods of exposure were arranged chronologically (Table II), it becomes apparent that roots exposed to 0.5 mg% reserpine contain a gradually increasing percentage of cells in mitosis and in metaphase reaching a maximum at 24 and 48 hours respectively and declining after 72 hours to a point near the figure observed for untreated root tips. It is interesting to speculate that this difference is due to accelerated growth development in the presence of the compound at specific concentrations. Reserpine phosphate increases mitosis by increasing prophase and metaphase stages.

TABLE I. Mitotic Index of Allium Root-Tip Cells Exposed 24 Hours to Reserpine.

mg %	Total mitosis %	Metaphase %
4	.63	.37
2	3.81	1.77
1.5	3.97	1.98
1	4.62	1.99
.5	5.93	2.10
.25	5.22	2.37
.125	4.57	2.10
.0 (control)	3.66	1.72

TABLE II. Mitotic Index of Root-Tip Cells Exposed to .5 mg % Reserpine.

Hr of exposure	Total mitosis %	Metaphase %
0	3.66	1.72
6	5.40	1.80
24	5.93	2.10
48	4.91	2.37
72	4.35	1.66

After 2 and 3 days growth in 2 mg% reserpine solutions (renewed every day), several roots showed vague swelling and distortion. Such roots replaced in water resumed normal growth, leaving the swollen or distorted area behind. Slight thickenings were also observed in root growth of radish seeds exposed to higher concentration of the alkaloid during seed germination studies. Although these macroscopic observations suggested similarities to the activity of colchicine as described by Levan(5), alteration of chromosome activity was not apparent in these swollen parts, upon microscopic examination. Neither were the colchicine-like effect of spindle solution or clumped condensed chromosomes—the latter condition following retarded prophase noted by Dustin(6) with certain "Radiomimetic" compounds—observed with reserpine treated roots. It may thus be presumed that the action of reserpine phosphate is different from that of colchicine.

Summary. The effect of reserpine phosphate on dividing onion root-tip cells showed that this alkaloid was toxic at 4 mg%. In lower concentration an increase in mitotic index was noted. A maximum ratio of dividing to resting cells was observed at 0.5 mg% after 24 hours exposure to drug. Although an intensification in metaphase was present, the compound was not capable of arresting the metaphase. Thus, reserpine is not a mitotic inhibitor of all cells. If it is possible to draw conclusions from these experiments upon the effect of reserpine on mouse tumors, one would think that regression of mouse tumor under high alkaloid medication is not due to

an intrinsic anti-tumor action of the drug.

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Serum Protein and Glycoprotein Alteration in Swine with Experimental Arthritis.* (24008)

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A naturally occurring inflammatory arthritis of swine which frequently persists following systemic infection by *Erysipelothrix rhusiopathiae* has been described(1-4). A consistent finding in patients with active rheumatoid arthritis is an increased concentration of carbohydrate-rich globulins (serum glycoproteins)(5). Serum glycoprotein levels have been utilized as objective means of evaluating current inflammatory activity of patients with rheumatoid arthritis(6,7). The present investigation is concerned with the study of serum protein and glycoprotein changes in swine following the production of chronic arthritis by injections of *Erysipelothrix rhusiopathiae.*

Method. The incidence of arthritis following acute infection by this organism can be substantially increased by first vaccinating the swine with bacterins and later administering an intravenous challenging dose of *E. rhusiopathiae.* Under such circumstances approximately 90% of surviving animals develop the characteristic inflammatory arthritis(4). Swine of both sexes weighing approximately 200 lb were used. After intravenous injection of 10 ml of a 24 hour culture of *Erysipelothrix rhusiopathiae* in semi-solid medium, all animals developed acute swine erysipelas with high fever followed by death of approximately half of the animals. Initial blood samples were taken from 16 surviving

TABLE I. Effects of Swine Arthritis and Acute Erysipelas Infection on Protein-Bound Carbohydrates and Proteins of Swine.

		Arthritic		
	Normal	Inactive	Active	Initial infection
No. of animals in group	11	4	12	16
Total serum glycoprotein (mg %)*	163 ± 6	162	229 ± 5 †	205 ± 4 †
Seromucoid fraction (mg %)*	12 ± .7	16	31 ± 1.6 †	29 ± 1.9 †
Total serum protein (g %)	7.17 ± .11	7.10	7.47 ± .12†	7.23 ± .09 †
(Total serum glycoprotein + total protein) × 100	2.27 ± .06	2.28	3.10 ± .06†	2.85 ± .06 †
[(Total serum glycoprotein-seromucoid) + total protein] × 100	2.10 ± .05	2.04	2.65 ± .04†	2.45 ± .03 †
Seromucoid + total glycoprotein	.075± .002	.099	.136± .006	.138± .007†

* Expressed as bound hexose (galactose-mannose).
† Significantly higher than normal value at 1% level of probability.

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