Studies on the Leaf Movement of *Aldrovanda vesiculosa* L.

II. Effects of mechanical, electrical, thermal, osmotic and chemical influences

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With 17 Text-figures

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Contents

Introduction

Although some effects of various agents upon the movements of *Aldrovanda* leaves have been introduced already in Part I of this study, the object of study in previous paper was to analyze the leaf movement itself and to demonstrate its mechanism. Additional facts remain to be described and discussed. They will be treated in this paper under six different categories :

1. Stimulating effects of deformation of various cells tested by means of the micro-manipulator.

2. The stimulative efficiency of an electric current with respect to its direction of flow (relative to the leaf), and the sensitivity of various parts of the leaf to electrical stimuli.

3. The stimulative effect of sudden changes of temperature. (This is especially interesting as the *Aldrovanda* leaf is particularly suitable material for the investigation.)

4. The time necessary for osmotically active solutions of diverse concentration to make leaves immovable, and the stimulative effects of a rapid increase or decrease of osmotic value of the environmental medium.

5. The stimulative and the immobilizing[1] effects of salt solutions, mixture of acetic acid and sucrose, and organic fluids ; and also the influence of external osmotic pressure on the permeability of cells.

6. Change, due to aging, of characters related to the leaf movement.

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    1) By " immobilization " is meant, in the present work, the act or process of destroying the power of movement, distinguished from anæsthetization.

Brief Notes on the Previous Paper, Part I.

In order to save readers the inconvenience of referring to the first part of this work, the writer's theory on mechanism of the leaf movement and some terms used there will be restated here briefly.

In the responsive movement of *Aldrovanda* (and *Dionaea*) leaves the writer discriminates two phases, the "shutting" which is passed over very quickly, and the "narrowing" which follows the former and proceeds very slowly. Both phases of the movement are caused by increase of curvature of the "motile zone"[1] of each lobe. (Hence in the discussion related to the motor mechanism, expressions such as "the inner, or the outer, epidermal cells" will mean those cells which belong to the motile zone).

When cells of the outer epidermis and the middle layer have turgor, they exert a force to increase their curvature, owing to a proper arrangement of cell walls of different extensibility. On the contrary, the inner epidermal cells are stiff in their own curvature when they are turgid, because their outer walls have as little extensibility as their inner ones. A leaf is in the "open stage" when all its cells are turgid, the inner epidermis supporting the outer two layers so as not to let them bend freely. And the outer two layers are permitted to bend, when the force of the inner epidermal cells (to keep themselves stiff transverse to the midrib) is lost, either from loss of turgor or contraction of protoplast. Thus the lobes are bent inwards until their margins touch against each other, the leaf coming into the "shut stage." In the case of strong excitation in the shut stage, the suction pressure in the outer epidermal cells increases gradually thereafter, making the lobes bend farther, and causing one of them evert. This is the narrowing movement which finally brings the leaf into the "narrowed stage."

Leaf whorls of every individual are numbered O, I,[2] II, III . . . . , from the one which has just grown up to be responsive basipetally towards the decaying end; hence the "whorl No." of a leaf is a measure of its age. The distance between the margins of two lobes at the middle, the "margin-distance," is maximum when the leaf is open, and becomes nearly nil when the leaf is shut. The "boundary-distance" is a similar measure, but it differs in that this is the distance from an "enclosure-boundary," the border of the three-layered and the one-layered regions (cf. Fig. 3, A), to that of the opposite lobe. The boundary-distance is maximum when the leaf is open, becomes smaller as it shuts and narrows, and attains minimum when it is quite narrowed.

Mechanical Stimulation

a. Sensitive Hair

It can be determined, by touching various parts of the leaf with a fine glass rod attached to a micro-manipulator, that no other parts and trichomes of the leaf than the long fine hairs, "the

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1) In Fig. 3, the shaded (in C, A′, B′, C′) or the darkened (in A, B) parts in the leaf represents the "motile zone."

2) The whorl No. I is the youngest, the one that stands apparently off from the apical bud, while the leaves of No. O are yet standing close to the bud.

sensitive hairs," on the inside are sensible to touch. Thus it is certain that small animals can stimulate the leaf in no other way than by striking against the sensitive hairs ; in spite of that, the leaf can be stimulated in various ways as will be stated in this paper. When the free end of a sensitive hair is pushed slightly, the hair curves along its whole length, making, however, a specially pronounced bend at the thin-walled joint, as was shown by FENNER ('04, Fig. 31a, Pl. XIII). The mechanical influence is certainly concentrated to this small part of the hair as HABERLANDT ('06, p. 133) states. The hair unbends elastically when released.

Leaves of Nos. II and III, which are usually the most irritable, commonly react when a hair is bent twice, or when a second hair is bent after another hair has been bent and released. However a few leaves are so sensitive as to react when a hair is bent only once, as in the case of *Dionaea* (cf. BROWN, '16; MACFARLANE, '19).

Though the results of the experiment are greatly diverse, perhaps due to large variation in the sensitivity of hairs, yet a tendency is apparently perceived, for the number of stimuli necessary to cause the shutting reaction to increase as the leaf grows older. Among the leaves older than No. V, there are found frequently those that do not shut even after being stimulated (bending and unbending, together) ten times or more.[1] With the increasing age of the leaf, the sensitive hairs themselves seem to become less sensitive, and in those hairs in which the joint cells look morphologically unsound also increase in number. This may be one reason why the number of stimuli necessary to cause the reaction increases with the increase of age, though a decrease in the activity in the latter parts of the reaction chain must also contribute to this cause.

Besides bending, excitation is also caused in the sensitive hair when its joint cells are deformed by pinching with fine glass rods. Sensitive hairs can be stimulated, not only by rigid agents, but also by an air bubble or a stream of water.

Repeated stimulation by a captured animal touching many sensitive hairs makes a shut leaf proceed into the narrowed stage (Part I, p. 230). If the tip of a pincette is thrust into the leaf

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1) In the experiments, however, the time interval between the two successive bendings was 5-7 seconds. Hence even the leaves that did not react after ten stimuli would have reacted if many hairs had been stimulated within a very short time, as in the case of stimulating with the tip of a needle.

cavity so as to stimulate continuously the sensitive hairs for one minute or so, the leaf enters into the narrowing phase after the pincette is withdrawn. But most leaves do not reach the ultimate narrowed stage due perhaps to a fading away of the excitation in the course of time and of lack of renewed stimulation, such as would be given incessantly if an animal were captured in the cavity. Repetition of electrical stimulation, however, can bring the leaf into the narrowed stage (I: p. 231).

b. Direct Perception by Motor Cells

The shutting reaction is called forth when cells in the three-layered region are deformed or hurt. Neither deformation nor a prick nor a cut of the cells in the one-layered region causes any sign of excitation, as in the case of the marginal glandless portion of a *Dionaea* leaf.

The following two observations were made to demonstrate that cells of the three-layered region are sensitive to deformation :

a) A leaf was laid on the bottom of a Petri-dish containing water, and a part of the three-layered region that was just in contact with the dish was pressed from above (from the concave side of the leaf) with the rounded tip of a glass rod, care being taken not to let the glass rod touch any one of the sensitive hairs during the procedure. Deformation of a few cells due to such pressing caused the leaf to shut. BROWN and SHARP have also observed that the *Dionaea* leaf is sensitive to mechanical pressure.

b) The two lobes of an open leaf were pressed from the out-side to bring them nearer together ; then the leaf suddenly shut by its own mechanism when the lobes approached to within a certain distance of each other.[1] In order to determine the threshold value of such deformation of lobes, the leaf was put between two fine glass rods in such a way that the tip of each rod touched the enclosure-boundary of each of the lobes. Then the rods were brought gradually nearer to each other pressing the lobes, and the distance between the rod-tips at the instant of reaction of the leaf was measured. This distance expressed in percentage of the boundary-distance in the open stage was 89.2% in the mean (extreme

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1) If a leaf is picked up slowly from the water directing its opening downwards, it shuts suddenly when some portion of it comes above the water surface. This is due to the same mode of stimulation, possibly accompanied by the excitation of sensitive hairs in some cases.

values, 79–94%) with leaves of Nos. II–V, at 20–25°, and 95.2%
(94–100%[1]) at 5–15°. The threshold was so low at the low tem-
perature that a very slight pushing together of lobes gave rise to
the reaction. This is in accordance with the LEPESCHKIN's ('27)
determination that the protoplasm of *Spirogyra* cells is very sensi-
tive to mechanical influences at low temperatures, and also with
HILL's ('35) observation that a chilled spot is much more sensitive
to mechanical stimulation than is a spot at room temperature.

Pushing outwards the two lobes from the inside so as to make
the leaf open wider than ever also forms a stimulus. But in this
case the deformation has to be so pronounced that the margin-
distance must be made twice as large as that in the open stage,
or even wider, viz. that the leaf is quite deformed, the midrib being
recurved. According to BÜNNING ('30), *Sparmannia* filaments the
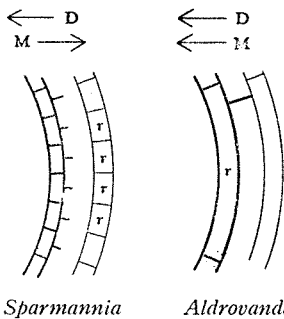responsive movement of which is directed outwards, are stimulated

more effectively when they are bent in-
wards, than outwards. This is contrary
to the case of *Aldrovanda* leaves, as
shown in Fig. 1, with respect to the di-
rection of effective deformation (D) in
relation to that of the reaction move-
ment (M) and to the site of reactive cells
(*r*). But both the cases are alike so far
as that an organ is stimulated more ef-
fectively when its inherent curvature is
made stronger, than when the curva-
ture is weakened.

If the two lobes of a leaf are pressed
so strongly together that the leaf shows
the narrowed stage, it remains in this
stage even after the pressure is with-
drawn, although with a little elastic re-
version at the instant of withdrawal of
the pressure. In this case, the leaf seems
to be brought into the half narrowed
stage without positive action of the mecha-
nism.

Fig. 1. Diagrammatic re-
presentation of the motile parts
of the *Sparmannia* filament and
the *Aldrovanda* leaf. The thick-
lined cell walls are less extensi-
ble than the thin-lined walls.
r : cells the loss of turgidity
of which causes the reaction
movement of the filament or
the leaf. M : direction of the
reaction movement. D : direc-
tion from which a slighter push
can cause irritation, than when
it comes from the reversed
direction.

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1) No measurable difference was observed in the distance between the rod-tips
before and after the stimulation.

c. Hydrostatic Pressure

When a hydrostatic pressure of about 2.5 atms. is applied to a leaf which is floating in a water-filled injection tube, it sinks slowly downwards ; after removal of the pressure, it begins to float upwards. But in both cases, no reaction is observed.

Similarly, when the pressure is lowered nearly to the vapour pressure of the water by a vacuum pump,[1] and also when the pressure is suddenly returned to normal, the leaf shows no action.

Electrical Stimulation

Electrical stimulation has offered a convenient means for registering or observing the leaf movements. But for the present it appears that no interesting studies can be made with *Aldrovanda* concerning the electrical effect itself, except as to the difference in stimulating effect regarding the direction of current flow through the leaf. This subject will be dealt with in this chapter, for it gives a clue to the site of perception of electrical stimuli.

a. Method

i. Electrode for stimulation

CZAJA ('24) used cut ends of paraffined wire as electrodes of very small surfaces, and assumed that the electric current flowed through the plant body between the points of contact of the wire ends. But according to the author's observation, it does not matter whether the electrodes are in contact with the plant body or not; a leaf can be stimulated electrically even if the straight line connecting the two electrodes may pass through no part of the leaf. When a potential difference is applied between such small electrodes, the strength of the electrical field set up there varies considerably, even in a small space. So if a leaf is put between or in the neighbourhood of the electrodes, the electric current passing through it may differ largely from part to part. Hence it is more convenient to use large electrodes and put the leaf in as uniform a field as possible, though the field may be somewhat distorted by the dielectric character of the leaf itself.

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1) If a trace of air remains dissolved in water, it comes out as if bursting when the pressure is lowered, the leaf being thus stimulated severely. Hence the air contained in intercellular spaces of the leaf as well as that dissolved in the medium water should be freed before the reduction of pressure. The air in the intercellular spaces can be dissolved out if the leaf is left in air-free (boiled) water, even if it is not left in the darkness (cf. SEN and BLACKMAN, '33). After the injection of water in the intercellular spaces, the leaf becomes somewhat transparent and almost loses its bouyancy. It will not float up even when the pressure is reduced.

Thus, aluminium plates[1], 2×2 sq. cms., were used as the electrodes. They were put in a water-containing Petri-dish (ca. 9 cms. in diameter and 1.7 cms. deep), perpendicular to the bottom and parallel to each other. The submerged portion was 1.5 cm. each, so the available area of each electrode was 2×1.5 sq. cms. The leaf to be stimulated was held by ball-and-socket joints roughly at the middle between the electrodes. The supporters of the leaf and of the electrodes were insulated from one another and from the ground. The distance between the electrodes was 8 cms. in most experiments. But they were put close to each other when very strong stimuli were wanted.

ii. Method of stimulation

Either constant or induction current can be used for stimulation. But as only a relative strength of the electrical influence was to be registered, an induction coil of DU BOIS-REYMOND's type was preferred for convenience' sake, instead of condensers and batteries. In the experiments, only break shocks were used, and the distance between the primary and the secondary coils ("coil dist."), or number of shocks successively given with a constant position of coils, were registered as the measure of stimulation. In the latter case, the interval between two successive shocks was 3 seconds, as the latent period of the reaction was generally shorter than 3 seconds.[2]

A device for attaining single induction shocks.—The author has contrived, and used in the experiments, a key which can afford a make or a break induction shock by a simple procedure (cf. Fig. 2). The four rails, A, A', B and B', are made of metal, while the other four, X, X', Y and Y', are of bakelite, all being fixed on an ebonite plate. To each arm of a bakelite lever, which can be turned around the axis O, is fixed a metal piece (M, M') in such a way that when the lever is put in a position as in L_1L_1', M connects B and B', and M' connects A and A' electrically.

Now let A and A' lie in the battery-primary-coil circuit (I), and B and B' in the circuit (II) of the secondary coil and the electrodes for stimulation. If the lever is turned clockwise starting from the position L_0L_0' where both of the circuits are open, the circuit I (AA') is closed at first, and then the circuit II (BB'), and after I is open, then also II. Thus one break-shock is afforded in the circuit II by turn-

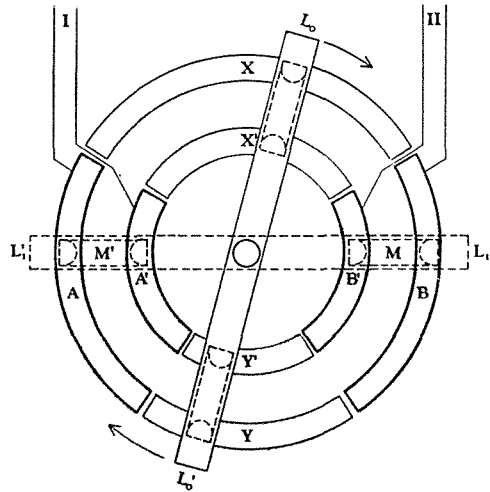Fig. 2. A key which produces single make or break induction shock. For explanation, see text.

