The control was of two kinds. One consisted of two 2-yr-old Coulter pine seedlings which had been well watered throughout their entire lives; the other consisted of an empty chamber.

The apparatus was checked before each experimental run for leaks and drifts in the relative humidity readings with time. When drifting did occur, more moist air was added to the empty chamber until readings remained constant for 6 hr; not until then was the chamber used.

Before each experimental run, both the test plants and the control plants were placed in front of a fan for 48 hr in order to assure a water deficit in the aerial part of the plant. Preliminary runs had shown that the same effect could be obtained by placing the plants in full sun for a week or more. The 48-hr exposure in front of the fan merely made possible a larger number of runs in the same period of time.

All experiments were carried out in diffused light at room temperatures which remained relatively constant (24.5° ± 0.7° C).

Five experimental runs were made with each test plant (A 1–5 and B 1–5 in Table 1). After each test plant a control plant was run; only six of these are shown (C 1–3 and D 1–3 in Table 1). Before each experimental run a blank run was made in which the chamber was empty; only three of these are shown (E 1–3, Table 1).

Results in Table 1 indicate that water was removed from the air by the aerial parts of the plants growing at the wilting point, but not by the control plants growing in well-watered soil. As a result of this negative transpiration, relative humidities as low as 84% were obtained in the test chamber.

Data in Table 1 also show that the test plants, which at first display "negative" transpiration, later display normal transpiration after a 24-hr period in the test chamber, even though the rate is extremely slow. This could be explained on the basis of a slow water uptake by the roots when in soil at the wilting point. When first placed in the chamber, the aerial portions of the plant remove water from the air until the vapor pressure of the water at the leaf surface is equal to the vapor pressure of the water in the surrounding chamber. As water is gradually removed from the soil by the roots, it passes through the conducting system into the leaf; then vapor pressure of the water in the leaf increases and water loss to the atmosphere begins, showing as normal transpiration.

Five-year averages of the relative humidity in the San Gabriel mountains of southern California during the period from July to October show that values of 90% or more were recorded an average of 18 days per month with an average duration of 8 hr, at 1,500 ft and 8 days per month with an average duration of 7 hr, at 2,800 ft. These are conservative figures (probably low), since they were taken in a standard shelter 4 ft above the ground in a cleared area, and not at the leaf surface where radiation effects are operative. Nevertheless, they do show that relative humidities are such during the summer that negative transpiration can occur under field conditions.

Calculations of the maximum absorption forces (suction pressures or diffusion pressure deficits) attained in each experimental run are shown in the last column in Table 1. Relative humidities of 98% and above are considered as 100%, because of the inaccuracy of the instrument in this range; absorption forces are consequently considered as 0 under such conditions. Calculations were based upon the relation existing between vapor pressure, relative humidity, and osmotic concentration of a salt solution contained in a closed vessel. At a specific temperature such a salt solution has a specific vapor pressure and the air above it a specific relative humidity. If air of a higher or lower relative humidity is introduced into the closed vessel, the original relative humidity is again attained after a short period of time; this will continue to happen as long as the concentration of the salt solution is not appreciably changed by the gain or loss of water in the form of vapor. Therefore, when the relative humidity above a solution is known, the osmotic concentration of the solution, which is identical to the absorption force for water, can be calculated. For example, in one of the experimental runs cited in Table 1, water at the leaf surface was in equilibrium with an atmosphere of 84% relative humidity. This relative humidity would also exist over a salt solution with an osmotic concentration of about 230 atm; hence, the absorption force developed at the leaf surface was considered to be 230 atm.

Since such large forces are developed, one would not expect leaves with large thin-walled cells, as on the sunflower plant, to display negative transpiration of a measurable magnitude, or to develop high absorption forces in the leaves. On the other hand, leaves of many of the desert and Mediterranean type plants are mechanically much stronger, and in them negative transpiration and high absorption forces under drought conditions may be expected. Even if this is found to be generally true, much more experimental work must be carried out to determine whether this absorption of water in itself is an important survival factor or whether it is merely a phenomenon that can occur in plant tissues rigid enough to resist collapse when desiccated.

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Disruption of Mitosis by Desiccated Thyroid Tissue

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It has been reported that numerous substances, notably colchicine (*1*), acenaphthene (*4*), salts of heavy elements (*5*), and ribose nucleic acid (*3*) disrupt onion root mitosis in producing polyploidy, c-mitosis, nondisjunction, and chromatin bridges. Except for colchicine and ribose nucleic acid, the substances used were mainly those not found in living organisms. With this in mind,

several preparations of organic substances that normally occur in mammals were used, namely, acetyl choline, adrenalin, insulin, parathyroid powder, dried thyroid tissue, and histamine. These substances were made up in concentrations from 0.001 g to 10 g/100 ml of H_2O. Onion roots were treated with these concentrations for 4–72 hr, and smears were made with aceto-brilliant cresyl blue following the procedure of Stewart and Schertiger (*6*).

