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Growth Effects of Pyridine, Piperidine, Atropine Sulfate and Thiamin Chloride on Stramonium Seedlings*

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Recent investigations on the treatment of plants with chemical substances have resulted in well-founded evidence that plants will respond to chemical feedings in varying ways. So numerous are the claims made that only a few, those pertaining to treatments of pharmaceutically important drug plants, can be cited here. Blakeslee, *et al.* (1), have shown that the alkaloid colchicine will produce marked effects on the chromosome arrangement and inhibitory effects on the growth habit of *Datura Stramonium* L. seeds and seedlings. Avery, *et al.* (2), have induced decided growth responses in *Nicotiana* species with the use of several auxins. Zopf (3) showed that indoleacetic acid, α-naphthaleneacetic acid, thiamin chloride, and ascorbic acid in varying combinations inhibited or accelerated growth responses in species of *Belladonna* and *Ricinus;* further, that simultaneous treatments with vitamin B_1 and vitamin C produced antidotal properties toward toxic doses of α-naphthaleneacetic acid.

On the basis of these and other findings (4) that indicate plants absorb chemicals in solution and are thus affected in growth, an experiment was planned to study the effects of pyridine, piperidine, atropine sulfate and thiamin chloride upon *Datura Stramonium* L. This experiment was to serve a two-fold purpose: (*a*) to determine to what extent certain concentrations of these chemicals when applied to growing stramonium seedlings would produce toxic effects, and (*b*) to determine what effect, if any, the feeding of chemical substances with structures similar to the atropine nucleus might have upon the yield of this alkaloid in the leaves of mature stramonium plants.

The procedure of the first part of this plan has been completed and is presented with its results.

EXPERIMENTAL

The chemical substances used in the experiment consisted of chemically pure pyridine, piperidine, atropine sulfate and thiamine chloride. The relative strength of each follows in the text. Soil cultures and pure quartz sand cultures were employed for growing media. Soil cultures consisted of a fine grade peat soil obtained from the University of Minnesota Medicinal Plant Gardens. This was thoroughly sifted and mixed. Several nutrient solutions for use in quartz sand cultures were tried during the preliminary investigations. These included Knop's modified solution and two nutrient solutions from Hoagland and Arnon (5). One

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modified formula devised from the latter was found best suited for use. This nutrient formula is given in the following tables.

TABLE I.—NUTRIENT FORMULA

	Concentration, Cc./L.
Ammonium acid phosphate, 1 *M*	1
Calcium nitrate, 1 *M*	4
Potassium nitrate, 1 *M*	6
Magnesium sulfate, 1 *M*	2

To this solution a supplementary solution supplying boron, manganese, zinc, copper and molybdenum was added (Table II).

TABLE II.—SUPPLEMENTARY SOLUTION

	Concentration, Gm./L.
Boric acid	2.86
Manganese chloride	1.81
Zinc sulfate	0.22
Copper sulfate	0.08
Molybdic acid	0.02

One cubic centimeter of this solution (Table II) was added for each liter of nutrient solution at frequent intervals. This resulted in the concentrations shown in Table III.

TABLE III.—APPROXIMATE CONCENTRATIONS

	Parts per Million of Nutrient Solution
Boron	0.5
Manganese	0.5
Zinc	0.05
Copper	0.02
Molybdenum	0.01

In order to supply necessary iron a 0.5% iron tartrate solution was added about once a week to the nutrient cultures. Additions of this depended upon the chlorotic appearance of the plants. The *p*H of the solution was adjusted to approximately *p*H 6.

Quartz sand used in the experiment was supplied by the Minnesota Quartz Company, Mendota, Minnesota, and was assayed by the University of Minnesota Geology Department. It consisted of 99.6% of SiO_2 and 0.4% of limonite, calcite and iron. This was thoroughly washed before using.

Growing seedlings approximately 2 and 3 cm. in height, without the appearance of the young leaves but with two cotyledonary leaves, were transplanted from soil to each of a series of soil- and quartz sand-containing jars, and treated under glass. Two-gallon glazed earthenware jars were used. Each was equipped with suitable drainage systems.

