A CONTRIBUTION TO THE KNOWLEDGE OF REGENERATION IN HIGHER PLANTS

(*With 12 Figures*)

By

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The causes of the regeneration and the reparation of tissues or organs of various plants have been studied by many authors. GOEBEL (1898) attempted to explain the formation of organs in plants on the assumption of internal correlation and external stimuli. SACHS (1892) assumed the special substances which cause the formation of shoots and roots; the prevention of the flow of these specific substances induces shoot formation at the apical part and root formation at the basal part of a regenerating piece. ERRERA (1905) suggested the idea of "Hemmungstoff" and Driesch "Entelechie." LINSBAUER, (1925) advanced a hypothesis of "äquipotentielles System" in tissue, according to which the harmony between tissues is the main factor concerned in regeneration. LOEB pointed out two factors which control regeneration; inhibiting effect and mass-relation. SETCHELL (1905) maintained, as well as LOEB, that regeneration is not caused by the existence of a specific stuff and its particular nature, but that the materials needed for regeneration are only the nutritive materials. According to SETCHELL the control of the direction of sap flow seems to be important in inducing regeneration. ROBERTSON (1923) stated that the inciting cause of regeneration is due to the removal of the products of the reaction which underlie and determine growth-rate, and the removal of these products, as in other chemical equilibria, reinaugurates the forward reaction by which regeneration takes place. HABERLANDT (1913, 1914 and 1921) advanced an opinion on regeneration based on the assumption of the formation of hormone, which is secreted from the vascular system, especially from phloem, and stimulates cell division.

The present writer has been engaged for the past few years in

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investigation on the inciting cause of regeneration in Bryophyllum, especially in relation to the catabolic products which accumulate in the regenerating pieces. One part of the experiments on this problem has already been accomplished and reported (1928 and 1930 a, b). MOLISCH (1931) favoured and introduced the writer's results and opinions with much interest. Others also expressed their approbation. HARIG (1931) and others in Ruhland's Institute, Leipzig, supported, as well as the present writer, a catabolic theory in regeneration, using the materials other than those which the present writer had employed. OSSENBECK (1927) and MEHRLICH (1931) attempted to induce regeneration on an attached leaf of Bryophyllum by warm-bath method. In both cases, however, they obtained negative results. Mehrlich criticized the results of the present writer (1928) and expressed contrary opinions. The writer carefully repeated the experiments in this direction after the publication of Mehrlich's work, and came to the conclusion that the warm-bath method is very successful to induce the regeneration of the attached leaf of Bryophyllum as stated in detail in later pages.

In the present work, further results supporting the writer's viewpoint in regeneration will be reported and discussed in detail. It is also confirmed that these results obtained in the case of Bryophyllum can be successfully applied also to the case of regeneration in the stem cuttings of various plants, so-called tumor formation and cell multiplication of other plants.

I. Regeneration in Bryophyllum

(1) Bryophyllum calycinum Salibs.

The Bryophyllum, like Begonia, regenerates new roots and shoots on an isolated leaf. This plant is a very interesting material for the study on the problem of regeneration. Therefore, since BERGE (1877), studies on regeneration in this plant have been carried out hitherto by many authors. In this plant, two oppositely growing leaves in the same node are produced at the same time, therefore they are of the same age and develop symmetrically in relatien to size and form. They will be called sister leaves. In the greenhouse the leaves of this plant are thin between May and September, and thick and very fleshy in the remaining part of the year. In the latter case they are called winter leaves. So far as the leaf of this plant is attached to the stem,

it performs the same functions as those of other common plants. But when it is isolated from the stem, it shows a strong power for regeneration. It is usual for this plant to be propagated in this way. The plants employed in the present investigation were propagated vegetatively by the writer in the greenhouse. Abnormal materials which are easily produced by insect parasites or other causes, were excluded in the present investigation, except the special case which will be described later.

(2) Regeneration in an isolated leaf of Bryophyllum calycinum

When a leaf, a notched piece of a leaf or a piece of a stem of Bryophyllum calycinum is detached from the plant and put on moist sand, it regenerates a complete plant. When a leaf is taken from the mother plant, root formation occurs first, followed by shoot formation at the notches. The regeneration in this case begins in a group of cell masses at the base of the notches, which is composed of many small isodiametric cells, possessing large nuclei and dense cytoplasm (Beals 1923, Howe 1931, Mehrlich 1931 and Naylor 1932). The present writer has carried out a similar observation. The notches were cut off from the isolated adult leaves, fixed in Nawaschin's fixing fluid, imbedded in paraffin, cut 10 μ in thickness, and stained with Delafield's iron-alum haematoxylin, gentian-violet and safranin. A small group of meristematic isodiametric cells with lage nuclei is found at the base of the notches. When regeneration takes place from a leaf, it occurs from this group of meristematic cells exogenously. The writer carried out microscopical observations in the other parts of the leaf (petiole, epidermis, vascular bundle, parenchymatous tissue etc.), using fixed materials of isolated and attached leaves of Bryophyllum calycinum. But any anatomical differences between isolated and control leaves could not be found. In the culture of small pieces of the leaf of Bryophyllum calycinum, LAMPRECHT (1918) found that even a piece of Bryophyllum leaf of 0.5–0.7 mm.^{2} was able to form the callus tissue on the wound surface, and cell division is limited only to the leaf piece which possesses vascular bundles.

(3) Regeneration in the attached leaf of Bryophyllum calycinum

When a leaf, or a notched piece of a leaf or a piece of the stem of Bryophyllum calycinum is detached from the plant and put on moist

sand, it regenerates a complete plant, i.e. roots and shoots. In regeneration, root formation occurs first at the notches of the leaf about 5 days after isolation at the temperature of about 25°C. Soon after, shoot formation occurs at the notches. However, regeneration in a piece of the stem is somewhat different. If a piece of the stem is detached from the plant, shoot formation at the apical node begins to occur soon after the stem was isolated. But there is a relatively long latent period before the basal roots appear.

The question now arises whether it is possible to induce such growth of new shoots and roots on the remaining stem or leaf without mutilation. To this question, LOEB (1924) gave an affirmative answer: "When a stem contains many leaves, when stem growth is stopped, or when a plant becomes old, the regeneration takes place at the notches of a leaf which is still attached to the plant." Further, some others showed that such growth of new shoots and roots on the leaf or stem of a normal and healthy plant of Bryophyllum calycinum could be brought about by giving it some particular treatment. CHILD and BELLAMY (1919 and 1920) succeeded in producing roots and shoots at the notches of a leaf of a healthy plant of Bryophyllum calycinum by certain procedures without taking off the leaf from the stem. The cooling of a certain zone of the petiole of this plant below a temperature of 2.5–4.0°C. for a few days, or pressing the petiole by a screw clamp to half its thickness is a very effective means of inducing the outgrowth of the leaf bud. In both cases, the treated, as well as the opposite untreated leaf, and often an adjoining untreated leaf show a sign of development, when the blade is submerged in water. Child and Bellamy in their papers have called this mode of procedure a physiological isolation. SMITH (1921) induced bud development on the attached leaves and stem of Bryophyllum calycinum by crown gall formation which was caused by inoculation with Bacterium tumefaciens. LEVINE (1919) secured negative results in a similar experiment. GOEBEL (1902) succeeded in inducing the formation of roots and shoots at the notches of the attached leaves of Bryophyllum plant by treating the whole plant body with ether vapour for 12–48 hours. This method of treatment has been already known as JOHANNSEN's "Ätherisieren" to force the bud development in the winter resting period. Goebel applied this treatment to regeneration in attached leaves of Bryophyllum plants. REED (1923) obtained a positive result in inducing roots and shoots on the attached leaves of Bryophyllum calycinum by placing the whole

plant body in a dark room. He placed the plant under natural conditions, and then deprived the plant of sunlight only. Two weeks later, regeneration at the notches of the attached leaves became visible.

The present writer (1928 and 1930 b) reported that warm-bath and some other treatments are very effective means to induce the growth of new roots and shoots on the attached leaves of Bryophyllum calycinum. The investigation on this problem including the preceding results will be described here in detail.

1. *Warm-bath treatment*

(a) The shoots of potted Bryophyllum plants, about 30–50 cm. in height and about one year old, were submerged in a warm-bath of about 30°C. for 8 hours or for a shorter time in the laboratory. After this warm-bath treatment the materials were placed in the greenhouse. Some of them were covered with bell jars to keep them in a more or less moist condition, and some others were so placed that the apices of the treated leaves were dipped in water. In both cases, the supply of fresh air was carefully considered. After 5–10 days (at 20°–25°C.), roots appeared at the notches of the treated leaves, except the very young leaves near the top, and the shoot formation soon followed. These regenerated young plants grew on for quite a long time. Some of them were planted in a pot after 3–4 weeks from the beginning of their development, together with their mother leaf, without taking it away from the stem. They lived for a long time. In some cases the untreated leaves of the same materials also produced a few small roots and shoots at the notches. However, roots and shoots produced on the untreated leaves were considerably fewer than those on the treated ones. In other cases, both in the treated or untreated parts of the stem of the same plants, adventitious roots only were formed. On the control material, such regeneration did not take place.

(b) Young plants 2–3 months old, still attached to the isolated mother leaves, were used. These young plants were immersed together with their mother leaves in the warm-bath of about 35°C. for 8 hours. Then the whole materials were put in a moist chamber in the greenhouse, keeping only the mother leaves in water. About 10–15 days later, regenerated roots began to appear at the notches of the leaves in the middle and the lower level of the young plants, sometimes accompanied by very tiny shoots at the same notches. But these regenerated parts soon disappeared.

(c) In this experiment, the writer submerged almost all parts of the shoot of the plant in the warm-bath of about 30°C. for 8 hours. Then the material was placed in the greenhouse. The treated plant was kept under normal conditions, immersing the treated leaves in water. About 5 days later, the treated leaves began to produce roots. This root formation occurred at first chiefly at the notches of the treated leaves which were attached to the upper part of the stem. After this happened, the leaves attached to the middle and the lower parts of the stem began to produce roots. Shoot formation also took place mainly on such leaves. Generally speaking, the regenerated roots dried off soon after their appearance, though the regenerated shoots grew and grew for a long time. In some cases, not only the formation of roots and shoots on attached leaves occurred, but also adventitious roots were produced in the lower part of the stem. This observation was made from June to September, 1928.

(d) Healthy one year old Bryophyllum calycinum plants which had been cultured in pots in the greenhouse, where the temperature was about 18°–26°C., were divided into two groups of equal members; one group was submerged in the warm-bath; the other was left in the air in the laboratory as control. The method of warm bathing was that almost all the parts of the shoots of each material to be treated were immersed into the warm-bath; the temperature of it was regulated by the electric heater to keep it 30°–32°C. constantly. The pots, in which the treeated materials were planted, were left in the open air in the laboratory. The duration of warm bathing in this treatment was two and ten hours respectively. After treatment, the treated and the control materials were put into a moist chamber (relative humidity, about 85% and temperature, 15°–27°C.). Two or three days later, the materials which had been treated for 2 hours began to produce new roots at the notches. But the materials which had been treated longer, namely for 10 hours did not show any regeneration, and moreover, such a longer treatment was harmful to the materials; that is to say, the young leaves attached to the upper and middle parts of the materials decayed. The control materials in the moist chamber never showed such an abnormal phenomenon. This experiment was carried out in November 1–10, 1931.

(e) In this treatment, healthy plants were put in the warm-bath of 27°C. for 5 hours. Then they were placed in the moist chamber as stated above. A few days later, the production of roots and shoots

at the notches of the treated leaves occurred vigorously. Control materials in the same moist chamber did not show such a regeneration. This experiment was carried out in March, 1932.

(f) A healthy Bryophyllum plant, which possessed five nodes in the stem and ten adult leaves and two very young leaves at the top of it, was used in this treatment. Only the adult leaves attached to the upper two nodes and the very young leaves at the top were put in the warm-bath and the other leaves were left in the air. In this state, the plant was left for 3 hours. The temperature of the warm-bath was 30°–32°C. and the room temperature 17°–23°C. After such treatment, the plant was placed in the moist chamber. About three days later, only the treated adult leaves began to produce roots at the notches (Fig. 1) and soon formed little shoots at the same notches.

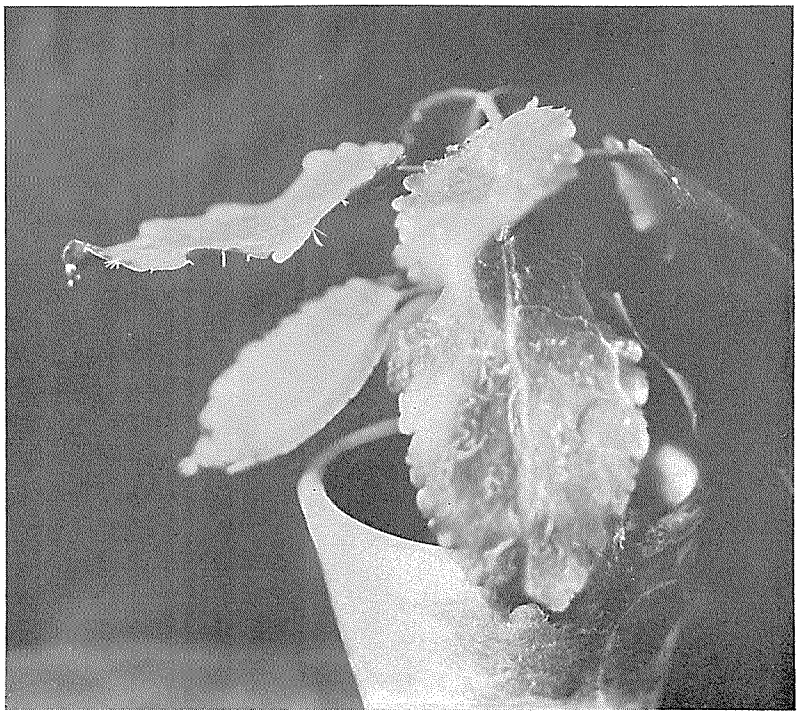


Fig. 1. The upper attached leaf of Bryophyllum calycinum which was treated in warm-bath for 3 hours produced roots, while the other untreated leaves did not.

The treated young leaves near the top did not produce roots or shoots. The untreated adult leaves of the same plant showed no sign of such development. The treatment was made in December, 1931.

(g) Healthy individuals, each with 8–10 nodes, were employed as materials. In this treatment, special care was taken not to soak the growing points of the materials in the warm-bath. That is, only the adult leaves attached on one side of a plant were dipped in the warm-bath, without immersing the top and the control leaves attached on the opposite side of the plant. The treatment continued for 3 hours in the warm-bath of 30°C. During the treatment, the growing points and the control leaves of the materials were kept in the air of room temperature. About 5 days later, some of the treated leaves began to produce little roots and shoots. The untreated control leaves of the same plant did not show such phenomenon. This experiment was conducted January 17–25, 1932. From above stated results, it may be asserted that MOLISCH's warm-bath method is a very effective means of producing roots and shoots at the notches of the attached leaves of Bryophyllum calycinum, not only in the treatment of the whole shoot but also in that of two or three attached adult leaves. And the optimum treatment of warm-bath to induce the root and shoot formation on the attached leaves Bryophyllum calycinum is secured by placing the material in the bath of about 27°–30°C. for 3–5 hours.

(h) Two or three adult leaves attached on one side of a plant only were soaked in the warm-bath of 30°C. for 3 hours, leaving the top and the leaves attached on the opposite side of the plant in the air of room temperature, as in the foregoing experiment. After the treatment, the materials were placed in the moist chamber. After 2–3 days, the treated leaves produced roots and shoots at the notches. After about 10 days from the beginning of the experiment, some untreated leaves near the treated ones began to produce also tiny roots and shoots at the notches, though very few in number. For instance, one of the treated leaves produced a total of 50 roots and 22 shoots at the notches, but untreated leaves attached to the opposite side of the treated leaves produced only 12 tiny roots and 8 tiny shoots at the same time. This may be due to the flowing of the mobile substances produced in abnormal metabolism (see the following chapters) in the treated leaves into the neighbouring untreated leaves. This experiment was carried out in February, 1932. This phenomenon seems to be very similar to the fact which was found by CHILD and

BELLAMY (1920). When they cooled the petiole of one of the attached leaves of Bryophyllum calycinum, not only the treated leaf but also neighbouring untreated leaves produced regeneration.

(i) Isolated leaves of Bryophyllum calycinum, which possessed young regenerated plants at their notches, were placed in the moist chamber in the greenhouse from October to December, 1926. In the beginning the weather was rainy or cold, but this was followed by a short spell of fine weather for about 10 days, during which the temperature in the moist chamber rose suddenly and remained at about 30°C. for some time. The young plants which developed on the isolated mother leaves, regenerated vigorous roots at the notches of their own leaves, chiefly in the middle and in the upper part of the stem. These regenerated roots soon drooped. Some other healthy plants of 15–20 cm. in height were placed near the hot water pipes in the greenhouse. They also regenerated roots and shoots at the notches of the leaves attached to the middle and lower level of the stem. These newly produced parts lived for a long time. The temperature in that corner of the greenhouse registered sometimes higher than 30°C. Child and Bellamy (1920) reported that a sudden rise of temperature from 15° to 20°C. in the saturated air usually induced outgrowth of some roots on the attached leaves of Bryophyllum calycinum, but the development of these regenerated roots was soon inhibited.

2. *Placing material in H_2- or N_2-gas*

The present writer reported (1928 and 1930 b) that inducing anaerobic respiration by placing the whole plant body of Bryophyllum calycinum in H_2-gas is a successful method for root and shoot formation on the attached leaves of healthy plants.[1)]

The experimental results of these papers can be summarized as follows: potted plants of Bryophyllum calycinum were placed under a bell jar, in which a stream of H_2-gas was constantly passing for 48–72 hours in the laboratory. The temperature was about 20°C. The plants, thus treated, were placed in a room of the laboratory, where no gas burner was used, and sunny illustration was good, and the temperature was kept between 15° and 24°C. They were covered

1) MEHRLICH (1931) placed Bryophyllum plants in N_2-gas for 124 hours, instead of in H_2-gas. In his experiment, however, MEHRLICH opposed the present writer's experimental result. But HARIG (1931) obtained the positive results in a similar experiment, using Cardamine.

with bell jars in order to keep them more or less in moist condition. The supply of fresh air was carefully considered. About 2 weeks later, regenerated roots appeared at the notches of the leaves of the treated plants, and the production of shoots soon occurred, chiefly on the leaves of the middle and lower levels of the stem. Sometimes adventitious roots appeared in the lower part of the stem. A similar experiment was repeated by the writer (1930 b) as follows: potted Bryophyllum calycinum were placed in H_2-gas for 48 hours, during which period the H_2-gas was constantly replaced by fresh qualities. After such treatment the plants were placed in the greenhouse (20–30°C.) and no bell jar was used to cover them. About 5 days later, regenerated roots appeared at the notches of the leaves of the treated plants except in very young leaves near the main tip; not later shoot formation occurred chiefly on the leaves which were attached to the middle and lower parts of the stem. In this case, however, the regenerated roots on the attached leaves dried off soon after their appearance. But new little roots were formed after old ones at the same notches of the treated leaves. This repeated root formation on the treated leaves continued for some time. Then it stopped and the regenerated roots disappeared. On the other hand, the regenerated little shoots on the treated leaves, which were produced chiefly on the leaves of the middle and lower parts of the stem grew and grew and lived for a long time.

Recently, similar experiments were repeated by the writer as follows: only one leaf attached to the middle part of a normal healthy and about one year old Bryophyllum calycinum, was closed within a leaf chamber which was 15 cm. × 8 cm. × about 2.5 cm. in size. This chamber consisted of two equal boxes, each having a glass plate at the base which was connected with the wooden frame by means of putty. Putty was used to coat also the inner side of the wooden part of the boxes. The two boxes were fitted together tightly closed with each other by means of screw clamps during the experiment. Between the two boxes there was a heavy rubber sheet in order to prevent the entrance of air. The leaf chamber was held in proper position by means of a stand and one leaf was closed within it when the measurement was made. Having closed a leaf within a chamber, the air in it was replaced by H_2-gas which passed through a gas wash bottle containing 0.1 normal K-permanganate which absorbed the O_2 and then through soda-lime and the solution of KOH which absorbed the CO_2 mixed in

the gas, before the gas entered the chamber. The leaf was placed thus in anaerobic condition for 48 hours, during which period fresh gas was constantly passing through the chamber. Then the leaf was returned to normal aerobic condition and kept in the greenhouse. About 4 days after the end of the treatment, roots appeared at the notches of the treated leaf. This experiment was carried out in December, 1928.

Another experiment was carried out, in which one adult leaf attached to a normal healthy plant was closed within the leaf chamber; the air in it was replaced by N_2-gas instead of H_2-gas. The leaf was placed in such condition for 24 hours at room temperature, then the leaf was returned to normal aerobic condition in the glass chamber. Soon after the treatment, the notches of the treated leaf had already become white, showing a sign of the development of root primordia, while the control leaves did not show such change. One or two days later, root regeneration at the notches of the treated leaf became visible, followed by little shoot formation. The control leaves never showed such phenomena. So far as the present writer's experimental results are concerned, N_2-gas is more effective than H_2-gas to induce regeneration on the attached Bryophyllum leaf. This experiment was carried out in January, 1932.

3. Placing material in cold place

Another experiment was carried out in which twenty healthy potted plants of about one year old were placed in a cold glasshouse where the temperature was kept below that of the normal greenhouse, at about 10°C. But other environmental conditions—light, aeration etc.—were almost equal to those in the normal greenhouse. About 3 weeks later, all these individuals formed vigorous roots and shoots on the attached leaves of the middle and lower parts of the stem.[1] In some plants, not only did roots and shoots develop on attached leaves, but also auxilliary buds in the node on the upper part of the stem began to develop, while the tops of the plants showed continuously almost normal growth. After the development of the newly produced parts, all the plants were returned to the normal greenhouse (about 20°–30°C.). The mother plants and also the newly regenerated parts

1) OSSENBECK (1927) reported that in Bryophyllum crenatum and Bryophyllum calycinum regeneration on the attached leaves of the stem was induced by cooling the pots in which the plants were planted.

lived for a long time. And some of the little newly regenerated plants on the attached mother leaves produced a little flower on their stems, while none of the mother plants produced flowers. This experiment was carried out from December, 1928 to January, 1929. It is to be noted that before this experiment was made, the weather had long been fine and accordingly the leaves of the materials used in this experiment became extraordinarily fleshy.

