sive accumulation of the drug in the plasma. For days 7 and 10 the overnight lows for NA in the six subjects ranged from 0.1 to 1.7 mcg./ml., and those for HNA ranged from 1.6 to 12.1 mcg./ml.[4]

(e) HNA represented a more constant constituent of the plasma than NA. Evidence for this is the fact that at practically every low point in the NA curves, the level of HNA was higher than that of NA, and *vice versa*. Thus, following each dose of NA the ratio NA/HNA increased to a peak of about 3/1, and then gradually decreased, so that by the time the next dose was taken the ratio was less than 1. (*Note:* at the equilibrium high the calculated plasma levels for subjects of the average weight used in this study would be: NA = 25 mcg./ml.; HNA = 7.5 mcg./ml.)

SUMMARY

The absorption and elimination of nalidixic acid have been studied in subjects receiving the drug four times daily on two different dosage schedules: "after meals" and "between meals." More satisfactory and predictable results were obtained when the doses were given at least 1 hr. before meals, rather than at variable times after meals. Plasma levels and excretion patterns of nalidixic and hydroxynalidixic acids resulting from the administration of 1 Gm. of the former four times daily for 10 days followed quite closely a model derived from single-dose studies. This dosage regimen did not result in any important change in the characteristics of the absorption, excretion, or metabolism of the drug, as based on a comparison of the data for the first, seventh, and tenth days of medication. The multiple dosage regimen resulted in some carry-over of naphthyridine in the plasma from one day to another, but the carry-over did not appear invariably to increase or decrease with the number of days on medication. Sensitivity of the plasma levels to small changes in the rate constants has been shown. The inverse relationship of plasma levels and body weights suggests that dosage should be adjusted to individual body weights, with the initial adult dosage regimen being approximately 15–16 mg./Kg. four times daily.

[4] Since the number of metabolic and excretion processes involved in the over-all picture is considerable, relatively small changes in the rates of several of these could grossly alter the overnight values. For example, a 30% decrease in the mean elimination rate constant for HNA (k_{d2}) would increase the overnight low from 0.8 to 1.9 mcg./ml., and would increase the 9.5-hr. value from 7.6 to 10 mcg./ml. A 1.5-fold increase in the average rate constant for NA → HNA (k_{M1}) without a corresponding change in k_d would increase HNA 1.5 times. Such differences in rate constants have been observed (2).

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Effects of the Ratio of Calcium to Potassium in the Nutrient Medium on the Growth and Alkaloid Production of *Atropa belladonna*

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The increase of calcium/potassium ratios in nutrient solutions reduces growth of belladonna plants. This is evident in the reduced elongation of all stems, particularly the sympodial flowering branches, and in the fresh and dry weights of all plant parts. There appears to be a concurrent increase in the proportion of leaf to total plant at the expense of stem and root. The increase in calcium/potassium ratio results in higher yields of total nitrogen and alkaloids.

THE IMPORTANCE of calcium and potassium in the nutrition of plants is well established (1–4). The authors' interest was directed to the effect of varying ratios of calcium/potassium concentration on the metabolic reactions in the alkaloid-producing plant *Atropa belladonna* L. The influence of the concentration of one of these cations on the other has been expressed in connection with: (*a*) their being bound on soil particles (5–7), (*b*) their absorption from the soil solution by roots (7), (*c*) their translocation through the plant (8), and (*d*) the rate of a number of metabolic reactions within the plant (9, 10).

Brown (11) reported that interactions involving nutrient elements may directly or indirectly affect all of the major metabolic pathways in plants and animals. The ratio of iron/phosphorus may drastically alter the ability of cells to grow by controlling oxidative mechanisms.

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Likewise, the calcium/boron ratio can markedly influence development of meristems and metabolism of sugars in all living cells.

James (12), in 1945, detailed a factorial experiment, involving additions of nitrogen, calcium, potassium, and phosphate to potted plants of belladonna. He reported that the application of nitrogen increased the total plant yield to its greatest magnitude. The addition of potassium and calcium increased the yield of alkaloids. Calcium was present at 40 Gm. of lime per plant, and potassium at 8 Gm. of potassium sulfate. This implies that a relationship of the two (Ca–K) exerted an effect on the plant in addition to that stimulated by the other nutrients.

