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

I. Process and Mechanism of the Movement

by

Joji ASHIDA

(Botanical Institute, Kyoto Imperial University)

With Plates IX-X and 36 Text-Figures

Contents

Introduction

The following characteristics may be singled out as making of *Aldrovanda*[1] specially good material for the study of the physiology of irritability :

1. Its responsive movement is of the quickest type found in plant movements.

2. Its leaf has a very simple structure, and is transparent.

3. As the whorls are arranged in order, the age of a leaf is known at a glance, and leaves of any desired age can be selected for observations. This is difficult with *Dionaea*.

4. It is a submerged plant. *Utricularia* and *Aldrovanda* are the only submerged irritable plants, and while the reaction of the former is not very conspicuous, that of the latter is easily recognizable with the naked eye. The submerged habit renders it convenient for study in a good many respects :

a. Regulation of temperature is easier and more accurate than in the case of land plants, as the specific heat of water is high. It is also no difficult task to subject the plant to sudden changes of temperature.

b. No attention need be paid to the atmospheric humidity.

c. Land plants must be in an abnormal condition when they are immersed in water, or in an isotonic solution, whereas submerged ones are quite normal in ordinary water. The latter are therefore more fitted for experiments with plasmolytic agents or chemical stimulants.

As a material for the study of irritability, however, the plant is not without defects. The most marked one is that the leaf recovers very slowly from the reaction. Though this character has teleological significance, it makes some of the tests which are commonly applied to animal muscles or *Mimosa* pulvinus, such as determination of the threshold stimulus etc., difficult.

Of the various irritable plants, *Mimosa* has been much studied by many authors, while the sensitive filaments or stigmata of *Berberis*, *Sparmannia* etc., have been precisely investigated by BÜNNING and others lately. These plants are utilized for the study of nastic movement, or even of irritability in general in the vegetable kingdom. Having the characteristics mentioned above, *Aldrovanda*, too, should serve for the same purpose. Little is known, however, about the

---

1) The writer will use the spelling *Aldrovanda* after LINNE's "Species Plantarum" instead of *Aldrovandia* which is grammatically correct as CASPARY ('62) points out.

process and the mechanism of the leaf movement, and also about the effects of various forms of stimulation. The aim of the present investigation, therefore, is to cast as much light as possible on the leaf and the leaf movement of *Aldrovanda*, so as to qualify the plant as a material for the study of irritability.

Authors have hitherto been inclined to deduce conclusions about *Aldrovanda* from results obtained in experiments with *Dionaea*. In the present studies, however, the contrary method is adopted; observations are made chiefly upon *Aldrovanda*, and the results are extended to the case of *Dionaea*. Thus, important features difficult to unveil from researches upon *Dionaea* alone are found out, and then inconsistent notions about the motor mechanism are corrected.

The scantiness of physiological studies upon *Aldrovanda* may be, at least partly, due to the difficulty of cultivating it. But the writer has succeeded in cultivating it in the laboratory all the year round, and so has been able to carry on the study of it with ease. Hence this paper deals first with the appropriate cultural conditions.

One of the most interesting parts of the research described in this paper is the measurement of the reacting force of the leaf; the changes in that force being traced, it is made clear that the responsive movement of the *Aldrovanda* leaf proceeds in two steps, the shutting and the narrowing, differing in strength and velocity. The process of the leaf movement and that of the force acting are also compared, and factors that hinder the movement, such as resistance of the lobe margins, water etc., are analysed.

The most probable mechanisms are proposed for both of the movements. Then, on the assumption that the same may hold good also for the *Dionaea* leaf, the experimental results obtained with it by many authors are quite clearly explained, as well as those obtained with *Aldrovanda* by the present author.

The effects of various forms of stimulation, and statistical studies on the latent period and on the reopening velocity at various temperatures will be given in Parts II and III, which will be issued before long.

Before going further, the author desires to express his sincerest thanks to Prof. Dr. K. KORIBA, for his careful guidance and criticism in the investigations. His gratitude is also due to Mr. TSUCHIYA, lecturer, on whose suggestion the recording of the quick phase of the shutting movement was undertaken.