TABLE 1

EFFECT OF VARIOUS CONCENTRATIONS OF THYROID POWDER ON ONION ROOT MITOSIS

Plant No.	Thyroid powder in g/100 ml of H_2O	Length of treatments in hr	Predominant types of abnormality
20	0.130*	48	nondisjunction
44	0.500	4, 8, 27, 72	nondisjunction c-mitosis chromatin bridges
59	0.700	24, 31	c-mitosis chromatin bridges
62 and 63	1.00	23, 41, 48	c-mitosis chromatin bridges shattered nuclei
64	1.00	24, 48	c-mitosis
65	1.00	24	c-mitosis
66	1.00	24	c-mitosis

* Concentrations below 0.130 g gave normal mitotic figures.

Thyroid powder (control number H17204 Armour and Company, Chicago) was the only one that produced abnormal mitotic figures in onion root cells. Of the 18 plants treated with thyroid powder, eight showed very definite abnormalities (see Table 1); root smears from four showed some mitotic irregularities; in three plants there were no abnormalities; and in three others the thyroid powder concentrations were below the threshold, 0.130 g/100 ml H_2O. The most common abnormality at higher concentrations was c-mitosis (Fig. 1), whereas at lower concentrations, below 0.7 g/100 ml H_2O, the most frequent abnormality was chromatin bridge formation (Fig. 2). The presence of c-mitosis indicates that a metaphase block and incomplete splitting of the chromosomes had occurred. Note in Fig. 1 that the chromosomes are in an extremely contracted state and that the partially split chromosomes are still attached at the centromere. Mitosis seems to have been stopped at this stage. The chromosomes resemble those found in the process of meiosis. The chromatin bridges, we believe, are due to the failure of chromosomes to separate completely. A chromosome may split partially so that a separate centromere is present in each product of the split chromosome, although the rest of the chromosome has not split. During anaphase these unsplit ends are, therefore, stretched between opposite poles. There is also a possibility that the bridges may result from somatic crossing over. The thyroid powder may affect the spindle, for several cells showing nondisjunction have appeared in smears.

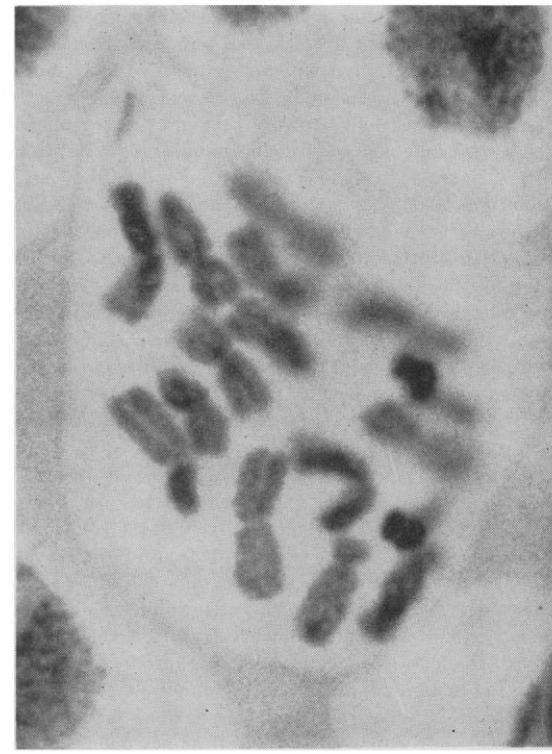

FIG. 1. C-Mitosis produced in onion root cell by treatment with 1 g thyroid powder/100 ml H_2O for 24 hr. Magnification x 2600.

Since thyroxin is a well-known constituent of thyroid tissue, it was suspected of being the causative agent of mitotic abnormalities. Concentrations of thyroxin (sodium salt) ranging from 1 mg to 40 mg/100 ml of buffer pH 7.8 were used. Mitosis in the onion root was normal. Thyroxin as the causative agent can, therefore, be ruled out, since 1 mg of crystalline thyroxin per 100

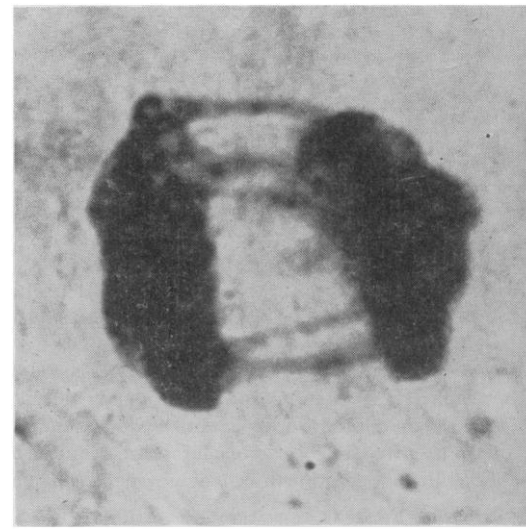

FIG. 2. Chromatin bridges produced in onion root cell by treatment with 0.5 g thyroid powder/100 ml H_2O for 8 hr. Magnification x 2600.

ml of distilled water is over 100 times the concentration of thyroxin found in 1 g of thyroid powder. In doses of 10 mg or higher of thyroxin, root growth ceased, and at a lower concentration growth was slowed down.