The experiment[1] was divided into two parts: Part I consisted of the treatment of seedlings with five different concentrations of each of four chemical substances. The purpose of these treatments was to determine the minimum toxic concentration, or that concentration at which plants would show definite toxic effects when regularly saturated with the chemicals. A period of twenty days was allotted for these treatments. Part II consisted of treating quartz sand and soil cultures with a single concentration of each of the four chemical substances. This concentration was chosen only after the results of Part I could be ascertained and was based on these findings. Saturations were made once every three days for the soil cultures and twice weekly for the sand cultures of Part II. A period of sixty days was allotted for these treatments. Untreated controls were run with each series. Before employing the chemical salts, a period of three days was allowed for seedlings to recover sufficiently from the transplantings. All had recovered by the end of this period.

PART I: DETERMINATION OF THE MINIMUM TOXIC CONCENTRATIONS

Five seedlings not exceeding 3 cm. in height were placed into each of 50 glazed jars. Twenty-five of these jars were used for soil cultures and 25 for quartz sand cultures. Each culture was denoted for convenience by a lot number, so that the soil cultures became lot 1 and the quartz sand cultures lot 2.

TABLE IV.—PART I, CHEMICAL TREATMENTS FOR DETERMINING THE MINIMUM TOXIC CONCENTRATION

Chemical Substance	Number of Seedlings Used	Concentrations: Per Liter of Solution[a]	Concentrations: Per Cent
Pyridine	50	5.00 cc.	0.5
		1.00 cc.	0.1
		0.50 cc.	0.05
		0.10 cc.	0.01
		0.01 cc.	0.001
Piperidine	50	5.00 cc.	0.5
		1.00 cc.	0.1
		0.50 cc.	0.05
		0.10 cc.	0.01
		0.01 cc.	0.001
Atropine sulfate	50	0.500 Gm.	0.05
		0.250 Gm.	0.025
		0.100 Gm.	0.010
		0.005 Gm.	0.0005
		0.001 Gm.	0.0001
Thiamin chloride	50	0.0250 Gm.	0.0025
		0.0100 Gm.	0.0010
		0.0050 Gm.	0.0005
		0.0010 Gm.	0.0001
		0.0025 Gm.	0.00025
Untreated (control)	50	.....	.....

[a] Distilled water was used for seedlings of lot 1. Nutrient solution was used for seedlings of lot 2.

[1] The authors are indebted to Dr. W. R. Lloyd for the photographic records of the treatments and to Dr. C. V. Netz for photographic film strips made of the experiment.

Varying concentrations of the chemicals to be used in lot 1 were made with distilled water (Table IV). Daily waterings using 150 cc. of each concentration were then applied. One liter of freshly prepared nutrient solution with varying concentrations of the same chemicals was added daily to each of the quartz sand cultures. The cultures were thoroughly drained before new treatments were made.

Pyridine in Soil Cultures (Lot 1).—A definite growth response was evident for all seedlings receiving different concentrations of pyridine C. P. This was noted in particular for stem elongations. Stem measurements showed an increase in growth from 1 to 2 in. and more for a period of eight days. Following this period those seedlings receiving high concentrations, such as 0.5% and 0.1% solutions, showed a tendency of retarded growth. Two plants developed epinasty of the leaves from which they did not recover. Cotyledonary leaves dropped off as early as the third day of treatment. Plants receiving 0.05%, 0.01% and 0.001% solutions remained vigorous for a period of eighteen days. Following this period these plants failed to grow as actively as did the untreated controls. It was decided to continue the seedlings of Part II with a 0.01% concentration of pyridine.

Piperidine in Soil Cultures (Lot 1).—The response to varying concentrations of piperidine C. P. was nearly uniform for a period of ten days. Those seedlings receiving concentrations greater than 0.01% were slightly retarded in growth. No signs of epinasty of top growth were observed. The growth measurements of stem elongations varied from 1 to 2 in. for the period of the first ten days of feedings. Following this period the seedlings showed a sudden definite retardation of growth. All survived, however (Fig. 1). A 0.01% concentration was used for Part II.

Atropine Sulfate in Soil Cultures (Lot 1).—A favorable growth response was noted for concentrations below 0.05% during a period of twenty days of treatments (Fig. 1). Seedlings treated with a range of concentrations below this strength responded better than did those for either pyridine or piperidine (Fig. 1). Growth compared favorably with that of the untreated controls. Stem elongations varied from 1 to $3\frac{1}{2}$ in. The best response was with concentrations of 0.0005% and 0.0001%. Leaves became broader with concentrations of this strength. Two seedlings treated with 0.05% and 0.025% showed a marked roughening of the leaves. This roughening resembled that reported by Blakeslee (1) for colchicine-treated stramonium seedlings. Seedlings treated with a 0.01% concentration surpassed the piperidine responses but did not equal those of the untreated controls (Fig. 1). This per cent solution was used for continuing seedlings with treatments in Part II.