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1) As these were coated by oxide film, liberation of metal ions seemed to be less than with silver plates.

2) The reaction time amounts sometimes even to 4 seconds when the leaf is very old or the temperature is low, but it seldom exceeds 1 second if the leaf is young and the temperature is optimal or high.

ing the lever clockwise 180°. On the other hand, single make-shocks are also given, if the lever is turned counter-clockwise, or by turning it clockwise if AA′ are laid in the secondary, and BB′ in the primary circuit.

Velocity of the turning of the lever was made roughly constant by practice, but it would be more convenient if it were made to turn by a spring. The metal rails (A, A′ B and B′) may be replaced by mercury pools, if the metal pieces (M, M′) be improved properly.

iii. Direction of the leaf to the electric current

Out of innumerable possible directions of a leaf relative to that of the electric current, three characteristic positions are preferred, *lateral* (Fig. 3, A), *dorsal* (B) and *longitudinal* (C). The relation of the leaf to the electric current is not changed even if the former be rotated with an axis parallel to the current flow, hence those three positions can be represented also by A′, B′ and C′.

b. Result

Leaves are less effected by electrical stimulation in the longitudinal position than in the other two, as seen in the left half of Table 1. On the other hand, difference between the lateral and the dorsal positions appear in the degree as noted in the right half of the table, only when the intensity of the stimulus is
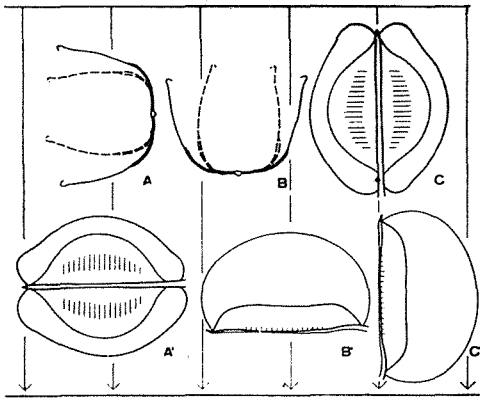
Fig. 3. The characteristic positions of the leaf, lateral (A, A′), dorsal (B, B′), and longitudinal (C, C′), the direction of the electric current being shown by the arrows. A and B represent cross-sections of widely open (unbroken lines) and half-open (broken lines) leaves, while C and A′ are back views, and B′ and C′ are lateral views of widely open leaves. The motile zones are darkened or shaded.

Table 1

Number of electrical shocks needed to stimulate the leaf, intensity of the shocks being constant.

Leaf position	Whorl No.			
Lateral (A)	1, 1	1, 1	1, 1, 2, 3	1, 1, 1
Dorsal (B)	1, 1	1, 1	1, 2, 3, 4	1, 2, 3, 3
Longitudinal (C)	3, >10	>10, >10		

quite suitable[1]. A less effect in the dorsal position however can oft-
en be known from the length
of the latent period[2], even in
cases when the numbers of
stimulating shocks are the
same for both of the positions.
The most striking example of
the difference was as shown
in Table 2.

Table 2

Leaf position	No. of shocks
Lateral (A)	1, 2, 2
Dorsal (B)	36, 37, 46
Longitudinal (C)	>120, >120

So far with fully-open leaves. With half-open leaves, however,
those in the dorsal position can be effected as easily as those in
the lateral position. Hence a summary of the order of effectiveness
of electrical shocks may be given as follows :

lateral (fully-open) > lateral (half-open), dorsal (half-open) ≧ dorsal
(fully-open) > longitudinal (fully- and half-open).

c. Discussion

As living cells have, perhaps, lower electrical conductivity than
the surrounding culture water[3], more electricity may pass sensitive
hairs when the leaf is in the longitudinal position than when it is
in the other two, for only in that position do the hairs stand directly
in the path of electric current and are not sheltered by the lobes.
That leaves are least effected in the longitudinal position, may lead,
therefore, to a conclusion that the hairs are not the most sensitive
receiver of electrical stimuli.

In order to find the part of leaf most sensitive to electrical
shocks, the following observation was made : One of the electrodes

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1) The minimal stimulus for the leaf movement varies largely from individual
to individual even if the whorl number be the same. Hence comparisons were made
only among the leaves from a single whorl. It was inconvenient that an intensity,
which had revealed the difference between the lateral and the dorsal position clearly
in one whorl, was weaker than the threshold of any leaf in another whorl, or was
so strong for still other whorls that all the leaves reacted to a single shock.

2) Steinhausen ('21) determined that the weaker the stimulating current, the
longer the latent period of sartorius.

3) The largest electrical resistivity that Fleischer ('25) measured with natural
water was 15,900 ohms (spring water). On the other hand, according to Blinks ('30),
the resistances of living *Nitella* cells are usually between 1,000,000 and 2,000,000 ohms,
with 1 cm. contacts of tap water and 1 cm. between contacts. As the resistance of
culture water must be lower than pure spring water, the assumption under discussion
is more probable.

was replaced by a platinum plate, 1 cm. square, and the other by a capillary electrode filled with the culture water. The leaf to be tested was fixed close to the plate electrode, directing its concave side towards this (as B in Fig. 3 inverted upside down), and the capillary electrode was put close to the midrib, or to the motile zone, on the convex side of the leaf. From number of shocks needed before the leaf shut, it was decided that the motile zone is more sensitive than the midrib (and very narrow zones on both sides of it).[1)] Putting the plate electrode on the convex side of the one-layered region, and the capillary electrode on the concave side, strong shocks was applied across this region, but only incomparably stronger shocks could stimulate the leaf. CZAJA made leaves shut by applying very strong shocks, with his wire electrodes, across the one-layered region, across a petiole, between petioles of neigh-bouring leaves, or across or along the stem. And he thought that the reaction of the leaf, or leaves, was caused by conduction of excitation from the part through which electricity flowed. But according to author's own observation, using electrodes similar to CZAJA's, the intensity of shocks necessary to cause the reaction depended not upon the part of the plant to which electrodes were applied, but upon the distance of the motile zone from the short-est line of current flow. That is the nearer the motile zone lay between the electrodes, the weaker were the shocks sufficient to stimulate the leaf. It is very probable that the above-mentioned parts, other than the motile zone, have no notable irritability.

Thus, the idea is shown to be most probable that *the motile zone is more sensitive to electrical shocks than any other part of the whole plant, even than the sensitive hairs.*

Let us now examine the efficiency of electrical shocks with respect to the leaf positions in detail. In the longitudinal position the electric current flows parallel to the leaf axis, that is through a large number of cells which have a higher resistance than the surrounding water, so that the current passing through the leaf

1)

Point of contact of capillary electrode	Number of shocks necessary to cause reaction							
Midrib	1	4, 5, 7	2	1	>40	2	2	1
Motile zone	1	1, 1	2	1	5	1, 1	2	5

tissue may be relatively small. While in the dorsal and the lateral positions, stronger current may flow through the tissue, as the number of cells to be passed through is smaller in these cases. This may be a possible reason why electrical shocks are least effective in the longitudinal position.[1]

If, then, any possible difference of the lateral and the dorsal position of fully- and half-open leaves be looked for, the inclination of the motile cells to the electric current may be taken into account. The longitudinal axes of the motile cells of fully-open leaves in the lateral position are nearly parallel with the current, while those of half-open leaves in the lateral and the dorsal positions are inclined to it, and those of fully-open ones in the dorsal position are nearly perpendicular to it. Hence it is conceivable, that *an electric current affects the cells of the motile zone more efficiently when it flows lengthwise than crosswise to them.*

Difference in the efficiency of electrical stimulation with respect to the leaf position, therefore, may be ascribed to two causes : 1) difference in electrical resistance of the leaf tissue as a whole, and 2) difference in the direction of current flow through every motile cell.

Thermal Stimulation

Temperature may influence " irritable " plants in the following three ways :

1) Extreme temperatures give injurious or paralysing effect ;
2) A sudden change of temperature has a stimulative effect ;
3) Temperature governs physiological (physical as well as chemical) processes and their equilibria, hence a change brings about alterations in threshold value, reaction time and velocity of the reaction and recovery movements.

In the present chapter, the first two will be dealt with.[2]

A. Extreme Temperatures

If *Aldrovanda* leaves are left at 40° or at higher temperatures,

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1) However, when a leaf is excited in this position by an electrical shock, we are not sure which of the two organs, sensitive hairs or cells of the motile zone, have been the chief cause of the reaction.

2) The last category will be discussed in a paper, which will be issued before long.

they shut " spontaneously " sooner or later. The time factor was studied :— Beakers containing boiled water[1] with *Aldrovanda* floating in it were put in a water thermostat of the same temperature as the culture medium, and then temperature of the thermostat was raised gradually. Table 3 represents the result with five individuals, when the water in the beakers was warmed from 27° up to 42° for 2.5 hours, and was maintained at this temperature.

Table 3

Effect of the high temperature on leaves.

Whorl No.	Number of open leaves at 27° (before warming)	% of open leaves		
		When the water temp. attained 42°	After 1 hr. at 42°	After 2.5 hrs. at 42°
I	23	100	0	0
II	27	89	0	0
III	27	70	0	0
IV	27	85	0	0
V	23	96	4	0
VI	21	95	33	0

All the leaves of the youngest four whorls shut within an hour at 42°, and some of older leaves left open at this time were shut after the following 1.5 hour.

The result of putting six individuals at 41° is shown in Table 4. At this temperature, the effect was slower to reveal itself ; less than 10% of the leaves shut during the first 7 hours, the greater part of young leaves shutting during the next 4 hours, while older ones were still later. But no leaves remained open after 24 hours.

At 40°, all the leaves of No. II were shut after 18 hours. The percentage of shut leaves decreased with the age of the leaves, 70% of those of No. VI shutting in the 18 hours. At 39°, 15–70% (Nos. II–VI) of the leaves were shut after 18 hours.

The shutting of leaves at high temperatures is perhaps due to the fact that the most sensitive inner epidermal cells are affected

1) Bubbles must not be formed when the medium water is warmed, lest the leaves should be stimulated by them. So the air dissolved in the water should be driven out beforehand.

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Table 4

Effect of the high temperature on leaves.

Whorl No.	No. of open leaves when the water temp. attained 41° (warmed from 27°)	% of open leaves		
		After 7 hrs. at 41°	After 11 hrs. at 41°	After 24 hrs. at 41°
I	23	91	30	0
II	27	93	30	0
III	19	95	21	0
IV	25	96	70	0
V	31	84	61	0
VI	36	92	69	0
Mean		91.8	46.8	0

sooner than cells of the outer two layers.[1] If, however, the permeability of cells of the outer two layers should be changed (to lose their turgor) not later than the inner epidermal cells, the shutting movement would not take place, just as when *Dionaea* leaves are put into boiling water. But such a case is not observed with *Aldrovanda*, as the reactive cells of this plant are very sensitive.[2] In the experiment, no leaf was found to be in the state of heat coma.

On the other hand, chill coma is induced: though even slow cooling down of leaf temperature made many leaves shut[3], some were left open even at 2', and were found to be non-reactive to tactile stimulation (cf. also p. 70).

B. Thermal Shock

The *Aldrovanda* leaf is a very good material for research of stimulating effect of sudden changes of temperature, thanks to the following characters: 1) Temperature of the protoplasm can be changed rapidly and definitely, since (a) the plant is submersed, and (b) the tissue is thin. 2) The reaction is easily observable to

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1) The leaves which were used for the observations represented in Tables 3 and 4 did not reopen. But whether or not a leaf just caused to shut by a high temperature can recover from the injury and reopen, if it is returned soon to a suitable temperature, is left for future observation.

2) Even *Dionaea* leaves shut when dipped into boiling water, if their irritability is raised beforehand by stimulating a sensitive hair weakly (cf. I: pp. 181–2).

3) Perhaps by the effect of thermal shocks due to irregular cooling.

the naked eyes. 3) The latent period is short and the reaction is very fast.

a. Method

Leaves were floated in beakers which were put in a water thermostat. They were kept at a constant temperature for a few hours, lighted by a 250-watt lamp from 20 cms. above, until they were carried one by one, with a small glass spoon, into beakers which stood in another thermostat. In this way, leaves were subjected to sudden definite changes of temperature.

In order to change leaf temperature as quickly as possible, the spoon with a leaf floating in it was thrusted about 2 cms. below the water surface; thus, in floating up to the water surface with its own bouyancy the leaf was washed by water of the second temperature. This procedure seemed not to give seismonic stimulation, for leaves which were transferred repeatedly from water to water of the same temperature in a more violent manner did not show any sign of reaction.

b. Result

In case the difference between the first and the second temperature exceeded a certain limit, all the leaves shut, stimulated by the shock of the temperature change. Leaves which reacted to thermal shocks were counted for every degree of the temperature difference, within the above-mentioned two limits. The number of shut leaves was expressed in percentage of the leaves tested. Table

Table 5

Leaves reacting to infra-optimal temperature-falls, expressed in percentage of the tried leaves, the initial temp. being 25°.

2nd temp.	Temp.-fall	Whorl No.					
		I	II	III	IV	V	VI
21°	4°	2	2	0			
20°	5°	15	5	3	0		
19°	6°	38	26	24	4	0	0
18°	7°	92	80	64	26	21	9
17°	8°	100	100	91	72	88	40
16°	9°			100	100	100	78
15°	10°						100

5 represents the result of exposing leaves, adapted at 25°, suddenly to lower temperatures.

The table is divided into two, in such a way that in the lower half more than 50% of tried leaves reacted to the thermal shock. Thus we see that a sudden temperature fall of 7° can stimulate more than a half of the leaves of Nos. I, II and III, while the fall should be 8° or 9° to cause similar effects upon leaves of Nos. IV and V or No. VI.

Analogous determinations as expressed in Table 5 were made with respect to every 5° change, from 10° to 40°, of the initial (adaptation) temperature. And falls of temperature just sufficient to make more than a half of the leaves shut were determined, they being shown in Table 6.

Table 6

Temperature-falls in degrees that can stimulate half the number of leaves, absolute values of the 2nd temps. being shown in brackets. Marks of degree omitted.