It is very reasonable to suppose that the above stated treatments, which have been carried out by many authors including the present writer to induce regeneration on the attached leaves of healthy Bryophyllum plants, may have altered the metabolism in a leaf or other parts of a plant body.

As we have seen above, the ether treatment,[1] warm-bath treatment, inducing anaerobic respiration, cooling, and sudden increase of temperature under aerobic condition etc., are all effective means to produce roots and shoots on the attached leaves of Bryophyllum. The ether treatment and warm-bath method are common procedures used to wake up the winter resting state of the buds. It is very interesting that other methods, which were attempted successfully in producing roots and shoots on the attached leaves of Bryophyllum calycinum, are the same or very similar to the treatments designed to wake up a resting winter bud. According to the investigation of BORESCH (1924, 1926 and 1928 a) the forcing action of the warm-bath method and other similar treatment upon the development of a resting winter bud is due to the fact that the warm-bath and other similar treatments cause intramolecular respiration in a bud. As a result of such catabolism, the intermediate and end products of alcoholic fermentation are accumulated in the bud, which may work as stimulant to force bud development. That the treatments, which are used to force the winter bud, may cause respiration of just the same kind in Bryophyllum plants and the products of such respiration or some related substances may stimulate the regeneration, is easily supposed.

4. Injection with substances which are assumed to be the products of incomplete or anaerobic respiration

In order to confirm the assumption stated above, the experiment

1) GOEBEL (1902) and MEHRLICH (1931).

of injection with such substances as organic acid, pyruvic acid, acetaldehyde and ethyl alcohol which are now believed to be the products of incomplete and intramolecular respiration, was carried out using the attached leaves of Bryophyllum calycinum as materials.

First, acetaldehyde, pyruvic acid, and ethyl alcohol were selected for injection. Each chemical was prepared in concentration of 0.5, 0.05 and 0.01%. About 0.3–1 c.c. of each of them was injected into the stem, the petioles, or leaf margin near the notches of different individuals. One group of plants was injected only once, and the other group was injected once every day, but continued for over 5 days. As the control water injection was carried out. The results which were obtained in September and October, 1927 are shown hereinunder: 1. A 0.5% solution of each chemical was injected into petioles once a day for 5 days. In this experiment, acetaldehyde induced roots and pyruvic acid, tiny shoots. This experiment was worked out in the greenhouse, September 29—October 20, 1927. 2. A 0.05% solution of each chemical was injected into the petioles of young plants only once. The results were that acetaldehyde and pyrubic acid induced roots. This experiment was performed in the moist chamber in the greenhouse.

Other experiments were carried out as follows: one pair of attached sister leaves on a healthy plant was selected out as materials. One of these sister leaves was injected with any one of the above mentioned chemical and the other with pure water as control. The injection was made once in the leaf blade near the notches. About 1–2 c.c. of the prepared chemical solutions was injected with a common injection needle. Care was taken to avoid injuring the vein of the leaf with the injection needle. After injection, the plant material was placed under normal conditions. All the experiments in this series were made in the greenhouse.

First, a 5% ethyl alcohol was used for injection. One of the sister leaves was injected with about the same amount of pure water as control. After this treatment the plant was put under normal condition in the greenhouse of about 20°–28°C. Two days later, regenerated roots began to appear at the notches of the leaf which was injected with ethyl alcohol. Soon shoot formation also occurred at the same notches. On the control leaf such regeneration did not take place. After a few days from the beginning of the regeneration of roots and shoots on the treated leaf, the roots were merely dipped in water, in

order to avoid the possibility of their drying off; the mother leaf and its regenerated shoots on the same notches were left in the air. The regenerated plants on the attached leaf grew and grew. This experiment was carried out in January, 1929.

Next, pyruvic acid, acetaldehyde and organic acids were also chosen for injection. Five-hundredth per cent solutions of acetaldehyde and pyruvic acid were prepared. About 1 c.c. of each kind of the solutions was injected once into the leaf blade of one of the sister leaves which were attached to the middle level of a healthy plant. In every case, water injection was carried out on the opposite leaf as control. In both cases of the treatments, tiny roots were regenerated at the notches of the injected leaves, but not in the controls. This experiment was done in January, 1929.

In another experiment, one leaf of one plant was injected with 5% acetaldehyde and the other plant with water as control. A few days later, some leaves near the leaf which was injected with acetaldehyde, produced shoots and roots vigorously at the notches. At the same time, the leaf which was injected with 5% acetaldehyde decayed, owing perhaps to its high concentration. The control plant showed no such result. This experiment was made in February, 1929.

The following preparations of organic acids were also made for the purpose of injection. Five c.c. of 0.1 M solutions of various organic acids were mixed with various amounts of a 0.1 N NaOH-solution and enough H_2O was added to bring these mixtures to 10 c.c. respectively, and the following series was made:

Malic acid,

No. 1	0.1 M malic acid	5 c.c.	pH 2.4	No. 4	0.1 M malic acid	5 c.c.	pH 3.8
	0.1 N NaOH	0			0.1 N NaOH	3 c.c.	
	Water	0			Water	2 c.c.	
No. 2	0.1 M malic acid	5 c.c.	pH 2.9	No. 5	0.1 M malic acid	5 c.c.	pH 4.3
	0.1 N NaOH	1 c.c.			0.1 N NaOH	4 c.c.	
	Water	4 c.c.			Water	1 c.c.	
No. 3	0.1 M malic acid	5 c.c.	pH 3.4	No. 6	0.1 M malic acid	5 c.c.	pH 4.7
	0.1 N NaOH	2 c.c.			0.1 N NaOH	5 c.c.	
	Water	3 c.c.			Water	0	

Control Pure water.

Citric acid,

No. 1	0.1 M citric acid	5 c.c.	pH 2.8	No. 2	0.1 M citric acid	5 c.c.	pH 4.6
	0.2 N NaOH	1 c.c.			0.2 N NaOH	3 c.c.	
	Water	4 c.c.			Water	2 c.c.	

No. 3 { 0.1 M citric acid 5 c.c. / 0.2 N NaOH 5 c.c. / Water 0 } pH 5.3

Control Pure water.

Tartaric acid,

No. 1 { 0.1 M tartaric acid 5 c.c. / 0.2 N NaOH 1 c.c. / Water 4 c.c. } pH 2.8

No. 2 { 0.1 M tartaric acid 5 c.c. / 0.2 N NaOH 3 c.c. / Water 2 c.c. } pH 3.6

No. 3 { 0.1 M tartaric acid 5 c.c. / 0.2 N NaOH 5 c.c. / Water 0 } pH 5.6

Control Pure water.

Oxalic acid,

No. 1 { 0.1 M oxalic acid 5 c.c. / 0.2 N NaOH 1 c.c. / Water 4 c.c. } pH 1.6

No. 2 { 0.1 M oxalic acid 5 c.c. / 0.2 N NaOH 3 c.c. / Water 2 c.c. } pH 2.8

No. 3 { 0.1 M oxalic acid 5 c.c. / 0.2 N NaOH 5 c.c. / Water 0 } pH 4.7

Control Pure water.

The solutions were each injected into one of the sister leaves of different individuals respectively. The other of each pair of sister leaves was injected with water as control respectively. The solutions No. 1, 2, 3, 4 and 5 of malic acid induced tiny roots at the notches of the injected leaves, but No. 6 solution of malic acid did not cause regeneration. The control leaf showed no sign of such development of roots. This was carried out from April 14 to May 15, 1928.

A similar experiment was worked out with citric acid. The notches of the leaf, which was injected with No. 1 solution of citric acid formed very tiny roots only. Injection with tartaric acid and oxalic acid did not induce regeneration. This experiment was conducted from May 22 to 30, 1928.

Ethyl alcohol, pyruvic acid, acetaldehyde, and some organic acids, which are believed to be the products of anaerobic or incomplete respiration, are effective to induce regeneration in the attached leaves of Bryophyllum calycinum. Of these substances, ethyl alcohol and acetaldehyde were found to work most effectively in this respect. From the experimental results of warm-bath treatment, inducing anaerobic respiration and injection with the end and intermediate products of intramolecular repiration, it is now known that the products of alcoholic fermen-

tation and some organic acids (mainly malic acid) may play a stimulative rôle in the inducing of regeneration in an attached leaf of Bryophyllum calycinum. Further it may be expected that the isolation of a leaf from the stem, which prevents direct flow of sap from the leaf into the stem and from the stem into the leaf similarly, causes in the isolated leaf some kinds of metabolism which are quite different from that of the normal attached leaf, and that the products in such case may stimulate regeneration in the isolated leaf. These points will be confirmed by the following experiments with regard to the products of respiration.

(4) Analysis of respiratory products

Throughout the present work, experiments on the estimation of CO_2, O_2, acidities, acetaldehyde and alcohol will be constantly met with. Determination of acidities was carried out in the following ways: the materials to be tested were mashed in a mortar by hand pressure, using approximately the same force in each experiment. The paste of the materials was filtered through the filter paper using BUCHNER's funnel. The juice, thus obtained, was used for the determination of acidities. A sample of 10 c.c. was used for each determination. Total acidity was estimated by titration with 0.1 N NaOH solution, and pH by using Clark and Lub's indicators.

The analyses of acetaldehyde and alcohol were carried out as follows: materials to be used were torn into small pieces which were distilled with boiling vapour, cooling with Liebig's condenser 1 m. in length, which was connected with a spiral cooler having a coil about 1 m. long, cooled with ice water. One or two hundred c.c. of the distillate, thus obtained, was used for each determination. Acetaldehyde was sometimes detected with SCHIFF's reagent, p-nitrophenolhydrazine, sodium nitroprusside and by GRIEBEL's method. The quantitative determination of aldehyde was done after the method of Neuberg and GOTTSCHALK (1924): When aldehyde reacts with hydroxylamine sulfate, free H_2SO_4 and oxim are produced. This free H_2SO_4 is titrated with NaOH. In the present experiments, 10 c.c. sample was taken and 0.2 c.c. of 4% hydroxylamine sulfate added to it. This mixture was kept at 30°C. for one hour. The aldehyde in the sample reacted with hydroxylamine sulfate, producing free H_2SO_4 and oxim as the reaction products. The free H_2SO_4 was titrated with a 0.1 N NaOH solution

by means of micro-burette using a methyl orange as the indicator. From the volume of 0.1 N NaOH solution used for the titration, the milligram of aldehyde was calculated.

The determination of ethyl alcohol was made according to NICLOUX's method. Ethyl alcohol solutions of various concentrations, i. e., from 0.01 to 2.0% were prepared. Five c.c. of each solution was put in the test tube and one c.c. of 2% potassium bichromate and 5 c.c. of concentrated H_2SO_4 were added to it. This series shows difference in colour. By the use of this series as the standard, the estimation of ethyl alcohol was colorimetricaly carried out. Five c.c. of the test material was taken in the test tube, and mixed with potassium bichromate and H_2SO_4. The colour which this mixture showed, was compared with that of the standard series. In this way, the volume percentage of ethyl alcohol in the sample was determined.

The volume of O_2 and CO_2 in respiration is estimated by the following two methods:

1. Using PETTENKOFER's tube.

The measurement of the volume of CO_2 produced was made by absorption in baryta water. One hundred c.c. baryta water was put into a Pettenkofer's tube. One end of the tube was connected with the plant container, into which only CO_2-free fresh air, which had been passed through soda lime and KOH-solution, came constantly. The other end of the Pettenkofer's tube was connected with a wash bottle containing baryta water and then with an air pump, by means of which a uniform air stream from the plant-container was kept through the PETTENKOFER's tube. About 1000 c.c. air stream per hour was passed constantly through the PETTENKOFER's tube during the experiment. The titration of baryta water was made with oxalic acid.[1)]

2. Using HEMPEL's apparatus.[2)]

RQ-[3)] value in respiration was determined by using HEMPEL's apparatus.

Gas analysis was made using 100 c.c. of gas, taken with gaspipette. The volume of O_2 in gas was determined by the absorption of alkaline solution of pyrogallol and that of CO_2 by the absorption of the KOH solution.

1) 5.635 g. dissolved in 1000 c.c. water.

2) As the purpose of the measurement of CO_2 and O_2-volume by this method was to secure the RQ-value, temperature and atmospheric pressure during the measurement was omitted.

3) RQ means the respiratory quotient CO_2/O_2.

Changes in the actual and the total acidities in the treated and control leaves of Bryophyllum calycinum during a day

WARBURG (1886) claimed that in the normal attached leaf of Bryophyllum calycinum acidity increased during the night and decreased as time of day advanced. Before WARBURG, similar results had already been obtained by HYNE, KRAUS, MEYER and others. A more detailed investigation in this direction was carried out more recently by GUSTAFSON (1925) and WOLF (1931). GUSTAFSON studied not only the diurnal changes of total acidity, but also the changes of pH in the leaf of Bryophyllum calycinum. The results of his experiments showed that both actual and total acidities increased steadily at night and reached the maximum at 10 o'clock in the morning and after that time both decreased to the minimum at about 4 o'clock in the afternoon and again increased until they reached the maximum on the following morning. In order to confirm whether or not such changes in acidities during a day occur in an isolated or experimentally treated leaf of Bryophyllum calycinum, the writer estimated the total acidities and pH-values in the pressed juice of isolated or other treated materials. A sample of 10 c.c. was used for each determination. All the determinations were made on fine days during a certain period, i. e., from March to August, 1928. All the experiments were conducted in the greenhouse.

For the purpose of the determination of the change of acidities in the isolated leaves, healthy, vigorous and about one year old sister leaves were chosen. One leaf of each pair was detached from the stem and put on the moist soil in a pot, in which the mother plant was planted. The other of these sister leaves remained on the stem of the mother plant as control. Therefore, light, aeration etc. were almost equal in both the isolated and control leaves. After a certain number of days the determination was made by using the isolated and control leaves. The results of the determination are represented in the TABLE I and II.[1]

On the day following isolation, acidities are higher in the isolated leaves than in the control. It is known that the origin of organic adid in Bryophyllum is the incomplete oxidation of carbohydrate owing to

1) The results of the estimation of acidities in Bryophyllum leaf were described graphically in the preceding paper (1930 b).

incomplete respiration. According to this fact, respiration in an isolated leaf may be more incomplete than in a normal attached leaf. However, generally speaking, after the third day following isolation, acidities were lower in the isolated leaves than in the control. This may show that organic acid once produced was consumed later on account of the recovered normal respiration..

In the attached control leaf, a certain correlation exists between pH-value and total acidity as GUSTAFSON described in his paper (1925). Both the actual and total acidities show the highest level at about 10 o'clock in the morning and as the time of day advances both decrease to the lowest level in the afternoon. After that lowest level, both increase again until they reach the highest level on the following morning as GUSTAFSON pointed out (1925). In the isolated leaf parallelism between the actual and the total acidities is also seen. The highest points of both acidities are found in the morning, after which both decrease. It is remarkable, however, that at the time, in which acidities in the control leaf show almost their lowest level in the afternoon, both the actual and total acidities in the isolated leaf suddenly rise to a relatively high level and after that point is reached, both decrease again (Fig. 2). And in the night both the actual and total acidities in the

Fig. 2 a.

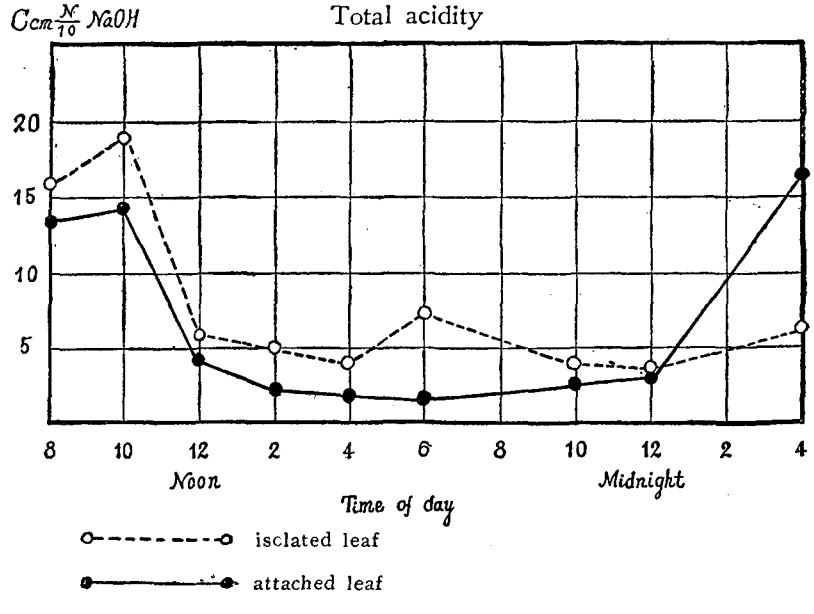


Fig. 2 b.

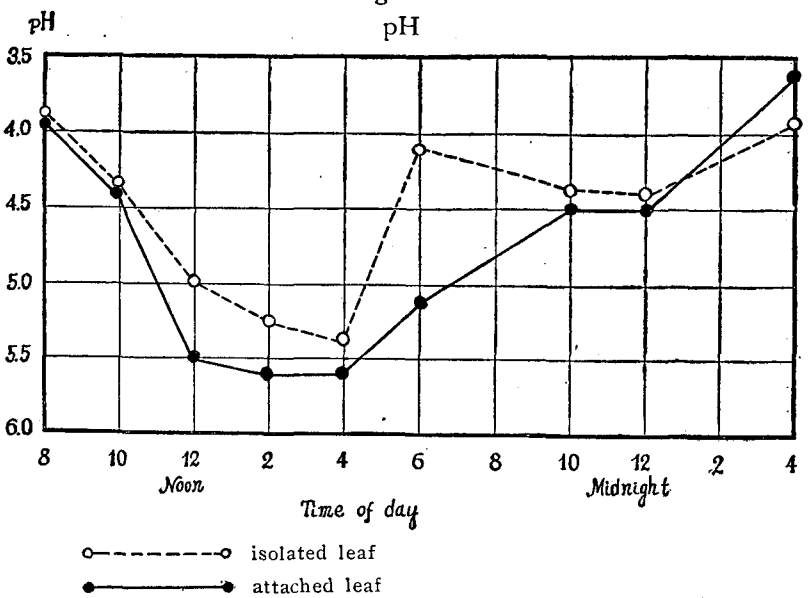


Fig. 2, a-b Acidities in the leaves of Bryophyllum calycinum on the following day of the isolation.

isolated leaf steadily increase again until they reach the maximum on the following morning.

Other experiments were carried out in which healthy, vigorous, about one year old potted plants were used as materials. They were divided into two groups. One group was immersed in the warm-bath of about 35°C. for 8 hours from 8 o'clock in the morning to 4 o'clock in the afternoon. The other group served as control. The measurements of acidities at various times during and after the warm bathing were made by using the leaves attached to the middle part of the stem. The results of this experiment are given in the TABLES III, IV and V. From these results it is indicated that during the warm bathing, both the actual and the total acidities in the treated leaves were very high and hardly any variation was seen.

Immediately after warm bathing, both the actual and the total acidities in the treated leaves decreased suddenly and then rapidly increased, and again decreased (Fig. 3). On the 2nd, 3rd and 4th day after the experiment, the variation of acidities during a day in a treated leaf was as follows: the actual and the total acidities both steadily

Fig. 3 a.

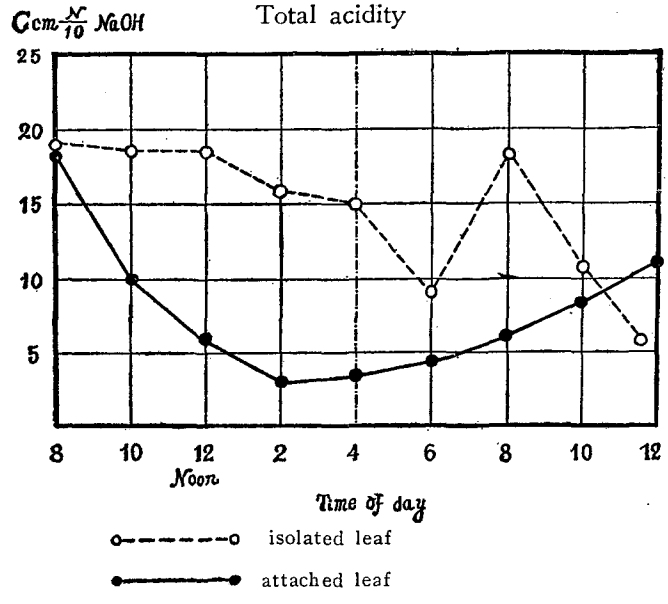


Fig. 3 b.

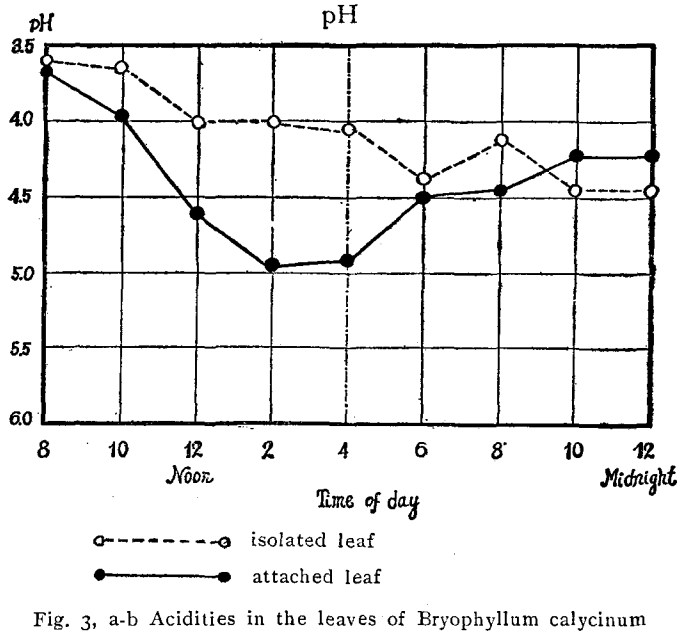


Fig. 3, a-b Acidities in the leaves of Bryophyllum calycinum immediately after warm bathing.

decreased in the morning and reached the minimum in the afternoon. At 6 o'clock p. m. both suddenly rose once, and then decreased again. At night both increased slowly. When warm-bath treatment was applied, parallelism between the actual and the total acidities was also seen in the treated leaves.