Thiamin Chloride in Soil Cultures (Lot 1).—The twenty-day growth response of seedlings treated with varying concentrations of vitamin B_1 as thiamin chloride was not uniform (Fig. 1). A greater response was observed among seedlings treated with concentrations lower than 0.250 Gm. per liter of solution. Stem elongations of these cultures measured from 2 to 5 in. Stem elongations ranged from $\frac{1}{2}$ to $1\frac{1}{2}$ in. longer than for the untreated controls and from 1 to 3 in. longer than for pyridine and piperidine treatments in twenty days. Leaf growth and stem growth at the concentrations of 0.001% and lower were found most satisfactory.

Controls in Soil Cultures (Lot 1).—Seedlings grown in untreated soil cultures (Fig. 1) and watered once every three days reached an average height of 3 to 4 in. for the period of twenty days. Leaf and stem growth was generally better than for pyridine, piperidine and atropine sulfate treatments. Stem elongations were slightly less than for thiamin chloride treatments of low concentration, but greater than those at strengths above 0.025%.

Pyridine in Quartz Sand Cultures (Lot 2).—Seedlings receiving 0.5% and 0.1% pyridine in nutrient solution for a period of eight days showed almost as vigorous a net growth response as did the controls

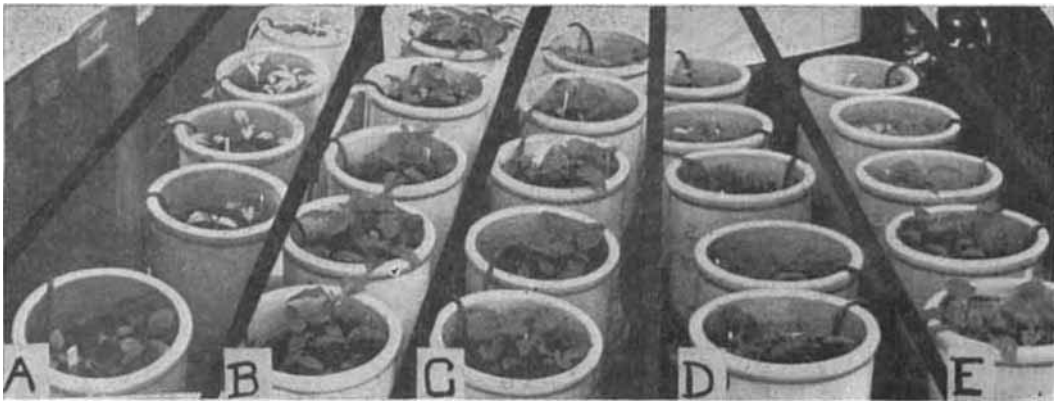

Fig. 1.—*Datura Stramonium* seedlings (Part I) after twenty days of daily feedings of the following chemicals added to soil cultures: (*A*) atropine sulfate in 0.05% to 0.0001% concentration, (*B*) untreated controls, (*C*) pyridine in 0.5% to 0.001% concentration, (*D*) piperidine in 0.5% to 0.001% concentration and (*E*) thiamin chloride in 0.0025% to 0.00025% concentration.

