Explanation of Plate VIII.

Tradescantia canariculata.

Figs. 1-4. Treated with the solution of iodine and potassium iodide. 1, microspore mother cell in early prophase stage. 2, tetrads. 3, one-nucleate young microspore. 4, two-nucleate microspores. Only a few cells contain starch grains. Figs. 5-6. Fresh materials, photographed with the LEITZ polarization microscope, a, without and b, with analyser. 5, one-nucleate microspore. 6, tetrads. Granules are in BROWNIAN motion. Figs. 7-8. Microspore mother cells, treated with acetocarmine. 7, before the treatment with heat. Granules are clearly seen. 8, after the heat-treatment. Granules became swollen. Figs. 9-11. Microspore mother cells and one-nucleate microspore, treated with acetocarmine 10 minutes after centrifugation (3100, 17 cm, 20 min., 27°C).

1-3, 5-11, $\times 900$. 4, $\times 400$.

On the Nature of Recretion from *Colocasia antiquorum* var. *esculentum*.

By

Tadayoshi Tazaki

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Introduction.

From the water pore at the leaf-tip of *Colocasia antiquorum*, water drops are excreted during the night or even during the daytime when the humidity is high. This excretion is effected passively by the root pressure and is called recretion by guttation (FREY-WYSSLING, 1935).

This phenomenon was already observed by MUNTINGH (1672) in *Colocasia*. Based on the precise observation of *Colocasia*, SCHMIDT (1831) compared this phenomenon to the excretion of animal. DUCHARTRE (1859) investigated this phenomenon closely, determining the velocity of exudation and the substances contained in the exudation sap.

In 1903, MOLISCH published a close investigation on the recretion of *Colocasia*, which will be referred afterwards. A little later, MÜLLER (1919) observed the hydathods of Aroidae, including *Colocasia*, and found that the sap was excreted from a water pore without epithem. In the same year, FLOOD (1919) proved that the recretion in *Colocasia* was a passive filtration by the root pressure. Also in the same year, STAHL (1919) attributed the significance of this phenomenon to the removal of no longer

useful substances whose accumulation inside the plant was more or less harmful and might cause a fatal result to the plant.

On the chemical nature of exudation sap, DUCHARTRE already found in it almost undefinably small amount of foreign substance (CaCO_3). UNGER (1919) obtained from the recreation sap of *Colocasia*, 0.056% of solid substance and 0.0027% of ash.

SABININ's school (1925) has recently made a remarkable contribution on the relation between the bleeding sap (not recreation sap) and the nutrient solution. PAVLINOVA (1926), a SABININ's associate, found in maize that the calcium concentration of recreation sap was directly proportional to the calcium concentration of the nutrient solution and is about $\frac{2}{5}$ of that of the latter.

Using *Phaseolus multiflorus* var. *coccineus* as the test plant, LAINE (1934) investigated closely on the absorption of electrolytes by the cut roots and the chemistry of exudation sap by bleeding. The external solutions were 10^{-5} — 10^{-2} normal solution of MnCl_2 , CaCl_2 and KCl .

From the investigation on recreation, GÄUMANN (1938) distinguished two groups of plants, that is, sensitive and unsensitive plants for temperature. In the former, recreation occurred when the soil temperature was equal or higher than the air temperature.

At the beginning of this year, SPEIDEL (1939) published a treatise on the physiology of bleeding in higher plants, in which he observed that the volume of exuded sap diminished as the calcium and potassium concentration in the nutrient solution become concentrated.

The object of this investigation is to determine the relation of the recreation sap and the nutrient solution and to elucidate the physiological significance of the phenomenon concerned.

Material and methods.

The test plant used was *Colocasia antiquorum* var. *esculentum*. Young plants of *Colocasia* were severed from the tuber and were planted in water-culture vessels, each filled with distilled water or tap water or KNOP's solution. The plants were put in a dark chamber shut tight. To sustain the relative humidity over 90%, the water-culture vessels in which the test plant had been planted, were put in a broad shale filled with water. The leaves whose recreation sap were collected, had been rolled, the tip of each leaf was led to a test tube.

Before the analysis of recreation sap, the volume of it was measured and in some cases, pH of it was also determined by colorimetric method. The microanalysis adopted for the analysis of calcium and potassium was PINCUSSEN's method (1937) a little modified.