Another experiment was conducted in which healthy one year old plants were used as materials. They were divided into two groups, in one of which, each plant was covered with a bell jar, and the air was replaced by H_2-gas. Into the jar a stream of fresh H_2-gas was constantly passed. The plants were kept in this state for 48 hours. After this treatment, the determination of acidity in the leaf was made for a certain number of successive days. The other group served as the control. Leaves to be used for the determination were those which were attached to the middle part of the stem of a plant. The results are described in TABLES VI and VII.

When the plants, which had been placed under anaerobic state for a certain time, were returned to aerobic state, acidities in the treated leaves suddenly decreased and then increased considerably and again decreased, as was seen in the case of warm-bath treatment (Fig. 4). That this phenomenon may be connected with the respiration of the plant will be confirmed by the experimental results described in the

Fig. 4 a.

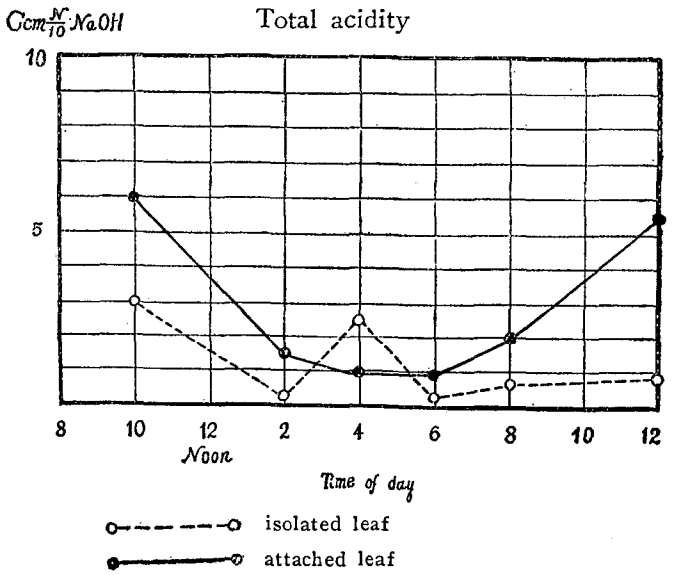


Fig. 4 b.

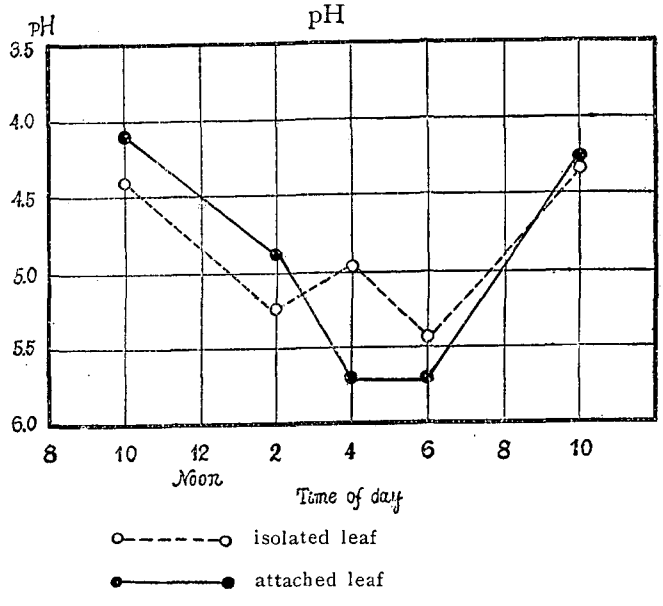


Fig. 4, a-b Acidities in the leaves of Bryophyllum calycinum immediately after keeping in anaerobic state.

following pages. Since the second day after the treatment, variation in total and actual acidities during one day was such that their maximum was seen in the morning, then they gradually decreased and at about 4 p. m. they rose again suddenly, and then decreased again. At night acidities increase. In case of isolation, acidities on the following day after isolation, were very much higher in the isolated leaf than in the control, but it so happened that from the third day they decreased especially during the night. In the case of warm-bath treatment, when Bryophyllum plants were put in the warm-bath, namely, in anaerobic condition, the acidities were very high during the treatment. But when the treated plants were placed in air after the treatment the acidities in the treated leaves became, generally speaking, lower than control. This fact is very similar to the case of isolation. The fluctuation of acidities during one day after warm bathing and after inducing anaerobic respiration was the same.

In connection with the acid metabolism in a Bryophyllum plant some of the experiments, which were carried out in the present work,

will be described hereunder. Some organic acids in the pressed juice of a leaf were examined. Malic acid was detected by colour reaction using the mixture of α- or β-naphtol and H_2SO_4 or of resorsin and H_2SO_4. Citric acid was detected by DEINIGE's reaction. Oxalic acid was proved by the production of precipitation of Ca-oxalate. Tartaric acid was detected by MÖHLER's reaction. Fumaric acid was tested by colour reaction, using the mixture of α-naphtol and H_2SO_4. The results were as follows:

	Malic acid	Citric acid	Oxalic acid	Tartaric acid	Fumaric acid
Isolated leaves	+	−	−	−	−
After placed in H_2-gas	+	−	−	−	−
After warm-bath treatment	+	−	−	−	−
Attached control leaves	+	−	−	−	−

The organic acid in the treated and control Bryophyllum leaves is mainly malic acid. According to KRAUS and WOLF (1931) organic acid in succulent plants is mainly malic acid.

Estimation of the volume of CO_2 output from the treated and control leaves of Bryophyllum calycinum

For the experiments on respiration about 1–1,5 year old, healthy 1,5 and vigorous plants were employed, and thick winter leaf attached to the middle part of a stem only was selected throughout this work. For the determination of CO_2 output from a leaf PETTENKOFER's tube and a leaf chamber were used as described in the foregoing page. During the experiments, the leaf chamber, in which the material was closed, was covered with a wooden plate, in order to keep the chamber always in the dark. All the experiments in this series were carried out on fine days between November, 1928 and June, 1929, in the greenhouse, except the special case which will be stated later.

One leaf of Bryophyllum calycinum was enclosed in the leaf chamber and the CO_2 output from it was measured on the first day of the experiment. This experimental result served as control. This

control experiment having been finished, the leaf was taken out from the leaf chamber and returned to the normal air. On the following morning, the leaf was again put in the leaf chamber and then the air in it was completely replaced by H_2-gas and the stream of H_2-gas was constantly passing in the chamber, that is, the leaf was in anaerobic condition. From the beginning of the treatment, the measurement of CO_2 was carried out resulting as follows:

		CO_2 c.c. 8 a.m.-1 p.m.	CO_2 c.c. 1 p.m.-6 p.m.	CO_2 c.c. 6 p.m.-11 p.m.
Control		6.5	7.0	2.5
In anaerobic condition	The 1st day	2.0	2.5	1.0
	The 2nd day	1.5	2.0	0.5

In anaerobic condition, the volume of CO_2 output was small, and generally speaking, it decreased as the days advanced.

This may be due partly to the incompleteness of gas exchange in the leaf in anaerobic condition.

In the next experiment, the CO_2 output from the leaf which had been placed in H_2-gas was measured. After the leaf had been placed under the anaerobic condition for 48 hours, it was returned to the normal aerobic state, under which condition the measurement of the volume of CO_2 was also made. The volume of CO_2 produced by the leaf immediately after treatment is represented in TABLE XI.

The leaf, which was returned to the aerobic state, produced considerable volume of CO_2 during the first five hours, and small volume during the next five hours, and then its volume increased again. If one considers this fact of fluctuation in the volume output of CO_2 from the operated leaf, compared with the change in acidities after treatment, the detail of which was described in the preceding chapter, the following interesting facts are to be recognized: acidities in the treated leaf decreased during the first four hours immediately after the treatment; on the contrary there was a remarkable production of CO_2 from the treated leaf during this period. During the next four hours acidities in the treated leaf suddenly rose, but the volume of CO_2 from the

taeated leaf suddenly fell during this time. Then the acidities in the treated leaf again decreased, while the volume of CO_2 emitted again increased.

The volumes of CO_2 output on the 1st, 2nd, 3rd and 4th days after treatment were as follows:

		Total CO_2 c.c. 8 a.m.-11 p.m.	Temperature
Control		18.1	20°–30°C.
After treatment	The 1st day	4.5	20°–30°
	The 2nd day	2.5	20°–30°
	The 3rd day	4.5	20°–30°
	The 4th day	6.8	20°–30°

The volume of CO_2 produced by the treated leaf was smaller than that of control, especially so on the second day after the treatment.

In the next experiment, other healthy and one year old plants were used as materials. The shoots of the plants were immersed in the warm-bath of about 35°C. for 8 hours, from 2 a. m. to 10 a. m. Immediately after that, one treated leaf which was still attached to the middle part of the stem was closed within the leaf chamber, and the determination of CO_2 output was made. The results are given in TABLE XII.

The volume of CO_2 produced on the first day after the warm-bath treatment was less than that of the control. And it is remarkable that the volume output of CO_2 from the treated leaf during 3 hours immediately after treatment was rather great, but it decreased during the next 5 hours, and then increased again. This phenomenon must be considered together with fluctuation in acidities in the warm-bath material. Immediately after warm-bath, acidities in the treated leaf suddenly decrease, during which period, however, CO_2 production from the treated leaf increases and then the acidities in the treated leaf rise considerably. On the contrary, the volume output of CO_2 from the treated leaf is very small. After this, the acidities again decrease, while the volume of CO_2 emission from the treated leaf becomes larger. In other words, after warm bathing, when acidities in the treated leaf

decrease, the volume output of CO_2 from the treated leaf increases, and vice versa. As we have seen in the preceding experiments, similar phenomena have already been seen in the case of placing the plant in H_2-gas.

These facts may well be explained by the fact reported by RUHLAND and WETZEL (1931) and WETZEL (1932) which will be cited in later pages.

The total volumes of CO_2 output from the warm-bath material on the 1st, 2nd, 3rd, and 4th days after treatment were as follows:

		Total CO_2 c.c. 10 a.m.-10 p.m.	Temperature
Control		28.0	22°–28°C.
After treatment	The 1st day	14.5	22°–30°
	The 2nd day	2.5	20°–30°
	The 3rd day	4.5	22°–30°
	The 4th day	6.0	20°–30°

The volume of CO_2 emitted from the treated leaf was small especially on the second day after warm-bath treatment.

The writer (1930 b) reported that when Bryophyllum leaf was detached from the stem the CO_2 production from it became small. The following repeated experiment will confirm this fact. At 8 o'clock a. m. on the first day of experiment, the leaf was detached from the stem and enclosed in the leaf chamber and CO_2 output from it was measured.

	CO_2 c.c. 8 a.m.-1 p.m.	CO_2 c.c. 1 p.m.-6 p.m.	CO_2 c.c. 6 p.m.-11 p.m.
Control	9.5	10.0	5.4
After isolation	The 1st day 9.0	0.5	0

The CO_2 production during the first five hours after isolation was almost equal to that of control in this experiment, but from the next five hours the CO_2 output from the isolated leaf suddenly decreased. In the next experiment the total volume of CO_2 output from isolated leaf on the 1st, 2nd, 3rd and 4th days after isolation was measured:

		Total CO_2 c.c. 8 a.m.-11 p.m.	Temperature
Control		28.5	20°-28°C.
After isolation	The 1st day	2.0	21°-28°
	The 2nd day	5.0	22°-28°
	The 3rd day	6.5	20°-28°
	The 4th day	7.0	20°-28°

The volume of CO_2 output from the leaf decreased after isolation, but the volume increased as days advanced. This point is very similar to the case of inducing anaerobic respiration.

In the next experiment, respiratory quotients in an attached and isolated leaf were measured. For the estimation of the volume of CO_2 and O_2 in this experiment, HEMPEL's apparatus was employed. Healthy, vigorous, about one year old plants were selected for materials. A potted plant was placed in the thermostat of 28°C. One leaf, attached to the middle level of this plant, was closed within the leaf chamber in air-tight condition. In the present case, however, the air in the leaf chamber was not replaced by fresh air during the experiment. After the leaf was closed within the leaf chamber, the whole plant body was kept in the dark in the thermostat for 24 hours. Gas analysis was carried out with the air which had been enclosed for 24 hours in the leaf chamber. One hundred c.c. of air was used for each determination. A gas pipette was used in order to remove 100 c.c. gas in the leaf chamber. One entrance of the leaf chamber was connected with the flask containing 20 c.c. of water, and the other entrance with the gas pipette containing about 20 c.c. of water. For the gas analysis exactly 100 c.c. gas was taken in the gas pipette, by driving the water from the flask into the leaf chamber. The results were collected in TABLE XIII.

The RQ-value in the attached control leaves of Bryophyllum calycinum is about 0.5 in the dark, while the value on the 3rd day after isolation is about 0.3. These facts may indicate that respiration in the isolated leaves was not the same as in the attached leaves.

Acetaldehyde and alcohol in the leaves of Bryophyllum calycinum

In intramolecular respiration various substances are produced, for

instance, methyl glyoxal, pyruvic acid, acetaldehyde, ethyl alcohol etc., of which acetaldehyde known as the precursor of ethyl alcohol in intramolecular respiration has a special physiological meaning. NEUBERG (1918), NEUBERG and GOTTSCHALK (1924), BORESCH (1926 and 1928 a), and NIETHAMMER (1928 a, b) and others showed this fact in their experimental results. As has been shown in the preceding pages, the occurrence of regeneration in the attached leaf of Bryophyllum calycinum is also concerned with the acetaldehyde formation.

In the following experiments the writer intended to determine the amount of acetaldehyde and alcohol which are produced in the leaves of Bryophyllum calycinum in various cases. The method employed in the quantitative estimation of aldehyde in this experiment was that of NEUBERG and GOTTSCHALK (1924) which was described in foregoing pages. The experiments in this series were worked out on fine days at the temperature of about 25°C. during April-July, 1929, except special cases which will be stated later. All the determinations were made at 2 o'clock in the afternoon throughout the experiments. Fresh leaves of 100 g. were used in each determination. They were torn into small pieces and distilled with boiling water. Exactly 100 c.c. of the first distillate was taken up for each experiment. The number indicated in mg. in the tables shows the total quantities of aldehyde in the original fresh weight of 100 g. leaves.

Detection of acetaldehyde in the leaf sap of Bryophyllum calycinum

The detection of acetaldehyde in the distillate was carried out by making a precipitate of acetaldehyde-p-nitrophenylhydrazine in it (after Kostychew 1913). When a few drops of the saturated solution of p-nitrophenylhydrazine in 15% acetic acid were added to the distillate, the yellow precipitate of acetaldehyde-p-nitrophenylhydrazine was produced in the existence of acetaldehyde. The distillate of the attached leaves and isolated leaves of Bryophyllum calycinum showed clearly the existence of acetaldehyde with this reagent, producing the yellow precipitate in the distillate. Similar reactions were also observed, using the distillate from the leaves treated with warm-bath, H_2-gas or N_2-gas respectively.

Further, the writer could detect acetaldehyde by the reaction of sodium nitroprusside and piperidine (after NEUBERG 1924) as follows:

The shoot of Bryophyllum calycinum was put in the warm-bath of 30°C. One hour after from the beginning of the treatment, some leaves of the treated materials were detached and distillated. The distillated became blue by adding sodium nitroprusside solution and piperidine to it, showing clearly the existence of acetaldehyde.

GRIEBEL (1924) detected acetaldehyde in some seeds and fruits by a micro-sublimation method, using p-nitrophenylhydrazine in acetic acid as a reagent. In the present experiment, the detection of acetaldehyde in the leaf of Bryophyllum calycinum by a modification of GRIEBEL's method was attempted. The procedure in the experiment was as follows: the whole plant body of potted Bryophyllum calycinum was placed in H_2-gas for 24 hours and then put in the moist chamber for 2–3 days. Some little pieces were made of the treated leaf and were put on the slide. Then the pieces were covered with a glass ring and a cover-glass. On the lower surface of the cover-glass one drop of the saturated solution of p-nitrophenylhydrazine in 15% acetic acid was hung down. This apparatus was warmed on the gas-burner, until the leaf pieces on the slide faded a little, and then it was cooled. A few minutes later, slender needle-shaped crystals of acetaldehyde-p-nitrophenylhydrazine were observed under the microscope. Therefore, acetaldehyde exists in the leaf of Bryophyllum calycinum, and the most part of aldehyde in the following quantitative analysis may be regarded as acetaldehyde.

Quantitative determination of aldehyde and alcohol in the leaves attached to the upper and middle parts of the stem (TABLE XIV)

In the very young leaves growing on the top of the plants, and in the adult, healthy, vigorous, about one year old leaves attached to the middle part of the stem, the quantities of aldehyde and ethyl alcohol were determined.

The results show that the quantities of aldehyde and ethyl alcohol are greater in the growing young leaves than in the adult ones. This experiment was carried out in May, 1929.

Quantitative determination of aldehyde and alcohol in the isolated leaves (TABLE XV)

Five hundred gram of fresh leaves, attached to the middle part of

the stem and about half one year old, were detached respectively in each experiment. These leaves were divided into 5 equal groups, each weighing 100 g. One of the groups (100 g.) was used for the estimation of aldehyde and ethyl alcohol immediately after isolation. This served as the control. The other four groups remained on the moist soil, the temperature 18–25°C. These four groups were used for the determination respectively at intervals of 24 hours for the successive 4 days after isolation.

In the isolated leaves, the weight of aldehyde increased 2–7 times and the volume of ethyl alcohol more than doubled during the first 24 hours after isolation, compared with those of the control, and then both decreased as the days advanced.

In the above experiment, the determination of acetaldehyde and alcohol in the leaves treated in various ways was carried out at a certain time of day, (namely in the above experiment, at 2 p.m.). Now the question arises how aldehyde in Bryophyllum leaf changes during a day or whether it does not change at all. In order to examine this point, the following experiments were carried out, using the isolated leaves of Bryophyllum calycinum on the following day after the isolation. The results were as follows; at 10 a.m. aldehyde 3.5 mg.; 12 a.m. aldehyde 4.3 mg.; 2 p.m. aldehyde 6.0 mg. and 4 p.m. aldehyde 1.7 mg.

In the morning the quantity of aldehyde was small, but gradually increased as the time of day advanced, and it reached the maximum points at about 2 p.m. and then again decreased.

Quantitative determination of acetaldehyde and alcohol in the leaves subjected to anaerobic respiration (TABLES XVI and XVII)

Potted plants, about one year old, were placed under a bell jar which was filled with H_2-gas and through which this gas was constantly flowing. The plants were kept in this state for 48 hours at the temperature of about 25°C. After this treatment, the plant materials were returned to the normal aerobic condition, and then the determination of aldehyde and alcohol was carried out in the leaves which were attached to the middle part of the stem (TABLE XVI).

The quantity of acetaldehyde immediately after treatment in H_2-gas is greater, and after that period it decreases as days advance. The

volume of ethyl alcohol in the treated leaf is large for 2 days after treatment, but on the 4th day after treatment it rapidly decreases.

Another experiment was carried out in which the whole plants were placed in N_2-gas, instead of in H_2-gas, for 24 hours. Then the plants were returned to the aerobic condition and the determination of acetaldehyde and alcohol was carried out (TABLE XVII).

The quantities of acetaldehyde and alcohol increase by placing the whole plant in N_2-gas, that is, in anaerobic state for a certain number of hours. This experiment was made in February, 1932.

Warm-bath treatment (TABLES XVIII, XIX and XX)

The shoots of plants, about one year old, were submerged in the warm-bath of about 30°C. for 5 hours. At a certain period of time during the treatment, the estimation of acetaldehyde was carried out. The results were collected in TABLE XVIII.

For the first two hours from the beginning of treatment, the formation of acetaldehyde was relatively considerable, but after about three hours from the beginning, it decreased a little, and after five hours it decreased remarkably. This experiment was carried out in Nov.-Dec., 1931. Bryophyllum plants were treated in the warm-bath for 5 hours and then they were placed in the greenhouse. After a certain number of hours, the determination of acetaldehyde in the leaves was carried out. From the results we can see that not only during the treatment, but also after treatment, the quantities of acetaldehyde were greater in treated leaves than in the control (TABLE XIX). This experiment was made in March, 1932. Similar results were obtained by an 8 hours' treatment (TABLE XX).

In another experiment, some leaves attached to one side of a plant only were soaked in the warm-bath of about 30°C. for 3 hours, and the remaining leaves and the tops were left in the air of room temperature during the treatment. Aldehyde and alcohol in the treated and untreated leaves were estimated after 24 hours from the end of the treatment. The results were as follows:

	Treated leaves	Untreated leaves	Control
Acetaldehyde mg.	1.76	0.44	0.44
Alcohol %	0.01	0.002	0

In the treated leaves a relatively considerable quantity of aldehyde and ethyl alcohol was found. But in untreated leaves, the quantity of aldehyde was almost equal to that of control. This experiment was carried out in February, 1932. Next, the writer used one plant, of which some adult leaves were treated in the warm-bath. About 2–3 days later, the treased leaves (a) produced roots and shoots at the notches, but about 10 days from the beginning of the treatment, tiny shoots and roots were produced also at the notches of the neighbouring untreated leaves (b). In these leaves the determination of aldehyde and alcohol was carried out.