Historical

The authors who have worked on *Aldrovanda* will be mentioned in chronological order :[1]

HAUSLEUTNER (1850) says that the method of cultivating this plant is a very simple one and requires little attention, and remarks on the necessity of protecting it from winds and direct sunshine. He says nothing, however, about the culture medium.

F. COHN (1850) describes, with several figures, the leaf, the stem and the hairs on them, as well as their ontogenesis ; and he makes it clear that the leaf is not a " vesicule " but is made up of two lobes.

Fr. SEYBOLD (1852) writes on the turion.

R. CASPARY (1859) mentions a great deal of taxonomic literature. Detailed morphological descriptions are given in his work, with more illustrations than ever, and the habits of the plant are also described. (His work is summarized again in " Flora, " 1859, pp. 140-144).

M. AUGÉ DE LASSUS (1861) was the first to discover that the leaf is open and irritable if the plant is vigorous. He also found that young leaves move more actively than old ones.

R. CASPARY (1862) introduces AUGÉ DE LASSUS's discovery. In continuation of his previous paper, he gives the ontogeny and the mode of life of the plant.

B. STEIN (1873) found out the irritability, independently of AUGÉ DE LASSUS. (Bot. Zeitg. 1874, p. 389).

F. COHN (1875) remarks that the long hairs on the leaf are sensitive to touch, this being suggested by analogy with the case of *Dionaea*. He also deals with the carnivorous mode of nutrition of this plant.

C. DARWIN (1875) compared the structure of the leaf with that of *Dionaea*, and made some experiments on the absorbing power of various processes on the epidermis.

S. KORZCHINSKY (1886) studied the anatomy and germination of the seed.

K. GOEBEL (1891) demonstrated the structure of the leaf with a half-schematic sketch of its cross section.

C. A. FENNER (1904) gives the newest and most precise morphological description of the leaf. He concerns himself chiefly with the ontogeny of the leaf and of the glands born on it, but without making any physiological experiments.

G. HABERLANDT (1906) examined the sensitive hairs in detail.

A. TH. CZAJA (1924) is perhaps the only one who has experimentally studied the irritability of *Aldrovanda*. He tried various kinds of stimulation, mechanical, electrical, thermal and chemical. But he is mistaken in various respects, and in general more investigations are needed. His results and conclusions will be discussed in their proper place.

P. A. NIKITIN (1927), comparing fossil seeds with the recent ones, came to the conclusion that *Aldrovanda vesiculosa* " is to be regarded as a relict, which is dying out."

F. E. LLOYD (1933) recently published a monograph concerning carnivorous plants, in which many fine pictures are given.

If the literature is scrutinized, it will be seen that the morphology and the ontogeny of the leaf and its appendages are known rather

---

1) Text-books which contain no original investigations (PFEFFER's, JOST's, ARBER's etc.) are not mentioned, nor are taxonomic papers.

well, whereas the irritability has been experimentally studied by no
more than one author, and hardly anything has been revealed about
the physiology of nutrition.

Conditions for Cultivation

Aldrovanda can not be cultivated well even if all the conditions noticed by Hausleutner
be fulfilled, and Caspary (*b*) regards the method of cultivation as one of the unrevealed
facts about this plant.　Czaja used water brought from the habitat, but according to the
present author's experience, *Aldrovanda* can not be kept in normal condition with this medium
for a long period of time.　And even if that were possible, it would be very troublesome to
bring much water from the locality to the laboratory.　Hence, in order to study the plant
experimentally, it is essential in the first place to discover a suitable medium and the other
conditions necessary for its cultivation.　The results of some tests made for this purpose
will be stated in this section.

i. Chemical nutrient solutions

If the plant is placed in water taken from the university mains, it grows weak, much
sooner than other water plants; the blades of the newly developing leaves become smaller,
and the apical bud grows slender and abnormally shaped, even within a week. The question
of nutriment is therefore the first that arises.