1st temp.	Whorl No.					
	I	II	III	IV	V	VI
40	10 (30)	10 (30)	10 (30)	10 (30)	11 (29)	12 (28)
35	7 (28)	7 (28)	9 (26)	10 (25)	10 (25)	12 (23)
30	7 (23)	7 (23)	8 (22)	8 (22)	8 (22)	9 (21)
25	7 (18)	7 (18)	7 (18)	8 (17)	8 (17)	9 (16)
20	6 (14)	7 (13)	7 (13)	7 (13)	7 (13)	8 (12)
15	7 (8)	7 (8)	7 (8)	7 (8)	7 (8)	7 (8)
10	7 (3)	7 (3)	7 (3)	7 (3)	7 (3)	8 (2)

The higher the initial temperature, and the older the leaf, the greater fall of temperature is needed to stimulate the leaf. Leaves which were first adapted to 15° or 10° and were then stimulated by transferring into lower temperatures often shut incompletely, and even if stimulated mechanically in this half shut stage they reacted no more. They may have been chilled.

In order to stimulate leaves by raising the temperature, difference in degrees should be larger than in the case of stimulating by lowering. Table 7 represents the result of bringing leaves adapted to 25° to higher temperatures. Leaves which reacted are expressed in percentage of the leaves used, just as in Table 5. Rises of temperature just sufficient to make more than a half of leaves shut were determined in the same manner as before, from

10° to 30° of the first temperature, and are represented in Table 8.

Table 7

Leaves reacting to temperature-rises expressed in percentage of the tried, the initial temp. being 25°.

2nd temp.	Temp.-rise	Whorl No.					
		I	II	III	IV	V	VI
36°	11°	16	12	1	0		
37°	12°	45	22	5	1	0	0
38°	13°	59	34	12	9	1	1
39°	14°	74	50	25	19	8	5
40°	15°	84	67	48	42	22	17
41°	16°	93	88	73	63	38	30
42°	17°	95	93	87	83	60	53
43°	18°	96	97	97	93	83	83
44°	19°	99	99	98	95	93	89
45°	20°						99

Table 8

Temperature-rises in degrees that stimulate half the number of leaves, the absolute heights of the 2nd temps. being shown in brackets. Marks of degree omitted.

1st temp.	Whorl No.					
	I	II	III	IV	V	VI
30	11 (41)	12 (42)	13 (43)	14 (44)	15 (45)	16 (46)
25	13 (38)	14 (39)	16 (41)	16 (41)	17 (42)	17 (42)
20	19 (39)	19 (39)	19 (39)	21 (41)	21 (41)	23 (43)
15	25 (40)	26 (41)	26 (41)	27 (42)	27 (42)	28 (43)
10	24 (34)	24 (34)	28 (38)	32 (42)	28 (38)	26 (36)

The lower the first temperature, and the older the leaf, the greater the rise of temperature necessary to stimulate the leaf.[1] In spite of that the temperature changes were limited chiefly within the infra-optimal range in the case of stimulation by temperature-falls (Table 6), many of the second temperatures in the present case were over 40°, namely in the injurious range of temperature. The effect of temperature-shock, however, can be discriminated from that of injury by limiting the observation to within 10 seconds

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1) Exceptional figures in the initial temperature of 10° may be due to insufficiency in number of the observed leaves.

after the temperature-change. A far longer time is needed for the temperatures, 40-45°, to cause leaves to shut by injury.

The thermal shock effect studi-
ed is summarized graphically in
Fig. 4. With respect to the initial
temperature, the largest and the
smallest temperature differences in
Tables 6 and 8 are represented with
arrows, the corresponding whorl
numbers being put beside them.
With this figure and the tables,
the following conclusions may be
drawn :

Fig. 4. Stimulating temperature change in relation to the initial temperature of the leaf. Roman numerals indicate whorl Nos.

1) *Cooling can stimulate leaves more easily than warming.* (Variations are also smaller in the former case).

2) *The higher the initial temperature, the more sensitive the leaf is to rises, and the less so to drops.*

3) *The older the leaf, the less sensitive it is to a thermal shock, either a rise or a fall.*

c. Discussion

The volume of air contained in intercellular spaces may change with the temperature. But that is not the cause of the stimulation, for, (a) leaves, the intercellular air of which has been previously dissolved out by once boiled water[1], can be stimulated by a thermal shock just as may normal leaves containing air ; (b) even if the air is so much compressed by pressure that bouyancy of the leaf is lost, or is expanded again, the leaf is not excited (cf. p. 61).

In literature, we have many examples of the physiological effect of change of temperature, but not a few of them are scarcely free from participation of injurious effect (reversible or irreversible) of supra-maximal temperatures :

After stimulating *Dionaea* leaves by falling drops of hot water, MACFARLANE ('92) observed that the spot where the water had been applied turned yellow and then brown ; when BOSE stimulated *Mimosa* with electrically heated wire (p. 25, and *Biophytum*, p. 286), injury from " scorching " was avoided, but we are not sure that
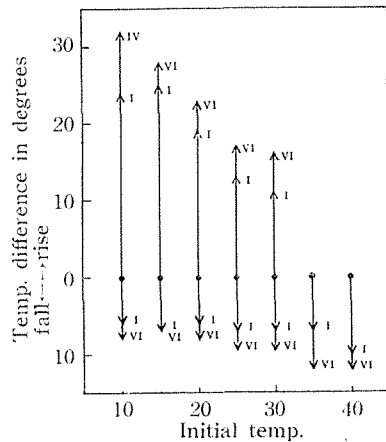
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1) Cf. the F. N., p. 61.

there was no injurious effect of supra-maximal temperatures; BÜNNING ('29 b) found that cooling stimulated filaments of *Centaurea,* etc. more easily than warming, using hot air of 50° (or a heated needle) to give the thermal stimulus, but a temperature of 50° is injurious as he himself recognized. Thus it is not certain yet whether those materials are really sensitive to temperature changes within infra-maximal range or not. However, application of such high temperatures is inevitable in those experiments, since otherwise the temperature of the plant body standing in the air cannot be changed rapidly enough not to let the stimulus "creep in" ("einschleichen"), since the specific heat and the thermal conductivity of air is low. JENSEN ('09) used isotonic solutions, etc. of as high temperatures as 47-81° to make frog sartorius contract. It must be a difficult task to raise the temperature of every fibre of the massive muscle quickly.

On the other hand, bending of *Drosera* tentacles when warmed to 40-50° in water (DARWIN, '75), and the marked change in velocity of anthocyan exosmosis from roots of *Beta* when cooled a little from high temperatures, 45-55°, (DE VISSER SMITS, '26) may surely be reactions of denaturating cells.[1] It may be due to a combined effect of thermal shock and injury that *Aldrovanda* leaves shut when dipped into boiling water. Frog nerve was excited by water drops of over 53-54°R, independent of the magnitude of temperature changes (ECKHARD, 1851).

The chief point of the present discussion, however, is in the physiological action of a sudden change of temperature within the infra-maximal range. And the following cases may fall under this category: A maximum and a minimum appeared in the growth rate of coleoptiles and a hypocotile after the temperature was raised or lowered suddenly (SILBERSCHMIDT, '25); temperature variations of ca. 1° caused synchronous nastic movement of *Phaseolus* leaves (STERN and BÜNNING, '29), and a rapid rise of temperature (even to 30°C) made leaflets "shut" (KOSANIN, '05); respiration was influenced by temperature changes[2] (PALLADIN, '99; MACK, '30; CROZIER and NAVEZ, '30–31); *Tulipa* flowers were made close by many authors by lowering the temperature[3]; drops of ice-cold water made *Dionaea* leaves shut (MACFARLANE, '92); sudden local chilling caused action currents to be set up in *Nitella* and in *Chara* (HILL, '35).

The thermal shock effect, however, has been studied most often

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1) That the exosmosis of anthocyan in question is owing to irreversible change of protoplasm is noticed by RAMAER in the postscript of DE VISSER SMITS' paper.

2) CROZIER, TANG and FRENCH ('34), however, state that respiration of *Chlorella* is influenced little (within the limit of error) by alternating temperatures.

3) Opening of tulip flowers by a rise of temperature is assumed by BÜNNING ('29a) not to be due to stimulation by thermal shock, though BÖHNER ('33) objects to that. Similarly, curling of tendrils induced by rise or fall of temperature was asserted by McDOUGAL ('93), contrary to CORRENS' ('96) view, that it was not due entirely to the special irritability. DOERFEL ('30) showed that favouring of germination by intermittent exposure of seeds to high and low temperatures was not owing to stimulative action of temperatures.

with protoplasmic streaming. Though some different results are reported by early authors, conclusions of the latest three, COOK (*Nitella clavata*, '29), ROMIJN (*N. flexilis*, '31) and BOTTELIER (*Avena*, '34), agree in that protoplasmic streaming are stopped or retarded temporarily, not by a rise, but only by a fall of temperature. This may be analogous with the fact that *Aldrovanda* leaves are stimulated more easily by a fall of temperature than by a rise. If the temperature change is limited strictly within inframaximal range, stimulating effect of rises of temperature upon *Aldrovanda* leaves is observable chiefly with youngest leaves.

The author can not find in literature any works in which have been studied the stimulative effects of thermal shocks more precisely than the above-mentioned, and accordingly any theoretical considerations sufficient to account for various facts in the present work.

HILL ascribes the action currents by chilling to a partial solidification of the non-aqueous protoplasmic surfaces which makes them susceptible to rupture by the protoplasmic streaming. But his theory cannot account for the stimulation by rises of temperature. And even in the case of fall of temperature, stimulation by putting in so high a temperatures as 30° (from the initial temperature of 40°) is difficult to be explained. In addition, presence of protoplasmic streaming in quite intact leaf cells of *Aldrovanda* is very doubtful. JENSEN's ('15) theory of "labile complex", on the contrary, concerns only the case of rise of temperature.

It may be natural, however, to take some disturbance of the protoplasmic state as the cause of excitation due to thermal shocks. The fact that cooling of leaves should be done very slowly if they are to be brought to a low temperature without causing excitation, may mean that a new equilibrium can be constructed very slowly at low temperatures. On the contrary, accomodation to a new temperature goes on quickly at high temperature,[1] so it is difficult to stimulate 100% of the leaves by rises of temperature, as seen in Table 7 (p. 71). Even if a change of temperature may be large enough for stimulation (and even though the second temperature is as low as 15°), a leaf is not stimulated if the transference is performed very gently (instead of thrusting it below the water surface as noticed on p. 69). Hence such "creeping in" of stimulus may be considered to occur in a case when the disturbance of the protoplasmic state proceeds so slowly as to be recovered on the other hand by the restitution process occurring at the same time.

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1) The author could determine the velocity of thermal accomodation at different temperatures. But he wants to publish the result with more numerical data.

Why, then, are falls of temperature more stimulative than rises (fact 1, p. 72)?   The slowness of accomodation at low temperatures may partly account for it, but we cannot be contented with this reason alone, for a drop from 40° to 30° can stimulate a leaf, whereas a rise from 10° to 30° can hardly do so, notwithstanding that the leaf temperature is lower throughout the procedure in the latter case.

The second fact (p. 72) may mean that to change the leaf temperature from ordinary to very high or low is more stimulative than to change it inversely.   As temperature rises are not very stimulative, the leaf is stimulated in this case not by a change from a very low to a medium temperature, but only by a change to a very high temperature ; whereas temperature falls can stimulate the leaf in either of the two cases, from a high to a medium, and from a medium to a low temperature as well.

It may be natural to consider that the slower the recovery of the normal state of protoplasm from the disturbed condition, the more easily the cells are stimulated by a thermal shock.[1]   If this assumption be true, falls of temperature from 10° may be expected to be more stimulating than falls from 15° or 20°.   According to Table 6 (p. 70), however, the same order of lowering is needed for the three cases, even greater changes being needed for the initial temperature of 10° and 15° than for 20°, in the cases of whorl No. I.   Therefore, it may be assumed further that starting from the protoplasmic disturbance primarily caused by a temperature-shock, the final increase of permeability (or contraction of protoplast) is attained through the intermediation of a chain of reactions which are retarded at low temperatures.   That the sensitivity of proto-plasmic streaming to a temperature-fall is greater at 20° than at a lower, as well as at a higher, temperature, may be analogous to this case.

The third fact (p. 72) may be due to the rise of threshold stimulus with aging of the leaf, just as was found with other means of stimulation.

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1) That the leaf is very susceptible to a mechanical deformation at low temper-atures (p. 60) may also be ascribed to the slowness of the reparation of the proto-plasmic disturbance at this condition.

Osmotic Influences

A.   Immobilization[1] by Sucrose Solution

i.   Immobilization time

Aldrovanda (as well as *Dionaea*) leaves lose their power of reaction when their turgidity is decreased to some extent by an osmotically active solution (I : p. 175), for the bending force of the outer epidermis and the middle layer is then lost.   In order to determine how quickly they were immobilized, individual cut off leaves were transferred one by one into sucrose solutions of various concentrations, in the same manner as they were transferred in the study of thermal stimulation (cf. p. 69).

The outer surface of the leaf comes in good contact with the new solution, while on the inside water is not replaced by the solution so rapidly and so completely as on the outside.   But in the present case, chiefly the medium on the outside comes into question, as the osmotic immobilization is due mainly to the shrivelling of outer epidermal cells.

The leaf reacts normally if it is stimulated soon after the immersion into a sugar solution.   However, the longer it is immersed, the less becomes the amplitude of its responsive movement, at last it loses the power of movement entirely.   The time necessary for sugar solutions to make leaves immovable, viz. the duration of time from the immersion of leaves into sugar solutions till they were able to respond to tactile stimuli with so slight movement as to be hardly discernible, was determined with respect to whorl number and concentration of sugar solution.   Each of A and B, Table 9, represents the result gained with an individual plant.

Table 9

Immobilization time in sugar solution in minutes, at 25°.

(A)	0.11 M	0.15 M	0.20 M	0.30 M	0.50 M	(B)	0.11 M	0.15 M	0.20 M	0.30 M	0.50 M
V	7.5	3.5	2.5	1.6	—	V	5	3	1.8	1.1	0.8
IV	7.0	3.5	2.5	1.5	—	IV	5.0	3.0	1.8	1.1	0.7
III	7.0	3.5	2.3	1.4	0.8	III	4.5	2.7	1.7	1.0	0.7
II	6.0	3.0	2.0	1.1	0.7	II	4.5	2.5	1.5	1.0	0.6
I	5.5	2.5	1.5	1.1	0.7	I	4.0	2.0	1.5	1.0	0.6
Mean	6.60	3.20	2.16	1.34	0.73	Mean	4.60	2.64	1.66	1.04	0.68

1)   Cf. F. N., p. 56.

As individual differences are rather large, the relation of immobilization time to the leaf age and to the concentration of the solution is understood more easily by this way of representation, unless the determination is performed with a very large number of individuals. Using a plant most leaves of which are open, it is not very difficult to determine the immobilization time of a whorl for five classes of concentration of the solution. The determination was made at 25°. The plant to be tested had been kept at the same temperature in order to avoid the temperature-shock.