Kodani (*3*) has demonstrated that a 2%–4% solution of ribose nucleic acid is the optimum range in which mitotic abnormalities are produced in onion root cells. In solutions of 0.05% and 0.1% of ribose nucleic acid growth was normal. In our laboratory we have found that a 2% solution of desoxyribonucleic acid (Lot No. DN4902, Schwartz Laboratories, New York City) induced mitotic abnormalities in onion root cells. Is there enough ribose nucleic and desoxyribonucleic acids in dry thyroid tissue to account for the abnormalities observed? Davidson and Waymouth (*2*) reported that in dry thyroid tissue from sheep there are 148 mg of nucleic acid P/100 g of tissue. Since P is approximately 10% (more likely 8.5%–9.5%) of nucleic acid molecules there is 0.014 g of nucleic acid/g of thyroid tissue. In 1 g of thyroid/100 ml of water, we then have 0.014% solution of nucleic acids. The concentration of thyroid tissue used in the present tests certainly does not contain enough nucleic acids to account for the abnormalities observed. Evidently there is some unknown factor in the thyroid powder which is responsible for the production of c-mitosis, chromosome bridges, and nondisjunction. The normal mitosis is upset. How this is done is unknown. This factor may either enter the cell or act upon another substance outside the cell, which in turn enters and affects the metabolism of mitosis. Since solutions of the thyroid powder that have been made up and placed in the refrigerator for 24–48 hr before use were usually more potent in producing abnormalities than fresh solutions, a decomposition product may be the causative agent.

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A Laboratory Lyophil Apparatus[1]

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During the course of work in the Gates and Crellin Laboratories, a need arose for a lyophil apparatus capable of handling liter quantities of solution. The apparatus of Campbell and Pressman (*1*) was inadequate, since the limiting capacity of this apparatus was about 400 ml of solution. Furthermore, the apparatus could be operated only intermittently, since several hours were required between lyophilizing operations for de-icing of the condenser surface. As a result, a modified apparatus was developed which obviated these difficulties and which had the additional advantages of low cost and ease of construction. This apparatus is shown in Fig. 1.

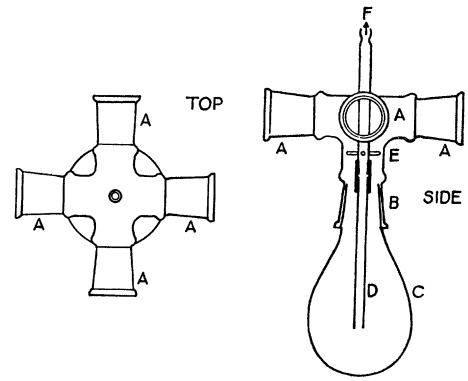

Fig. 1. Lyophil apparatus. A, ports, ST 34/45, for flasks; B, ST 34/45, for receiver flask; C, receiver constructed from 800-ml Kjeldahl flask; D, removable tube connected to center tube with rubber tubing; E, three glass pin supports arranged symmetrically on center tube; F, to vacuum.

The apparatus consists essentially of five female standard taper 34/45 joints. Four of the joints, which are arranged at right angles, serve as ports for the insertion of lyophil flasks. The flasks were constructed from male 34/45 joints and Kjeldahl flasks as described previously (*1*). The joints are arranged as compactly as possible in order to shorten the path of water vapor from the lyophil flask to the receiver; however, apparatus with joints having arms of 2 in. to 3 in. still operate effectively. The fifth joint accommodates the receiver for condensing moisture. The receiver is simply another lyophil flask constructed from an 800-ml Kjeldahl flask and is replaced periodically during operation of the apparatus.

TABLE 1

Operation time (hr)	Percent total water collected
0.9	21.1
2.0	44.5
3.2	64.4
4.3	80.2
5.9	97.2

The operation of the apparatus was similar to that of Campbell and Pressman (*1*). The four flasks were filled with solution and frozen by turning in a bath of methyl cellosolve and dry ice. The flasks were attached to the apparatus after lubrication of the joints, the receiver was put in place, and the apparatus was moved downward until the receiver was immersed to within a few inches of the joint in a dry ice-methyl cellosolve bath. The cooling mixture was conveniently contained in a 1-gal wide-mouthed Dewar flask. The system was then evacuated with an efficient vacuum pump (a Hyvac was satisfactory). In order to replace the receiver, air could be admitted into the system through an auxiliary manifold system. The

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