Various well-known inorganic mixtures for water culture were tried for the plant, but
they brought about no better results.　For example, in Benecke's solution which Pringsheim
recommends for the culture of fresh water algae, the leaf-blades and buds became abnormal,
and after a week, some plants even perished with the leaf-blades turned black.　Unsuitable
as the solution was, yet that with a total salt concentration 0.04% was, so far as the author's
tests went, rather better than the 0.08% solution, and this again was less harmful than 0.12%
or 0.01% solutions.

Thus, no inorganic culture medium could be found which would keep the plant in the
normal form even for a week.　The addition to it of some organic matter, however, such as
asparagin, peptone or the like, inevitably causes putrefaction of the medium.　Though it is
not definitely known whether these nitrogenous compounds can nourish the plant or not, those
media which putrefy in the unsterilized culture of the plant are of practically no use.

ii. Imitation of the habitat water

In the natural location,[1] *Aldrovanda* is chiefly found floating among *Typha*, *Zizania*
or *Phragmites*, but seldom among *Nelumbo* and others.　This suggested to the author that
there might be some material relationship between those plants and *Aldrovanda*, though
Hausleutner thinks that the helophytes merely protect *Aldrovanda* from the sun and wind,
but, acting on the idea that dead leaves and stems of helophytes belonging to the Gramineae
or Typhaceae may have made the water suitable for *Aldrovanda*, the author dipped remains
of those plants in culture basins and large beakers.　The result of this trial culture was

---

1) We have plenty of the material in two marshy ponds, *Ogura* and *Mizoro*, in the
suburbs south and north of the city of *Kyoto*. The plant was originally found in the former
and then transplanted into the latter, where it grows luxuriantly just as in the former, except
that the plants in the latter have rather longer internodes and look somewhat more slender
than those in the former.

satisfactory. In the water thus prepared, *Aldrovanda* grew quite normally, flowered in season and even bore seeds.

It should be noted, however, that the plant remains to be used for the culture must be sufficiently decayed, or they will rot in the culture vessel, and the contamination will injure *Aldrovanda*. Old leaves or stems of *Typha*, left sunk on the bottom of a pond for a long time, may be most suitable for the purpose. The medium remains perfectly good however much of this decayed matter may be put into the culture vessel, while, on the contrary, it does not act well if not enough is added.

Besides the plant remains from the habitat, an infusion of peat may also be used, though the result is not so satisfactory. Decayed leaves and stems of meadow grasses also seem to suit the purpose, though it is difficult to gather sufficiently weathered materials at times. Soil rich in organic matter is not always favourable. Various concentrations of Merck's fumic acid gave no better results than a very dilute infusion of *Typha*.

A decoction obtained by autoclaving decayed leaves of *Typha* is not so good as the infusion of raw material, whereas autoclaving such leaves with a small amount of KOH, followed by neutralization with HCl, furnishes a good medium. However, these decoctions are of no use for the present purpose, for their preparation is troublesome, and worst of all, they inevitably putrefy in a few days.

It is quite unknown why the infusion of plant remains is favourable for the life of *Aldrovanda*. Whether it serves as nutriment, or is important in buffering acidity, or rather is necessary for maintaining an ecological balance of micro organisms or of animals of prey is an open question. When *Aldrovanda* cultivated in a large beaker, the wall of which is covered with a layer of paraffin, is compared with that cultured in one not covered with paraffin, the latter may manifest an injurious effect due to the glass if tap water or an inorganic nutrient solution only is used as the culture medium. But if the medium is the infusion above recommended, *Aldrovanda* grows quite normally whether the glass wall is covered with paraffin or not. The cause of this fact will also be cleared up when the questions mentioned above are solved.

iii. Suitable culture conditions

The culture of *Aldrovanda* was tried with success in three different conditions, namely (1) in a dark room in which experiments were carried on with electric illumination, (2) outdoors in the shade and (3) in the sun, using the above mentioned medium.