From the table it is concluded that *the more concentrated the sucrose solution, and the younger the leaf, the more readily the immobilization occurs.*

Most leaves were immobilized by the saturated sucrose solution within 15–20 seconds. In a 0.10 M solution, some leaves were immobilized in 6–8 minutes, some after more than 10 minutes, and some reacted to stimulation even after 24 hours, though the movement was very slight.

ii. *Immobilizing concentration*

CZAJA'S method, in which a quantity of concentrated solution is added to a vessel containing a known quantity of water and an individual plant with open leaves, is convenient when a large number of leaves are to be tested, though the result is not so accurate as by the method used above, for the solution can be mixed up neither so rapidly nor so thoroughly.

An individual plant from which whorls older than No. XI are cut off, is floated in 30 c.c. of water, contained in a glass case 2.4×6×2.5 c.c. After making a note of open leaves, a proper quantity of 1.0 M sucrose solution is poured into the vessel, and the plant body is moved gently to and fro with a pincette to stir the medium and make it as uniformly concentrated as possible. After the plant is left in the solution for 30 minutes, it is lifted above the surface and put back soon into this again. The leaves which are yet capable of reaction are shut, stimulated by deformation of the lobes (cf. p. 59, b), while those which have lost the power of movement remain open even after this procedure.[1] By observing the grade of reaction (open, half-shut, or shut) of the leaves which have been open before, Table 10 was made.

Possibly the mixing does not proceed uniformly enough, so that some leaves may be in contact with a more concentrated solution, and some others with more diluted solution than that of the described molarity. Such may be a cause of the presence of scarcely immobilized (C) leaves in the concentrated solutions, and the presence of completely immobilized ones in the diluted.

Though Table 10 indicates only the general tendency, it is clear that in a 0.09 M (and 0.08 M) sucrose solution completely im-

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1) Non-reactive leaves are also made to close more or less by cohesion of the solution while they are brought up in the air, but they become open again as soon as they are returned to the solution.

Table 10

Immobilizing effect of sucrose solutions in 30 minutes at ca. 25°.
Leaves of whorls I–X used.

No. of leaves observed		0.15 M	0.12 M	0.11 M	0.10 M	0.09 M	0.08 M
		820	411	161	641	354	115
% of leaves	A) Completely immobilized	91.1	86.2	75.2	39.8	9.6	16.5
	B) Partially immobilized	8.9	13.6	24.2	51.3	70.6	57.4
	C) Scarcely (or not) immob.	0.2	0.2	0.6	8.9	19.8	26.1

mobilized leaves are only a few. Hence it may be concluded that solutions more diluted than this can hardly immobilize leaves.

Immobilizing effect of glycerin solutions was also determined in the similar way (Table 11).

Table 11

Immobilization in glycerin solution, comparable to Table 10.

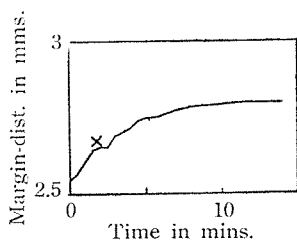| No. of leaves observed		0.15 M	0.12 M	0.11 M	0.10 M
		206	399	116	184
% of leaves	A) Completely immobilized	97.1	82.2	83.9	31.8
	B) Partially immobilized	4.4	16.3	12.9	53.0
	C) Scarcely (or not) immob.	2.5	1.5	3.2	15.2

Fig. 5.   Change of opening of a leaf of whorl No. III after the immersion into a 0.2 M sucrose solution. The leaf is supposed to be immobilized somewhere near ✕ in the curve.

iii.   Immobilized leaves

When a leaf is immersed in a sugar solution, its opening gets slowly a little larger. This may be caused by weakening of the bending force of the outer epidermis and the middle layer due to decrease in turgor of the cells. Fig. 5 represents the relation between the change of the margin-distance and the immobilization time, the latter being assumed from Table 9 and shown by the cross mark in the figure.[1]

Leaf lobes are yet elastic to some extent even after the leaf is immobilized by a

1) Loss of turgor of cells of the one-layered region also affects the opening of the leaf, the irregular movements due to this being especially prominent when the solution is more concentrated than 0.2 M.

sugar solution, hence if they are pressed nearer together and then freed, they spring back nearly to the orginal form. But if they are pressed together so far as to show the narrowed stage, they cannot return to the original position, but stay somewhat at a half-shut stage. This may be due to a plastic extension of the walls of the outer epidermis (cf. I : p. 184).

B. Recovery of the Power of Movement

i. *Recovery of the Power in Solutions*

After the observation of the immobilization (Table 11), in-dividuals which were dipped in the 0.15 M glycerin solutions were left as they were, and their leaves were observed after 6 hours. Results are given in Table 12.

Table 12

Leaves observed	200
A) Open leaves	148 (74%)
B) Half-shut leaves	30 (15%)
C) Shut leaves	22 (11%)

Table 13

Leaves observed	148
A) Open leaves	0 (0%)
B) Half-shut leaves	27 (18.3%)
C) Shut leaves	121 (81.8%)

Compared with the second column of Table 11, the number of half-shut (B) and shut (C) leaves has increased. This indicates that some of the leaves closed more or less while they were still floating in the solution.

Then the plants were lifted above the surface again, and all the leaves which had been open were seen to have reacted in various degrees, as shown in Table 13.

Hence the open leaves left in the 0.15 M glycerin solution regain-ed (at least partially) their reactivity within 6 hours. Perhaps penetra-tion of glycerin into (and anatonosis occurring in) the cells, especially of the outer epidermis and the middle layer at and near the motile zone, may have been the cause.

Also in sucrose solutions the power of movement is recovered, though not so markedly as in glycerin solutions. Fig. 6 represents the movement of a leaf of No. III stimulated very strongly 21 minutes after the immersion in 0.2 M sucrose solution. Fig. 7 represents the movement of a leaf which belongs to the same whorl as the one used in Fig. 6, stimulated in the same way, but differing only in the time of stimulation, viz. 5 hours after the immersion in the solution. By comparing the two figures, it may be seen how the movement is faciliated during 5 hours.

ii. Returning to water

Leaves immobilized in a sugar solution recover their reactivity
if they are returned to water.   In such cases, however, some leaves
shut "spontaneously," while some others remain open ready to
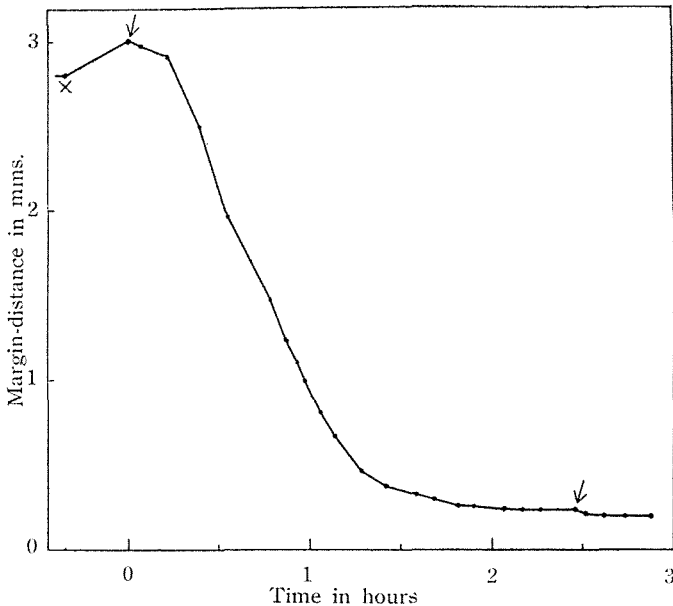react to stimulation, and the shorter the time of immersion in the

Fig. 6. Movement of a leaf dipped in a 0.2 M sucrose solution at ×, and then
stimulated with three successive strong break shocks (coil dist.=0, electrode dist.=2
cms.) at each of the arrows. (When the leaf was returned to water after the ob-
servation, it narrowed roughly at the same velocity as the normal narrowing move-
ment.)

solution, the more liable is the latter case to ensue : Leaves seldom
shut "spontaneously" after they are transferred into water from
a sucrose solution more dilute than 0.15 M ; but the stay in the
solution should not exceed 2 minutes if it is 0.3 M, or 50 seconds
if it is 1 M, if the "spontaneous" shutting is to be avoided.   Such
a relation may indicate that leaf cells are excited if they are returned
to water after they have received, from a solution of high osmotic
value, an alteration over a certain limit, the degree of the alteration
depending on concentration of the solution and on duration of stay
in the solution.   The "spontaneous" movement therefore is caused
by *excitation due to a rapid change of environmental osmotic pressure*.
There are two types of movement in leaves stimulated by a

decrease of environmental os-
motic pressure; (1) the "quick"
one, just as in the normal re-
action, and (2) the "slow" one.
At the present stage of investi-
gation, it is impossible to predict
which movement would ensue.
But whichever of the two modes
of movement the leaf shows,
it will eventually enter the
narrowed stage afterwards.

An example of change of
opening before and after that
quick "spontaneous" move-
ment is shown by Fig. 8; the
opening becomes wider when
the leaf is dipped in the sucrose
solution (a–), it becomes smaller
when it is returned to water
(b–), and shuts abruptly (c),
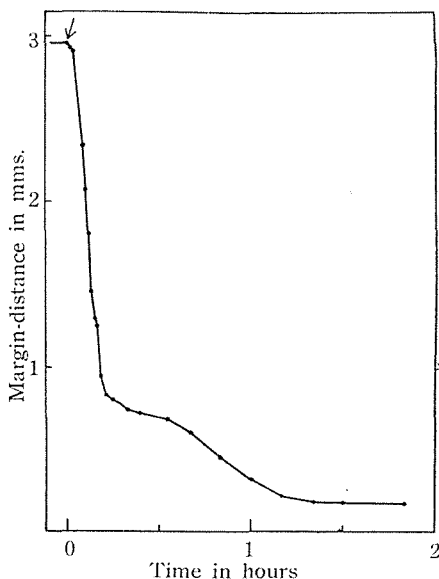followed by the narrowing

Fig. 7. Movement of a leaf stimulated
with three successive strong break shocks
(coil dist.=0, electrode dist.=2 cms.) at
the arrow, after the immersion in a 0.2 M
sucrose solution for 5 hours.

movement (c'–d'–
e'). Other types
of change of
opening after re-
turning to water
are represented
in Fig. 9, A, B
and C. Leaves
which are trans-
ferred from a
concentrated so-
lution often open
wider in water
(C), perhaps by
irregular un-
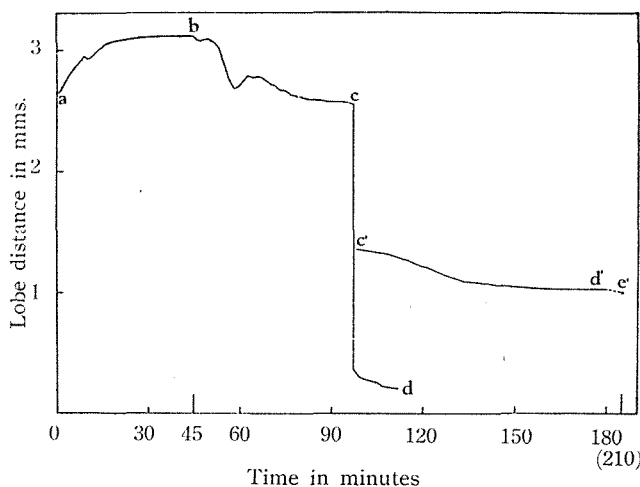bending of the
one-layered re-
gion.

The slow

Fig. 8. Change of opening of a leaf before and after the
reaction due to osmotic stimulation. a: transference from
water to 0.3 M sucrose solution; b: from this solution back
to water (at 45th min.). a–d: margin-distance measurements;
c'–d'–e': boundary-distance measurements (representing the
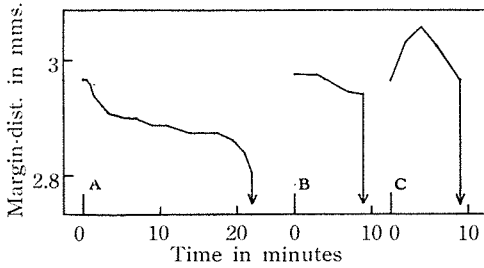narrowing movement). The leaf is of No. IV.

Fig. 9. Slight changes of opening of leaves before the reaction by osmotic stimulation. The leaves are returned to water, (A) from a 0.05 M sucrose solution after a stay of 8 hrs.; (B): from a 0.3 M solution after a stay of 26 mins.; (C): from a 0.3 M solution after a stay of 33 mins.

motility before it shows the "spontaneous" movement.[1] A leaf which is slowly shutting reacts to tactile stimulation at any phase of that slow movement.

C. Stimulating Effect of Osmotic Changes

Though most of the leaves are immobilized in sugar solutions more concentrated than 1.0 M, a few of them shut in such solutions without receiving any seismonic stimuli. Change, i. e. increase in the present case, of the environmental osmotic pressure seems to excite the leaf[2]. Thus the effect of osmotic stimulus[3] is observed with *Aldrovanda* leaves

type of movement generally takes a course as shown in Fig. 10. In such cases leaves often enter into the narrowing phase, hardly pausing at the shut stage as seen in the figure.

If a leaf is stimulated 6 or 7 minutes after it is brought back from 0.3 M sucrose solution into water, it reacts with a normal shutting movement. In other words, it recovers its
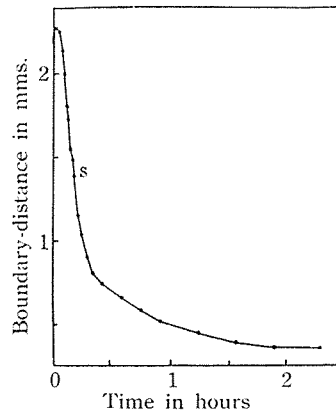

Fig. 10. The slow type of movement due to osmotic stimulation. The leaf (II) is returned to water at time 0 after staying in 0.3 M sucrose solution for 44 mins. The shut stage of the leaf is passed at s.

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1) In Fig. 9, B and C, the leaves react 9 minutes after the transference.

2) DARWIN showed (p. 237) that not the inner epidermal cells but sensitive filaments of *Dionaea* leaves were irritable to osmotic extraction of water. But such differentiated application of a sugar solution is impossible with *Aldrovanda* leaves.

3) "Als *osmotischer Reiz* kann jede Einwirkung auf lebende Substanz betrachtet werden, die eine Aenderung des osmotischen Druckes des die lebende Substanz umgebenden Mediums hervorruft." (BROEMSER, p. 289).

both when they are immersed in a sugar solution and when they are returned from this to water (cf. p. 80). The reaction due to osmotic stimulation takes place but rarely in the former case, for the leaf is commonly immobilized before it reacts ; a leaf may shut " spontaneously " in a sugar solution only in the case where the leaf is stimulated before it is immobilized. One cannot find the concentration in which the quick shutting movement occurs without fail, for the velocities of the osmotic stimulation and the immobilization depend on the concentration of the solution in parallel with each other.

