XXXIV. THE INFLUENCE OF THE CARBONATES OF THE RARE EARTHS (CERIUM, LANTHANUM, YTTRIUM) ON GROWTH AND CELL-DIVISION IN HYACINTHS.

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The effects of various organic and inorganic bodies on growth and cell-division in both plants and animals have been studied by several researchers in this laboratory during the past few years. Moore, Roaf, and Whitley [1905] investigated the action of acids and alkalis on the development of the fertilized eggs of the sea-urchin, *Echinus esculentus*. Their inquiry was directed to the effects of variation in hydrogen and hydroxyl ion concentration in the medium (sea-water) in which the eggs were allowed to develop, and they found that within certain narrow limits of concentration, alkalis favoured cell-division, whereas acids were invariably fatal. Small additions of acids inhibited cell-division and growth, and at a concentration of 0.001 molar practically all cell-division was stopped. A slight increase in alkalinity favoured development, at the same time producing irregularities in cell-division. Beyond the optimum concentration of alkali, exceedingly irregular division, resulting in particular in the production of multi-nucleated cells, was observed. At a concentration of 0.0015 M. of caustic alkali, however, cell-division was completely arrested.

Whitley [1905] observed similar effects on the eggs of plaice, and also noted the action of the indicators phenolphthaleïn and dimethylaminoazobenzene on the eggs both of plaice and echinus. He found that while phenolphthaleïn was deadly to the eggs of echinus, it was harmless to those of the plaice. On the other hand, the azobenzene derivative quickly killed the latter, and appeared, if anything, to have a favourable effect on the development of the former.

Moore, Knowles, and Roaf [1908] extended the observations to plants, using for their experiments the common hyacinth. They obtained similar

results in regard to the influence of acids and alkalis, and they also showed that the cation had a specific effect, potassium being much more stimulating than sodium to both rootlets and foliage leaves. The phosphatic ion also had a special effect on the flower, causing an increase in size at optimum concentration, and at higher concentrations an irregular inflorescence, with packed florets on a dwarfed stalk.

Histologically they observed depression of nuclear division with acids, and thickening of the cell walls; with alkalis, increased nuclear division, changes in chromosomes and irregular figures, while the cell outlines became obscured.

Coppin [1912] studied the effect of allantoin and other purine derivatives on the growth of hyacinths, and also salts of organic acids such as sodium huminate, sodium malate, and sodium oxalate.

These latter substances, as well as sodium urate, had a stimulating effect on the growth of the hyacinths, but allantoin and the other purine substances inhibited both growth and cell-division.

Working on somewhat different lines, Ransom [1912] observed the action of caffeine upon the germination of seeds. He used a large number of different seeds, and his method consisted in soaking the seeds for a short time in his caffeine solutions before sowing them in the usual manner. He found that caffeine, even in a very dilute solution, had a powerful effect in retarding germination and growth; while a concentration of 1 per cent. in many cases completely inhibited germination.

In the present inquiry, salts of the rare earths were used to test whether these produced any physiological effects on growth and cell-division. For this purpose the carbonates of cerium, lanthanum, and yttrium were selected.

Preparation of carbonates.

The carbonates were prepared, according to Moissan, in the following ways:

(1) Cerium. A solution of cerium nitrate was treated with ammonium carbonate, and the precipitate of cerium carbonate filtered off, and thoroughly washed.

(2) Lanthanum and Yttrium. The hydroxides of the metals were taken, suspended in water, and thoroughly saturated with carbon dioxide over a period of several hours. The precipitates were then filtered off.

Preparation of the carbonate solutions.

Owing to the very slight solubilities of the carbonates of cerium, lanthanum and yttrium, some difficulty was experienced in preparing solutions. Finally two or three grams of each of the respective carbonates were suspended in about two litres of Liverpool tap-water¹, and carbon dioxide was passed into the bottles for one or two hours, thus ensuring complete saturation. After being allowed to stand overnight, the undissolved residues were filtered off.

These clear filtrates were the actual solutions used in the experiments. Their concentrations were estimated by taking a measured volume of each, evaporating to dryness, and weighing the residue. The following figures were obtained:

Cerium	...	...	...	0.007 %
Lanthanum	...	...	...	0.01 %
Yttrium	...	...	...	0.017 %

In all probability they contained a mixture of the respective carbonates and bicarbonates.

Effect on the growth of hyacinths.

The plants used were a common variety of hyacinth. Healthy bulbs of as nearly uniform size as possible were selected. They were placed in hyacinth glasses which had been blackened on the outside with black lacquer to prevent action of light upon the rootlets. The hyacinth glasses held about 450 cc. and the solutions mentioned were filled in until they just wetted the bulbs. In addition, controls were grown under precisely similar conditions in Liverpool tap-water. Water was added to the glasses from time to time to make up for the loss due to evaporation. A few of the ends of the growing rootlets were cut off on the twenty-fifth day for the purpose of studying the effects of the solutions on cell-division and nuclear changes. These were all immediately fixed in Flemming's solution, cut in paraffin, and stained for nuclear figures by Heidenhain's iron-alum, haematoxylin method.

On the twenty-fourth day, a measured volume of the fluid was removed from each of the different solutions, filtered, and evaporated to dryness, organic matter being removed by ashing with ammonium nitrate. The residues were then weighed. Results:

¹ Liverpool tap-water is very pure surface water and practically free from inorganic salts.

	24th day	Original
Cerium	0.0068 %	0.007 %
Lanthanum	0.0076 %	0.01 %
Yttrium	0.0066 %	0.017 %

Thus in the case of the cerium carbonates there had been little or no absorption; but in the other two cases, particularly in that of yttrium, there had been marked absorption.