	Treated leaves (a)	Untreated leaves (b)	Control
Acetaldehyde mg.	1.32	0.50	0
Alcohol %	0.03	0.02	0

Not only in the treated leaves, but also in the neighbouring untreated leaves on which tiny shoots or roots began to appear, aldehyde and alcohol were produced more or less, while they were not detected in the control plant which was kept in the moist chamber. This experiment was carried out in February, 1932.

Isolation, inducement of anaerobic respiration, and warm-bath treatment caused the accumulation of a relatively considerable amount of acetaldehyde and alcohol in the leaves of Bryophyllum calycinum.

(5) Warm-bath treatment of the isolated leaves of Bryophyllum calycinum

Isolated adult leaf of Bryophyllum calycinum was immersed entirely into the warm-bath of 25°–30°C. for 12 hours. After the treatment, it was enclosed in an Erlenmeyer flask (250c.c.) containing normal air for 2 weeks in the laboratory at room temperature. During this period, the air in the flask was not replaced with fresh air. A week later, the regeneration of roots and shoots began at the notches, as on the normal isolated leaves. It was also noticeable that in the isolated leaf treated in warm-bath little white callus-like cell masses began to appear at the notches and they grew day after day. The development

of the cell masses was limited to some notches and to the leaf edges where slender leaf veins end. At some notches both callus and regenerated roots were formed and at the other notches regenerated roots and shoots only were produced (Fig. 5). Callus development was restricted to the back of the leaf. After two weeks from the beginning of the experiment, the air in the flask contained 10.4 c.c. of CO_2 and only 5.8 c.c. of O_2. Anatomical observations on the notches of the leaves treated in warm-bath showed that regeneration occurred from the little cell group near the vascular bundle at the notches as in the normal isolated leaf, and the callus-like cell masses developed from the parenchymatous tissue near the notches.

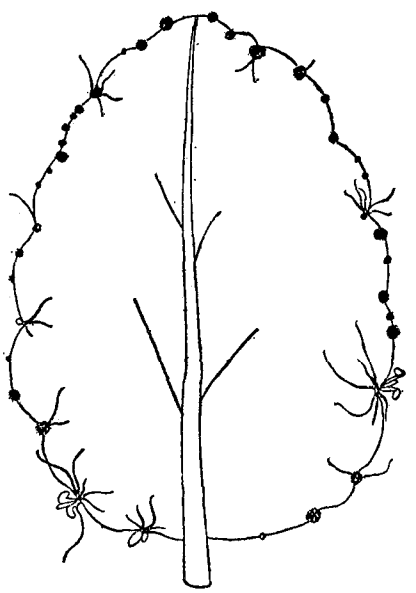


Fig. 5

Development of callus-like cell masses at the notches of the isolated leaf of Bryophyllum calycinum which was placed in anaerobic condition.

(6) Regeneration in Bryophyllum crenatum

Bryophyllum crenatum is also a tropical succulent and has a slender unbranched stem bearing two leaves situated oppositely in each node as in Bryophyllum calycinum. The two leaves in each node are also called here the sister leaves as in the case of Bryophyllum calycinum. The adult leaves are very fleshy and can be easily removed from the stem. The detached leaves regenerate shoots and roots at the notches, not at the wound portions, under adequate temperature and moisture. As stated before, in the leaf of Bryophyllum calycinum, the regeneration of roots first occurs at the notches followed by shoot formation, while in Bryophyllum crenatum shoot formation occurs first at the notches of an isolated leaf followed by root formation. This is a point of difference in regeneration between Bryophyllum crenatum and Bryophyllum calycinum. Many authors have studied regeneration in this plant hitherto. An isolated leaf, as stated above, has a character

to regenerate shoots and roots at the notches, but it is also possible to induce such growth of new shoots and roots at the notches of the leaf still attached to the stem, by some treatments or changes of the environmental condition.

GOEBEL (1908) showed that in Bryophyllum crenatum, if all shoot buds are removed, new bud formation ought to take place at the notches of the attached leaves of this plant and the separation of one part of the midrib also had the same effect. GOEBEL (1902) attempted to produce new shoots and roots on an attached leaf by ether treatment. He placed the whole plant body in ether vapour for 14–48 hours, succeeding to produce new plants on the leaves. OSSENBECK (1927) reported that by cooling the pot, in which Bryophyllum crenatum was placed, the formation of new shoots and roots at the notches of attached leaves and of adventitious roots at the lower part of the treated plant can be induced. OSSENBECK placed the pot in an ice box and the surface of the pot and the ice box were covered, in order to cool only the root, but to avoid the cooling of the shoot. After 7 days, regeneration in the attached leaves became visible. The present writer (1930 b) reported that early in June, 1929, all healthy potted plants of Bryophyllum crenatum in the greenhouse produced shoots and roots at the notches of the attached leaves of the middle and lower level of the plants, when the temperature suddenly rose, so that the temperature in the greenhouse reached 30°C. or over. A similar observation was made in the same season in 1931. DOSTAL (1930) found that in Bryophyllum crenatum injected diastase induced the increased formation of regenerated plants from the leaf. HARIG (1931) secured a positive result in the experiment in inducing regeneration on the attached leaves of Bryophyllum crenatum by the treatment of warm-bath and HCN treatment. Recently the present writer obtained a similar result by placing the whole plant of Bryophyllum crenatum in N_2-gas for 24 hours. The materials were put in N_2-gas for 24 hours, then they were removed to the greenhouse. A few days later, the leaves attached to the upper parts of the stem produced little shoots. The leaves on control materials did not show such development. Thus by inducing anaerobic respiration, the production of shoots on the attached leaf of Bryophyllum crenatum were caused. It is known that the respiration in succulents is somewhat incomplete in nature. The writer's experimental results in Bryophyllum calycinum showed that by the isolation of a leaf from the mother stem, the production of incomplete and

intramolecular respiration in the leaf becomes more remarkable. In order to see whether the similar things happen in Bryophyllum crenatum, the following investigations were carried out. Many pairs of sister leaves, attached to the middle level of the healthy and potted plants, were selected for this purpose.

Changes in the actual and total acidities in the isolated and attached leaves of Bryophyllum crenatum during a day

According to the investigations of GUSTAFSON (1925) and the present writer (1930 b)[1] the diurnal changes of actual and total acidities in the leaf of Bryophyllum calycinum showed their highest level in the morning and they decreased to the lowest level at about 4 p. m. and then both steadily increased again until they again reached the maximum on the following morning. It is necessary to ascertain whether or not such changes of actual and total acidities during a day are also seen in the case of Bryophyllum crenatum. The writer examined the diurnal changes in acidity, using the leaf sap of the attached leaves of Bryophyllum crenatum which was expressed at various times during a day. The TABLE VII represents the results.

The highest point of the total acidity was found in the morning; after that point it decreased to the lowest level in the afternoon and then it increased again. The highest actual acidity was reached in the morning and it decreased, as the time of day advanced, to the lowest level in the afternoon, and again it rose gradually. From above stated results, it may be said that there is a parallelism between the diurnal changes of total acidities and those of actual acidities in the leaf sap of Bryophyllum crenatum. These results with Bryophyllum crenatum are very similar to those which GUSTAFSON (1925) and the writer obtained with Bryophyllum calycinum.

In order to see the diurnal change of the total and actual acidities in the isolated leaf of Bryophyllum crenatum, the writer carried out the following experiments. Many pairs of sister leaves, attached to the plants, were selected. One leaf of each pair of sister leaves was detached from the stem and put on the moist soil in the pots in which the mother plants grew. The other leaf of each pair of sister leaves was left on the stem as control. After a certain number of days the determi-

1) See also the preceding chapter.

nations of acidities in the isolated and attached (control) leaves were carried out and compared with each other. The results of this experiment are shown in TABLE IX.

On the day following isolation, both acidities are higher in the isolated leaves than in the control. It is known that the origin of organic acids in succulents is due to the incomplete oxidation of carbohydrate owing to incomplete respiration, and it may be assumed that the respiration in isolated leaves is more incomplete than in the control. On the 4th day after isolation, acidities became lower in the isolated leaves than in the control. This may be caused by the use of accumulated organic acid by respiration in the isolated leaf.

In isolated leaves, the maximum point of the total acidity is found in the morning and after that point it decreases as in the control. However, it is remarkable in the variation of the total acidity in the isolated leaves that at the time when the total acidity in the control shows its lowest level in the afternoon, the total acidity in the isolated leaves rises suddenly to the highest level and then decreases again.

The highest point of the actual acidity in the isolated leaves is found in the morning, after that point it decreases as seen in the control. However, at the time when the actual acidity in the control shows its lowest level in the afternoon, the actual acidity in isolated leaves rises suddenly, then decreases and again increases. There is parallelism between pH and the total acidity in the isolated leaf of this plant. All the experimental results obtained in the case of the isolated leaves of Bryophyllum crenatum are the same as those which have been obtained in the isolated leaves of Bryophyllum calycinum by the present author.

In order to see the changes of total and actual acidities after anaerobic respiration, another experiment was carried out. Healthy and potted plants of Bryophyllum crenatum were divided into two groups, one of which was covered with a bell jar, and the air was replaced by H_2-gas. Into the bell jar, a stream of fresh H_2-gas was constantly passing and the plants were kept in this state for 48 hours. The other group served as control. After treatment, the determination of actual and total acidities in the treated and control leaves was made. The results are given in TABLE X.

In the leaves of Bryophyllum crenatum, which had been left in anaerobic condition, the maximum of the actual as well as total acidities was found at 10 o'clock in the morning as in the control, after which

they decreased until 4 o'clock in the afternoon; then both acidities in the treated leaves suddenly rose, and decreased again. This parallel fluctuation of both kinds of acidity in the treated leaves was in agreement with that of isolated leaves, as stated in the preceding pages.

Quantitative determination of aldehyde and alcohol in the leaves of Bryophyllum crenatum

Fresh leaves to the amount of 500 g. were detached from the middle part of the stem. These were divided into 5 groups, each having 100 g. of leaves. One of the groups was used for the estimation of aldehyde and alcohol immediately after isolation. This served as control. The other four groups remained on the moist soil in the greenhouse. The quantity of aldehyde and ethyl alcohol in the four groups was determined successively at intervals of 24 hours for 4 days after isolation. The results of the experiment are given in TABLE XXI.

In the control leaves a slight quantity (0.5 mg.) of aldehyde appeared, while no alcohol was detected. In the isolated leaves, 1–2 days after the isolation, a relatively considerable quantity of ethyl alcohol and about 6 times that of aldehyde are formed, compared with those in the control. This experiment was carried out in June, 1930.

From the experimental results above described, relating to respiratory products, i.e. from the estimation of aldehyde, alcohol and acid metabolism, we now see that more products of incomplete and intramolecular respiration are produced in the isolated leaves. According to the writer's experimental results regeneration in Bryophyllum calycinum is concerned with the products of respiration, especially aldehyde or some other related substances. HARIG (1931) obtained, as stated above, positive results in inducing regeneration on the attached leaves of Bryophyllum crenatum by HCN treatment.[1] HARIG reported that warm-bath treatment is also effective to induce adventitious shoots and roots on the attached leaves as in the case of Bryophyllum calycinum reported by the present writer. Further, the present writer succeeded in inducing adventitious shoots on the attached leaves of Bryophyllum crenatum by inducing anaerobic respiration by placing the plants in N_2-gas.

In the next experiment, the writer carried out the injection of the

1) It is known that HCN depresses oxydative phase in respiration remarkably and has practically very slight effect on the fermentation.

products of intramolecular respiration, especially of acetaldehyde using the attached leaves of Bryophyllum crenatum.

Artificially induced regeneration in Bryophyllum crenatum by the injection of acetaldehyde

The writer succeeded in artificially inducing regeneration at the notches of the leaves which were still attached to the healthy plants, by the injection of 0.05% acetaldehyde. One pair of sister leaves, which was attached to the stem at the third node from the top, was used as material. One of the sister leaves was injected with about 0.3 c.c. of a 0.05% acetaldehyde solution and the opposite one was injected with the same amount of pure water as control. The injection was made at the leaf blade near the notches, once at the beginning of the experiment. The utmost care was taken to avoid the injury of the leaf vein by the injection needle when injection was carried out. After a few days, the leaf which had been injected with acetaldehyde, began to produce some new shoots vigorously at the notches. In the control leaf which was injected with water such a phenomenon did not take place. This experiment was carried out in the greenhouse in March, 1931.

The products of incomplete and intramolecular respiration, for instance, organic acid, aldehyde and ethyl alcohol, are very much increased in the isolated leaves of Bryophyllum crenatum, and injection with acetaldehyde can induce artificial regeneration on the attached leaves of the same plant. From the above stated facts, the occurrence of regeneration in the isolated leaves of Bryophyllum seems to be connected with the products of intramolecular respiration or other related substances. This problem will be discussed in detail later in this paper.

II. Regeneration in stem cuttings of various plants[1]

In a preceding paper, the writer (1930 a) studied the acceleration of the formation of adventitious shoots and callus in stem cuttings. In the present work, further studies on this problem were carried out. For years, many authors have been engaged to find some method of

1) In the present work the polar character of regeneration in stem cuttings was not touched.

accelerating root formation in stem cuttings. For instance, DACHNOWSKI (1914) pointed out that the growth of roots in tomato cuttings was remarkably increased by the addition of glycocoll in acid and alkaline solutions. In his experiments using solutions of various chemical compounds CURTIS (1918) came to the conclusion that root regeneration in the stem cuttings of Ligustrum ovalifolium and other woody plants was stimulated when the cuttings were treated with a solution of potassium permanganate. CONNORS (1924) obtained a similar result with carnation cuttings. POPOFF and GLEISBERG (1924) found that the immersing of twigs of willow, apples, pears, mulberry and Cryptomeria in the solutions of Mg- and Mn- salts and KBr produced a significantly increased root formation. BIERMANN (1926) examined the effects of the mixed solutions of various concentrated solutions of $MgCl_2$, KCl, KBr, $MnSO_4$ and $Mn(NO_3)_2$ upon root regeneration in stem cuttings, but the results obtained by him were inconclusive. WINKLER (1917) indicated that oxdizing reagents considerably stimulated roots and callus formation in the cuttings of vine, when the basal end of the cuttings were immersed in the solution of these reagents. ISHII (1929) obtained a similar result with the cuttings of a grape by such treatment, as well as by warm-bath treatment. The present writer (1930 a) reported that warm-bath method has a very stimulative effect upon callus formation and shoot regeneration in stem cuttings of Populus nigra. RICHTER (1930) reported similar results in the formation of roots in cuttings treated with warm-bath and the solutions of some salts. The present writer (1930 a) found that the inducement of anaerobic respiration in the stem cuttings of Populus nigra by placing them in H_2-gas or in vacuum caused very much accelerated shoot and callus formation in them. EMIL (1925) stated that treatment with CO_2-gas is very effective to stimulate root regeneration in the stem cuttings of Chrysanthemum and Dahlia. BOEHM (1867) examined the influence of atmospheric pressure upon the rooting of cuttings and found that the pressure of 3–6 atmosphere inhibited root regeneration. SWINGLE (1924) and SAKURAI (1927) reported a beneficial effect of using a special bed for the rooting of orange cuttings.

It is very important to see whether such kinds of treatment stimulate the formation of roots or whether such treatment is useful to the further growth of newly regenerated roots in the cuttings.

In applying the facts and the theory in the investigation with Bryophyllum in the preceding pages, the writer came to find some new

methods for the acceleration of regeneration of roots in stem cuttings. At first, the forcing action of warm-bath and other similar treatments upon the rooting of stem cuttings of various plants will be mainly described.

Twigs of about 50 cm. or shorter ones of various plants were cut off from the stem. Each of the detached twigs was cut transversely into two parts in the middle so that the two halves measured almost equal. After cutting, the weight of those two halves, which came from one original twig, were regulated by weighing and whittling. Thus many pairs of twigs were prepared. One member of each pair was experimentally treated and the other served as the control. Practically, however, the following two ways were adopted in treating the cuttings throughout the experiments. All pairs of cuttings were divided into two groups in each experiment. In one group, one member of each pair, which had originated from the apical part of the twig, was experimentally treated, and the other taken originally from the basal part, was kept as the control. In the other group, the basal ones were treated and the apical ones served as the control.

In the stem cuttings of Populus nigra, it required about 4 weeks to see regeneration at room temperature in summer (in Sapporo). However, when the stem cuttings of this plant were experimentally treated for a certain number of hours, immediately after isolation from the mother plants, as will be hereunder described, the apical adventitious buds commenced to grow out soon after the treatment, and soon the basal root formation followed. In the stem cuttings of various other plants, similar results could be obtained.

(1) Warm-bath treatment

The present writer (1930 a) reported that shoot regeneration and callus formation in Populus nigra was very much stimulated by the treatment of warm-bath. In the present work, experiments on the acceleration of root regeneration by warm-bath treatment were carried out using the stem cuttings of Populus nigra and other plants. The materials were immersed in the warm-bath of about 30°–35°C. for a certain time. The control materials were placed in the laboratory during the experiment. After the warm-bath treatment, all the materials (both the treated and the control) were placed in the greenhouse or in the laboratory, where the temperature was kept at about 20°C. The basal

ends of the materials were put in water, and the remaining portions were kept in the air in vessels (Fig. 6). After twenty or thirty days from the beginning of the experiments, all regenerated organs in the treated and the control materials were separated from the cuttings, and dried in the oven electrically heated for about 24 hours. The dry weight of the regenerated organs in the treated and in the control was compared with each other.

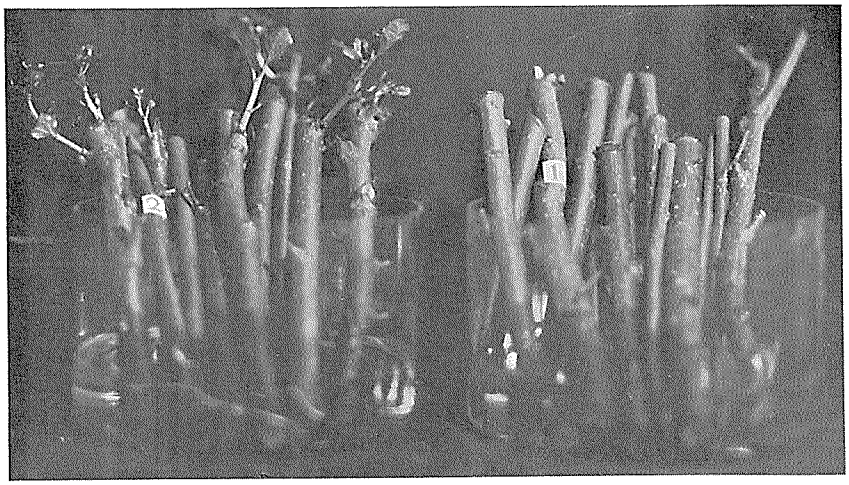


Fig. 6. Regeneration of adventitious shoots and roots in the stem cuttings of Populus nigra: the cuttings (2) treated in warm-bath, produced more vigorous formation of shoots and roots than the control (1).

a) Populus nigra (TABLES XXII and XXIII)

Stem cuttings of Populus nigra, about 25 cm. long and 1–2 cm. in diameter, were immersed entirely in a warm-bath for 8 hours. The regenerated shoots began to develop at the upper parts of the treated cuttings soon after the treatment, followed by root formation at the basal parts of the same cuttings (Fig. 2). Regeneration in the control cuttings occurred later. Thirty days after the beginning of the experiment, the dry weight of regenerated shoots and roots in the treated and in the control materials was measured. The dry weight of shoots and roots was greater in the treated cuttings than in the control. Regenerated organs appeared earlier and were heavier in the warm-bath treated materials than in the control. This experiment was carried

out from November 20 to December 20, 1930 and in October, 1931. Similar results were obtained in March, July, September, and October 1930, in December 1931, and in February 1932.

b) Syringa vulgaris (TABLE XXIV)

Stem cuttings of Syringa vulgaris were immersed entirely in the warm-bath for 8 hours. Regenerated adventitious buds at the upper parts of the stem cuttings began to appear soon after the treatment, while, generally speaking, it required relatively a longer time to see regeneration in the control. After twenty days from the beginning of the experiment, the weight of the regenerated parts was compared. The shoot formation occurred more vigorously in the treated cuttings than in the control. Root formation did not occur in either the treated or control materials in this experiment.

c) Salix sp. (TABLE XXV)

The lower half of each stem cutting of Salix, about 25 cm. in length and 1–2 in diameter, was immersed at its basal end into the warm-bath for 5 hours. One or two days later, regeneration occurred in the treated cuttings, but there were a few days before shoot formation took place in the control. After ten days from the beginning of the experiment, all the regenerated parts were separated from the treated and control materials and compared with each other.

The dry weight of the regenerated shoots was greater in the warm-bath materials than in the control. This experiment was made from Sept. 20 to Oct. 6, 1931. A similar result was obtained in another experiment, in which the entire cuttings were immersed in the warm-bath of 30°C. for 5 hours. This experiment was carried out in November, 1931.

d) Pelargonium zonale (TABLE XXVI)

Stem cuttings of Pelargonium were immersed entirely in the warm-bath of about 30°C. for 3 hours. The regeneration of root occurred in the treated materials earlier than in the control. Two weeks after the beginning of the experiment, the regenerated roots in the treated and control cuttings were measured. The results are given in TABEL

XXVI. The dry weight of the regenerated roots was greater in the treated cuttings than in the control.

Generally speaking, apical adventitious buds in the warm-bath treated cuttings begin to develop soon after the treatment, and soon basal root formation follows. Such phenomena were not seen in the control. A certain number of days after the beginning of the experiment it was shown that the dry weight of the regenerated organs was greater in the treated cuttings than in the control. These facts indicate that the warm-bath can serve very effectively to force the development of adventitious roots and shoots in the stem cuttings of various plants.

In the following experiment, a treatment similar to the warm-bath, for instance, the inducement of anaerobic respiration or the placing in vacuum was carried out using the stem cuttings of various plants.