Similarly to the case of returning leaves from a solution to water, either quick or slow type of the movement may be discerned also in the present case.

Fig. 11. Movement of a leaf (No. III) after immersion into a 0.5 M sucrose solution. (Since the one-layered region became gradually wrinkled from dehydration of the cells, values of the margin-distance as represented are not strictly comparable to those of turgescent leaves).
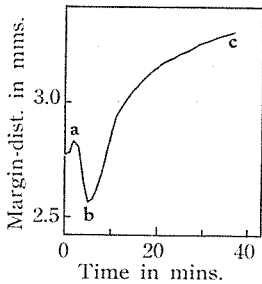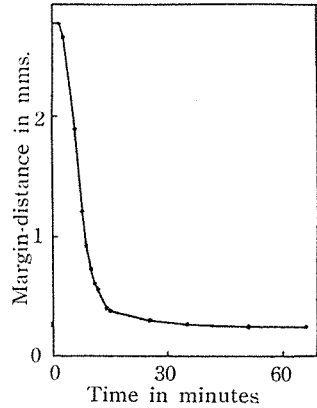

Fig. 12. Change of opening of a leaf (No. II) after immersion into a 0.5 M sucrose solution, indicating the reaction movement due to osmotic stimulation (a–b), and the retrogression of the movement (b c) due to loss of turgor of cells. The latter part of the curve is influenced considerably by shrinking (unbending) of the one-layered region.

Though the quick type of movement looks just like the normal shutting movement, hardly any leaf reaches the closely-shut stage, for leaves are already more or less immobilized when they react to the osmotic stimulation. And the more delayed the reaction, the less is the magnitude of the movement, and no leaf will shut after it is immobilized.

We are not certain, therefore, how many leaves are excited osmotically among those " completely immobilized " in Table 10. Among the " partially " and " scarcely immobilized " leaves of the table, are included leaves which reacted to the osmotic stimulus, that is those which had closed before the plant was picked up.

An example of slow type of the movement is represented in Fig. 11. This type is observed chiefly in concentrated solutions. Leaves which have once half-shut with the " spontaneous " quick movement, too, often continue shutting in this slow type.

Fig. 12 represents a complicated move-

ment of a leaf, which at first began to open wider but started to shut slowly after a few minutes, this movement being overwhelmed then by the widening tendency (cf. p. 78). This is an example which may indicate that two antagonistic forces, one caused by osmotic stimulation and one due to osmotic immobilizing or widening effect, are working in the leaf at the same time.

Influences of Chemical Agents

Submersed plants are generally more fitted for studies of effects of chemical solutions than aerial plants, as the latter must be in an abnormal condition if used in water as a control. For the present, however, *Aldrovanda* does not seem to be a very good material for the purpose so far as the following circumstances are concerned: 1) As the leaf is very sensitive and cannot be transferred from one medium to another by picking out, it should be brought with a quantity of the first medium, being floated in with this. Hence the two media are necessarily mixed together. To make it worse, the leaf cavity cannot be washed quickly with the streaming second medium, so that the instant when a leaf is quite soaked with the second medium being indefinite, exact determination of the reaction time is impossible. 2) Local application of a chemical solution is impossible, for the leaf cannot be laid out of water, and hence it necessarily diffuses here and there. 3) Whatever influence be exerted, leaves can only react by shutting, and varied forms of the reaction (such as opening or closing as with stomata, or change of velocity as with protoplasmic streaming) cannot be observed. 4) Reactivity is lost from the osmotic effect of the solutions.

Hence in studying the action of salts and acids on *Aldrovanda* leaves, no better theme was found than the interference of the osmotic effect with the chemical stimulative effect.

While sucrose and glycerin produce only an osmotic influence, there are many substances which give chemical influences, too. According to the mode of influencing *Aldrovanda* leaves, these can be classified as follows:

1. Chemicals which have the effect of osmotic immobilization, besides the chemical stimulation— salts (neutral, acidic, basic); some acids (as boric).

2. Chemicals which destroy the power of movement of the leaf by injuring, before stimulating it—formalin; diluted alcohol.

3. Chemicals which stimulate leaves to shut, before the immobilizing effect (osmotic or injurious) of themselves appears— acetic acid; HCl; NaOH; ethyl alcohol (above 20%).

4. Chemicals which stimulate leaves without injurious effect— water-soluble nitrogenous matter.

A.   Neutral Salts

With neutral salt solutions, CZAJA found no other effect than the immobilization as with sugar solutions.   He used only dilute solutions (more diluted than 0.091 M, according to the present author's calculation) and perhaps for no long duration of time.   But with more concentrated solutions a stimulative effect appears besides the immobilizing.   The former may be a chemical action of the salt and the latter may be due to its osmotic pressure.

Cut off leaves were transferred one by one from the culture water to salt solutions with a small glass spoon, as mentioned before.   Though the leaves were moved about in the salt solutions, the water in the leaf cavity was certainly not replaced by the new solution in an instant.   And as the chemical stimulus that causes the shutting movement[1] is perceived mainly at the concave side of the leaf (cf. p. 93), the real reaction time must be somewhat shorter than that represented in the present determination, viz. the duration of time from the dipping of a leaf into the solution till the shutting movement of it.

The reaction time defined above is determined with respect to

Table 14

Stimulating effect of KCl-solution—Mean reaction time in seconds
(Extreme variations in brackets).

Whorl No.	Saturated	2.0 M	1.0 M	0.5 M
I	1.6 (1.0—2.2)	5.1 (3.0—8.5)	8.7 (2.8—16.6)	14.7 (8.9— ∞)
II	1.4 (0.8—2.3)	2.4 (1.5—5.6)	8.0 (2.5—14.6)	16.8 (8.2—17.5)
III	2.6 (1.5—4.0)	2.8 (1.5—5.9)	7.8 (3.2—15)	18.5 (7—25.6)
IV	2.3 (1.0—3.5)	5.5 (1.8—12)	11.2 (4.2—20)	28.1 (5—33.2)
Mean	1.98	3.95	8.93	19.53

1) Salt solutions generally cause the narrowing movement, too.   But chiefly the effect causing the shutting movement is studied for the present.

the concentration of KCl-solution and the age of the leaf, at 25',
as shown in Table 14.

The great deviations as shown in the table may be due partly to the inconstancy
of the flowing velocity of salt solutions into the leaf cavities, and partly to variations
inherent in the leaves.

In general, the older the leaf, the longer is the reaction time,
except that leaves of No. I are more slow to react than of No. II.
Many leaves of No. I do not shut in 0.5 M solution, and they are
found then to be non-reactive also to tactile stimuli ; in other words,
though the leaves which have short reaction time shut before they
are immobilized, those which are slow to be excited are immobilized
before the reaction.   The reaction time of No. I in 0.5 M in the
table is the average, excluding those immobilized, viz. slowly re-
acting, leaves.   Hence the value is naturally smaller than it would
be if the immobilization should not take place, thus the value of
No. I is smaller than No. II only in 0.5 M.   While the immobili-
zation occurs quickest in premature leaves, the reaction time of
these is a little longer than mature but young leaves (No. II).   It
is owing to this difference between the immobilization and the
stimulation that premature leaves are immobilized by salt solutions
far more frequently than older leaves.

The more diluted the solution, the more leaves are immobilized
before the reaction occurs, especially among young leaves. In 0.1
M solution and using whorls of Nos. I–IV, the leaves that shut and
that do not shut number roughly the same, most of the former
leaves showing incomplete shutting, viz. being partially immobilized.
The mean and extreme reaction times of the leaves of Nos. I–IV
that react at all (even if slightly) are as follows :

<div align="center">

Table 15

Time in seconds

</div>

	0.4 M	0.3 M	0.2 M	0.1 M
I–IV	25.4 (11−45)	33.1 (9−90)	38.5 (9−70)	44.2 (10−120)

When the solution is diluted more than 0.1 M, its immobilizing
power decreases more abruptly than its stimulating effect ; the leaf
is not completely immobilized in 0.05 M solution, and even partial
immobilization does not occur in 0.02 M solution.   That is, the leaf
can move in 0.02 M solution ; the leaf shuts completely within a
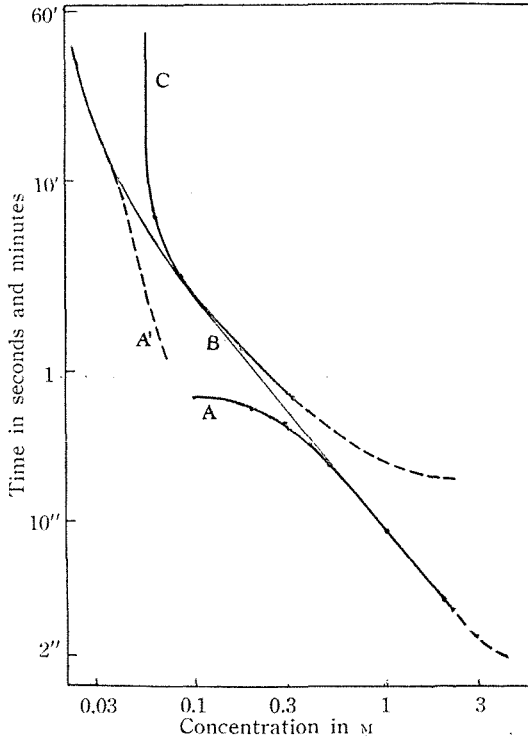few hours after being stimulated by the solution, and finally it

Fig. 13. Time relation of stimulating and immo-
bilizing effects of KCl-solution. A (A') represents the
log. of the *observed* mean reaction time plotted against
the log. of concentration of the solution. B : *presumed*
real curve for A. C : log. of immobilization time
plotted against log. of conc. of KCl-solution, recal-
culated from the data with sucrose (Table 9). (The
lines are broken where variations of individual obser-
vations are too large for the mean value to be relied
upon).

enters the narrowed stage after a number of hours have passed. Naturally, stimulating power also disappears if the solution is diluted much : in 0.002 M solution, some leaves are made to shut in a day, while some others are open and irritable after three days.

Slow type of the move-
ment, as observed among
osmotically stimulated leaves
(cf. Fig. 10), was seen from
time to time in cases when
the reaction took place very
late. There were even the
cases in which quick and
very slow movements alter-
nate.

Summarizing the above-mentioned obser-
vations, the following is concluded : KCl-solu-
tions more concentrated than 0.002 M can stimu-
late the leaf, but the reaction movement is often prevented, com-
pletely or partly, in 0.5-0.05 M, most often in 0.1 M solution. When a leaf is stimu-
lated before it is immobilized by the osmotic action of the selfsame solution, it may cause the reaction movement ; but if a leaf has been already immobilized when the chemical stimulating action attains the threshold, it does not move at all.[1]

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1) If, however, leaves are left in KCl-solutions for long time, the injurious effect
will bring them into the narrowed stage at last.

Why the reaction time in Table 15 is comparatively small may be explained with Fig. 13.[1] All the leaves show the reaction in concentrated solutions (1 M and 2 M), as the chemical action appears much sooner than the immobilization. But as the effecting time of the two actions draws nearer to each other with dilution of the solution (0.5-0.1 M), some leaves which are slow to be excited may be immobilized before they react. Hence the reaction times measured are only those of highly irritable (viz. quickly reacting) leaves. Therefore, the averaged values of reaction time represented in Table 15 are smaller than the real values, so that the curves A and A′ come below the curve B. That the curves, B and C, almost touch each other at x=0.1 M, accords with the fact that the number of leaves that react and that are immobilized are roughly the same. And the deflexion of A and A′ from B is maximum at this point, since the curves, B and C, diverge again from here towards the more dilute region. (Near the concentration of 0.056 M which is considered to be isosmotic with 0.1 M sucrose, the mean immobilization time may become infinitely large—cf. Table 10. But the chemical effect still remains in more diluted solutions —cf. p. 87).

Similar to the case of the KCl-solution, simultaneous action of stimulative and immobilizing influences are found also with a solution of NaCl, LiCl, CaCl$_2$, MgCl$_2$, and KNO$_3$, though the relation between the velocities of the two kinds of influence is somewhat different for each of the salts.

B. Acids and Alkalies

a. Reaction Time

Acetic acid stimulates *Aldrovanda* leaves very soon at low concentrations, so that the osmotic immobilization effect does not come into question. Table 16 represents the reaction time of leaves of a single plant, the average with respect to the whorl and the concentration of acid being shown in brackets.

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1) The OSTWALD equation of toxicity (OSTWALD u. DERNOSCHEK, '10) is introduced here to faciliate the graphic representation, it being aside from the question whether the equation holds for the stimulating and the immobilizing effect of KCl-solution or not. The equation has the form, $\frac{1}{T}=K \cdot (c\text{-}n)^m$, where T is *the duration of life (the reaction time or the immobilization time)*, c is the concentration of *the toxic substance (KCl or sucrose)*, n is the greatest possible concentration of this substance which does not yet produce *death (excitation or immobilization)*, K and m are constants. Without the correction factor, n, viz. if the initial equation, $\frac{1}{T}=K \cdot c^m$ (OSTWALD, '07), is used, the plotted ourve of log T against log C is not a straight line but bends towards the origin. As the value of n differs for each of the stimulative and immobilizing effects, the curves, B and C, are plotted without this correction factor, for the relation of the two are difficult to understand if such corrections of different values are introduced.

Table 16

Stimulating effect of acetic acid—Reaction time in minutes.

Whorl No.	Acid conc. 0.05 N		(Mean)	0.01 N		(Mean)	0.005 N			(Mean)
VI	2	2.9	(2.5)	7.4	17.5	(12.5)	40	44		(42)
V	3.2		(3.2)	14.2		(14.2)	20.7			(20.7)
IV	1.9	2.3	(2.1)	10.8	18.7	(14.8)	25.8	37.3		(31.6)
III	2.0 2.0	2.5	(2.2)	11.2	12.8	(12.0)	24.2	40.3		(32.3)
II	2.4	2.6	(2.5)	10.2 12.1	13.0	(11.8)	18.7	26	32	(25.6)
I	1.8	2.0	(1.9)	12.0	13.0	(12.5)	17.3	22		(19.8)
Average of the means			2.40			12.97				28.67

Table 17

Variations of reaction time in the two acids.