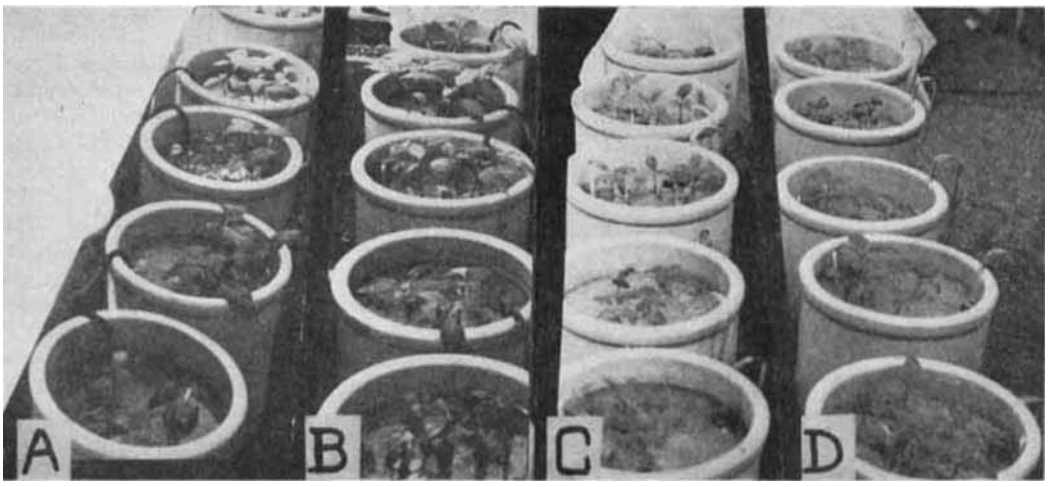

Fig. 2.—*Datura Stramonium* seedlings (Part I). Growth response of quartz sand cultures to (*A*) thiamin chloride (twenty days), (*B*) pyridine (twenty days), (*C*) thiamin chloride (eight days) and (*D*) pyridine (eight days). Concentrations given in Table IV.

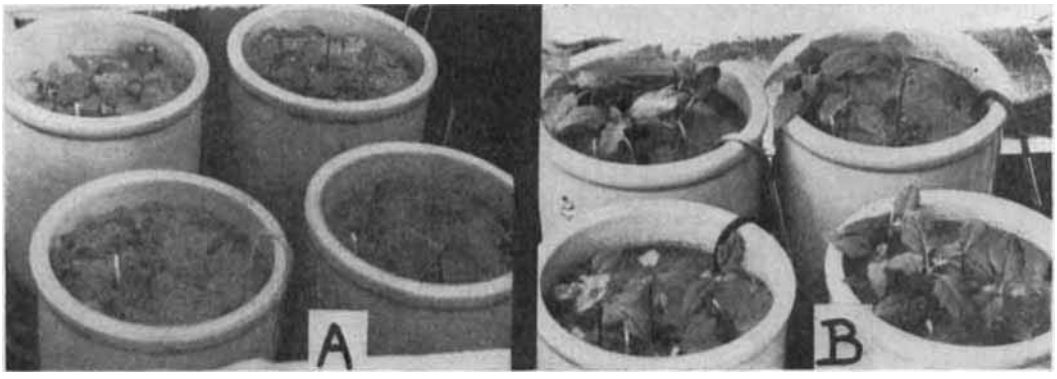

Fig. 3.—*Datura Stramonium* seedlings (Part I). Growth response of quartz sand cultures to piperidine in 0.5% to 0.001% concentration (*A*) after eight days and (*B*) after twenty days of daily feedings.

(Fig. 2). Cotyledonary leaves dropped off soon after treatments were started. Three plants developed a roughening of the leaves from which they recovered. Most seedlings receiving 0.5% solutions showed an epinastic condition following eight days of feedings (Fig. 2). Lower concentrations such as 0.05%, 0.01% and 0.001% responded favorably to the twenty-day treatments. Concentrations of 0.05% and 0.01% showed the best stem elongation and leaf development. This compared satisfactorily with that of the nutrient control. A 0.01% concentration was adopted for Part II treatments.

Piperidine in Quartz Sand Cultures (Lot 2).—All seedings responded favorably to this treatment in nutrient solution (Fig. 3). A definite stem elongation was evident. This measured from 1 to 2 in. for a period of eight days. The growth was slightly slower among seedlings treated with higher concentrations (above 0.05%). No toxic responses were observed.

Atropine Sulfate in Quartz Sand Cultures (Lot 2).—Plants in nutrient solutions responded vigorously to the addition of all concentrations of atropine sulfate used. Stem elongations ranged from 2 to 4 in. for a twenty-day period. Leaf curling was observed among some of the plants receiving concentrations above 0.05%. Three of the 25 seedlings showed a slight degree of epinasty following twelve days of feedings. A concentration of 0.01% was chosen for Part II treatments.

Thiamin Chloride in Quartz Sand Cultures (Lot 2).—Concentrations lower than 0.025 Gm. (0.0025%) in nutrient solution produced a vigorous growth for the twenty-day period (Fig. 2). Stem elongation was rapid and surpassed most of the nutrient control seedlings. The lowest concentrations produced the best response. High concentrations, above 0.002%, remained less active until after approximately ten days of treatment. Measurements of 4 and 5 in. were recorded for plant growth. A concentration of 0.001% was chosen for Part II.