Olsen (13) studied the effects of calcium and potassium on the growth and production of dry matter on *Sinapis* and *Hordeum* in nutrient solutions. He found the accumulation of dry weight greatest when the plants received Ca–K in a ratio of approximately 10:1.

It was the purpose of this study to determine what effects the ratios of concentrations of calcium to potassium have on the growth of the belladonna plant and its alkaloid production. In order to eliminate effects of soil binding of ions, plants were grown in nutrient solutions. Thus, concentrations of each of the ions available to the plant could be controlled.

EXPERIMENTAL

Plants and Containers—Seeds of a selected strain of *A. belladonna* were scarified by exposure to concentrated sulfuric acid for 1 min., followed by vigorous rinsing with tap water. They were then germinated in a sand–vermiculite mixture (1:1) maintained at 70°F. Germination occurred within 3 weeks. Following germination, the young seedlings were transferred to a full nutrient solution (14) for 3 weeks. They had developed 5 leaves, 2–3 cm. tall, averaging 2 Gm. fresh weight. The plants were then transferred to the experimental treatments.

Plants were grown in the greenhouse in glazed crocks which contained 12 L. of nutrient solution. The plants, which were supported on specially prepared Masonite covers, were lightly fastened by plant ties to a heavy wire frame. Four plants were grown in each of the four crocks used, making a total of 16 plants for each treatment.

Nutrient Solutions[1]—Solutions were prepared from reagent grade chemicals and distilled water on a milligram-equivalent per liter basis. All solutions contained the following ions in milligram-equivalents per liter: magnesium, 4 ml.; phosphate, 1 ml.; nitrate, 20 ml.; sulfate, 4 ml.; iron as EDTA, 1 ml.; and trace elements,[2] 1 ml. Experimental solutions contained, in addition to these, quantities of calcium and potassium in mg.-equivalents/liter, as adapted for each of the treatments.

Distilled water was added periodically to maintain the crock volumes at the same level as that at the beginning of the experiments. The pH of the solutions was adjusted weekly to 5.8 by the addition of dilute hydrochloric acid.

Nutritional Treatments—Three experiments were performed. In the first, the ratio of calcium to potassium was varied by maintaining the potassium concentration at the same level as that of the full nutrient solution. The following seven treatments were selected: Ca–K: 30:4, 20:4, 10:4, 5:4, 4:4, 2:4, and 1:4.

In the second experiment, the ratio was varied by maintaining the calcium level but by introducing a wide range of potassium concentration. The following eight treatments were chosen: Ca–K: 10:0, 10:1, 10:2, 10:4, 10:5, 10:10, 10:20, and 10:30

In the third experiment, five ratios were selected. Based on the observations of the preceding two experiments, it was found that they would support growth satisfactorily. They were, as follows: Ca–K: 1:10, 1:4, 4:10, 4:4, and 10:4.

Measurement and Sampling—Measurements of the length of the primary shoot and of the first sympodial flowering shoot were made weekly during the growth period. Plants were harvested 35 to 42 days after having been transferred to the nutrient solutions. Fresh weights of separated parts were made at harvest; dry weights were recorded after the parts were air-dried and then oven-heated at 100°C. for 1 hr.

All of the dried leaves of a single treatment were combined and made into a homogeneous powder (20 mesh). Three-gram samples of these were taken and prepared for total nitrogen (Kjeldahl) determinations. Two separate determinations were conducted, and agreed within 3%.

In order to determine the total alkaloids, the authors followed the method of Brown, Kirch, and Webster (15), which involved aliquot portions from an extract of a 10-Gm. sample. However, they modified it by utilizing only 2-Gm. sample extractions for each determination, and chromatographing the total extract.

RESULTS

Growth Responses

Shoot Elongation—(Table I)—With an increase of the calcium/potassium ratio, there was a progressive reduction of primary shoot length. In experiment *A*, the growth of plants was greatest at the lowest ratios of Ca–K: 1:4 and 2:4. In experiment *B*, ratios of 10:20 and 10:4 produced optimum growth. In experiment *C*, selected ratios of 1:10 and 1:4 resulted in the best growth. Otherwise, fairly uniform growth was observed.