	CH₃COOH	(Maxim. deviatn.)	HCl	(Maxim. deviatn.)
V	2.7 5.2	(2.5)	10.2 34	(24.8)
IV	1.9 2.2 2.3	(0.4)	1.3 8.3 25	(23.7)
III	1.7 2.1 2.7	(1.0)	1.1 1.3 32	(30.9)
II	2.1 2.2	(0.1)	0.5 1.5	(1.0)
I	0.5 0.5	(0.0)	0.7 0.4	(0.3)
0	0.5		0.4	

If HCl is used as the stimulant, the reaction time deviates considerably from leaf to leaf, even in one whorl. Table 17 represents the result when leaves of an individual plant were acted upon by 0.05 N acetic acid and some other leaves of the same individual by 0.05 N HCl. Variation of reaction time is larger also with NaOH or KCl than with acetic acid.

b. Influence of Osmotic Pressure of the Solution

As acetic acid is suitable for chemical stimulation, relation of the stimulation to osmotic immobilization was studied with this acid : Sugar solutions of varied concentration were prepared using 0.05 N acetic acid as the solvent, and the reaction time of leaves in these solutions was determined. Table 18 shows a result with an individual plant. The leaves shut just as in the normal reaction, except in the 5 cases, *α–ε*. But all the leaves, including these, came sooner or later to the narrowed stage afterwards, and at last died, losing their green colour and turgidity.

The leaf of *β* and one of the two leaves of *δ* had been partially immobilized when they reacted to chemical stimulation, and the

Table 18

Reaction time in sucrose-acetic mixture, in seconds. Each figure is
the reaction time of one leaf.

	a	b	c	d	e
{Acetic acid }Sucrose	0.05 N 0	0.05 N 0.001 M	0.05 N 0.05 M	0.05 N 0.1 M	0.05 N 0.3 M
VII	188	— (α	91	67	48
VI	182	87	76	59	45
V	137	158	77	63	52
IV	171	126	72	66	50
III	155	101	98	69	55
II	128	119	106	83 (β	56 ∞ (δ
I	124	104	102	— (γ	∞ ∞ (ε
Mean (II–VI)	154.6	118.2	85.8	68.0	51.6

(α Slow shutting.

(β Almost shut but not completely, followed by a very slow movement.

(γ Started to move slowly after 2 minutes and became completely shut
in 1 minute.

(δ One leaf shut quickly but only a half way. The other one did not move
at all and was irresponsive to tactile stimuli.

(ε Two leaves showed no movement.

other one of δ and two of ε were completely immobilized. A 0.3
M sucrose-solution in 0.05 N acetic acid (the vertical column, e)
has an osmotic value a little higher than pure 0.3 M sucrose solu-
tion, hence leaves may be immobilized a little sooner in the former
solution than in the latter, viz. the immobilization time of the leaf
of No. I or II in the former solution may be somewhat less than
1 minute (cf. Table 9). In Table 18 a tendency is perceived that
in solutions of higher osmotic value (columns, c, d and e), the
younger the leaf, the larger the reaction time, contrary to the
tendency in the pure acetic acid solution (column, a). It must be
noted here that the leaves older than No. III, which have shorter
reaction time, show complete shutting in the 0.3 M–0.05 N sucrose-
acetic mixture (e), whereas the leaves of the youngest two whorls
that have a reaction time of nearly 1 minute are immobilized in the
same mixture. By this experiment is proved indirectly the sugges-
tion (cf. p. 87) that sucrose or salt solutions have two contrary actions,
stimulating and immobilizing, and *whether a leaf will be shut or
be immobilized in the open stage depends upon which of the two
actions affects the leaf first.* Behavior of boric acid, KH_2PO_4
and Na_2CO_3 belongs to the type of neutral salts, for the osmotic

pressures of themselves interfere with the responsive movement.

Besides proving the above-mentioned idea, Table 18 indicates an interesting fact, viz. *the more sucrose is contained in acetic acid solution, the shorter is the reaction time.* There may be two possible ways of explaining this fact :

1. Though the osmotic excitation itself can take place only in concentrated solutions, weak osmotic stimulation below the threshold may be able to help and accelerate the chemical stimulation of acid, working additively.

2. Permeability of cells for acetic acid may be raised while the protoplast is changing its volume owing to exosmosis of water.

The first notion does not hold, for both osmotic and chemical stimulation occurs quicker in younger leaves. Hence the additive effect of the two may also be quicker in younger leaves, whereas the three columns, c, d and e, of the table show the reversed tendency.

On the other hand, the second idea is made probable by the following experiment, which shows that leaves left in a glycerin solution for hours are excited more readily when transferred into pure acetic acid solution than when transferred into a glycerin-acetic mixture which has nearly the same osmotic value as the previous glycerin solution. As examples, results of two individuals are introduced in Table 19, A and B, the former being laid beforehand in 0.1 M glycerin solution for 6.5 hours, while the latter in 0.15 M in the same way for 5.5 hours.

Glycerin solutions denoted as 1.0 M and 1.5 M in the present case, are prepared by diluting a glycerin solution which has a freezing point of -1.002°, 5 and 5/3 times, respectively.

In case a figure represents a mean of two observed values, the deviation is shown after the sign, ±. The vertical columns, a and b, (of both of A and B of the table) correspond to a and d of Table 18, respectively, though glycerin takes the place of sucrose in the present case.

In both of A and B, the mean reaction time of the column, a, is larger than b, and that of d is larger than c. This fact indicates that acetic acid influences irritable cells (cf. p. 94) of leaves more quickly while the cells are giving out or taking in water to or from the surrounding medium. As the rapidity of chemical action may depend upon the velocity of its penetration, it may be concluded that the permeability[1] for acetic acid of irritable cells is increased

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1) The chemical stimulation may perhaps occur when the acid affects the plasm-layer even if it does not enter into the vacuole, so that it may be more proper to use the term " intrability " in place of " permeability," if the conception of the latter term is strictly limited after Höfler ('34).

J. ÂSHIDA

Table 19

Reaction time in seconds.

(A)

From	Water		0.1 M glycerin	
	a	b	c	d
To	0.05 N Acetic acid	0.1 M-0.05 N Glyc.-Acet. mix.	0.05 N Acetic acid	0.1 M-0.05 N Glyc.-Acet. mix.
VI	223	101	96.5±18.5	253.5±11.5
V	128	125±1	104	221.5±28.5
IV	182	110	143	119
III	154	128	97	147.5±35.5
II	147	156	109	56
I	131	130.5±2.5	78	97.5±12.5
Mean	160.8	125.1	104.6	147.5
Index	**100**	77.8	**65.0**	**91.7**

(B)

From	Water		0.15 M Glycerin	
	a	b	c	d
To	0.05 N Acetic acid	0.1 M-0.05 N Glyc.-Acet. mix.	0.05 N Acetic acid	0.1 M—0.05 N Glyc.-Acet. mix.
VI	273	98	109	232
V	268	187.5±54.5	135.5±0.5	200±60
IV	207	162±40	120±33	89.5±11.5
III	136.5±6.5	120	81±39	85
II	190	131	98	103±2
I	33	126	68	82±7
Mean	184.6	137.4	101.9	131.9
Index	**100**	74.4	**55.1**	**71.4**

while the protoplast is changing its volume as SCARTH ('27) has proved with *Spirogyra* cells and acid dyes.[1] ·As briefly noticed before (I: p. 175), cells in the motile zone do not normally show plasmolysis even in a solution of higher osmotic values. Hence the

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1) HUBER u. SCHMIDT ('33) and some authors cited by them determined that the permeability was not influenced, or was lowered, by the occurrence of plasmolysis, but it was the entrance of a rather large quantity of substances into cells in the many hours that they tested. On the contrary, the determination of SCARTH, WEBER and STRUGGER that the permeability was raised by plasmolysis, concerned the entrance

increase in permeability is caused, in the present case, not by tearing off of protoplast from the cell wall, thus differing from the determination of STRUGGER ('32) that the increase of permeability occurs just when plasmolysis appears. The only observation comparable to the present result was made by SCARTH, in whose experiments even solutions without hypertonicity had influences upon the permeability of dyes.

WEBER ('31) asserts that permeability of a plasm membrane which is not easily plasmolyzed on account of high viscosity, increases much at the plasmolysis. But in the cells of the motile zone, the absence of plasmolysis is perhaps due to the long flexible cell walls easily adhering to the contracting plasmalemma, rather than to high viscosity of plasm. The present case seems, therefore, to be analogous to another case he assumed ('33), viz. that the permeability of yeast cells is raised in hypertonic media, though they show no true plasmolysis but "Plasmorrhyse." He thinks that sucrose, differing from neutral salts, has no influence upon the permeability of the cells, but the present idea is not opposed to his, as entrance of a small quantity of an injurious substance may be detected far more sensitively by the excitation of irritable cells than by morphological change of ordinary cells due to injury.

The averaged reaction time (more remarkably, its index) of the column, c, of B is smaller than that of the corresponding column of A, perhaps because the change of the environmental osmotic pressure is greater in the case of B. In comparing d's of A and B, the stimulative effect is larger in the case of B than A; in spite of that the osmotic pressure of the medium is not changed in B (from 0.15 to 0.15), while it is increased in A (from 0.1 to 0.15). Hence *a change in concentration of glycerin alone seems to be a more important factor than the change in the entire concentration, glycerin plus acetic acid.* With sucrose-acetic mixtures (cf. Table 18) we see a tendency for the influence of osmotic value upon the reaction time to become greater as the leaf grows older. But with glycerin-acetic mixtures, such a tendency is less apparent (b and c of A), or even reversed (b and c of B). The discussion in this paragraph, however, is not quite valid, for quite a number of exceptional individuals were found.

c. Site of Perception of Chemical Stimuli

If a small quantity of acetic acid is ejected from a micropipette at the outside surface of a leaf (close to the midrib or to one of the motile zones), or into the cavity between the lobes, the shutting reaction occurs sooner in the latter case, suggesting that the chemical stimulus is perceived more quickly on the inside rather than on

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*of a small quantity of substance *during, or within a short duration of time after, the plasmolysis* or attempted plasmolysis. The result of the first-mentioned determinations, therefore, may be accounted for by restoration of disturbances due to plasmolysis during the long time of the test. Stimulation by acetic acid, as in the present case, may enable the detection of entrance of the smallest quantity that ever has been detected with respect to the question of "plasmolysis-permeability," hence change of permeability (or intrability) during the dynamic phase of plamolysis or deplasmolysis may be detected most sensitively using excitation as the criterion.

the outside. But the author cannot determine what part on the
inside is the most sensitive, for even if a very small quantity of
acid be poured upon the joint cells of a sensitive hair, the water
in the leaf cavity turns quite acidic before the shutting movement
occurs.[1]   It is probable, however, that thin-walled joint cells perceive
chemical influences sooner than the stiff parts of hairs, and also
quicker than the inner epidermal cells which are also thicker-walled
all over. If this reasoning be correct, the expression, irritable cells,
used in discussing chemical stimulation (p. 91, etc.) should mean
these joint cells. In *Dionaea*, osmotic stimulation cannot be perceived
by any other parts than the sensitive hairs (or the cells of lobes
close to their bases), for concentrated glycerin spread over the leaf
surface does not stimulate the leaf, if the hairs are free from it.

C. Organic Matters
a. Formalin

If an open leaf is dipped in undiluted commercial formalin and
is moved about in this so as to be acted upon very quickly, the
leaf is generally found to be irresponsive to tactile stimuli after
35–45 seconds. But if the leaf is stimulated earlier, it reacts and
shuts, though not completely. It should be noted that older leaves
are immobilized sooner than younger ones, quite contrary to the
case of osmotic immobilization. Hence mechanism of immobilization
may differ in the two cases, as might be presumed from the low
osmotic value of the formalin used. Injury of outer epidermal cells
may, perhaps, be the cause in the present case.

Not all leaves treated as above, however, are immobilized, but
some of them close by stimulative action of formalin. These leaves
move generally 15–30 seconds after the immersion, but they do not
attain the completely-shut stage as they are immobilized already to
some extent. Here again we find an example of simultaneous action
of stimulation and immobilization, though here both are due to
injurious effects. If formalin is diluted, leaves are not immobilized
before they shut.[2]   This may be explained as follows : It takes

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1) For the observation, culture water is coloured beforehand with a coloured
acid indicator.

2) Even with undiluted formalin, leaves are often stimulated instead of being
immobilized, if they are left quiet after they are put in formalin, that is if they are
not in contact with strong formalin immediately. In this case, the effect must be
similar to the case of applying diluted formalin.

more time for diluted formalin to injure outer epidermal cells and make them lose turgor, than to stimulate sensitive hairs chemically. When leaves are immersed in various toxic solutions, not only of diluted formalin, but also of acids, alkalies, heavy metal salts or alkaloids, they close, viz. sensitive hairs are stimulated before the turgor of the outer epidermal cells are lost, though the latter are in contact with a far more concentrated solution than the former, at least for a while.   The immobilization before the shutting can take place only with solutions, the toxicity of which is so strong that outer epidermal cells are already injured while only a small amount of the toxic substance enters into the leaf cavity where sensitive hairs stand.   Undiluted formalin may have such specially strong toxicity.

If a leaf is brought back to pure water after staying in undiluted formalin for 45 seconds, which is the least necessary time for immobilization by this liquid, the leaf regains reactivity to tactile stimuli in about 20 minutes.   Hence the injury in the outer epidermal cells may have been reversible to some extent.   But the leaf thus treated shuts and narrows afterwards without receiving any external stimuli, perhaps because of progressing injury from remaining traces of formalin.   Leaves left in formalin for 1 minute (hence immobilized) shut " spontaneously " 5–10 minutes after being returned to water, some quickly and others slowly, followed by the narrowing movement.   If a leaf is left in formalin for several minutes it is entirely killed in the open stage, and does not close even if returned to water afterwards.

b.   Ethyl Alcohol

Generally leaves are made to shut by alcohol.   As the reaction time varies much from one individual to another, the result of

Table 20
Reaction time in alcohol in minutes.

Whorl No.	Concentration of alcohol in vol.-%			
	40	30	20	10
V	6	9.5	26	—
IV	3.5	8.5	27	90
III	2.3	10	23	—
II	1.3	3	15	—
I	0.8	1	6	—

measurement with leaves of a single individual is shown in Table 20. In 10% alcohol, leaves die in the open condition rather often.
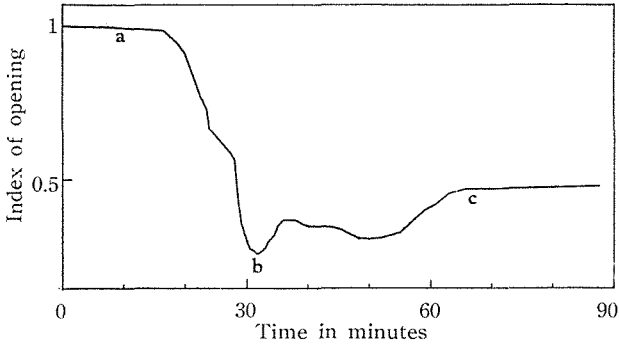

Fig. 14. Change of opening of a leaf (No. II) after immersion into 20% alcohol at 25°. The margin-distance in the open stage is made unity, and that in the shut stage 0.

Though most leaves shut quickly as in the normal reaction, some leaves shut slowly as shown in Fig. 14, a–b. But even if a leaf may shut quickly or slowly (completely or partially), an irregular movement generally follows (as Fig. 14, b–c, for example), perhaps owing to contraction or shrinking of cell walls in various parts of the leaf[1].