(2) Influence of anaerobic respiration on regeneration in stem cuttings

1. *Placing the cuttings in H_2-gas*

The stem cuttings were placed under a bell jar, the base of which was closed with vacuum cock grease of Kahlbaum. A stream of H_2-gas was constantly driven through the bell jar in order to keep the materials always free from oxygen. In this state, the cuttings were kept for 48 hours in the laboratory. The control materials were also placed near the treated materials and covered with a bell jar, filled with moist fresh air. After treatment, the materials were returned to a normal state, their basal ends being put in water and the remaining portions kept in the air. A certain number of days after the treatment, all regenerated organs were isolated from the cuttings, and dried in an oven electrically heated to about 100°C. for about 24 hours. The dry weight of the regenerated organs of the treated and control materials was measured.

a) Populus nigra (TABLES XXVII and XXVIII)

Stem cuttings of Populus nigra, about 25 cm. long and 1–2 cm. in diameter, were divided into two groups; one was placed in H_2-gas and the other was placed in moist air for 48 hours. After treatment,

all the materials were placed in the laboratory (at about 15°–20°C.). The regeneration in the treated cuttings began to take place soon after treatment and further development occurred better than in the control. Generally speaking, the formation of the regenerated shoots and roots occurred much more vigorously and earlier in the treated cuttings than in the control. Similar experiments were carried out, using 10 pairs of Populus cuttings. In this experiment, the materials after treatment were placed in the greenhouse, at about 20°–30°C. The results were as follows: the regenerated organs appeared earlier and developed better in treated cuttings than in the control. In this experiment it was remarkable that the callus formation at the basal cut surface of the treated cuttings was very vigorous (Fig. 7).

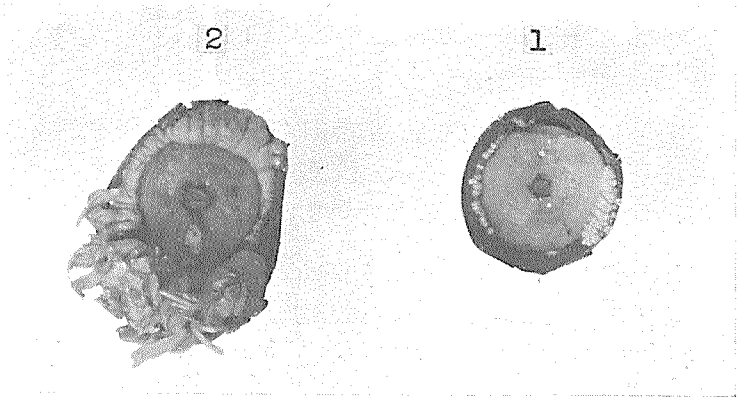


Fig. 7. More vigorous callus formation at the basal end of a cutting of Populus nigra which was placed in anaerobic condition (2) than the control (1)

b) Tropaeolum majus (TABLE XXIX)

Stem cuttings of Tropaeolum were made; one group of them was placed in H_2-gas and the other in moist air for 48 hours. Then they were placed in the laboratory, dipping their basal ends in water. As a whole, root formation occurred earlier and better at the basal ends of the treated cuttings than of the control. Callus formation at the basal cut end of Tropaeolum majus was vigorous in the treated cuttings.

c) Solanum Lycopersicum (TABLE XXX)

Stem cuttings of Solanum Lycopersicum were divided into two groups; one was treated and the other served as control. The treated cuttings had already begun to produce roots about 24 hours after the treatment, but in the control several days were necessary to see such root regeneration. Nine days later, the dry weight of the regenerated roots was compared. It was greater in the tread cuttings than in the control, that is to say, the root formation in cuttings was much accelerated by the treatment.

The treated cuttings of Solanum vigorously produced callus at the basal end.

d) Rosa microphylla (TABLE XXXI)

Shoot regeneration in the treated cuttings of Rosa was better than in the control. The callus formation at the basal end of the treated Rosa cuttings was better.

e) Syringa vulgaris (TABLE XXXII)

The dry weight of the regenerated shoots shows that regeneration was much accelerated by the treatment.

f) Hydrangea opuloides var. japonica (TABLE XXXIII)

The dry weight of the regenerated shoots was greater in the treated cuttings than in the control. This indicated that the shoot formation in the cuttings was accelerated by the inducement of anaerobic respiration.

g) The so-called "main roots" (adult hypocotyls) of Raphanus sativus ("Hatukadaikon") all whose hair roots were cut off, were placed on 2% agar-jelly in the germinating vessels. The vessels were divided into two groups, one was placed in N_2-gas and the other was placed in the laboratory for 48 hours. Then all the vessels were placed in the laboratory. The treated materials produced little roots at the cut surface of the lower part of the main roots much earlier and more vigorously than the control at the room temperature. This experiment was carried out in May, 1932. From these facts, it may be clear that anaerobic respiration stimulates not only regeneration of roots in the other parts but also reparation in the wounded portion itself.

Generally speaking, it may be concluded that the inducement of anaerobic respiration causes a vigorous formation of regenerated roots, shoots or callus in the stem cuttings of various plants.

2. *Placing the cuttings in vacuum*

The present writer (1930 a) reported that the development of adventitious shoots[1] and callus in the stem cuttings of Populus nigra was stimulated by placing the cuttings in a vacuum for a certain length of time. In the present work other experiments on the development of adventitious roots and shoots etc. in the stem cuttings of various plants are reported.

The materials were divided into two groups of equal numbers. Each group was closed in flasks respectively. Air in one of the flasks was sucked out by the rotary pump to almost vacuum (70 cm. Hg.). The flasks were placed in the laboratory at room temperature for about 5 hours. After treatment, both the treated and the control materials were placed in the normal atmosphere in the greenhouse. The basal ends of the materials were kept in water, (except the special cases which are stated later). About 3–4 weeks after the beginning of the experiment, the dry weight of the regenerated roots mainly were compared.

a) Populus nigra (TABLES XXXIV and XXXV)

Many pairs of stem cuttings about 20 cm. long were made. They were divided into two groups. One group was placed in vacuum for 5 hours at the temperature of 14–17°C. The regeneration of shoots in the treated cuttings took place soon after the treatment and was followed by root formation, while it was relatively a long time before regeneration took place in the control. The dry weight of the regenerated roots and shoots was greater in the treated cuttings than in the control in the same duration of time. The callus formation at the basal ends of the treated materials was very vigorous. This experiment was carried out from Oct. 8 to Nov. 5, 1931. A similar experiment was made in October and November, 1931, using Populus cuttings. The treatment was carried out at 11°–13°C. The results were that

1) Similar results could be obtained in regeneration in the stem cuttings of *Syringa japonica*, *Morus bomcina*, and *Salix* sp.

the regeneration of shoots and roots took place earlier and they developed better in treated cuttings than in control. Generally speaking, the dry weight of the regenerated shoots and roots was greater in the treated cuttings than in the control. These facts indicated that the regeneration of roots and shoots in the treated cuttings was much stimulated by the treatment.

In the next experiment, the writer used three flasks, in which equal numbers of Populus cuttings were respectively closed, and the air in them was sucked out with a rotary pump. Each flask was placed at different temperatures for 5 hours, namely 10°C., 20°C. and 30°C. After this, all the materials were taken out and removed to the greenhouse of about 20°–30°C. The result of this experiment showed that there was practically no great difference of regenerated organs among them. Similar results could be obtained in the cuttings of Syringa japonica.

b) Coleus Blumei (TABLE XXXVI: Fig. 8)

Cuttings with leaves of Coleus Blumei were made in the manner of the horticultural practice. One member of each pair of cuttings of about the same age was taken up as control. The other of each pair

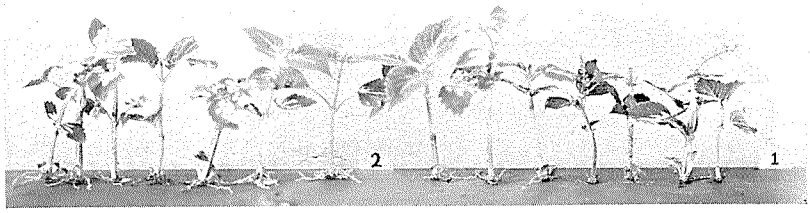


Fig. 8. Root regeneration in the cuttings of Coleus Blumei which were placed in vacuum for a certain number of hours (2) and control (1). The cuttings (2) produced more vigorous formation of roots than the control (1).

was treated for 5 hours. The temperature during the treatment was 15°–20°C. After the treatment, all the materials were planted in the moist sand bed in the greenhouse. About 3 weeks later, the regenerated roots of each pair were compared. The regeneration of roots in the treated cuttings was much stimulated by the treatment.

c) Saintpaulia ionatha

The leaves were detached from the stems at the base of the petioles and placed in vacuum for 3 hours at room temperature. Then all the materials were taken out and placed in the moist sand bed in the greenhouse. Generally speaking, regenerated roots appeared earlier in the treated leaves than in the control. This experiment was conducted from Oct. 6 to Nov. 19, 1931. The formation of roots in stem cuttings or leaf cuttings is stimulated by placing the cuttings in vacuum (about 70 cm. Hg. and at room temperature).

As stated above, the occurrence of root formation in the stem cuttings of various plants is very much accelerated by placing them in anaerobic condition for a certain time (in warm-bath treatment about 8 hours at about 30°C. and in vacuum treatment about 5 hours at room temperature), immediately after isolation from the mother twigs. In some cases, as stated in the preceding experiments, callus formation was also stimulated by such treatment. This fact seems to be in agreement with one of the methods in horticulture. A gardener sometimes covers the operated portion with a wax, a paraffin paper or clay when grafting is carried out. On the one hand, this covering is done in order to protect the graft from drying out. On the other hand, it is very probable that such treatment causes an anaerobic condition in some degree, which may benefit the regeneration on both contact surfaces. On the whole, regeneration in the stem cuttings of various plants is stimulated by inducing anaerobic respiration. From this fact, it seems very reasonable to suppose that the occurrence of regeneration in the stem cuttings of various plants may be connected with the anaerobic respiration.

(3) Respiration of stem cuttings

a) A certain part of the twig which was attached to the mother plant was closed within a hollow cylinder of nickel-plated copper of 5 cm. diameter and 17 cm. length, for 24 hours, during which time the air in it was not replaced with the fresh. After 24 hours, the decrease of O_2 and the increase of CO_2 in the hollow cylinder, caused by the respiration of the stems was measured with HEMPEL's apparatus. The respiratory quotients in this case were almost 1. This determination served as the control. After the control determination a stem cutting (17 cm.) was made from that part in the closed state in the hollow

cylinder. Then the air in the hollow cylinder was replaced by fresh air. The stem cutting was then closed in the hollow cylinder for 24 hours. The decrease of O_2 and the increase of CO_2 in the hollow cylinder caused by the respiration of the stem cuttings during 24 hours was determined. Gas sample in the hollow cylinder was taken out, using a gas pipette as follows: one entrance of the cylinder was connected with this gas pipette and the other entrance of the cylinder was connected with a water reservoir of 20°C. Water in the reservoir came into the hollow cylinder and drove out as much gas as was to be used for analysis. The analysis was carried out in the intervals of 24 hours. At the end of each analysis, the air in the hollow cylinder was replaced by the fresh. The results were collected in TABLES XXXVII, XXXVIII, XXXIX, XL, XLI, XLII and XLIII. They indicate that the respiratory quotients in the stem cuttings of various plants were greater than 1, especially for the first few days after isolation. This shows that breakdown occurs in stem cuttings as a result of cutting off.

In the cases of the cuttings of Populus and Solanum the quotients which once became greater than 1, decreased lower than 1, as the days advanced after isolation. This decrease after increase may be due to the influence upon respiration by some of the products which were accumulated in the cuttings (see RUHLAND and WETZEL 1931).

b) In another way, two twigs of about the same age and each about 1 m. long were selected. The middle part of one twig was covered with the first hollow cylinder for 24 hours, while that of the other twig was cut off and put in a second hollow cylinder for the same duration of time. The measurements of CO_2 and O_2 in the control twig and the stem cutting were carried out after 24 hours. For measuring 100 c.c. of gas were taken from both cylinders and the increase of CO_2 and the decrease of O_2 were determined respectively. The results were given in TABLE XLIV.

In this experiment, the respiratory quotient was also larger in the stem cuttings than in the control twigs. The values of the respiration of stem cuttings were greater than 1.

c) The respiratory quotients in variously treated cuttings were determined. Many pairs of stem cuttings were made, each pair consisting of two cuttings almost equal in age, length and diameter etc. One member of each pair was experimentally treated, and the other of

each pair served as control. The treatments in this case were warm-bath and vacuum. The duration of warm-bath was 8 hours and that of vacuum was 5 hours. Then all the materials were closed in different hollow cylinders and a RQ-values after 24 hours were measured. The results were given in TABLES XLV and XLVI.

The respiratory quotient was larger in the treated cuttings after warm-bath and vacuum treatments than in the control.

The respiratory quotients in the stem cuttings became greater than 1 (in Salix, Solanum, Syringa, Pelargonium etc.) and the cuttings treated with warm-bath and vacuum became still greater. This fact may be due to the vigorous occurrence of anaerobic respiration or breakdown in them.

(4) Respiratory products in stem cuttings

The experimental results in the preceding pages showed that the inducement of anaerobic respiration very much stimulated the occurrence of the formation of adventitious roots and shoots in stem cuttings. Moreover, the respiratory quotient of stem cuttings was higher than 1 and that of the cuttings treated with warm-bath or vacuum was higher than in control cuttings. In such a case, the aldehyde and alcohol were examined in this experiment using Populus nigra. Stem cuttings (about 1–2 cm. diameter and 20 cm. long) to be tested were detached from the plants and placed in the greenhouse at about 20°–30°C., their basal ends (about 3 cm.) being dipped in water. As the control, twigs of about the same age were selected. The control materials remained on the mother twigs. In the experiment, the materials to be used were pulped by a masticator. From the pulp, thus prepared, alcohol and aldehyde were distilled by means of boiling vapour. Two hundred c.c. of the first distillate were used in each determination. One hundred g. of fresh materials were used in each experiment. Acetaldehyde was detected and determined with p-nitrophenylhydrazine in acetic acid and SCHIFF's reagent. The results in this estimation were as seen in TABLE XLVII.

There was more aldehyde and alcohol in the stem of Populus nigra in cuttings than in the control twigs.[1]

The determination of aldehyde and alcohol which were formed in

1) Similar results were obtained, usirg the stem cuttings of Syringa vulgaris, Syringa japonica, Pirus malus, Pirus serotina and Epiphyllum truncatum.

variously treated stem cuttings was carried out. A number of stem cuttings was made of some twigs of about the same age, and they were divided into three groups. Two groups were treated experimentally, namely by vacuum (for 5 hours) and warm-bath treatment (for 8 hours) respectively, while the other group served as control. The results of this estimation were shown in TABLE XLVIII. The cuttings treated with warm-bath or vacuum produced greater quantities of acetaldehyde and alcohol than the control cuttings did after 24 hours from the end of the treatment (TABLE XLVIII).

Acceleration of the formation of adventitious roots in the cuttings by the treatment with acetaldehyde vapour

Some pieces of main roots of Raphanus sativus (" Hatukadaikon ") all whose hair roots had been cut off, were put on a 2% agar-jelly, the surface of which was wet with 0.01% acetaldehyde solution. These materials produced little roots at the cut surfaces of the lower parts earlier and more vigorously than the control materials which were put on agar-jelly without acetaldehyde. This experiment was carried out in May, 1932.

From the results stated above it seems very probable that the occurrence of regeneration in stem cuttings is concerned with the products of anaerobic breakdown.

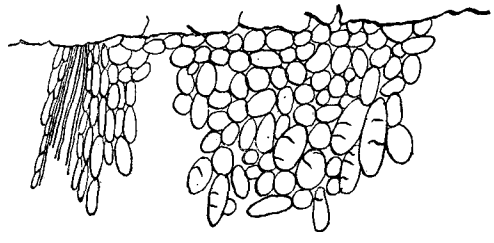
III. Stimulation of cell multiplication on the cut surface of roots of Beta vulgaris, Brassica Rapa and Raphanus sativus

As has been shown in the preceding pages, aldehyde or other related substances stimulate the development of adventitious shoots and roots. Now, the writer intends to ascertain whether acetaldehyde plays a stimulative rôle in the cell multiplication of permanent tissues. In connection with this question, the writer calls attention to HABERLANDT's experiments concerning wound hormone (1921), especially his results with kohlrabi: If the cut surface is washed with water in order to remove the wound hormone from the surface, the activity of cell multiplication on it is remarkably depressed. This fact can be demonstrated by using also the roots (hypocotyls) of Beta vulgaris, Brassica Rapa and Raphanus sativus, as will be stated in the following pages.

In the present work, other experiments were carried out which showed that very vigorous cell multiplication occurred even on the completely washed cut surfaces of the pieces of the roots of Beta vulgaris, Brassica Rapa and Raphanus sativus as results of some treatments as follows:

Fig. 9. Cell multiplication on the cut surfaces of the root pieces of Beta vulgaris.

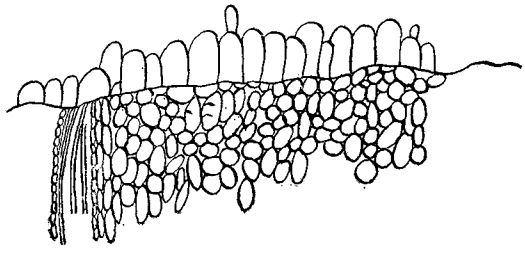
a



a. Washed cut surface. No cell multiplication.

1. The roots of Beta vulgaris, Brassica Rapa and Raphanus sativus were cut into small pieces and placed on a 2% agar-jelly in the germinating vessel at room temperature. Four or five days later, the cell multiplications (callus formation) on the cut surface began to take place.

b

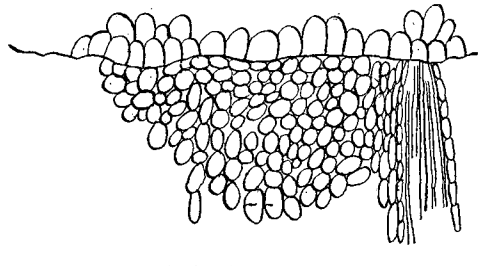


b. Cell multiplication on the washed cut surface which was afterward treated with acetaldehyde.

2. The cut surface of the small pieces of Beta vulgaris, Brassica Rapa and Raphanus sativus were thoroughly washed with redistillated water and the film of the water on the cut surface was absorbed slightly with a piece of charpie, and they were placed on a 2% agarjelly in the germinating vessel. No sign of cell multiplication was observed on such cut surface (Fig. 9 a).

3. Three equal small pieces were made of the roots of Beta vulgaris, Brassica Rapa and Raphanus sativus respectively. One piece from each root was placed on a 2% agarjelly in the germinating vessel as the control without treat-

c



c. Cell multiplication on the washed cut surface which was placed afterward in anaerobic state for a certain hour.

ment. The other two pieces of each root were washed with redistillated water; the film of the water on the cut surface was lightly absorbed with a piece of charpie. A washed piece from each root, that is three pieces in all, was treated with acetaldehyde vapour in the following way: the washed pieces of the materials were put on agar-jelly, the surface of which had been wet with a 0.001% solution of acetaldehyde, so that the vapour of the dilute acetaldehyde filled the germinating vessels. The other washed three pieces were put on agar-jelly in the germinating vessels without any treatment. A few days later, the cell multiplication on the cut surfaces of the treated materials as well as on the control materials began to take place (Fig. 9 b) except a part of the surface which was closely in contact with the agar. Cell multiplication in the treated piece of the root of the beet took place over an area about 50–80% the size of the area, over which the multiplication took place in the control, and in the other two materials, it occurred to the same degree as in the control. The washed pieces of each material without any treatment did not show such phenomenon (Fig. 9 a). This experiment was carried out at room temperature.

4. The washed pieces of the roots of Beta vulgaris, Brassica Rapa and Raphanus sativus were placed under a bell jar, into which a stream of H_2-gas was constantly passing and were kept for 24 hours at room temperature. After treatment, the materials were returned to aerobic state and kept on a 2% agar-jelly in the germinating vessel. A few days later, the cell multiplication began to occur at the washed surface of the roots (Fig. 9 c).

As we see in these experiments, cell multiplication can be induced on the washed cut surface of the roots of Beta vulgaris, Brassica Rapa and Raphanus sativus by treatment with acetaldehyde vapour, or by placing the pieces of the roots in H_2-gas, that is, in anaerobic condition. Further, in the pieces of the roots of Beta vulgaris, Brassica Rapa and Raphanus sativus, kept on agar-jelly, the existence of acetaldehyde was shown microchemically by SCHIFF's reaction.

Respiratory quotients of the pieces of the roots of Beta were measured, using Hempel's apparatus, as follows:

	O_2 c.c.	CO_2 c.c.	RQ
The 3rd day after the cutting	12.0	15.5	1.3
The 4th day after the cutting	8.1	10.0	1.2
The 6th day after the cutting	12.8	19.0	1.5

The respiratory quotients of the pieces of roots of Beta on agar-jelly without any treatment were larger than 1. This result indicates the occurrence of intramolecular respiration (breakdown) in the pieces of root as a result of cutting off. The appearance of aldehyde in the pieces may be due to such catabolism and aldehyde treatment stimulates very much the cell multiplication on the washed cut surface. From these facts it seems very probable that acetaldehyde or other related substances may play a rôle just like wound hormones in this case.

When a piece of the root of Beta vulgaris was placed on the agar-jelly in the germinating vessel, callus formation soon took place on the cut surface, as described above. When callus formation went on for a good while, the following facts on the cut surface were observed. The development of the cell masses, like callus formation in woody plants, began under the cut surface at the meristematic zone. These callus-like cell masses (perhaps a kind of tumor)[1] consisted of many irregularly formed parenchymatous cells and contained tracheids abnormally arranged in the center. The piece of the root of Beta vulgaris on the agar-jelly showed the existence of aldehyde as stated

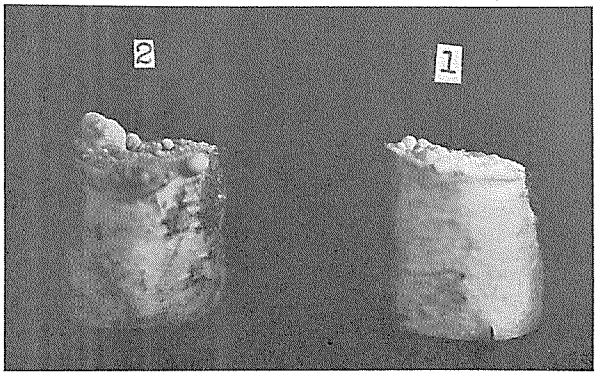


Fig. 10. Tumor development on the cut surfaces of the root pieces of Beta: left (2), treated with ethyl alcohol vapour; right (1) control.