Sympodial Flowering Branches—(Table I)—Differences in elongation of the sympodial flowering branches were of greater magnitude than those of the primary shoot, with the greatest elongation occurring at the intermediate ratio of 5:4 in experiment *A*.

[1] These nutrient solutions were prepared from gram-equivalent stock solutions of the following salts: calcium nitrate, potassium nitrate, potassium acid phosphate (Sorensen's), magnesium sulfate, and iron ethylenediaminetetraacetate. The final solutions were made by adding 1.0 ml. of the stock solution per liter to a large volume of distilled water, and then filling each crock to the desired level with distilled water.

[2] The trace elements were prepared according to the Hoagland and Arnon (14) formula and added weekly.

In experiment *B*, due to the low potassium concentration (1 or 2 mg. equivalent/liter), the growth of the sympodial flowering branches was not so great as that of the primary shoot.

TABLE I—EFFECT OF CALCIUM/POTASSIUM RATIOS ON SHOOT LENGTH IN THE NUTRIENT MEDIUM

Expt.	Ratio Ca:K	Length of Main Erect Primary Stem, cm.	Length of First Sympodial Flowering Branch, cm.
A[a]	1:4	61.5[b] ± 1.7[c]	63.4[b] ± 9.1[c]
	2:4	60.9 ± 2.2	86.4 ± 16.7
	4:4	57.0 ± 4.3	85.3 ± 9.3
	5:4	57.6 ± 4.3	94.6 ± 8.4
	10:4	54.6 ± 1.6	93.3 ± 8.8
	20:4	53.7 ± 1.7	69.3 ± 11.7
	30:4	49.5 ± 2.4	65.6 ± 9.6
B[d]	10:30	55.4 ± 6.0	39.3 ± 6.2
	10:20	61.5 ± 4.8	39.6 ± 9.7
	10:10	60.8 ± 3.0	43.3 ± 10.3
	10:5	61.5 ± 6.3	54.4 ± 7.3
	10:4	62.0 ± 2.3	53.7 ± 12.5
	10:2	57.5 ± 2.6	59.5 ± 15.2
	10:1	54.5 ± 1.7	34.0 ± 9.1
	10:0	22.3 ± 3.4	...
C[e]	1:10	54.2 ± 1.6	48.2 ± 5.9
	1:4	53.6 ± 1.3	43.1 ± 7.0
	4:10	51.7 ± 2.2	52.3 ± 11.0
	4:4	50.2 ± 1.6	44.4 ± 7.6
	10:4	51.4 ± 1.6	32.0 ± 5.8

[a] Experiment *A*, 6 weeks of growth. [b] Shoot lengths are mean of 16 plants. [c] Standard error of the mean. [d] Experiment *B*, 5 weeks of growth. [e] Experiment *C*, selected ratios.

In experiment *C*, the length of the sympodial flowering branches at the intermediate ratio of Ca–K 4:10 was significantly greater than the others ($p = 0.001$).

Fresh Weight—(Table II)—Increase of the Ca–K ratio resulted in decreased growth as measured by fresh weight of leaves, stems, and total plant at harvest. This was especially noted in experiment *C*, when selected ratios were used.

Fresh weights of roots appeared to be less responsive to the effect of this environmental ratio. The heaviest root systems were obtained at the intermediate ratios.

Dry Weight—(Table III)—In experiment *C*, increased Ca–K ratios produced less reduction in dry weights than was observed in fresh weights. On a percentage basis, the decrease in dry weights and in fresh weights was essentially the same.

Ratios of Plant Parts to Each Other—Increase in the Ca–K ratio resulted in a greater proportion of leaf to total plant with a corresponding decrease in stem. The ratio of shoot to root increased slightly, but none of the results were statistically significant.