Controls in Quartz Sand Cultures (Lot 2).—The twenty-day growth response of seedlings regularly treated with fresh nutrient solution (Tables I, II and III) was in general slightly greater than for this solution with each of the four chemical substances added. A less vigorous response was noted when compared with vitamin B_1 treatments.

PART II: SIXTY-DAY CONTINUOUS TREATMENTS

This portion of the experiment was carried out in much the same fashion as Part I. Concentrations now used were selected from the established results of growth responses in Part I. It was decided to select as closely as possible similar concentrations in order that a correlation on this basis might also be determined. Two seedlings not exceeding 3 cm. in height were placed into each of 50 glazed jars and a suitable time allowed for recovery from transplanting. Soil cultures were classed as lot 1 and quartz sand cultures as lot 2. Soil cultures were saturated once every three days with chemical solutions in distilled water. Sand cultures were completely changed twice a week with freshly prepared chemicals in nutrient solution. These treatments were continued from July 10 to September 9. Concentrations of chemical substances used are tabulated in Table V.

TABLE V.—PART II, CHEMICAL TREATMENTS FOR SIXTY-DAY PERIOD

Chemical Substance	Number of Seedlings Used	Concentration—Per Liter of Solution[a]	Per Cent
Pyridine	10	0.1 cc.	0.01
Piperidine	10	0.1 cc.	0.01
Atropine sulfate	10	0.1 Gm.	0.01
Thiamin chloride	10	0.01 Gm.	0.001
Untreated (control)	10	.....	.....

[a] Distilled water was used for seedlings of lot 1. Nutrient solution was used for seedlings of lot 2.

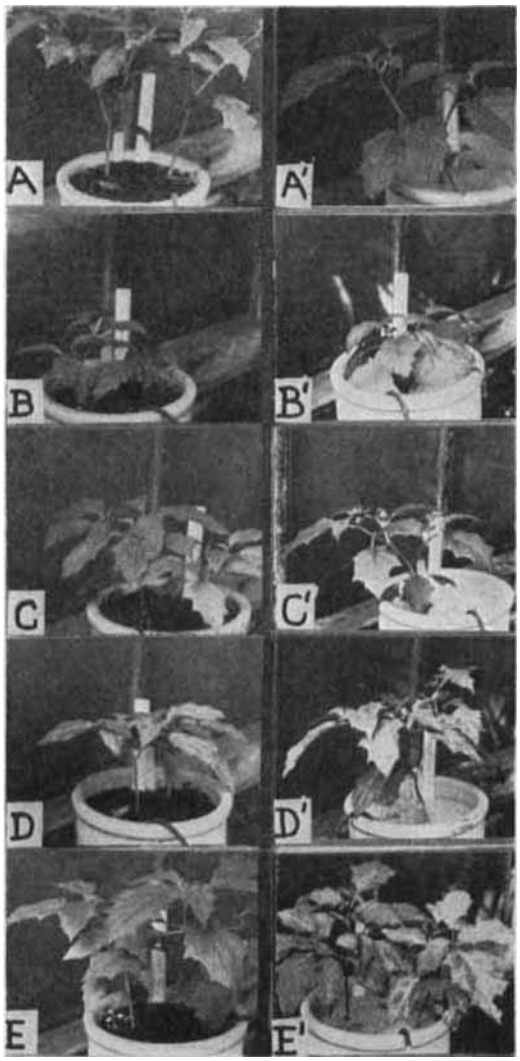

Fig. 4.—*Datura Stramonium* seedlings (Part II). Growth response after sixty days of feedings (left, soil cultures; right, quartz sand cultures): (*A*, *A'*) untreated controls, (*B*, *B'*) pyridine, (*C*, *C'*) piperidine, (*D*, *D'*) atropine sulfate and (*E*, *E'*) thiamin chloride. Concentrations given in Table V.

Pyridine in Soil Cultures (Lot 1).—Growth responses with a 0.01% solution of pyridine were slow from the beginning of the sixty-day period. After thirty days of feeding most seedlings reached a height of about $6^1/_2$ in. Leaves developed normally without any evident epinasty. The width of all leaves examined equaled that of the control plants. Following forty-five days of treatments stem elongation measured approximately 2 in. shorter than that of the untreated seedlings. A greater increase was noted in width of leaves, but little stem elongation continued. In sixty days most plants had reached an average height of 10 in., but with well-developed stems and leaves (Fig. 4). No apparent toxicity could be ascertained. Flower buds developed on many of the plants.