Experimental part.

The concentration of calcium in the exudation sap was much different by individual even if the nutrient solution was the same. So the mean value of the concentration of calcium was adopted in the following data.

TABLE 1.

5K indicates the nutrient solution whose concentration is five times the usual KNOP's solution, 1/16K the nutrient solution whose concentration is a sixteenth of the usual KNOP's solution and so on.

T indicates tap water, D indicates distilled water and S indicates soil.

Nutrient solution.	5K	2K	K	1/2K	1/4K	1/8K	1/16K	T	D	S
Ca-concentration (mg per 100 c.c.) of exuded sap.	9.90	3.46	3.24	2.93	2.98	3.02	3.22	1.89	1.34	1.94
Number of parallel experiments.	1	3	45	41	25	17	4	30	31	128

As is shown in Table 1 and Fig. 1, the calcium concentration of exudation sap was almost constant, about 3 mg per 100 c.c., when the nutrient solution was from 1/16 K to K. When the nutrient solution became so

concentrated as 5K, the calcium concentration of exudation sap became so high as 9.9 mg per 100 c.c. But the plant perished sooner or later in this case. On the contrary, low concentration of calcium such as in tap water (1.22 mg per 100 c.c.) or in distilled water (0.31 mg per 100 c.c.) made the plant survive for a long time. And in such low concentration of calcium in the nutrient solution, the concentration of calcium in the exudation sap suddenly fell. The concentration of calcium in the 1/16 K solution being 1.50 mg per 100 c.c. (This value was deter-

mined by analysing 1/16 K solution by PINCUSSEN's method) the lower limit of nutrient solution that cause the constant concentration of calcium in the exudation sap (3 mg per 100 c.c.) seems to exist between tap water and 1/16 K solution whose concentration of calcium is respectively 1.22 mg and 1.50 mg per 100 c.c.

This concentration curve resembles the curve suggested by LAINE in the relation between the concentration of bleeding sap and the external solutions in *Phaseolus*. The linear relation between the two proposed by PAVLINOVA was not seen in *Colocasia*.

The concentration of calcium in the exudation sap is lower than that of the nutrient solution when it was 5 K, 2 K, K, 1/2 K and 1/4 K and higher when it was 1/8 K, 1/16 K, tap water and distilled water. This lower concentration of calcium in the concentrated nutrient solution seems to be due to "the excessive concentration of nutrient solution" assumed by SABININ. In his case, this phenomenon was already seen when the nutrient solution was 0.001 normal regarding calcium ion, but in the recretion of *Colocasia* this was seen when the nutrient solution was about 0.003 normal (1/4 K). Such high concentration of calcium in the recretion sap by SHARDAKOFF (1928) in Brussels cabbage (9.1–12.5 mg per 100 c.c.) was not seen in *Colocasia*.

The relation between the amount of calcium exuded per hour and the concentration of calcium in the nutrient solution resembles the above mentioned concentration relation (See Table 2).

TABLE 2.

Nutrient solution.	5K	2K	K	1/2K	1/4K	1/8K	1/16K	T	D	S
Amount of calcium (mg) exuded per hour $\times 10^3$.	7.50	0.90	4.20	4.00	4.00	4.10	3.80	2.00	1.30	9.00
Number of parallel experiments.	1	3	45	41	25	17	4	30	31	128

The amount was almost constant, about 4×10^{-3} mg per hour, when the nutrient solution was between K and 1/16 K. The irregular value in the high concentration of KNOP's solution is due to the small number of parallel experiments.

The relation between the volume of recretion sap in unit hour and the concentration of nutrient solution is shown in Table 3.

The volume of exudation sap per hour was almost constant when the nutrient solution was more concentrated than K or more diluted than 1/8 K.

TABLE 3.

Nutrient solution.	5K	2K	K	1/2K	1/4K	1/8K	1/16K	T	D
Volume of exuded sap. per hour (c.c.)	0.08	0.04	0.13	0.13	0.12	0.12	0.11	0.09	0.08
Number of parallel experiments.	1	3	52	43	25	18	4	13	34

This volume curve is different from that of LAINE. According to him, the volume of exudation sap increased as the nutrient solution became diluted. And such decreased volume of exudation sap by the low concentration of nutrient solution was never seen in his experiment. After all in the recreation of *Colocasia*, the concentration of calcium, the amount of it and the volume of exudation sap are almost constant in certain range of KNOP's solution.