In addition measurements and observations were made at intervals of the growth and conditions of the plants, the points noted being the length of the green leaves, length and condition of the rootlets, and the condition of the florets and the flower spike. These observations and measurements will be found set out in the accompanying Table (p. 353).

While the results obtained were not in all cases concordant, a few definite points may be made. In the first place, all the bulbs in the experimental solutions reached maturity before the controls in water.

Secondly, the plants in the cerium carbonate solution were the first to attain maturity, though their development was not so marked as that of those in the lanthanum carbonate, which followed next in point of time.

The effect of the yttrium solution was somewhat anomalous, since while all the plants matured a few days before the controls, the rootlets were dwarfed, and looked yellow and unhealthy from the beginning.

The lanthanum ion seems to have a special effect on the flower stalks, resulting in very tall plants. No irregularities manifested themselves in the inflorescences.

HISTOLOGICAL INVESTIGATION OF GROWING ROOT-TIPS UNDER THE INFLUENCE OF THE ABOVE REAGENTS.

The varying effects produced by the different metallic ions are best seen from the accompanying photomicrographs (Plate I). Speaking generally it may be stated that the cerium and lanthanum ions have a decidedly stimulating effect on cell-division in the rootlets, while that of yttrium has a deleterious effect.

The following are brief notes of the histological examination of slides prepared from each of the twelve plants as mentioned in the Table. They were stained by Heidenhain's iron-alum, haematoxylin method.

1. *Cerium carbonate.* (Nos. 2 and 3.) (See photomicrograph 2.)

In both these preparations a noticeable feature is the beautiful regularity of the arrangement of the cells, the sections showing a large number of

Measurement of roots and leaves in cm.

Started Dec. 16th	Jan. 8, 23rd day	Feb. 8, 54th day		Feb. 11, 57th day		Feb. 15, 61st day		Feb. 24, 70th day		Feb. 28, 74th day		Mar. 4, 78th day	
		Roots only	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves
Cerium Car- bonate 0.007%	1	1	1	1	4.5	No development							
	2	1	Look rotten	1	7	1	7	—	—	—	—	—	—
	3	7.5	12	15	15	All florets open		15	17.5	—	—	—	—
	4	12.5	15	15	17	15	17	—	—	—	—	—	—
Lanthanum Car- bonate 0.01%	5	7	7.5	8	12.5	8	15	—	—	—	—	—	—
	6	4	4	5	10	5	12.5	5	12.5	—	—	—	—
Yttrium Car- bonate 0.017%	7	2.5	4	4	10	6	12.5	—	—	—	—	—	—
	8	2	2	2	12.5	2	12.5	2	12.5	2	15	—	—
	9	2	2.5	3	7.5	3	7.5	3	7.5	3	8	—	—
	10	7	10	10	12.5	10	12.5	10	15	10	15	12	16
Controls Water	11	4	6	6	11.5	6	12	7.5	12.5	7.5	15	7.5	15
	12	2	4.5	5	10	5	10	7.5	12.5	Withered			

rather small, closely packed, square cells, containing many dividing nuclei. The cell-walls are clearly defined, and the cytoplasm is granular and stains well. In many cases the nuclei are elongated, and show one or two nucleolus-like chromatin dots surrounded by a clear space.

2. *Lanthanum carbonate.* (Nos. 4, 5 and 6.) (See photomicrograph 3.)

The same regularity of the arrangement of the cells is seen in these sections, but it is not quite as marked as in the cerium preparations. Many dividing nuclei are seen, and the nucleolus-like dots above referred to are also conspicuous. The cytoplasm is faintly granular, and the cell walls are less sharply defined than in Nos. 2 and 3 (cerium).

3. *Yttrium carbonate.* (Nos. 7, 8 and 9.) (See photomicrograph 4.)

In this series hardly any dividing cells are to be seen. The arrangement of the cells is irregular. The nuclei are very deeply stained, and irregular in size and shape. The cell walls are not distinct, and the cytoplasm is scanty, ill-defined, and very faintly staining. The whole appearance is that of an irregular mass of cells, with scattered deeply-stained nuclei, and presents a very different picture from the compact and regular arrangement shown in the cerium and lanthanum series.

4. *Controls—tap-water.* (Nos. 10, 11 and 12.) (See photomicrograph 1.)

In these sections no great amount of cell-division is noticeable. The cells are fairly regularly arranged, and the nuclei are deeply stained. They are for the most part in the resting condition, and many exhibit the darkly staining dots resembling nucleoli, each surrounded by a clear space.

CONCLUSIONS.

1. Marked effects are produced upon the dividing cells of hyacinth rootlets by the addition of the carbonates of cerium, lanthanum and yttrium to the medium. The concentration of these substances necessary to produce physiological effects is very small.

2. The cations produce diverse effects; lanthanum especially, and cerium being favourable to growth and cell-division, while yttrium is unfavourable.

3. The lanthanum ion has a special effect on the flower stalk, causing an increase in length.

Fig. 1.
Control—tapwater. $\times 460$ diameters.

Fig. 2.
Cerium carbonate $\cdot 007\%$. $\times 460$ diameters.

Fig. 3.
Lanthanum carbonate $0\cdot 01\%$. $\times 460$ diameters.

Fig. 4.
Yttrium carbonate $\cdot 017\%$. $\times 460$ diameters.

4. The cytological effects are best seen in the accompanying photographs. In the cerium and lanthanum preparations there is a marked increase in cell-division, accompanied by a beautiful regularity in the arrangement of the cells. With yttrium there is a diminution in cell-division, and the cells are irregularly arranged.

I should like to take this opportunity of thanking Professor Benjamin Moore for suggesting the line of research, and for his kindly help and criticism throughout the work.

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