Piperidine in Soil Cultures (Lot 1).—A 0.01% solution of piperidine fed once every three days to soil cultures produced as vigorous a response as that for pyridine. The stem elongation of most plants equaled that of the untreated soil control for the first six days of feedings. Following this period stem elongations were slower and became generally from one-half to three-fourths the size of the untreated controls for a period of twenty-five days. Plants reached a height of nearly 6 in. at the end of thirty days. During the last week of treatment some of the leaves developed white spots. None of the plants failed to survive. A maximum stem elongation of 12 in. was recorded for several plants (Fig. 4). This was about 6 in. shorter than the average stem elongation of the untreated controls. The shortest plant measured $6^1/_2$ in. Leaves were well developed and a few floral buds developed on the more mature plants.

Atropine Sulfate in Soil Cultures (Lot 1).—Shortly following the first twenty days of feedings in soil

cultures with atropine sulfate (a 0.01% solution), stem elongations suddenly exhibited a retarded growth. In thirty days this elongation became notedly less than that of the control plants. Most stems did not increase in length after this period by more than $3^1/_2$ in. The leaves of three plants showed a marked roughening (see Part I, lot 1). Two of the plants recovered from this condition. Several plants failed to recover from a condition of epinasty and white marks (Fig. 4) developed on the leaves of four plants. The leaves of most of the surviving plants developed as large a growth as the untreated controls (Fig. 4).

Thiamin Chloride in Soil Cultures (Lot 1).—Treatments of 0.001% thiamin chloride once every three days produced vigorous growth. Stem elongations ranged from 2 to $4^1/_2$ in. in eight days of feedings. At the end of thirty days most seedlings had attained a size of from 4 to $6^1/_2$ in. In forty-five days of treatments growth reached 12 in. and at the conclusion of the experiment average stem elongations (Fig. 4) were about 15 in. No plants developed white marks or epinasty. Flower buds were numerous and the leaves of all plants were seemingly more vigorous than those developed on control untreated plants.

Controls in Soil Cultures (Lot 1).—These plants were treated with tap water once every three days. Moisture accumulating in the jars was allowed to drain in the same manner as in the chemical treatments. Steam elongations ranged from $2^1/_2$ to 5 in. in a twenty-day period and reached an average height of 7 in. in thirty days. At the conclusion of sixty days of treatments the average size of the untreated control plants was from 15 to 18 in., (Fig. 4). No epinasty or leaf curling developed among the untreated plants. Flower buds were numerous.

Pyridine in Quartz Sand Cultures (Lot 2).—Seedlings in sand cultures treated twice weekly with a 0.01% pyridine in nutrient solution showed a slow growth response. Shortly following the start of the treatments several plants showed epinasty of the leaves. Most recovered from this; and stem elongations of 6 in., $2^1/_2$ in. shorter than for the untreated quartz sand control plants, were observed at the end of thirty days. The growth seemed to be concentrated in the leaves. In sixty days of feedings the maximum stem elongation was 8 in. (Fig. 4). This was about one-half the size of the nutrient untreated controls. Flower buds developed on four of the six remaining plants.

Piperidine in Quartz Sand Cultures (Lot 2).—Sand cultures treated with a 0.01% piperidine in nutrient solution exhibited a growth response similar to that of the nutrient quartz sand control plants. Stem elongations and leaf size were on the average larger than for pyridine-treated seedlings of lot 2. In thirty days several stem elongations of 7 and 8 in. were recorded. Growth continued at a slower rate than that of the untreated controls following the next five days of feedings. In sixty days (Fig. 4) elongations of 11 and 12 in. were observed. These growth measurements equaled approximately three-fourths those of the controls. Several flower buds were formed. One plant after forty days of treatments developed a marked roughening of the leaves. It did not recover completely from this, but did bear flower buds.

Atropine Sulfate in Quartz Sand Cultures (Lot 2).—A 0.01% concentration of atropine sulfate in nutrient solution produced a favorable growth response during the early feedings. Stem elongations and leaf developments were as fast as those of the controls at first. However, following the first thirty days of treatments this growth became noticeably slower. Four plants showed an epinastic condition but recovered from it. At the end of this period stem elongations measured from 4 to $7^1/_2$ in. This equaled the average size as recorded for the nutrient control plants for the same period. After sixty days of feedings (Fig. 4) plants measured from 10 to 12 in. in height or almost 4 in. less than the control plants. Many flower buds were produced and some plants showed white marks on the leaves. Leaves compared favorably in size with the controls.