Total Nitrogen (Kjeldahl) of Leaves—(Table IV)—Increase of the ratio of calcium/potassium resulted in a distinct rise in the total nitrogen content in the leaves of the plant, except in the case of the lowest ratio, 1:10. This increased amount of nitrogen per gram of dry tissue was in direct contrast

TABLE II—EFFECT OF CALCIUM/POTASSIUM RATIOS ON FRESH WEIGHT OF PLANT PARTS IN THE NUTRIENT MEDIUM

Expt.	Ratio Ca:K	Fresh Wt., Gm. Leaves	Stems	Roots	Total Plant
A[a]	1:4	71.9[b] ± 4.3[c]	56.1[b] ± 9.2[c]	44.3[b] ± 2.6[c]	189.3[b] ± 16.1[c]
	2:4	64.1 ± 4.5	51.0 ± 4.5	56.1 ± 5.1	171.3 ± 14.1
	4:4	55.7 ± 2.8	53.6 ± 4.8	55.6 ± 7.7	161.4 ± 15.3
	5:4	63.0 ± 3.4	60.8 ± 3.4	59.1 ± 3.2	183.0 ± 10.0
	10:4	69.3 ± 4.3	57.5 ± 4.0	78.4 ± 3.2	186.0 ± 11.5
	20:4	52.0 ± 3.7	46.8 ± 4.1	48.1 ± 3.7	147.0 ± 11.5
	30:4	44.0 ± 4.3	41.4 ± 5.5	35.4 ± 3.7	120.9 ± 13.5
B[d]	10:30	73.8 ± 7.1	47.4 ± 7.2	36.2 ± 4.1	157.2 ± 19.0
	10:20	75.8 ± 7.0	54.7 ± 5.9	36.5 ± 3.6	166.8 ± 15.6
	10:10	80.8 ± 6.5	63.6 ± 6.2	40.9 ± 3.4	185.4 ± 16.1
	10:5	58.8 ± 4.0	52.2 ± 3.5	37.3 ± 2.5	148.5 ± 9.4
	10:4	59.9 ± 4.6	49.7 ± 4.6	44.6 ± 4.6	153.9 ± 13.8
	10:2	53.3 ± 4.5	40.6 ± 4.7	37.8 ± 4.3	134.7 ± 13.5
	10:1	43.9 ± 3.1	37.2 ± 2.7	39.0 ± 3.6	120.3 ± 9.4
	10:0	8.6 ± 0.57	6.8 ± 0.48	7.2 ± 1.4	21.5 ± 3.4
C[e]	1:10	78.3 ± 3.5	60.8 ± 3.2	42.5 ± 2.5	181.6 ± 9.0
	1:4	69.5 ± 8.9	51.0 ± 3.6	34.8 ± 2.4	155.3 ± 9.5
	4:10	65.6 ± 6.3	51.1 ± 6.1	35.4 ± 4.1	152.0 ± 16.6
	4:4	65.2 ± 4.5	43.5 ± 4.0	37.4 ± 3.1	146.4 ± 11.3
	10:4	54.9 ± 3.8	38.0 ± 3.0	27.4 ± 2.7	120.3 ± 9.2

[a] Experiment *A*, 6 weeks of growth. [b] Weights are the means of the plant parts of 16 plants. [c] Standard error of the mean. [d] Experiment *B*, 5 weeks of growth. [e] Experiment *C*, selected ratios.

TABLE III—EFFECT OF SELECTED CALCIUM/POTASSIUM RATIOS ON DRY WEIGHT OF PLANT PARTS IN THE NUTRIENT MEDIUM (EXPERIMENT *C*)

Ratio Ca:K	Dry Wt., Gm. Leaves	Stems	Roots	Total
1:10	7.0[a] ± 0.07[b]	5.4[a] ± 0.35[b]	2.4[a] ± 0.04[b]	14.8[a] ± 0.59[b]
1:4	6.5 ± 0.32	4.8 ± 0.43	2.0 ± 0.08	13.3 ± 1.21
4:10	6.8 ± 0.49	4.7 ± 0.59	1.8 ± 0.06	13.3 ± 1.26
4:4	6.4 ± 0.39	4.4 ± 0.52	2.3 ± 0.06	13.1 ± 1.05
10:4	5.3 ± 0.32	3.3 ± 0.08	1.4 ± 0.003	10.1 ± 0.59

[a] Weights are the means of plant parts of 16 plants. [b] Standard error of the mean.