When the nutrient solution was simple KH_2PO_4 solution instead of KNOP's solution, the volume of exudation sap was almost constant, about 0.1 c.c. per hour when the nutrient solutions were 0.0001%, 0.001% and 0.01% solutions of KH_2PO_4 (See Table 4.).

TABLE 4.

Concentration of KH_2PO_4 (%).	D	0.0001	0.001	0.01	0.025	0.050	0.075	0.1
Volume of exuded sap. per hour (c.c.).	0.099	0.113	0.092	0.109	0.061	0.060	—	—
Number of parallel experiments.	15	13	26	17	3	3	3	10

Recreation ceased when the concentration in the nutrient solution became more concentrated than 0.075%. And in this experiment, the vessels with nutrient solution and test plants were immersed into hot water whose temperature was 40°C. But no effect was observed by this high temperature of nutrient solution. The volume of exudation sap showed no difference from the experiment of KNOP's solution whose vessels were immersed in cold water. As the concentration of KH_2PO_4 in 5 K solution is 0.125% where recreation occurs, and no recreation occurred when the nutrient solution became more concentrated than 0.075% in the simple solution of KH_2PO_4 , it is conjectured that the volume of exudation sap has not to do with the sum of the salts contained in it, when the nutrient solution is KNOP's solution. For if the sum of the concentration of each salt has to do with the volume of exudation sap, there must be no recreation in such a high concentration as KNOP's solution, whose concentration of salt is about

0.162%.

Now the relation between the concentration of potassium in the exudation sap and the nutrient solution will be shown in Fig. 1 and Table 5. The concentration relation showed equal tendency with calcium. But it must be noted that the concentration of potassium was higher than that of calcium, which is contrary to the analysis of SHARDAKOFF (1928) in the recretion sap in Brussels cabbage (Calcium concentration was in his data 9.1–12.5 mg per 100 c.c., potassium concentration 1.29–7.5 mg per 100 c.c.).

TABLE 5.

Nutrient solution.	2K	K	1/2K	1/4K	1/8K	D
K-concentration (mg per 100 c.c.) of exuded sap.	6.13	5.68	7.98	3.48	3.06	1.86
Number of parallel experiments.	2	13	9	6	9	9

In some individuals such high concentration of potassium as 26 mg per 100 c.c. was obtained. Such high concentration was not observed in calcium. And this extremely high concentration appeared when the volume of exudation sap was great, that is, when the recretion was vigorous.

The amount of potassium exuded per hour was not constant in some range of nutrient solution as calcium, but decreased as the concentration of nutrient solution became diluted (See Table 6.).

TABLE 6.

Nutrient solution.	2K	K	1/2K	1/4K	1/8K	D
Amount of potassium (mg) exuded per hour $\times 10^3$	1.90	9.10	6.50	3.70	3.30	2.00
Number of parallel experiments.	2	13	9	6	9	9

In individual plants, the variation of the concentration of calcium and potassium took equal tendency. In Fig. 2 is shown the concentration change of calcium and potassium in an individual as the nutrient solution changed.

In soil-cultured plants, the recretion was more vigorous in comparison

Fig. 2.

with that of the water-cultured plants. But such an enormous bulk as was observed by MOLISCH (92 c.c. in a night) could not be collected in a night.

There was no difference in the concentration and amount of calcium in the recretion sap and the bleeding sap from the cut end of leaf-tip (See Table 7.), but when the lamina was taken off and the bleeding sap was collected from the cut end of petiole, the bleeding sap became much concentrated and therefore exuded much amount of calcium. This shows that epithem does not take part in the absorption of calcium.

The concentration of calcium in the recretion sap of soil-cultured plants was lower than that of the water-cultured plants, but the amount of calcium exuded per hour was greater than that of the water-cultured plants on account

of the large volume of the exudation sap.

TABLE 7.