Thiamin Chloride in Quartz Sand Cultures (Lot 2).—A very noticeable increase in growth was noted for seedlings treated with 0.001% thiamin chloride in nutrient solution. Stem elongations of from 5 to $8^1/_2$ in. were noted in thirty days of continuous feeding. This was increased to from 10 to 17 in. in sixty days (Fig. 4). No epinasty developed. No signs of leaf curling were evident. White marks did occur on some of the more mature leaves (Fig. 4) during the final week of the treatments. Flower buds developed on eight of the ten plants.

Control Seedlings in Quartz Sand Cultures (Lot 2).—A modified nutrient solution (Tables I, II and III) was added to each jar in the same manner as for the chemical treatments. Following twenty days of growth, stem elongations measured up to 5 in. for most of the control plants. This increased to $9^1/_2$ in. at the end of thirty days of treatment. The average untreated control attained a height of 16 in. (Fig. 4) at the conclusion of the experiment. No epinasty or leaf roughening was noticed. White marks did develop on the leaves of a few plants during the final six days of growth. In general, control untreated plants (Fig. 4) grew larger in sand cultures than did the chemically treated plants in the same culture media. They attained a height and leaf growth only slightly less than plants treated with vitamin B_1 as thiamin chloride.

SUMMARY

Several concentrations of chemically pure pyridine, piperidine, atropine sulfate and thiamin chloride have been applied in continuous feedings to growing stramonium seedlings in soil and pure quartz sand cultures.

In general, the lower the concentration of these chemical substances, the better was the growth response. Concentrations of 0.025% or greater were found to produce the most toxic effects.

Pyridine, piperidine and atropine sulfate induced marked inhibitory stem growth responses during a period of sixty-day treatments. However, leaf growth was not appreciably impaired during the same period. A condition of roughened leaves has been described for some seedlings treated with piperidine and atropine sulfate. This response was found to be similar to that previously reported for stramonium seedlings treated with colchicine (1).

Epinastic responses and other toxic manifestations varied according to the size and the rate of growth of the seedlings.

Thiamin chloride accelerated growth responses. Concentrations of 0.001% and 0.0025% produced the most vigorous growth activity.

The fact that stramonium plants are influenced to some extent in stem and leaf growth by those substances which have a chemical structure related to the atropine nucleus and that they can tolerate small concentrations of these chemicals indicates the possibility that by feeding the plant continuous tolerated doses, the alkaloid content in the leaves might also be influenced. This phase of the experiment is being continued and will be reported subsequently.

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A Note on the Healing Action of Allantoin, Allantoin Dipiperazine and Urea*

Preliminary Report

By Frederick R. Greenbaum

The reintroduction of allantoin clinically occurred in 1935 through the work of Robinson (1) who demonstrated that one of the active principles of the excretion of maggots is allantoin. Greenbaum (2) reported on the healing action of allantoin in various clinical cases. In another publication by Robinson (3) it was shown that urea is present in the extracts of maggots, and that it also possesses healing action. Since this publication appeared, several investigators have tried urea to stimulate healing in chronic purulent wounds and in infected wounds (4, 5, 6, 7).

Urea is inexpensive, and therefore some clinicians might prefer to use urea provided it is as effective as allantoin. Holder and Mackey (7) used a 40% urea solution and also crystals of urea. Forty per cent urea solution has a tendency to dry and cake on occasion, and there is difficulty in maintaining it in proper contact with the tissue at times. The use of concentrated urea solution is frequently accompanied with pain so pronounced that it is necessary that a local anesthetic be added to the concentrated urea solution.

EXPERIMENTAL

The purpose of this investigation was to demonstrate by means of animal experiments the relative healing properties of allantoin and urea. We also included in this investigation a combination of allantoin with piperazine, which is more soluble than allantoin. This combination is a double salt of allantoin with piperazine. It was prepared by dissolving 2 moles of piperazine in water and 1 mole of allantoin. The maximum solubility of

* This work was carried out at the Research Laboratories of the National Drug Company, Philadelphia, Pa.