1) Such pathological growth of plant tissue has often been called "tumor" by many authors. In the present work this word will be practically used without any consideration, whether this means "tumor" in the strict pathological sense.

above, but the tumor on the cut surface of the root of Beta vulgaris showed a stronger reaction of aldehyde with SCHIFF's reaction. Further, it was observed that the tumor formation was stimulated by the treatment of acetaldehyde or ethyl alcohol vapour as follows: two equal pieces of Beta were made and one of them was put on the agar-jelly which was wet with a dilute solution (0.001%) of acetaldehyde. Another piece was put on agar-jelly without acetaldehyde. The tumor formation in the former case was very much accelerated. A similar result was also obtained using a dilute solution (0.01%) of ethyl alcohol (Fig. 10) instead of acetaldehyde.

From these results it may be said that the tumor formation on the cut surface of Beta was very much accelerated by treatment with acetaldehyde or ethyl alcohol, namely, with some of the products of anaerobic respiration.[1]

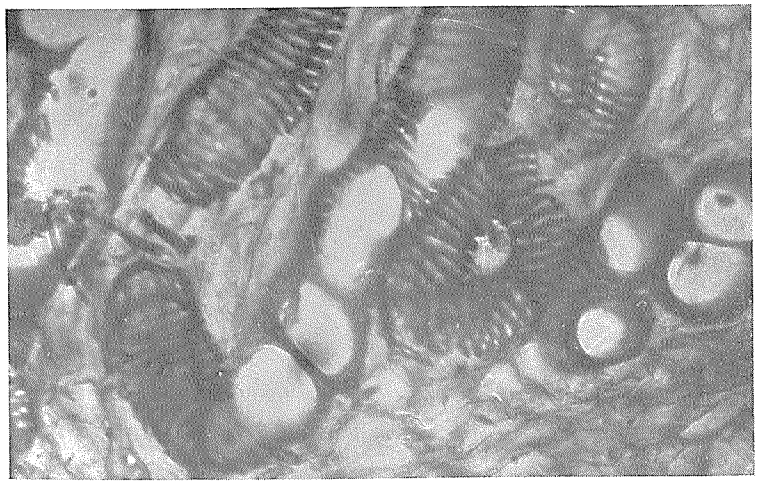
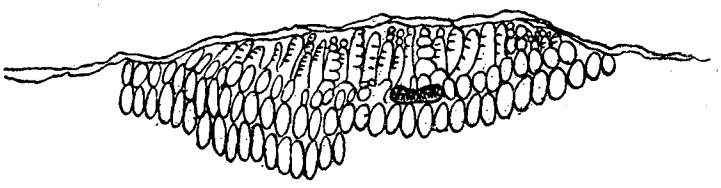


Fig. 11. Abnormally arranged tracheids in the tumor of a Beta-root.

1) REHWALD (1927) obtained the negative results in inducing tumor formation on the cut surfaces of roots of Daucus and other materials by the treatment of lactic acid or other organic acids. But before him, AULER (1925) said that tumor formation in plants concerned with anaerobic fermentation.

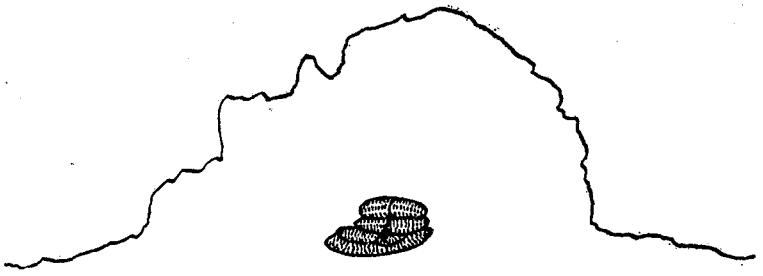
Fig. 12. The processes of the development of the tracheids in the tumor on the cut surface of a Beta-root.

a



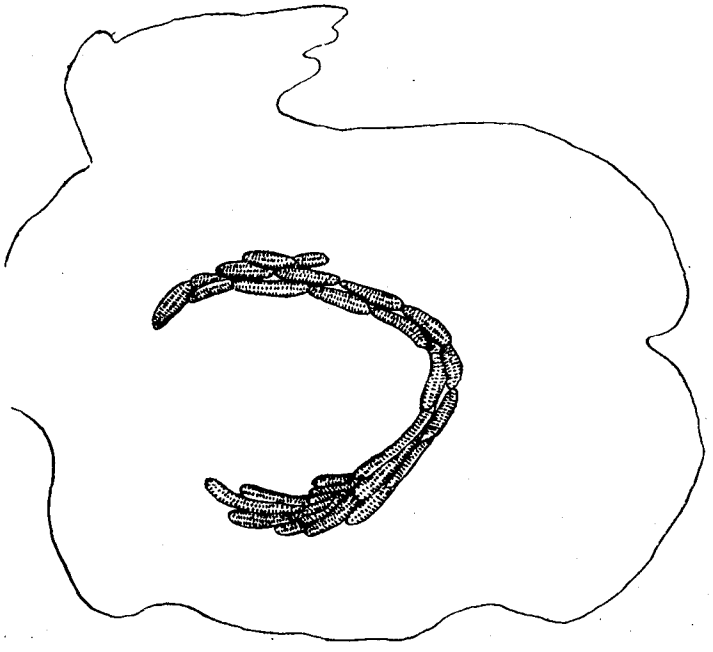
a. The first stage in which the tracheid appeared as one piece.

b



b. The second stage; the tracheids developed into a manifold form.

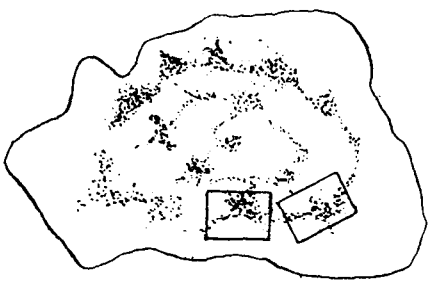
c



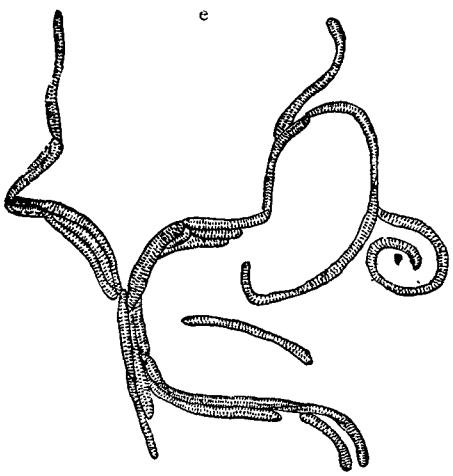
c. The third stage; the tracheids arranged in the form of a horseshoe in a polar view.

In the development of tumor on the cut surface of the piece of the root of Beta vulgaris, the process of the development of tracheids in it was very remarkable (Figs. 11 and 12). In the very young stage of the development of the tumor, the tracheid appeared only as one piece (Fig. 12 a). Next, it developed into a manifold form, that is, one tracheid piling after another (Fig. 12 b) or coiled in a comma-chaped way. The third stage of development was that many tracheids arranged themselves in the form of a horse's hoof in polar view (Fig. 12 c). At the fourth stage, the tracheidal elements of the hoof were irregularly scattered by the development of the tumor (d, e and f in Fig. 12), and each space between tracheids was filled with slender parenchymatous cells. These experiments were carried out from May to November, 1931.

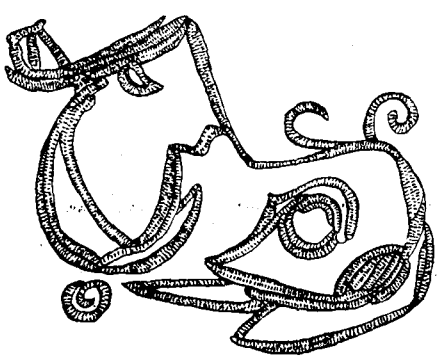
d



e



f



d, e, f. Later stages; the tracheids scattered about.

IV. Discussion

The cause of the occurrence of regeneration in Bryophyllum plants has been studied by many authors. GOEBEL (1908) manitained, from his experimental results of cutting off a part of mid-vein in the leaf and removing or arresting the top of a plant and other similar treatments (using Bryophyllum crenatum mainly), that correlation existed

between the growth of the growing point and bud formation on the attached leaf. So, according to GOEBEL, if the growth of the growing point is depressed or arrested, bud formation on the leaf ought to take place. With Bryophyllum calycinum, however, WAKKER (1885), de VRIES (1890) and others obtained negative results. CHILD and BELLAMY (1920) pointed out how the growth of the top inhibits the growth of a bud on the leaf of Bryophyllum calycinum and also the fact that regeneration on the leaf was caused by blocking this action. And they stated that this inhibiting action is dependent on some sort of effect transmitted physiologically through the living active protoplasm. OSSENBECK (1927) recognized correlation between a growing point and the formation of shoots and roots on the leaf, as well as the specific substance which is concerned with this correlation. WENT (1930) suggested some substance which is concerned with the formation of roots in Bryophyllum. REED (1923) attempted to apply the hypothesis of formative stuffs, which had been proposed by SACHS and GOEBEL, to regeneration in Bryophyllum calycinum. But REED (1923) concluded that there were no indications of dormancy in the meristems in the leaf, because of the facts that the meristems were deprived of formative stuffs or specific substances and the dormancy or the development of the meristems in the leaf was an expression of the metabolic condition of the organ of which they were a part. Further, REED said that the development of meristems in the leaf was due to physical and chemical changes within the organ. MEHRLICH (1931), from the view of enzymic actions, offered an opinion on regeneration. SMITH's experimental results (1921) were especially interesting. As stated in the preceding pages, according to SMITH, regeneration on an attached leaf or a stem was induced by making a crown gall by the injection of Bacterium tumefaciens. Further, he found out that a crown gall on potato tubers secreted aceton, aldehyde and ammonia etc.

According to the investigation of BEALS (1923), regeneration at the notches of an isolated leaf of Bryophyllum calycinum takes places from the division of the phloem cells of the veins, that is, its regeneration occurs from the small and actively dividing cells of the phloem of the veins of the leaf: thus the regeneration is developing endogenously. HOWE (1931) states that the growth in the notches proceeds from a meristematic group of cells which is present in the very young leaves and that growth in the notches is exogenous. MEHRLICH (1931), NAYLOR (1932) and the present writer's results are practically the same as that

of HOWE (1931). On the origin of a group of meristematic cells in the notches, BEALS (1923) states, as described above, that the origin was in the vein, namely, in the phloem. But this opinion was criticized by MEHRLICH (1931) as follows: "no evidence had yet been presented to demonstrate beyond doubt that these veins and meristems might not have wholly separate origin." HOWE (1931) said that a group of small cells in the notches appeared distinctly different from the other cells of the leaf and apparently had no connection with the veins. NAYLOR (1932) was of a similar opinion.

MEHRLICH (1931) said that the foliar meristems were at any time more than embryonic. NAYLOR (1932) stated that the primordias of entire new plants were already present in the notches of the adult Bryophyllum leaf. HOWE (1931) observed the following facts: the group of cells in the notches gradually multiplied, producing a slight protuberance into the notches. In a few cases the protuberance was slightly lobed. Development beyond this took place after the leaves were removed from the plant.

As is seen above, in the notches of adult leaf of Bryophyllum, the primordia of the new plants were already formed. Concerning this point, meristems in the notches are very similar to winter resting buds.

Since the present writer (1928) reported that MOLISCH's warm-bath treatment was effective to induce new plants at the notches of the attached leaves of Bryophyllum calycinum, BORESCH (1928 a, b) and others in their papers acknowledged the possibility of such result, and MOLISCH (1931) was much interested in the result of the present writer. HARIG (1931) also obtained positive results in the experiment of warm-bath treatment to induce adventitious roots and shoots on the attached leaves of Bryophyllum crenatum and Cardamine pratensis. On the contrary, MEHRLICH (1931) was against the result and the opinion on warm-bath treatment. He said "The experiments performed by the writer fail to support two of the fundamental bases[1] of KAKESITA's hypothesis of regeneration (1928)."[2] MEHRLICH has treated Bryophyllum plant in the warm-bath of 30°–35°C. for 10 hours, and he did not succeed in regeneration on the attached treated leaves. But difference between MEHRLICH's experiment and that of the present writer lay in the methods of treatment, that is, MEHRLICH treated the materials

1) Warm-bath treatment and inducement of anaerobic respiration.

2) MEHRLICH (1931, p. 137).

longer than the writer did. That a treatment of too long duration and high temperature of warm-bath is harmful to the materials was recognized by HARIG (1931) in Cardamine pratensis. And the present writer's results on Bryophyllum confirmed that no regeneration took place as a result of too long treatment and such treatment was harmful to the Bryophyllum plant too. Consequently, when time and temperature are taken into consideration, warm-bath treatment is after all effective to induce adventitious roots and shoots on the attached leaves of Bryophyllum plants.

HARIG (1931)[1] criticized the experimental results of the present writer on the warm-bath treatment of Bryophyllum calycinum (1928 and 1930 b) and said that the results of the warm-bath treatment of Bryophyllum calycinum obtained by the present writer (1928) might show that the growing point of Bryophyllum was injured by the warm-bath treatment, and as a result, the so-called " Pseudostimulation " took place and this may have caused regeneration at the notches. In order to avoid this possibility in the present work such experiment as was shown in the preceding experiment on the warm-bath treatment was carried out, i.e., the growing points of the materials were not soaked in the warm-bath. In that treatment only some adult leaves attached to one side of a plant were soaked in the warm-bath of 30°C. for 3 hours. During this treatment the growing point of the same plant was carefully kept in the air at room temperature. About 5 days later, the leaves only, thus treated, produced roots and shoots at the notches. From this experimental result, it can be clearly seen that stimulative action for root and shoot formation on attached leaves by a warm-bath method, is not due to the break of correlation between the growing point and the adult leaf. In the treatment of whole plant body with H_2-gas for 48 hours or 12 hours a positive result was obtained by the present writer (1930 b). Later, HARIG (1931) put the whole body of Cardamine pratensis in H_2-gas for 24 hours and obtained the same positive result. In the present work such regeneration was also secured by the treatment of any one adult Bryophyllum leaf only, instead of the whole shoot, in H_2-gas or N_2-gas for 24 or 42 hours. From the facts above described it may be seen that, the occurrence of adventitious roots and shoots on the attached leaves of Bryophyllum plants may be connected with incomplete or anaerobic respiration.

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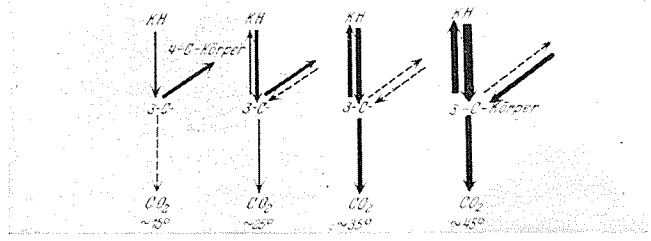
1) HARIG (1931, p. 67–68).

On the formation of organic acid (mainly malic acid) in the Bryophyllum leaves, WARBURG (1885) said that sugar which had been formed by assimilation in the day time is oxidized into organic acid by respiration in the night and on the following day, the organic acid is further oxidized to CO_2 and H_2O. GUSTAFSON (1925) stated that the formation of organic acid is a normal phenomenon in Bryophyllum calycinum and undoubtedly due to incomplete respiration, caused by lack of oxygen and perhaps by the slow escape of CO_2. From the writer's examinations on the measurement of the changes of acidities and those of CO_2 output of the leaf in anaerobic respiration induced by warm-bath or by placing the plant in H_2-gas, as described in the preceding pages, it may be clear that acid formation in Bryophyllum leaf is connected with respiration, showing that there is a reverse relation between the production of CO_2 and acid. This relation was already reported by the present writer (1030 b). WOLF (1931) also reported that the maximum of acid formation in Bryophyllum plants corresponds to the minimum of CO_2 output, and he ascertained that malic acid formation in Bryophyllum plants was concerned with the metabolism of carbohydrate.

WOLF (1931) further stated that PASTEUR's reaction existed in the respiration of carbohydrate in Bryophyllum and this is, according to WOLF, parallel to glycolysis in mammalian tissues, as follows:



Furthermore, according to WOLF, the respiration with the malic acid formation and O_2-respiration in Bryophyllum produces almost equal quantity of energy.

According to REED (1923), when Bryophyllum calycinum was placed in the dark in natural conditions, the formation of adventitious roots and shoots occurred at the notches of the attached leaves. MEHRLICH (1930) obtained the same result in respiration of the same experiments. In this case, the utmost probability exists of a relation

between the regeneration and formation of organic acid. It is a well known fact that the acidities became very high when Bryophyllum plants were placed in the dark (WARBURG 1886). Moreover GUSTAFSON (1925) pointed out that on a cloudy day the acidities in Bryophyllum calycinum are very high even in the day light hours. The results of the present writer showed that acidities in the isolated leaves are very high on the following day after isolation, and also in the attached leaves in anaerobic condition; for instance, in warm-bath treatment. It is known that anaerobic respiration of carbohydrate proceeds as follows: Hexose→ methyl glyoxal→ pyruvic acid→ acetaldehyde→ ethyl alcohol. Concerning malic acid formation in Crassulaceae, recently RUHLAND and WETZEL (1931) pointed out that it obeys the following process: fermentation sugar becomes pyruvic acid through the stage of methyl glyoxal as in the above formula, and pyruvic acid becomes acetaldehyde by the action of carboxylase. The activity of carboxylase is depressed by the accumulation of aldehyde (RUHLAND u. WETZEL, 1931 and WETZEL, 1932), resulting in the change of pyruvic acid into malic acid in this case (RUHLAND u. WETZEL 1931). That is to say, there is an intimate relation between malic acid formation and aldehyde formation through the function of carboxylase.

When regeneration takes place, for instance, in the case of isolation, the treatment of warm-bath or the keeping in N_2- or H_2-gas, the formation of aldehyde is also very considerable. Furthermore the injection of acetaldehyde or related substances is very effective to induce regeneration at the notches of the attached leaves of Bryophyllum. From these facts it can be undoubtedly seen that alcoholic fermentation and acid formation vigorously occur in the isolated as well as in the experimentally treated leaves, which cause the regeneration of new plants at the notches.

The chemical change between acetaldehyde and pyruvic acid in anaerobic respiration concerns with the function of carboxylase as stated above, and considerable CO_2 is formed in this reaction. As stated above, WETZEL (1932) and RUHLAND and WETZEL (1931) reported that the acitivity of carboxylase is remarkably depressed by the accumulation of aldehyde. In this case the CO_2 production becomes small. In such case, in Crassulaceae, according to RUHLAND and WETZEL (1931), pyruvic acid changes into malic acid and CO_2 production becomes small. Concerning this fact WOLF (1931) proved experimentally that the minimum of CO_2 production corresponds to the maximum of acid

formation. A similar relation between CO_2 and acid formation was found by the present writer (1930 b) in the case after treatment in anaerobic condition. The formation of aldehyde, of organic acid and of CO_2 in Crassulaceae is closely connected. If this relation is applied to the fluctuation of acidities and the accumulation of aldehyde in the isolated leaves during one day, the following fact will be apparent. As stated in the preceding pages, in the isolated leaves it is remarkable that the acidities rise once suddenly to a high level at about 4 o'clock in the afternoon. On the other hand, the maximum quantity of aldehyde in the isolated leaves is seen at about 2 o'clock in the afternoon. This extraordinarily accumulated aldehyde in the isolated leaves may depress the function of carboxylase at that time, and as a result, malic acid may suddenly be accumulated at about 4 o'clock. Similar statement may be also possible as to the fluctuation of acidities in the leaves after treatment with warm-bath or H_2-gas. Especially the relation between CO_2 and acid formation in this case may prove this statement true, because, after treatment, the minimum of the CO_2 output corresponds to the maximum of the acid formation. A similar relation between acetaldehyde and organic acid formation is seen during warm-bath treatment of Bryophyllum calycinum, that is, during the treatment, especially for the first 2–3 hours from the beginning the quantity of acetaldehyde is very large, and during the same period the total acidities are very high.

As is seen in the results of the preceding experiment, the question arises why the acid metabolism and other phenomena in an isolated leaf agree with the case in which anaerobic respiration is caused. In this relation, the accumulation of aldehyde in the isolated leaves may be very significant. In the isolated leaves, the quantity of aldehyde increased about 24 hours after isolation and then gradually decreased. The total acidities were very high on the following day after isolation. The CO_2 output was small at the beginning of isolation, but increased in volume as the days advanced. In short, at the early stage after isolation, the quantities of aldehyde and organic acid are large and CO_2 production is the minimum. From these facts, the accumulation of respiratory products may become very important, because the above stated phenomena in isolated Bryophyllum leaf are very similar to those which occur when the carboxylase is influenced by the accumulation of aldehyde.