From time to time in alcohol solutions more concentrated than 40%, the shutting movement proceeds in two steps: for example, 2 minutes after the immersion into a 40% alcohol solution, a leaf

Table 21

Reaction time and pause in seconds, in the two-stepped shutting movement in alcohol.

Concentration of alc. (vol.-%)	Whorl No.	Reaction time of the 1st movt.	Pause in the intermediate stage
40	II	120	50
	II	100	25
60	II	25	25
	II	20	17[2a]
	III	30	15
	III	28	14[2b]
80	III	16	14
	IV	10	10

1) The shutting movement is due to the action of alcohol upon protoplasm, while minor irregularities during and after such action is due to the action upon cell walls.

2a, b) These two leaves showed slow movement in the second step. The duration of the second movement was 9 seconds in a, and 2 seconds in b.

of No. II suddenly closed half-way, and after a pause of 50 seconds it started again to shut completely, each step of the movement being performed rapidly just as in the normal shutting movement.   Re-action time of the first step and time of the pause in the intermediate stage are shown in Table 21.

Each step of the movement has a tendency to occur sooner, as the alcohol is concentrated.

c.   Chloroform

If leaves are dipped in saturated chloroform water, most of them close within a minute : some shut very quickly, some slowly, and some others start to shut quickly but the movement is retarded about the half-way through.   Even if leaves are brought back to water, as soon as, or even a little earlier than, they are stimulated by chloroform-water, they die afterwards, after attaining the ultimate narrowed stage or some intermediate stage in the narrowing phase.

A few leaves become, however, non-reactive in the chloroform water, though it is not decided whether this is due to loss of irritability or loss of motility.

If leaves floated in water are exposed to chloroform vapour for hours, most leaves shut, slowly or quickly, and then narrow. Those left open under this condition are still responsive to stimulation, even after a few hours, though their reaction is not always complete.

d.   Nitrogenous matter

A solution of peptone or egg-albumin added to the culture medium causes the leaves to shut (in an hour or so, the time differing according to the concentration) and then narrow.   If a piece of meat or dried fish is put in a leaf, it immediately shuts from the mechanical stimulation and narrows gradually afterwards by the chemical stimulus of the animal matter.   On the contrary, if a small block of gelatine-gel or coagulated egg white is given, the leaf reopens from the shut stage holding the block upon the inside. These materials seem not to produce stimulative substances, just as DARWIN said when he tested them upon *Dionaea*.

Physiological Changes Due to Aging

As the leaf whorls of *Aldrovanda* are arranged in their developmental order, a steady change of physiological behaviors due to aging can be observed.   In case the leaves of a shoot which de-

velops through a long period, as for instance that of a cereal (Richards, '34), comparison of leaves of different ages at the same time may often lead to a wrong conclusion concerning physiological changes related to the age of leaf, because there is a seasonal change of the shoot itself. But in *Aldrovanda* the development of successive whorls is very quick, ten to thirteen of them coming forth in ten days under good condition[1], hence differences in leaf behaviors from whorl to whorl may be attributed chiefly to the aging of leaves.

Though the data in the present chapter was obtained with out-door material, originally used for other researches, yet the facts may serve, to some extent, to illustrate the effect of aging.

Leaves of No. 0 are apparently premature, the three-layered regions being quite narrow, and those of No. I are also rather premature in general.[2] They seem to be mature in Nos. II and III, and to get senile gradually thereafter. Some of physiological changes due to aging may be ascribed to the alteration of proto-plasmic natures, and some others to a decrease in extensibility of cell walls.

1. Threshold of stimulation. Leaves become less sensitive to sudden changes of temperature as they grow older (II: Tables 6, 8). Number of stimuli upon a sensitive hair necessary to cause the reaction increases as the leaf grows older (II: p. 58).

Czaja ('24; electrical stimuli upon *Aldrovanda*) and Bose ('13, '28; electrical stimuli upon *Mimosa*) also affirmed that the sensitivity decreases as the leaf grows older. Both of them noted that pre-mature leaves are less sensitive than the fully grown young ones. So the writer wants to make a further study (using leaves of No. 0) on this point, to learn whether the same holds for the thermal stimulation.

Threshold value may depend solely upon protoplasmic nature.

2. Reaction time. Reaction time is measured by stimulating leaves with a constant current of 70 volts at various temperatures (10°–40°), the movement being recorded on a moving picture film. For every temperature the mean reaction time of every whorl is

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1) Under suitable conditions the plant grows continuously and has the same ap-pearance throughout the year; new whorls develop incessantly while old ones fall off in turn. Thus aging of the shoot hardly comes into question.

2) The whorl number of a leaf roughly indicates the number of days that have passed since they became reactive.

expressed in percentage of the mean of whorls I–V, and the values are averaged with respect to the whorl (Fig. 15). The period is the shortest with Nos. II and III. Probable error of the mean of No. 0 is the greatest among the six whorls, suggesting that the nature of leaf is most indefinite in this growing stage. That the reaction time becomes larger as the leaf grows older than No. V can be recognized even with mere observation. Similar determination is also given by CZAJA.
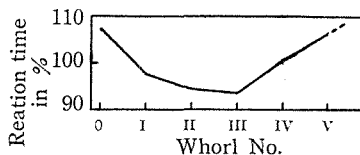

Fig. 15. Change of reaction time in relation to the age of the leaf, the average value being made 100.

As seen in Table 14, the time needed for KCl-solutions to stimulate leaves is least for whorl No. II (Saturated and 2.0 M solutions) or No. III (1.0 M)[1] and grows longer with their age. Reaction time of stimulation by acetic acid solution depends upon age in a similar way, except that the value of No. II is larger than No. I in some cases (Table 16).

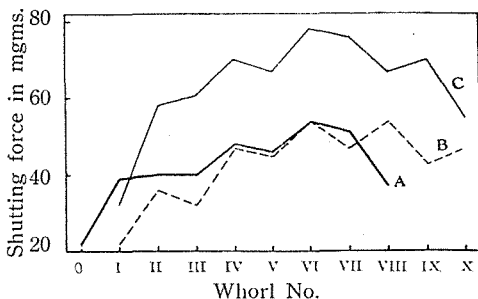Duration of the reaction time may also be dependent upon the protoplasmic state.

Fig. 16. Change of shutting force in relation to the age of the leaf, A, B and C representing three different individuals.

3. Shutting force. Three series of shutting force measurements by silica dynamometers (I: Tables 3, 4) are plotted in Fig. 16. In the case of B and C (from Table 4), the whorl numbered I is just about to part from the bud, hence it is in the state between Nos. 0 and I. The shutting force grows stronger with age and the maximum is reached when leaves become rather old, say No. VI. This maximum is shifted from the maximum of irritability which is represented by the threshold stimulus and the latent period.

As the shutting force may depend upon the turgor force of the outer epidermis (and the middle layer) and the degree of dif-

1) In the case of No. I in the 0.5 M solution, slowly reacting leaves are immobilized and are rejected from the mean, hence the value appears smaller than No. II, this being an exceptional case.

ferentiation in cell wall extensibility between the walls parallel to the lobe surfaces, either or both of the following two causes may account for the shifting of the maximum : 1) The turgor pressure does not weaken (but perhaps grows stronger) until the leaf becomes rather old, even though the irritability will decline early. 2) The walls of the inner epidermis, which resist the expansion of the outer epidermis to bend the lobe, become less and less extensible rather quickly, while the outer epidermal walls do not lose their high extensibility so soon[1]. And the decrease of the shutting force when the leaf grows older than No. VII may be ascribed to a decrease in the turgor of cells and a decrease of extensibility of the outer epidermal walls.

Velocity of the shutting movement depends largely upon the shutting force. Slowness of the shutting of very old leaves and of immature ones (I: Fig. 25A) is due to weakness of this force.

4. Reopening velocity. The time which elapsed between the instant when the leaf shut and the time when the lobe margins began to part was measured at various temperatures (10°–38°). Averaging the values with respect to the whorl, just as in the case of the latent period, the graph in Fig. 17, is gained. As the reopening is a growth movement, it is natural that the younger the leaf, the more quickly it reopens.
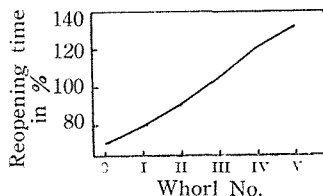

Fig. 17. Change of the reopening time in relation to the age of the leaf, the average value being made 100.

5. Other reactions. The younger the leaf, the sooner the immobilization by sugar solutions takes place (II: Table 9), and the slower the immobilization by formalin. It is already stated (p. 94) that the discrepancy may by due to the difference in the immobilizing mechanism.

When shut leaves are immersed in acetone solutions, the younger the leaf, the sooner occur both the narrowing movement and the vesiculation (I: pp. 190–). Young leaves may narrow very soon as the one-layered regions are more pliant than those of older leaves, the resistance against pressing out of water and the one due to the

1) The tendency for the extensibility of cell walls to decrease with age can be shown by measuring the extensibility (with a horizontal microscope) and the yielding tension of leaves of various ages (by the method as described on I: p. 167). The same tendency in the walls of the outer epidermis only, can be tested with acetone which makes the walls vesiculate out (I: p. 191).

evertion of the margins (I: p. 225, etc.) being smaller. Readiness to vesiculate depends on the extensibility of the outer epidermal walls.

General Conclusion

Aldrovanda and *Dionaea* have a special contrivance for capturing prey, the " steel trap type " of mechanism, called after LLOYD ('33), which is notable for its quickness of the movement. Among two submersed carnivores, *Aldrovanda* and *Utricularia*, it is only the former that is undoubtedly irritable, for the door function of the latter is asserted by LLOYD and some others to be mechanical.[1] Thus, *Aldrovanda* is a unique and interesting hydrophyte, the physiological behaviors of which are to be studied not only monographically, but also from a general standpoint.

At the start of the studies, a simple culture method was contrived, by which material was always at hand in the laboratorium throughout the year (I: p. 146). This helped the studies much.

With varied methods, the author could observe detailed processes of the leaf movement. As the mode of movement depends upon the age of the leaf and the temperature, the movement will be described here only with respect to the most active leaves, namely of the whorl No. III (II or IV), at 30°: —

In the open stage of the leaf, the margins of the two lobes stand about 3 mms. apart from each other at the middle. When the leaf is stimulated electrically, duration of the reaction time varies largely according to the intensity of stimulus, from 0.02 seconds when stimulated strongly, to nearly 1 second when stimulated very weakly. After this period the leaf shuts, the greater part —"the quick phase"— of this " shutting " movement being performed within 0.02 second or even sooner. The angular velocity of the movement is roughly 30-40 degrees per 0.02 second, or 1500–2000 degrees per second, in the mean, it being slow at the start and then accelerated. The quickest record of the movement of the lobe margin the author has ever measured was 16.1 cms. per second; at such an instant the angular velocity of the lobe must

1) Even if the door mechanism should be irritable in its nature, the door plays only an accessory role in the function of the utricle, thus it is proper to cross out the sentence, " *Utricularia* and *Aldrovanda* are the only submersed irritable plants, ..." from the fourth item on I: p. 143.

be greater than the above-mentioned average value. Then the shutting movement is much retarded when the lobe margins are about to touch each other, since pressing out of the water from the closed lobes become more and more difficult —"the slow phase."

The shutting movement occurs ordinarily when a small aquatic animal enters the leaf cavity and touches a few of the sensitive hairs. About a half of the water held between the lobes must be forced out when the leaf shuts, but the water at the bottom part of the cavity where the sensitive hairs stand, seems not to stream out much. Hence the animal which has touched the sensitive hairs is not carried out by the drift. Sometimes the drift or its active swimming towards the outside is obstructed by the marginal folds of the lobes. While the captured animal is swimming about in the closed leaf cavity, many sensitive hairs are stimulated repeatedly. Thus the leaf being excited strongly, the "narrowing" movement begins and proceeds gradually.

The narrowing movement is a bending movement of the lobes which renders the volume of the leaf cavity minimal, the one-layered region of the free-side lobe (I : p. 149) being everted and laid closely upon the corresponding region of the opposite lobe. A half to a few hours is needed for this slow movement to be completed, making a striking contrast with the shutting movement.

Leaves stay in the narrowed stage while they are stimulated by (captured) animal matter, but those which have been stimulated electrically (not very strongly) begin to "rebulge" after a pause of a half to several hours in the narrowed stage. The rebulging movement takes the reversed course of the narrowing, and at last the leaf becomes open through the "reopening" movement, which occurs continuously after the rebulging. Leaves which have been stimulated not so strongly as to cause the narrowing, reopen directly from the shut stage.

That the midrib is not the seat of the responsive movement is shown by comparing the back and the lateral views of open, shut and narrowed leaves (I : p. 159). With cross-sections of living leaves imbedded in agar-gel, it is determined that the narrowing movement is caused by the more and more acute bending of the lobes at a zone near the midrib in each·of them—"the motile zone" (I : Fig. 11). That the shutting movement is also carried out by this zone is understood if a shut leaf is observed from proper directions, as compared with an open leaf.

The site of the motile zone also can be observed in other ways, as it is differentiated from the other parts of the lobe in many respects: the outer epidermis of the zone is undulated when the leaf is open (I: Fig. 2, Pl. IX; Fig. 13); blisters are raised on the outer surface of this zone by the action of acetone or ammonia (I: Figs. 3-5, Pl. IX); the zone is blackened most deeply by osmic acid as, perhaps, tannin is contained richly in the cells (I: Fig. 14); the zone is coloured most lightly by iodine as the starch content is the least.

The walls of the inner epidermis have small extensibility, while the " outermost wall," and next to it " the outer subepidermal wall " (I: p. 152), is easily extensible in the motile zone. Such an arrangement of walls of different extensibility is essential for the responsive movement.

Little consideration has hitherto been paid to the motor mechanism of the *Aldrovanda* leaf. The movement has even been believed to be caused by the midrib. As to *Dionaea*, some theories have been advanced, but not one of them is satisfactory. Such misinterpretation as that the bending of a sectioned piece of a leaf is caused by the shutting mechanism, may have hindered the correct consideration of the fact. The author's studies have indicated that it is not the shutting, but the narrowing mechanism that is working in that case. And an adequate theory is advanced.

Though both the shutting and the narrowing movements are due to a bending of the lobes, it is clearly established from the process of exertion, as well as the absolute strength, of the causative force (I: Fig. 29, A, B), that the two movements are due to different mechanisms. The writer's theory (briefly delivered on p. 57) will be discussed here.

Possible mechanisms which have a seat in the outer epidermis or the middle layer may be four in all:—

1. Increase of osmotic value of cell sap,
2. Decrease of solute permeability,
3. Increase of extensibility of cell walls, and
4. Swelling of plasm-colloids.