Table IV—Effect of Selected Calcium/Potassium Ratios on Total Nitrogen (Kjeldahl) and Total Nitrogen in the Leaf in the Nutrient Medium (Experiment *C*)

Ratio Ca:K	Total Nitrogen, mg./Gm. Dry Wt.	Alkaloids, mg./Gm. Dry Wt.
1:10	44.8[a] ± 1.4[b]	1.03[a] ± .0017[b]
1:4	36.4 ± 1.3	0.91 ± .0014
4:10	44.1 ± 0.25	0.94 ± .0009
4:4	46.45 ± 0.16	1.35 ± .0010
10:4	48.2 ± 0.03	1.05 ± .0015

[a] Mean of 4 determinations. [b] Standard error of the mean.

to the amount of growth obtained with the same ratios.

Total Alkaloids—(Table IV)—The pattern of alkaloid yield in general followed that of the total nitrogen synthesized in leaves. The 1:10 ratio revealed an alkaloid yield similar to that of the 10:4. It should be noted that on a mg./Gm. dry weight basis, the amount of alkaloids at the lowest ratio was similar to that produced at the highest ratio.

DISCUSSION

The use of nutrient solutions for growing plants for this type of study is advantageous. It eliminates the problem of binding of solute ions on soil particles which affects their availability to the plant. Soluble salts were used in this work because absorption of calcium by roots in the soil is slower than that of potassium. Except where noted, ratios were unreasonably low in calcium or potassium ions. No morphological deficiency symptoms were observed in any of the plants.

The upper sympodial flowering shoots were more responsive to the different nutrient treatments than were the main primary shoots. This might be partially explained on the basis that the primary shoot had already begun its elongation pattern at the beginning of the experimental treatment, whereas the sympodial branches had not yet been initiated. However, many experimental treatments were so drastic that the few centimeters of shoot length at the beginning of an experiment were not significant, considering the results. Lack of responsiveness of the main primary shoot would then indicate a certain inherent genetic quality which to a large extent operated independently of environmental nutritional stimuli. By the time sympodial branches were in the process of developing, growth had been more profoundly influenced by the nutritional supply.

Elongation of both primary and secondary stems appeared to be somewhat retarded by increased ratios of calcium/potassium. Percentage increases which occurred in the second, third, and fourth weekly measurements were greater at the higher ratios. This is in contrast to the general tendency of the shoot which would normally approach its maximum length at progressively slower rates.

Effects attributed to the ratio of concentrations might be considered in terms of one of the following: (*a*) the result of the calcium concentration irrespective of the potassium concentration, or *vice versa;* (*b*) the difference between hydration of the tissue at the lower ratios, or less hydration and more woodiness at higher ratios; or (*c*) the result of total concentration of solutes in the solution.

In experiment *A* when plants were grown at varying calcium concentrations but with potassium constant, the differences in succulence *versus* woodiness were not apparent even at high concentrations of calcium. Likewise in experiment *B*, where the potassium concentration was varied, differences in succulence were not apparent. This indicated that the effect was due to the ratio of these ions to each other, rather than to either ion by itself.

When considering the water content in the tissue, the total range from the lowest to the highest ratio indicated a variation of less than 0.5%. Likewise, the effect of total ion concentration on hydration of the tissue resulted in a difference of 0.6% between the lowest and the highest ratios of calcium/potassium.

Except for the samples determined at the lowest ratio (Ca–K 1:10), the total nitrogen and the alkaloids present in the leaves of the plants resulted in a higher yield, as the ratio of calcium/potassium increased. Because the results of determinations were surprisingly high at the low ratio, they were redetermined and verified. High percentages of nitrogen and total alkaloids were also found by Schermeister in work done in this laboratory (16).

Ratios of leaf to stem, and shoot to root, showed that higher calcium/potassium ratios favored leaf development and decreasing stem. They also tended to favor shoot over root. The smaller plant at the higher calcium/potassium ratio produced more leaf or photosynthetic material, and less stem and root structure.

CONCLUSIONS AND SUMMARY

Growth of belladonna is affected by the ratio of calcium to potassium in the nutrient medium. Production of all plant parts is reduced as the ratio of calcium/potassium is increased. Reduced elongation occurred in the sympodial flowering shoots to a more marked extent than in the primary erect stems. Fresh and dry weights of all plant parts were lower as the ratio increased.

As the calcium/potassium ratio increased, the yield of total nitrogen and alkaloids was higher. There was one exception: the 1:10 ratio.

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