It must be noted that while the respiratory quotient of control attached leaves of Bryophyllum is 0.5, that of isolated leaves decreases

to 0.3, as the results of the preceding experiment show. After the present writer (1930 b) had reported this fact, RUHLAND and WETZEL (1931) in their work on the formatian of malic acid in Crassulaceen discussed this point as follows: "Besonderes Interesse für unsere Frage besitzt jedoch ein Hinweis von KAKESITA auf Gegenwart von Acetaldehyd in sukkulenten Crassulaceen. Dabei stellt der japanische Forscher fest, dass Verminderung der O_2-Tension, wie sie durch Einbringen der Pflanze in eine Wasserstoffatmosphäre oder such durch das Warmbad erreicht wird, den Aldehydspiegel im Gewerbe stark austeigen lässt. Selbst wenn man annehmen wollte, dass der Aldehyd ganz gleichmässig im Gewebe verteilt wäre, was indes durchaus unwahrscheinlich ist, würde die dadurch erreichte Carboxylasehemmung nach unseren Erfahrungen an der Hefencarboxylase bereits eine Aktivitätsverminderung von über 50% betragen. Ganz unseren Vorstellungen entsprechend nimmt gleichzeitig mit dem Aldehydanstieg auch der Gehalt an Apfelsäure stark zu, während die CO_2-produktion sehr erheblich zurückgeht, so dass Atmungsquotienten bis zu 0.3 herab gemessen wurden."[1]

The statement of RUHLAND and WETZEL may be summarized as follows: aldehyde formed in Bryophyllum leaf depressed the function of carboxylase remarkably, and in consequence of it, CO_2-production became very small, until the quotient declined as low as 0.3.

From the facts stated above, it becomes clear that the catabolism in an isolated leaf is different form that in the attached leaf and the products of such catabolism (perhaps, organic acid or acetaldehyde or other related substances) probably act as stimulants for regeneration in Bryophyllum. That is, when regeneration takes place in Bryophyllum catabolism in the leaf is influenced first, and next, its products act on the meristematic tissues of the notches for the development.

On the occurrence of regeneration in stem cuttings FAIVRE (1871) found that the starch content in the cuttings decreased when regeneration began. Similar observations were made by CARLSON (1929), using the cuttings of two rose varieties. ZIMMERMAN and HITCHCOCK (1929) found that the stem cuttings of Ilex opaca increased their starch content and that keeping the cuttings of Ilex crenata in the dark causes them to lose starch regularly for 6 weeks and did not cause root formation, but the control in the light increased starch and developed root formation. SMITH (1928) found that the total carbohydrate in the stem

1) RUHLAND und WETZEL (1931, p. 570).

cuttings of COLEUS BLUMEI influences the formation of regenerated roots in them. ZIMMERMAN and HITCHCOCK (1929) found that in the stem cuttings of Ilex opaca, sugar which was fairly plentiful at the beginning decreased to only a trace at the end of the experiment, accompanied by the increase of reducing substances.

From the results of many authors it will be seen that the root formation in stem cuttings depends on the stored starch (FAIVRE 1871, SMITH 1928, and ZIMMERMAN and HITCHCOCK 1929). According to the results of the present writer, as described in the preceding pages, respiratory quotient in the stem cuttings became greater than 1, especially in the first few days after isolation. On the other hand, the increase of acetaldehyde and alcohol in the stem cuttings was observed after isolation. Further, it was seen that when stem cuttings were treated with warm-bath or vacuum in order to cut off the O_2-supply of the stem cuttings, the acceleration of regeneration in them was induced. EMIL (1925) stated that CO_2-gas was very effective to stimulate root regeneration in the stem cuttings of Chrysanthemum and Dahlia. In the stem cuttings kept in warm-bath or vacuum, according to the present writer's result, respiratory quotient became greater than in the control cuttings, and the quantities of acetaldehyde and alcohol became greater also, and such treatments were very effective to induce regeneration in the cuttings. From these facts, the occurrence of regeneration in stem cuttings seems to be concerned with some of the chemical changes of carbohydrate, especially with anaerobic respiration.

SWINGLE (1929) stated: "In treatises on horticulture but two categories of cuttings are generally recognized—those of softwood and those of hardwood cuttings may, in turn, be divided into two categories —those that have preformed small but unmistakable root primordia (already present when the cuttings are removed from the original plant) and those that do not possess such primordia." He showed that such root primordia were formed by the cambium and apparently only at certain places where branch or leaf trace parenchyma or primary or secondary rays cross the cambium. The occurrence and formation of root primordia in certain varieties of apples, and some willows etc. have been reported by TRECUL (1846), VÖCHTING (1878), van der LEK (1925), SWINGLE (1925 ab, and 1927), PRIESTLEY (1926) and others. The writer's results show that the inducement of anaerobic respiration stimulated root, shoot or callus formation not only in hardwood cuttings, but also in softwood cuttings which possessed no root primordia.

Furthermore, the regeneration of roots at the cut surface of the root of Raphanus sativus was also stimulated by anaerobic respiration or by treatment with acetaldehyde vapour. From these results, the regeneration in the cuttings with or without root primodia may be concerned in the inducement of anaerobic respiration, and the products in such metabolism may stimulate regeneration in the cuttings.

On meristematic activity in permanent tissues, PRIESTLEY and WOFFENDEN (1922 and 1923) showed that the first stage in the process of wound healing in parenchymatous tissue is the formation of a suberian block, the products of fatty acid released from the tissue just below the cut surface. In this case, according to them, starch disappears and fatty products appear.

The writer's results showed that acetaldehyde or related substances played a stimulative rôle, like wound hormones, in the meristematic activity in the permanent tissues of the roots of Beta etc. Moreover, some of the products of anaerobic respiration caused not only the cell multiplication on the cut surfaces of permanent tissues, but also stimulated the development of the tumor on the cut surfaces. It is already known that aldehyde stimulates the meristematic activity of embryonal tissues, for instance, NIETHAMMER (1928 a, b) showed that acetaldehyde accelerated the germination of some seeds. BORESCH (1928 a) came to the conclusion that acetaldehyde, which appeared in buds as a result of warm-bath or other similar treat ments awakened the development of resting winter buds. Further on the anaerobic respiration, the following facts are known: according to the investigation of MORINAGA (1926) the reduction of oxygen pressure had a very beneficial effect upon the germination of intact cat-tail seeds and Bermuda grass seeds. NAKAJIMA (1927) described a similar fact in the germination of seeds of some water plants. GENEVOIS (1927) found that germinating plants showed a stronger fermentation than older plants did. BORESCH (1924) showed a similar effect upon the development of resting winter buds by placing the buds in vacuum for certain number of hours as well as MOLISCH's warm-bath (1908). From his result, BORESCH (1924) concluded that the forcing action of warm-bath treatment upon the development of resting winter buds consists of the combination of anaerobic condition and adequate temperature. It is known that a very small quantity of hydrogen cyanide depresses the oxydative phase of respiration remarkably, but that it has practically no effect on fermentation. GASSNER (1925) and MATSUBARA (1931) found that the development of winter buts of some

plants was stimulated by a treatment with HCN. HARIG (1931) secured a result that HCN treatment induced regeneration on the attached leaves of Bryophyllum crenatum and Cardamine pratensis. WARBURG (1926) found that tumors on mammalien tissues obtained the energy by respiration and by fermentation in natural condition. Similarity between the development of resting winter buds of plants, which was caused by warm-bath treatment and that of experimentally formed tumors on mammalian tissues was pointed by BORESCH (1928 b).

The writer's experimental results in the preceding pages show that the inducement of anaerobic respiration by placing Bryophyllum plants in the warm-bath is a successful method to induce the formation of roots and shoots in the attached leaves. This case is very similar to the awakening of winter buds. Further, similar results are obtained by the inducement of anaerobic respiration by placing the attached leaves in H_2- or N_2-gas. Regeneration in the stem cuttings of various plants is also accelerated by the warm bathing and by the inducement of anaerobic respiration, as has been shown in the preceding experiments. In the case of the isolation and inducement of anaerobic respiration in the attached leaves of Bryophyllum, the writer proved quantitatively the accumulation or formation of aldehyde, alcohol and organic acid. And the accumulation of the products of anaerobic respiration is also seen in the stem cuttings of various plants. Using the attached leaves of Bryophyllum the writer succeeded in causing regeneration artificially at the notches by the injection of acetaldehyde, ethyl alcohol and other substances which are assumed to be the products of incomplete and anaerobic respiration. Among these substances, acetaldehyde and ethyl alcohol are most effective in producing adventitious roots and shoots.

The isolated leaf of Bryophyllum calycinum when placed in anaerobic state produced not only regenerated roots and shoots at the notches, but also tumor-like cell masses at the leaf margin. Further, the formation of adventitious roots in the stem cuttings of hard and soft woody plants with or without root primordia was accelerated by placing them in warm-bath or vacuum, that is, in anaerobic condition. And callus formation on the washed cut surface of the roots of Beta vulgaris, Brassica Rapa and Raphanus sativus was caused by a treatment with acetaldehyde.

These facts show that the products of anaerobic respiration stimulate not only the development of the embryonal tissue or bud, but also the cell multiplication of the permanent tissues.

V. Summary

1. Regeneration in Bryophyllum calycinum, Bryophyllum crenatum, stem cuttings and cell multiplication were investigated experimentally.

2. Warm-bath treatment, placing the plants in anaerobic condition etc. are very effective to induce regeneration at the notches of attached leaves, and in some cases, adventitious roots in the stem of Bryophyllum.

3. Injection with the products of incomplete or anaerobic respiration into the attached leaves of Bryophyllum calycinum and Bryophyllum crenatum brought about similar results. Especially acetaldehyde or ethyl alcohol were effective in this respect.

4. Some examinations on catabolic products in Bryophyllum plants were made, showing that the catabolism in the isolated leaves was very similar to that which occurred in the attached leaves when anaerobic respiration was induced.

5. The types of CO_2 output from the isolated leaves and the experimentally treated attached leaves of Bryophyllum calycinum were very similar.

6. The relation between CO_2 output and the respiratory products was examined. The results obtained in this case may be explained by the theory of RUHLAND and WETZEL (1931).

7. Regeneration in the isolated leaves of Bryophyllum calycinum and Bryophyllum crenatum is concerned with the formation or the accumulation of the end or intermediate products (aldehyde, alcohol, malic acid etc.) of incomplete or intramolecular respiration.

8. The warm-bath treatment of cuttings or placing them in anaerobic state for a certain number of hours, stimulated the formation of adventitious shoots and roots and callus in various plants.

9. Quantitative changes of respiratory products in the stem cuttings and in variously treated cuttings were studied. The occurrence of regeneration in the cuttings of various plants seems to be concerned with the products of anaerobic respiration.

10. Acetaldehyde may play a rôle, like the wound hormone of HABERLANDT.

11. Formation of tumor-like cell masses on the cut surface of the roots of Beta was accelerated by treatment with some of the products of anaerobic respiration.

The writer wishes to take this opportunity to express his hearty thanks to Prof. T. SAKAMURA for his guidance in this work. He also desires to state his acknowledgement to Emer. Prof. K. MIYABE for his encouragement during the work and his kindness in letting him use his library. And the accomplishment of the present work was much facilitated by the scholarship of the University, for which the writer thanks to Prof. S. ITO and Prof. K. SUDA. He also expresses his sincere gratitude to Director ASANO for his generosity in allowing him to continue his stay in the University from May to August, 1932, for the accomplishment of the present work.

VI. Protocols

TABLE I

Total acidity (expressed in c.c. N/10 NaOH) in the isolated leaves of Bryophyllum calycinum after isolation.

(March—August, 1928)

		8 a.m.	10 a.m.	12 a.m.	2 p.m.	4 p.m.	6 p.m.	10 p.m.	12 p.m.	4 a.m.
The following day of isolation	1	24.3	9.0	6.5	2.0	1.5	2.5	1.5	—	—
	2	19.5	10.2	4.0	4.1	2.4	4.0	2.0	—	—
	3	16.0	19.0	7.3	5.0	3.5	6.5	4.0	3.5	5.5
Control	1	18.5	8.0	9.0	0.75	1.0	1.8	1.8	—	—
	2	13.5	10.0	4.0	1.8	1.6	1.6	2.0	—	—
	3	13.0	14.0	3.5	2.0	1.5	1.0	2.5	3.0	16.0
The 3rd day after isolation	4	14.5	10.5	5.4	4.2	2.5	4.8	4.0	—	—
	5	10.2	9.6	5.0	2.0	6.0	2.0	4.4	—	—
	6	15.0	14.5	7.0	2.5	5.0	2.0	4.8	—	—
Control	4	22.8	11.5	3.5	3.0	1.5	1.0	4.3	—	—
	5	13.5	10.0	4.5	2.0	2.2	2.0	4.3	—	—
	6	14.0	10.5	3.0	2.0	1.5	2.0	6.0	—	—
The 4th day after isolation	7	11.0	8.4	5.8	4.0	4.6	2.8	4.0	—	—
	8	12.8	14.8	5.5	2.3	3.5	2.0	5.0	—	—
	9	18.0	10.0	5.9	2.5	5.5	3.0	0.5	—	—
Control	7	13.6	11.2	6.0	2.8	3.0	3.5	4.3	—	—
	8	12.4	10.0	4.0	0.8	1.0	1.5	6.5	—	—
	9	12.0	11.5	3.5	1.5	1.0	0.5	4.0	—	—
The 5th day after isolation	10	17.8	9.0	8.0	2.0	3.5	1.2	4.3	—	—
	11	12.8	6.0	3.5	2.5	3.5	3.0	5.0	—	—
	12	11.0	5.0	4.5	7.5	3.0	3 5	5.0	—	—
Control	10	22.3	7.8	7.8	5.4	1.0	2.0	4.2	—	—
	11	12.6	7.8	4.8	3.1	1.5	1.5	4.5	—	—
	12	20.0	4.0	3.5	0.5	2.0	3.0	6.0	—	—

TABEE II

pH-value in the leaf sap of Bryophyllum calycinum after isolation.

(March—August, 1928)

		8 a.m.	10 a.m.	12 a.m.	2 p.m.	4 p.m.	6 p.m.	10 p.m.	12 p.m.	4 a.m.
The following day of isolation	1	4.1	4.3	4.6	5.3	5.3	5.5	4.0	—	—
	2	4.0	3.9	5.0	5.3	4.3	4.4	4.1	—	—
	3	3.9	4.4	5.0	5.3	5.4	4.2	4.4	4.5	4.9
Control	1	4.5	4.3	5.5	5.6	5.6	5.3	3.9	—	—
	2	3.9	3.9	5.6	5.6	5.2	4.5	3.7	—	—
	3	3.9	4.4	5.5	5.7	5.7	5.2	4.5	4.5	3.7
The 2nd day after isolation	4	3.9	4.2	4.5	4.9	4.5	5.5	5.0	—	—
	5	4.1	4.4	4.3	5.1	4.5	5.4	4.3	—	—
	6	3.9	4.1	4.2	5.1	4.5	5.4	4.3	—	—
Control	4	—	4.0	4.2	5.5	5.2	4.7	4.5	—	—
	5	4.0	3.9	4.1	4.6	5.5	4.1	4.0	—	—
	6	4.0	4.2	4.7	5.5	5.5	5.5	4.2	—	—
The 4th day after isolation	7	4.0	3.8	4.1	4.5	4.2	5.3	5.0	—	—
	8	3.8	4.0	4.3	5.2	4.7	4.7	4.3	—	—
	9	3.6	3.8	4.5	4.9	4.5	4.9	4.7	—	—
Control	7	3.9	4.1	4.5	4.8	5.5	5.1	5.0	—	—
	8	3.7	4.1	4.7	5.1	5.0	4.9	4.3	—	—
	9	3.8	4.0	5.7	5.4	5.5	5.2	4.6	—	—
The 5th day after isolation	10	3.8	4.2	4.4	5.1	5.0	5.1	—	—	—
	11	3.8	4.4	4.6	4.5	4.6	4.3	4.3	—	—
	12	3.7	5.3	4.8	4.5	5.5	5.5	4.3	—	—
Control	10	3.7	4.2	4.5	5.5	5.5	5.3	—	—	—
	11	3.7	4.5	4.5	4.8	4.7	4.7	5.0	4.3	—
	12	3.7	5.3	5.5	5.8	5.6	5.5	4.3	—	—

TABLE III

Acidities in the leaves of Bryophyllum calycinum during warm-bathing.

(August, 1928)

			8 a.m.	10 a.m.	12 a.m.	2 p.m.	4 p.m.
Total acidity c.c. N/10 NaOH	Treated	1	19.0	18.5	18.3	16.0	15.0
		2	18.5	18.0	17.9	15.0	13.0
	Control	1	19.0	10.0	6.5	3.0	3.5
		2	18.6	7.0	4.2	1.5	2.5
pH	Treated	1	3.7	3.7	4.0	4.1	4.1
		2	3.6	3.7	4.0	4.0	4.0
	Control	1	3.7	4.0	4.7	4.8	4.8
		2	3.6	5.2	5.5	5.4	5.4

TABLE IV

Total acidity (expressed in c.c. N/10 NaOH) in the leaves of Bryophyllum calycinum after warm-bath treatment.

(August, 1928)

		8 a.m.	10 a.m.	12 a.m.	2 p.m.	4 p.m.	6 p.m.	8 p.m.	10 p.m.	12 p.m.
Immdiatly after treatment	1	in warm-bath				21.4	16.7	20.5	14.3	10.2
	2					7.1	5.0	0.5	1.5	0.45
	3					15.0	8.0	18.0	11.0	6.5
Control	1					8.0	10.0	6.4	7.2	11.0
	2					3.1	3.5	3.7	7.5	11.5
	3					3.5	4.5	7.0	8.0	11.0
The 2nd day after treatment	4	5.5	2.75	—	0.5	1.5	0.5	—	1.75	—
	5	3.5	7.5	—	2.5	4.5	2.0	—	2.5	—
Control	4	19.9	11.5	—	6.5	8.5	10.5	—	12.0	—
	5	13.0	12.0	—	6.0	2.5	0.5	—	3.0	—
The 3rd day after treatment	6	2.25	5.0	2.0	0.5	1.5	0.35	—	0.4	—
	7	1.5	5.0	2.0	3.0	1.5	0.5	0.3	—	—
Control	6	10.5	17.0	9.0	3.4	2.6	2.0	—	2.0	—
	7	10.5	16.0	9.8	9.5	3.5	3.0	2.5	5.0	—
The 4th day after treatment	8	3.6	6.0	—	2.0	2.3	1.0	—	2.0	—
	9	3.5	7.5	4.5	1.5	3.0	0.5	—	3.0	—
Control	8	12.5	12.0	—	6.0	1.5	0.75	—	2.0	—
	9	13.75	13.25	8.0	5.5	2.0	0.5	—	3.0	—

TABLE V

pH-value in the leaf sap of Bryophyllum calycinum after warm-bath treatment.

(August, 1928)

		8 a.m.	10 a.m.	12 a.m.	2 p.m.	4 p.m.	6 p.m.	8 p.m.	10 p.m.	12 p.m.
Immediately after treatment	1	in warm-bath				3.7	3.9	3.8	4.1	4.1
	2					3.8	3.9	4.3	4.2	4.4
	3					4.1	4.3	4.1	4.4	4.4
Control	1					5.6	4.8	5.2	4.3	4.3
	2					4.9	4.8	5.2	4.3	4.3
	3					4.8	4.5	4.4	4.3	4.3
The 2nd day after treatment	4	4.1	4.6	—	5.3	5.2	5.5	—	4.5	—
	5	4.2	4.1	—	5.3	4.8	5.5	—	5.2	—
Control	4	3.5	3.9	—	4.3	4.3	4.1	—	4.0	—
	5	3.5	3.7	—	7.4	5.3	5.1	—	4.6	—
The 3rd day after treatment	6	4.5	4.7	—	5.5	5.0	5.4	—	4.5	—
	7	4.8	4.3	4.7	5.5	5.2	5.5	—	5.5	—
Control	6	3.3	4.2	—	4.4	4.8	4.5	—	4.5	—
	7	4.1	3.7	3.9	5.1	5.1	5.5	—	4.6	—
The 4th day after treatment	8	3.9	4.2	—	5.3	5.1	5.5	—	5.1	—
	9	4.3	4.1	4.5	5.3	5.0	5.5	—	5.3	—
Control	8	4.0	4.2	—	5.6	5.3	4.7	—	4.8	—
	9	3.5	3.7	4.0	4.7	5.2	5.1	—	4.6	—

TABLE VI

Total acidity (expressed in c.c. N/10 NaOH) in the leaves of Bryophyllum calycinum after being kept in H_2-gas.

(July, 1928)

		8 a.m.	10 a.m.	12 a.m.	2 p.m.	4 p.m.	6 p.m.	8 p.m.	10 p.m.	12 p.m.
Immediatly after treatment	1	—	4.0	—	0.8	1.3	0.4	—	—	—
	2	—	3.1	—	1.5	2.0	0.5	—	—	—
	3	—	3.0	—	0.5	2.5	0.5	0.8	—	1.0
Control	1	—	5.8	—	2.5	1.0	1.3	—	—	—
	2	—	5.5	—	3.0	2.4	5.5	—	—	—
	3	—	6.0	—	1.5	1.0	1.0	2.0	—	5.5
The 2nd day after treatment	4	—	2.5	—	0.75	1.8	0.8	—	0.75	0.75
	5	—	2.0	—	0.75	1.5	0.5	—	—	—
	6	3.25	1.25	0.5	0.25	1.0	0.5	—	1.25	—
	7	3.5	1.5	0.5	1.5	1.0	0.5	—	1.5	—
Control	4	—	6.0	—	2.5	1.0	1.0	—	2.0	5.0
	5	—	10.50	—	5.0	3.2	4.1	—	—	—
	6	8.5	5.0	1.7	1.0	1.0	1.5	—	4.0	—
	7	10.50	3.0	1.5	1.0	1.5	—	—	5.5	—
The 3rd day after treatment	8	—	0.5	—	0.5	0.8	0.4	—	—	—
	9	3.3	0.8	—	0.5	1.0	0.5	—	1.25	—
	10	3.7	1.5	0.3	0.5	2.0	0.5	—	1.0	—
Control	8	—	4.0	—	2.0	1.3	1.5	—	—	—
	9	10.25	2.6	—	1.5	1.0	1.3	—	5.3	—
	10	19.9	11.5	8.0	6.5	8.5	10.5	—	14.5	—
The 4th day afrer treatment	11	3.7	1.8	—	0.5	1.0	0.4	—	1.0	—
	12	3.25	0.5	—	0.5	1.5	0.5	—	0.75	—
	13	2.0	3.3	0.5	1.5	0.5	0.7	—	1.8	—
Control	11	12.0	4.0	—	1.8	1.3	1.5	—	4.7	—
	12	6.8	1.6	—	1.0	1.7	2.5	—	5.5	—
	13	4.7	6.8	1.6	1.7	2.5	3.5	—	4.5	—

TABLE VII

pH-value in the leaf sap of Bryophyllum calycinum after being kept in H_2-gas.