	Concentration of Ca (mg per 100 c.c.)	Amount of Ca exuded per 24 hours	Concentration of K (mg per 100 c.c.)	Amount of K exuded per 24 hours
Recretion sap.	1.94	0.236	2.33	0.277
Bleeding sap. (Leaf-tip).	2.01	0.213	2.71	0.221
Bleeding sap. (Petiole).	3.22	0.334	11.42	2.568

It is to be noted that the concentration of calcium was not always decreased but often increased when the volume of exudation sap was great, that is, when the recretion was vigorous. This shows that the strong recretion exudes larger amount of calcium than weak recretion. This tendency was seen in soil-cultured plants as well as in water-cultured plants.

The maximum volume of recretion sap in soil-cultured plant was 38 c.c.

in a night and the maximum volume of bleeding sap was 27 c.c. which was smaller than that of recretion. This may be due to the hindrance of mucilaginous substances at the cut end.

Potassium concentration and amount were almost the same in recretion sap and in bleeding sap from the cut end of leaf-tip, but the bleeding sap from the cut end of petiole was exceedingly concentrated concerning potassium (See Table 7).

The pH values of bleeding sap were lower than that of the recretion sap (Bleeding sap pH 6.3, recretion sap pH 6.6–6.9).

Discussion.

From the recretion sap and the bleeding sap from the cut end of leaf-tip in natural plant, it was confirmed that epithem did not take part in the filtration of cations. And seeing that the volume of exudation sap showed no great difference between the recretion sap and the bleeding sap, recretion in *Colocasia* is not effected by the active function of epithem, or cells at the hydathods, but may be effected passively by root pressure. After all in the recretion of *Colocasia*, epithem seems to have no significance in recretion. The hydathods of *Colocasia* is only the place from which the recretion sap is excreted.

The cations absorbed from the root of *Colocasia* seems to be partly consumed inside the plant and partly thrown out in the recretion sap. In the case of calcium, it may be consumed chiefly for the removal of oxalic acid by forming calcium oxalate crystals. The exceedingly high concentration in the bleeding sap from the cut end of petiole and greatly diminished concentration of it in the bleeding sap from the cut end of leaf-tip show that potassium is much absorbed in lamina.

It is to be noted that in spite of the lower concentration of potassium than calcium in the nutrient solution, the amount of potassium absorbed by root was much greater than calcium, and the diluted nutrient solution became often potassiumless in few days. From this and the above mentioned absorption of potassium in lamina, it seems that potassium has greater significance in the life of *Colocasia*.

The high concentration of calcium and potassium by vigorous recretion seems to suggest that the significance of recretion is to excrete unavailable substances.

Summary.

1. Calcium concentration of recretion sap was nearly constant in certain range of the concentration of KNOP's solution. When the nutrient

solution became diluted, the calcium concentration in the recretion sap suddenly fell. When the nutrient solution became concentrated, the concentration of calcium in the recretion sap also rised. The calcium concentration in the exudation sap was lower than that of the nutrient solution when the nutrient solution was comparatively concentrated and higher when the nutrient solution was comparatively diluted.

2. The amount of calcium exuded per hour was almost constant in certain range of KNOP's solution and diminished in the low concentration of calcium in the nutrient solution.

3. The volume of exudation sap per hour was almost constant in certain range of KNOP's solution and diminished when the nutrient solution was more concentrated than natural KNOP's solution or more diluted than 1/8-KNOP's solution.

4. When the nutrient solution was simple KH_2PO_4 solution, recretion ceased when the solution became more concentrated than 0.075%. As the concentration of salt in KNOP's solution is much higher than this, it seems that recretion has not necessarily to do with the sum of the concentration of salts in the case of KNOP's solution.

5. The relation between the potassium concentration in the exudation sap and the nutrient solution showed equal tendency with calcium. But it must be noted that the potassium concentration was higher than that of calcium. The amount of potassium exuded per hour was not constant in some range of KNOP's solution as calcium, but decreased as the concentration of nutrient solution became diluted. In individual plants, the variation of the concentration of calcium and potassium took equal tendency.

6. In soil-cultured plants, the recretion was more vigorous in comparison with that of the water-cultured plants.

7. From the results obtained in *Colocasia*, the absorption of salts seems not to take place in epithem.

8. The concentration of calcium and potassium was not always low but often high when the volume of exudation sap was great. So great amount of calcium and potassium was excreted in strong recretion.

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