But it is improbable that any one of them plays an active part in the quick shutting movement:—

1) From the curve in Fig. 8, the leaf cells of *Aldrovanda* may be regarded as no exception to plant cells in general in which water osmosis goes on only slowly. Even the quickest change of cell volume in HUBER and HÖFLER's protocols is 9% in 3 seconds,[1] this

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1) Deplasmolysis of *Hookeria* cells in 0.3 M NaCl-solution, after a stay in hypertonic 0.40 M NaCl-solution, p. 479, '30.

time is a hundred times as long as is required for the shutting movement of *Aldrovanda* leaf, even though the permeability may be increased to some extent in their case[1]. Hence it is unreasonable to expect semipermeable protoplast to let water in quickly enough for the movement.

2) Permeability for solutes and for water is considered to alter in parallel with each other (cf. LEPESCHKIN, '34), so the case is less probable than the foregoing.

3) It may be difficult to assume that cell wall extensibility is altered in so short a time as the latent period of the movement.

Moreover, intake of water seems not to take place in the case of *Dionaea*, for when a lobe is cut off quickly from the leaf, it curves just to the extent it might show after the normal shutting movement, showing that the shutting movement can take place without any supply of water from outside of the lobe.

4) As the plasm-layers are thin, it cannot be expected that they may *stretch* themselves along the long axes of the cells, over-coming the great resistances of water working against the quickly moving lobe, and of the turgidly standing inner epidermis.

On the other hand, the following two facts are favourable to the idea of ascribing the shutting movement of *Dionaea* to a sudden change in the inner epidermal cells : a) According to BURDON-SAN-DERSON ('88), change of electro-motive property due to excitation has its seat at or near the upper surface. b) Whereas a slight wounding of a few of the inner epidermal cells with a razor causes excitation of the leaf, deeper cutting into the outer side does not. Hence the inner epidermis is irritable to traumatic stimuli, while the outer epidermis is not.

Thus it may be understood that it is not the outer epidermis that gives a start to the shutting movement, though the turgor force of its cells is indispensable in bending the lobe when the statical equilibrium is released by the reaction of the inner epidermis.

With respect to the shutting movement, the leaf may be comparable to a steel trap : the open stage is the set condition of the trap, the outer epidermis and the middle layer together serving as the spring and the inner epidermis as the prop, and when the prop becomes no more sustainable the trap springs at once. The arrange-

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1) According to GAHLEN ('34), for example, NaCl made protoplast of *Helodea* very permeable to it sooner or later.

ment of cell walls of different extensibility in the motile zone is in accordance with the theory. The theory accounts best for the mode of reaction, viz. the short reaction time followed by the instantaneous exertion of the maximal force which lasts almost unchanged for about half an hour (I : Figs. 24B, 25B). The loss of sustaining force of the inner epidermis may be caused either by loss of turgidity of the cells due to a great increase of permeability, or by the contraction of the protoplast (without change of cell volume), and it is not decided for the present which of the two cases actually takes place in the reaction.[1]

Even Guttenberg ('25) was disposed to take a theory similar to the present one, though he gave it up since he thought that the following facts found with *Dionaea* were inconsistent with the theory. But it may be easily understood that the latter is not the case :—

a) The lower epidermis of *Dionaea* leaf is less extensible than the parenchyma layer just underlying it.—But the swelling tissue as a whole bends inwards even if the lower epidermis covers the outer (convex) surface of it (I : p. 179, c).

b) According to Brown the inner epidermis hardly shrinks when the leaf shuts. —But the data given by him are shown only in his Table III ('16). Leaves which have undergone the operation of making a window in a lobe to observe the inner surface of the opposite lobe, can react in a far smaller degree than normal leaves. Hence the shrinkage of 4.5 or 2.5% (in his table) is not small, shrinkage "between extreme dots" having little meaning, since the region of a lobe where the greatest changes are observed is important for the movement.[2]

c) A leaf does not shut when its upper surface (except the sensitive hairs) is covered with glycerin.—If only the cells near the upper surface lost turgor, the movement of lobes would take place, but the fact that the responsive movement becomes very weak after such treatment, shows that the treatment gives rise to a general decrease in turgor in the whole tissue of the leaf (see also I : p. 181, b).

d) A leaf does not shut when immersed into boiling water suddenly.—This cannot be unfavourable to the theory of attributing the movement to a loss of turgor

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1) The movement of *Berberis* filament is ascribed to a mechanism like the former by Bünning ('29), and to one like the latter by Colla ('33). *Berberis* filaments become shorter as a whole when it reacts, while such contraction is not observed with *Aldrovanda* and *Dionaea* leaves. But the difference is due only to the fact that the walls of the reactive cells (viz. the inner epidermal cells) of *Aldrovanda* leaf is not easily extensible and shows little elastic contraction when the turgor disappears.

2) Roughly speaking, the inner subepidermal wall of *Aldrovanda* leaf may not change its length (transverse to the midrib), and as the proportion of the thickness of the inner epidermis to that of the outer two layers is 3 : 16, the outer surface is stretched 16/3 times as much as the contraction of the inner surface. Expansion of the outer surface may be due both to stretching of the undulation of the outer surface (I : p. 164-5), and to plastic extension of the wall, while contraction of the inner surface may be caused by undulating of the wall rather than by elastic contraction of it.

of some cells, for it is the case only when the *Dionaea* leaf is so dull that the parenchyma cells are injured by heat not later than are the inner epidermal cells. *Aldrovanda* leaves and sensitized *Dionaea* leaves react and shut when dipped into boiling water (I: p. 181, c).

Moreover, GUTTENBERG's theory of swelling tissue can no longer be asserted because two of the four facts supporting that theory were observed with sectioned leaves, namely with those in the narrowing phase, while the other two are in accordance also with the present writer's theory.

The writer ascribes *the narrowing movement* to the mechanism which GUTTENBERG asserted to be the cause of the quick closing movement, viz. the present "shutting" movement: the "narrowing" movement of the *Dionaea* leaf is caused by an increase in turgor of the parenchyma cells. In the *Aldrovanda* leaf, the increase in turgor takes place chiefly in the outer epidermis. The theory is supported by the following facts found with *Aldrovanda* leaves.

a) The narrowing movement is due to a sucking in of water by some cells in the leaf: for, the movement ceases almost as soon as the leaf is soaked with a plasmolytic solution (I: p. 189), this being quite different from the case of the shutting movement (II: p. 76).

b) Suction pressure of the cells which exert the force to bend the lobe is higher in the narrowing phase than in the shutting phase: for, turgidity of cells sufficient to bend the lobe is lost by a 0.15 M sucrose solution when the leaf is causing the shutting movement, but the cells in question can suck in water from the same solution when the narrowing movement is going on (I: p. 190).

c) The movement can occur if the turgor pressure of the outer epidermis (and perhaps also of the middle layer) is raised: for, the movement proceeds very quickly if the outer surface of a shut leaf is soaked with an acetone solution (I: p. 190).[1]

d) The movement can proceed no more if the turgor of outer epidermal cells is lost from vesiculating or bursting due to entrance of much acetone (I: p. 191).

e) That the turgor of outer epidermal cells is raised after the leaf is excited strongly, is suggested from their swelling form when the narrowing movement is restrained (I: Fig. 18A), while the

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1) Cells of the motile zone of *Aldrovanda* leaf are to be classified with "the expansion type" ("Ausdehnungstypus") of ZEHETNER ('34).

puckered form of the inner epidermal cells when the leaf is narrow-
ed indicates that these cells are bent passively (I : Fig. 18B).

The following two facts are also favourable to the theory. (1) When a lobe is
cut off from a *Dionaea* leaf it bends a little, but never more until it is supplied with
water. This shows that water supply is indispensable for the narrowing movement,
being quite different from the case of the shutting movement (II : p. 104). (2) The
bending movement of *Drosera* tentacles, which is considered to be analogous to the
narrowing movement of *Aldrovanda* and *Dionaea* leaves, is caused by growth on the
convex side and the median section, after HOOKER's measurement (I : p. 195).

Both of the recovery movements, *rebulging* and *reopening*, are
ascribed to an accelerated growth of the inner side : for, the great
extension of the walls on the outer side is chiefly of plastic (ir-
reversible) nature ; BROWN has shown this to be true by measuring
the elongation of the lobe surfaces ; and " the boundary-distance "
at the shut stage becomes larger after the rebulging movement,
than before narrowing.

Questions remaining are : a) To what extent does the middle
layer of *Aldrovanda* leaf play an active part in the narrowing
movement (though it may be less active than the outer epider-
mis) ? b) Are the cells which react actively at the shutting move-
ment localized solely in the inner epidermis or also in a few
layers beneath it, in the case of *Dionaea* ? and c) Do the outer and
the inner halves of the parenchyma of a *Dionaea* leaf act differ-
entially in the narrowing phase ?

In the latter half of Part I, the processes of the movement and
of the force causing it are compared with each other, and hin-
drances against the movement causing the difference between each
process are elucidated.

In the present part of the studies, effects of various agents are
studied mostly in connection with the shutting movement, little
observation being made for the narrowing movement, as it takes
much time and trouble owing to the slow progression of the move-
ment, though it should be studied some day, of course.

The investigation which can be done easily only with *Aldrovanda*,
and perhaps never so precisely with other plant, is that of the effect
of thermal shocks. For this investigation, the submersed habits,
the shortness of the latent period, and the conspicuous manner of
the reaction of the leaf are excellent. It has required a series of
only simple experiments to determine how the threshold value of
thermal stimulation changes with change of initial temperature.
And the writer wishes to contribute to the question of thermal
adaptation with this material, in the near future.

Changes of a physiological nature due to aging may also be studied with this material rather conveniently. In the present paper, some changes related to irritability are dealt with, only to the extent of orienting the question in certain respects.

The submersed habit of this plant is convenient also for studies of osmotic and chemical influences. So an example of osmotic stimulation, which has been unknown among plants, is found: Leaves are stimulated when they are transferred from water to a plasmolyticum, or from this back to water, though the reaction often does not occur in the first case as the solution has the immobilizing effect. Increase of permeability due to a change of environmental osmotic pressure (when there occurs no plasmolysis) is observed.

With respect to chemical influences, however, the leaf has some drawbacks as a material for experiments, so that no interesting studies have been done in this field. The writer has only been able to classify chemical substances according to the way in which they affect the leaf, and to analyze the interference of an immobilizing (osmotic) effect of a chemical solution with the stimulating (chemical) effect of the solution itself. Thus the complicated behavior of leaves when dipped in salt solutions become clearly understood.

The leaf is useful for the purpose to determine whether a certain solution is injurious to the plant or not, since the leaf shuts when even a very slight intracellular change takes place. The method however is possible only when the solution has not so high an osmotic value as to immobilize leaves.

No characters of the leaf have yet been found especially excellent for studies of mechanical and electrical stimulation.

Summary

VIII. Mechanical stimulation

1. Deformation of joint cells of sensitive hairs (p. 58), or of cells of the motile zone in the leaf lobe (pp. 59–60), causes irritation in the leaf.

2. The leaf does not usually react to a single seismonic stimulus upon a sensitive hair, but to repeated stimulation upon a hair, or single stimuli upon a few hairs (p. 58).

3. Changes in hydrostatic pressure do not stimulate the leaf (p. 61).

IX. Electrical stimulation

4. A key which can afford single make or break induction shocks has been devised (p. 62).

5-a. Stimulative effect of induction current differs according to its direction in relation to the leaf (p. 63).

–b. Sensitive hairs are not the chief receptor of electrical stimulus (p. 64), and cells of the motile zone are more sensible than any other cells of the plant (p. 65).

–c. Cells of the motile zone seem to be more sensitive to electric current when this flows lengthwise than when it flows across them (p. 66).

X. Thermal stimulation

6. Most young leaves close within an hour at 42˙ (Table 3), but only 10% of them shut within 7 hours at 41˙ (Table 4).

7-a. The leaf is stimulated by a sudden rise or fall of temperature. Difference of temperature just sufficient to cause stimulation is determined (Tables 5–8):

–b. Falls of temperature make the leaf shut more easily than rises.

–c. The higher the initial temperature, the more sensitive is the leaf to a rise of temperature, and the less so to a fall.

–d. The older the leaf, the less sensitive it is to a thermal shock, either a rise or a fall.

8. Stimulation by a temperature change is not due to a change of volume of intercellular air (p. 72).

XI. Osmotic influences

9. The leaf receives from sucrose or glycerin solutions two antagonistic effects: 1) they are made immotile (pp. 76– ; the opening being made a little wider at the same time—p. 78), and 2) they are stimulated by a sudden increase or decrease of the environmental osmotic pressure—the osmotic excitation (pp. 80, 82).

10. There are two types, quick and slow, in the movements of leaves which are osmotically stimulated (Figs. 8–11).

XII. Influences of chemical agents

11. Chemical substances may be classified into four categories, according to their mode of affecting *Aldrovanda* leaves (pp. 84–5).

12-a. Neutral salts stimulate the leaf (Tables 14, 15), though their osmotic pressure works to immobilize it at the same time (p. 86).

–b. Time relation of the two effects is shown graphically (Fig. 13).

13-a. As acetic acid, HCl and NaOH stimulate the leaf strongly, interference of osmotic immobilization is not perceived with their solutions (p. 88). (With boric acid, KH_2PO_4 and Na_2CO_3, however, the interference is observed—p. 90).

–b. Variation in the reaction time of stimulation by acetic acid is smaller than by HCl (Table 17), NaOH and KCl (p. 89).

14. Increasing the osmotic pressure of acetic acid solution by addition of sucrose (Table 18), the following is verified : Whether a leaf is stimulated by the mixture and shuts, or whether it remains open, immobilized by it, depends on which of the two effects, stimulation or immobilization, appears sooner (p. 90). The same question must be considered also when leaves are immersed in solutions of sugar or neutral salts (pp. 83, 87).

15-a. Permeability (or " intrability ") for acetic acid of irritable cells is raised when their environmental osmotic pressure is changed (p. 91), though no plasmolysis occurs in them (p. 92).

–b. With glycerin-acetic mixtures, a change in concentration of glycerin alone is more effective to raise permeability than a change in the entire concentration, glycerin plus acetic (p. 93).

16. A chemical stimulus seems to be perceived in the first place by sensitive hairs (p. 94).

17. The leaf is injured by concentrated formalin, passing a stage in which it is made reversibly non-reactive to seismonic stimulation (pp. 94-5).

18. Ethyl alcohol makes leaves shut, in a quick or a slow movement, or in two steps (pp. 95-6).

19. Chloroform makes leaves shut and narrow, and injures them, the state of narcotization being difficult to observe (p. 97).

20. Water-soluble nitrogenous matters make the leaf shut and narrow, but water-insoluble ones do not (p. 97).

XI. Effects of aging

21. Changes, in relation to leaf age, of threshold stimulus (p. 98), reaction time (Fig. 15), shutting force (Fig. 16) to which velocity of the shutting movement depends, and velocity of the reopening movement (Fig. 17) are reviewed.

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