(July, 1928)

		8 a.m.	10 a.m.	12 a.m.	2 p.m.	4 p.m.	6 p.m.	8 p.m.	10 p.m.	12 p.m.
Immediately after treatment	1	—	4.1	—	5.6	5.3	5.5	—	—	—
	2	—	3.9	—	4.0	3.8	4.0	—	—	—
	3	—	4.4	—	5.3	4.9	5.4	—	4.5	—
Control	1	—	4.1	—	4.5	5.4	5.2	—	—	—
	2	—	4.0	—	4.8	4.8	4.7	—	—	—
	3	4.1	5.2	5.4	5.6	5.5	5.3	—	4.3	—
The 2nd day after treatment	4	—	4.4	—	5.1	5.1	5.4	—	4.5	4.9
	5	—	4.5	—	5.4	4.8	5.3	—	—	—
	6	4.2	5.3	5.7	5.0	5.5	5.7	—	5.2	—
Control	4	—	4.1	—	4.8	5.6	5.7	—	4.7	4.45
	5	—	4.5	—	5.3	5.4	5.0	—	—	—
	6	4.1	5.2	5.4	5.6	5.5	5.3	—	4.3	—
The 3rd day after treatment	7	—	4.3	—	5.2	5.0	5.4	—	—	—
	8	4.2	5.0	—	5.7	5.6	5.7	—	5.1	—
	9	4.3	4.5	5.7	5.4	5.2	5.4	—	4.7	—
Control	7	—	4.1	—	4.8	5.7	5.1	—	—	—
	8	4.0	5.3	—	5.5	5.6	5.4	—	4.3	—
	9	3.5	3.9	4.1	4.3	4.3	4.1	—	4.0	—
The 4th day after treatment	10	4.0	4.8	—	5.3	5.2	5.4	—	4.7	—
	11	4.3	—	5.7	5.4	5.0	5.2	—	4.0	—
	12	4.3	4.5	5.4	5.1	5.4	5.0	—	4.5	—
Control	10	3.9	4.1	—	4.8	5.7	5.2	—	4.3	—
	11	3.5	—	3.9	4.3	4.3	4.1	—	4.0	—
	12	4.2	4.5	5.0	5.4	4.8	5.3	—	5.1	—

TABLE VIII

Acidities in the leaves of Bryophyllum crenatum during a day.

(August—September, 1930)

		8 a.m.	10 a.m.	12 a.m.	2 p.m.	4 p.m.	6 p.m.
Total acidity c.c. N/10 NaOH	1	8.5	3.5	1.8	0.7	1.5	2.0
	2	10.0	12.0	5.5	1.0	1.5	3.0
	3	1.3	2.7	1.0	0.75	1.0	2.5
pH	1	4.0	4.2	4.8	5.2	4.9	4.7
	2	3.8	3.7	3.9	4.9	4.8	4.5
	3	4.2	4.0	5.0	5.3	4.8	4.8

TABLE IX

Acidities in Bryophyllum crenatum after isolation.

(September, 1930)

		10 a.m.	12 a.m.	2 p.m.	4 p.m.	6 p.m.
Total acidity c.c. N/10 NaOH	The following day of isolation ...	14.5	10.0	5.0	13.5	3.5
	Control	12.0	5.5	1.0	1.5	3.0
	The 4th day after isolation.....	5.5	3.5	2.5	5.0	1.5
	Control	10.5	4.0	2.5	1.0	3.0
pH	The following day of isolation ...	3.5	3.9	4.1	3.9	4.3
	Control	3.7	3.9	4.9	4.8	4.5
	The 4th day after isolation.....	4.6	5.0	5.1	4.1	5.0
	Control	3.6	3.8	4.5	4.9	4.5

TABLE X

Acidities in the leaves of Bryophyllum crenatum after inducing anaerobic respiration in H_2-gas.

(September, 1930)

		8 a.m.	10 a.m.	12 a.m.	2 p.m.	4 p.m.	6 p.m.
The 1st day after treatment	Total acidity c.c. N/10 NaOH	5.5	7.0	2.0	1.5	3.5	1.0
	pH	4.0	3.9	4.6	5.6	5.2	5.5
Control	Total acidity c.c. N/10 NaOH	9.0	13.5	3.0	2.5	1.5	6.0
	pH	3.9	3.8	4.7	5.6	5.6	5.1

TABLE XI

The volume of CO_2 output from the leaf of Bryophyllum calycinum immediately after it was returned from H_2-gas to the air.

(March, 1929)

		CO_2 c.c. 8 a.m.—1 p.m.	CO_2 c.c. 1 p.m. —6 p.m.	CO_2 c.c. 6 p.m.—11 p.m.	Total CO_2 c.c.
Control	1	7.5	8.5	2.1	18.1
	2	5.0	6.0	1.1	12.1
Treated leaf	1	2.5	0.5	1.5	4.5
	2	3.5	1.5	2.0	7.0
The temperature in the greenhouse		23–25°C.	25–30–20°C.	20°C.	

TABLE XII

CO_2 production from the leaf of Bryophyllum calycinum after warm-bath treatment.

(March, 1929)

		CO_2 c.c. 10 a.m.—1 p.m.	CO_2 c.c. 1 p.m.—5 p.m.	CO_2 c.c. 5 p.m.—10 p.m.	Total CO_2 c.c.
Control	1	11.0	12.0	5.0	28.0
	2	10.0	11.5	4.0	22.5
Treated leaf	1	6.5	2.5	5.5	14.5
	2	2.6	1.5	2.0	6.1
Temperature in the greenhouse		25–30°C.	30–22°C.	22–20°C.	

TABLE XIII

RQ in the isolated leaf of Bryophyllum calycinum.

(January—February, 1930)

		O_2-decrease durning 24 hours c.c.	CO_2-increase durning 24 hours c.c.	RQ
Control	1	8.8	4.3	0.5
	2	6.8	3.7	0.5
	3	6.0	3.0	0.5
Isolated leaves on the 3rd day after isolation	1	3.0	1.0	0.3
	2	3.0	0.9	0.3
	3	2.2	0.6	0.3

TABLE XIV

Acetaldehyde and alcohol in the leaves of Bryophyllum calycinum.

(May, 1929)

		Acetaldehyde mg.	Alcohol %
Very young leaves at top	1	11.20	0.112
	2	0.50	0.11
	3	9.54	0.33
Adult leaves	1	0.85	0.038
	2	1.70	0.04
	3	1.11	0.045

TABLE XV

Acetaldehyde and alcohol in the isolated leaves of Bryophyllum calycinum.

(May—July, 1929)

		Control	After 24 hours	After 2 days	After 3 days	After 4 days
Acetaldhyde mg.	1	2.0	4.0	3.0	3.2	2.2
	2	1.0	5.2	4.0	4.3	3.8
	3	1.5	3.2	—	—	—
	4	1.5	3.2	—	—	—
	5	0.7	5.2	—	—	—
Alcohol %	1	0.056	0.10	0.065	0.077	0.08
	2	0.04	0.085	0.07	0.065	0.065
	3	0.04	0.09	0.08	0.07	0.077
	4	0.035	0.07	—	—	—
	5	0.04	0.095	—	—	—

TABLE XVI

Acetaldehyde and alcohol in Bryophyllum calycinum after being placed in H_2-gas.

(June, 1929)

		Immediately before treatment	Immediately after treatment	2 days after the end of treatment	4 days after the end of treatment
Acetaldehyde mg.	1	0.44	5.42	2.2	1.7
	2	0.82	9.5	2.5	2.3
Alcohol %	1	0.02	0.081	0.06	0.01
	2	0.025	0.080	0.06	0.02

TABLE XVII

Acetaldehyde after being placed in N_2-gas.

(February, 1932)

		Immediately after treatment in N_2-gas	Control
Acetaldehyde mg.	1	2.64	0
	2	3.84	0
Alcohol %	1	0.01	0
	2	0	0

TABLE XVIII

Acetaldephyde in Bryophyllum calycinum during warm-bath treatment.

(Nov.—Dec., 1931)

		Immediately before treatment	During warm-bath		
			After 2 hours from the beginning of treatment	After 3 hours	After 5 hours
Acetaldehyde mg.	1	0.4	3.43	3.08	0.88
	2	0.88	4.90	2.40	1.32
	3	0.88	3.65	1.32	0.88
	4	0.44	4.64	2.64	0.46
	5	0.50	3.70	2.84	0.88

TABLE XIX

Acetaldehyde after warm-bath treatment (for 5 hours).

(March, 1932)

			After 24 hours	After 48 hours
Acetaldehyde mg.	Treated	1	1.75	0.88
		2	2.32	1.76
	Control	1	0	0
		2	0	0

TABLE XX

Acetaldehyde after warm-bath treatment (for 8 hours).

(May, 1929)

		Control	After 24 hours	After 4 hours
Acetaldehyde mg.	1	0.8	3.1	3.08
	2	1.7	5.0	3.2
	3	1.5	4.5	2.2
Alcohol %	1	0.04	0.08	0.02
	2	0.04	0.14	0.02
	3	0.03	0.09	0.05

TABLE XXI

Acetaldehyde and alcohol in the leaves of Bryophyllum crenatum.

(June, 1930)

	Control	After 24 hours	After 2 days	After 3 days	After 4 days
Acetaldehyde mg.	0.5	3.70	3.08	2.50	1.00
Alcohol %	0	0.04	0.035	0.02	0.01

TABLE XXII

Stem cuttings of Populus nigra.

(Nov.—Dec., 1930)

	Warm-bath treatment		Control	
	Regenerated shoots g.	Regenerated roots g.	Regenerated shoots g.	Regenerated roots g.
	0.834	0.013	0.070	0
	0.428	0.005	0	0
	0.336	0.011	0	0
	0.496	0	0	0.03
	0.250	0.010	0.187	0
	0.175	0	0	0
	0.261	0.030	0	0
	0.385	0.027	0.037	0
	0.692	0	0	0
	0.185	0	0	0
Total	4.042	0.096	0.294	0.03
Mean	0.404	0.010	0.029	0.003

TABLE XXIII

Stem cuttings of Populus nigra.

(October, 1931)

	Warm-bath treatment		Control	
	shoots g.	roots g.	shoots g.	roots g.
	0.152	0.065	0.007	0
	0.074	0	0	0
	0.006	0.003	0.004	0
	0.044	0.011	0	0
	0.179	0.006	0.011	0
	0.125	0.103	0.06	0
	0.028	0.057	0	0
	0.006	0	0	0
	0.445	0.015	0	0
	0.009	0	0	0
	0.205	0.002	0	0
	0.073	0	0	0.07
	0.015	0.004	0.05	0
	0.43	0	0	0
	0.117	0.081	0	0
	0.038	0	0	0
Total	1.572	3.47	0.33	0.07
Mean	0.093	0.204	0.019	0.004

TABLE XXIV

Stem cuttings of Syringa vulgaris.

(Sep.—Oct., 1929)

	Warm-bath treatment	Control
	Shoots g.	Shoots g.
	0.112	0.010
	0.126	0.014
	0.040	0.155
	0.139	0.011
	0.088	0.028
	0.163	0.123
	0.018	0
	0.103	0
	0.115	0
	0.077	0
Total	0.981	0.341
Mean	0.098	0.034

TABLE XXV

Stem cuttings of Salix sp.

(Sep.—Oct., 1931)

	Warm-bath treatment	Control
	Shoots g.	Shoots g.
	0.210	0.035
	0.165	0
	0.181	0.078
	0.298	0
	0.360	0.011
	0.050	0.038
	0.062	0.020
	0.030	0
	0.021	0.008
	0 011	0
Total	1.388	0.190
Mean	0.139	0.019

TABLE XXVI

Stem cuttings of Pelargonium zonale.

(Sep.—Oct., 1929)

	Warm-bath treatment	Control
	Roots g.	Roots. g.
	0	0
	0	0
	0.664	0.050
	0.132	0
	0	0
Total	0.796	0.050
Mean	0.080	0.005

TABLE XXVII

Stem cuttings of Populus nigra.

(April—May, 1932)

	Anaerobic respiration		Control	
	Shoots g.	Roots g.	Shoots g.	Roots g.
	0.018	0.007	0.01	0.002
	0.107	0.004	0.0565	0
	0.075	0	0.036	0
	0	0.013	0	0
	0.070	0	0	0
	0.062	0	0	0
	0	0.015	0	0.007
	0.1065	0	0.087	0
	0	0.0065	0.038	0
	0.060	0	0.029	0.001
Total	0.5885	0.0455	0.2475	0.01
Mean	0.0589	0.0046	0.0248	0.001

TABLE XXVIII

Stem cuttings of Populus nigra.

(August, 1929)

	Anaerobic respiration		Control	
	Shoots & roots g.	Callus formation	Shoots & roots g.	Collus formulation
	0.150	+++	0	—
	0.113	++	0.045	—
	0.141	++	0	—
	0.220	+	0	+
	0.015	++++	0	+
	0.100	++	0	—
	0.357	++++	0	++
	0.219	++++	0.015	+
	0.142	++	0	+
Total	1.587		0.060	
Mean	0.159		0.006	

\+ The smallest callus formation.

++ Callus formation occurs on one half of the cut surface of the basal end.

+++ Caullus formation occurs on the whole of the cut surface of the basal end.

++++ The strongest callus formation.

TABLE XXIX

Stem cuttings of Tropaelum majus.

(July, 1929)

	Anaerobic respiration		Control	
	Roots g.	Callus formation	Roots g.	Callus formation
	0.037	+	0	—
	0.010	+	0	—
	0	+	0	—
	0.004	+	0	—
	0.006	+	0.003	—
	0	+	0	—
	0.058	+	0.002	—
Total	0.115		0.005	
Mean	0.016		0.001	

TABLE XXX

Stem cuttings of Solanum Lycopersicum (Tomato).

(August, 1929)

	Anaerobic respiration		Control	
	Roots g.	Callus formation	Roots g.	Callus formation
	0.012	—	0	—
	0	+	0	+
	0.245	+	0.015	+
	0.052	++	0.007	—
	0.050	+	0.053	—
	0.047	+	0.009	—
	0.064	+	0.031	—
Total	0.470		0.115	
Mean	0.067		0 016	

TABLE XXXI

Stem cuttings of Rosa microphylla.

(August, 1929)

	Anaerobic respiration		Control	
	Shoots g.	Callus formation	Shoots g.	Callus formation
	0.021	+	0	—
	0.008	+	?	?
	0.007	+	0	—
	0.062	+	0	—
	0.054	+	?	?
Total	0.152			

The materials, indicated by the "?" were dried off during the experiment.

TABLE XXXII

Stem cuttings of Syringa vulgaris.

(August, 1929)

	Anaerobic respiration	Control
	Shoots g.	Shoots g.
	0.043	0
	0	0
	0.005	0
	0.142	0.007
	0.038	0
	0.027	0.005
Total	0.255	0.012
Mean	0.038	0.002

TABLE XXXIII

Stem cuttings of Hydrangea opuloides var. japonica.

(August, 1929)

	Anaerobic respiration	Control
	Shoots g.	Shoots g.
	0.027	0
	0.009	0
	0	0
	0.013	0
	0.008	0
Total	0.057	0
Mean	0.011	0

TABLE XXXIV

Stem cuttings of Populus nigra.

(Oct.—Nov., 1931)

	Vacuum		Control	
	Shoots g.	Roots g.	Shoots g.	Roots g.
	0.093	0	0	0
	0.070	0.007	0.0310	0
	0.0275	0	0.0200	0
	0.0350	0.017	0.0004	0
	0.008	0.0005	0.0010	0
	0.0380	0.0080	0	0
	0.0180	0	0	0
	0.0680	0.0180	0	0
	0.0085	0	0	0
	0.0050	0	0	0
	0.0035	0	0	0
	0.0327	0	0	0
	0	0.017	0	0
	0.0100	0	0	0
	0.0900	0	0	0
Total	0.5072	0.0675	0.0524	0
Mean	0.0338	0.0045	0.0035	0

TABLE XXXV

Stem cuttings of Populus nigra.

(Oct.—Nov., 1931)

	Vacuum		Control	
	Shoots g.	Roots g.	Shoots g.	Roots g.
	0.110	0.0012	0	0
	0.1392	0.0070	0	0
	0.2493	0.0195	0.050	0
	0.1040	0	0	0
	0.1820	0.0110	0	0
	0.0600	0	0	0
	0.0490	0.0070	0	0
	0.0540	0.0140	0	0
	2.2010	0.0045	0	0
	0.1350	0.0150	0	0
	0.0970	0	0	0
	0.1570	0	0.010	0
	0.0500	0.0070	0.0070	0
	0.0310	0.0065	0.0062	0
	0.0490	0.0190	0	0
	0.0280	0	0	0
	0.0200	0.0110	0	0.0030
	0.0180	0	0	0
Total	1.7335	0.1227	0.0732	0.0030
Mean	0.0963	0.0068	0.0041	0.0002

TABLE XXXVI

Cuttings of Coleus Blumei.

(Nov.—Dec., 1931)

	Vacuum	Control
	Roots mg.	Roots mg.
	1.9	0.6
	2.0	0.8
	2.6	0.75
	1.59	0.25
	1.3	0.1
	1.8	0.8
	1.5	0.2
	1.1	0.05
	2.2	0.11
	1.25	1.2
	1.8	0.03
Total	19.04	4.89
Mean	1.73	0.43

a)

TABLE XXXVII

Salix sp.

(September, 1930)

		Control twigs	Stem cuttings	
			3rd day after isolation	4th day after isolation
CO_2c.c.	1	4.5	3.0	19.0
	2	11.0	9.5	4.5
O_2c.c.	1	4.5	2.0	14.0
	2	11.0	8.2	4.0
RQ	1	1.0	1.5	1.4
	2	1.0	1.2	1.1

TABLE XXXVIII

Syringa japonica.

(August, 1930)

		Control twigs	Stem cuttings		
			2nd day after isolation	3rd day after isolation	5th day after isolation
CO_2c.c.	1	12.0	10.0	4.0	6.0
	2	4.0	4.0	4.5	16.5
O_2c.c.	1	11.0	9.0	2.0	0
	2	4.0	2.0	2.0	2.0
RQ	1	1.1	1.1	2.0	
	2	1.0	2.0	2.3	8.3

TABLE XXXIX

Populus nigra

(July, 1930)

	Control twig	Stem cutting	
		1st day after isolation	3rd day after isolation
CO_2c.c.	2.0	10.0	0 3
O_2c.c.	2.0	3.0	9.0
RQ	1.0	3.3	0.03

TABLE XL

Pelargonium zonale

(July, 1930)

	Control twig	Stem cutting 1st day after isolation
CO_2c.c.	4.0	10.5
O_2c.c.	3.5	5.0
RQ	1.1	2.1

TABLE XLI

Solanum Lycopersicum.

(September, 1930)

	Control twig	Stem cutting	
		1st day after isolation	2nd day after isolation
CO_2 c.c.	7.0	8.5	—
O_2 c.c.	5.0	5.0	10.0
RQ	1.4	1.7	—

TABLE XLII

Morus bomcina.

(July, 1930)

	Control twig	Stem cutting 3rd day after isolation
CO_2 c.c.	10.2	11.3
O_2 c.c.	9.5	8.5
RQ	1.1	1.3

TABLE XLIII

Pirus malus.

(July, 1930)

	Control twig	Stem cutting 5th day after isolation
CO_2 c.c.	14.4	13.0
O_2 c.c.	14.6	1.0
RQ	1.0	13.0

b) TABLE XLIV

Syringa japonica.

(November, 1931)

		2nd day	3rd day
Control twigs	CO_2 c.c.	5.0	9.5
	O_2 c.c.	5.0	7.0
	RQ	1.0	1.4
Stem cuttings	CO_2 c.c.	16.4	4.0
	O_2 c.c.	2.0	2.0
	RQ	8.2	2.0

c) TABLE XLV

Syringa japonica.

(December, 1931)

		Control cuttings	Treated cuttings
Warm-bath	CO_2 c.c.	5.0	11.0
	O_2 c.c.	5.0	1.0
	RQ	1.0	11.0
Vacuum	CO_2 c.c.	2.5	12.1
	O_2 c.c.	2.5	2.0
	RQ	1.0	6.1

TABLE XLVI

Salix sp.

(December, 1931)

		Control cuttings	Treated cuttings
Warm-bath	CO_2 c.c.	4.5	7.40
	O_2 c.c.	4.5	4.0
	RQ	1.0	1.6
Vacuum	CO_2 c.c.	4.5	12.5
	O_2 c.c.	4.0	6.0
	RQ	1.1	2.0

TABLE XLVII

Cuttings of Populus nigra.

(March, 1930)

	Stem cuttings		Control twigs(1)	
	Aldehyde mg.	Alcohol %	Aldehyde mg.	Alcohol %
3 days after cutting	2.20	0.01	0.35	0.0002
6 days after cutting	0.70	0.15		

(1) Average of 4 twigs.

TABLE XLVIII

Cuttings of Populus nigra.

(October, 1931)

	Control twigs(1)		Stem cuttings		Treated stem cuttings			
					Warm-bath		Vacuum	
	Aldehyde mg.	Alcohol %	Aldehyde	Alcohol	Aldehyde	Alcohol	Aldehyde	Alcohol
24 hours after treatment	—	—	0.70	0	5.28	0	2.78	0
	—	—	0.80	0	4.41	0	1.95	0
	—	—	0.21	0	2.88	0	5.14	0
	0.32	0.0004	0.45	0	0.9	0	2.57	0
	—	—	0.73	0	0.14	0	9.25	0
	—	—	0.72	0.01	2.00	0.10	7.20	0.15

(1) Average of 10 twigs.

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