1) Earthen ware basins, ca. 50 cms. in diameter and 20 cms. in depth, was filled with tap water and plant remains were placed in the bottom to a depth of about 10 cms., 100–250 watt electric lamps being used as the source of both light and heat. The distance between the water surface and the lamp was adjusted so as to maintain the water temperature near 25°C. Tap water was added from time to time, and if the water surface became covered with a film of bacteria, this was removed as far as possible, ladled out with a beaker. In this way, it is possible to have *Aldrovanda* in normal state throughout the year. Even in winter, the plant can be supplied for experimental use by putting turions into the basins, for the turions thus brought into the warm water at any time commence to grow immediately, and some whorls with sensitive leaf-blades appear in a week or so. This fact, together with the CASPARY's note (a) that *Aldrovanda* does not form turions in India, demonstrates that the rest period of the plant in winter in temperate regions is only a forced one.

Some individuals bear flowers in winter, most often in March, but the blooms do not open out wide, and there is no typical postfloral bending of the pedicels. This may be conditioned by the temperature, for in nature the blooming occurs at midday in summer when the water temperature is over 30°C. If the culture of *Aldrovanda* is left quite still for a

few weeks, every individual directs its apical bud towards the centre of the basin, above which the electric lamp hangs, owing to a phototropic growth.

2) Culture vessels were prepared in the same way as in the foregoing case, but were put outdoors on the north side of a building. The plant grows under diffuse sun light. Rain supplies water and cleans the water surface.

3) For the cultivation in the sun, rather deep pots were used. A few stocks of *Zizania* or *Typha* planted in the pots constantly supply the necessary constituents. It is better to bury the pots in the ground to avoid over-heating in summer. In these pots, *Aldrovanda* repeats its complete seasonal life for years, just as in its habitat.

In the other two cases, in which *Typha* or the like is not planted living, attention must be paid at times to the condition of the medium ; for example, plant remains must be supplied anew when the culture water becomes less brownish. If, by chance, insufficiently weathered plant remains are introduced, putrefaction of the medium will ensue. Thus, the cultivation of *Aldrovanda* without living *Typha* has more chances of being interrupted than that with living *Typha*. And the cultivation in the sun with living *Typha* seems to be as safe as leaving the material in the habitat. The author has been cultivating it in this way for the past three years, without taking any care of it except to reduce multiplying individuals.

Each of the cultures in the three different conditions, however, has its own advantages. The material cultured in the room is used for observations during the cold season, namely from October to April ; that in the sun is available during the warm seasons ; whereas that in the shade is useful in midsummer, when the material in the sun seems to be somewhat weakened by overheating.

Many other water plants and algae seem also to find this medium suitable. *Spirogyra*, for example, which is said to be difficult to cultivate for long, grows luxuriantly in it. Care must therefore be taken not to introduce even a few filaments of this alga, or of *Cladophora*, *Hydrodictyon* etc. into the vessels, because they will make it awkward to take out the very sensitive *Aldrovanda* from the vessel without irritation. Culture in an aquarium in a green house may be possible if some helophytes belonging to the Gramineae, Cyperaceae or Typhaceae are grown sufficiently in it.

Morphological Notes

Though studies upon the morphological nature of the *Aldrovanda* leaf have been given by many authors, yet they are not sufficient if one wants to describe and analyse the leaf movement minutely. Hence morphological features which are directly concerned with the present studies will be given with some new definitions.

i. The leaf-blade in relation to the axis

The leaves are arranged in whorls round the stem. The petioles are thin and broad, and terminate in a leaf-blade and four to six bristles[1]. The relation of the leaf-blade and the bristles to the petiole,

---

1) HEGI has collected the opinions of various authors as to whether the so-called " petiole, " " leaf-blade " or " bristles " are homologous organs or not. But, in spite of these morphological discussions, the commonly used names are employed in the present studies.

and of the petiole to the axis is shown in Fig. 1.

Fig. 1 A is the apical view of a whorl, five out of eight leaves being cut off at their petioles. The three leaves, *a*, *b* and *c*, represent the three characteristic stages of the reaction which will be described later. The petioles exhibit their whole breadth in this view. Fig. 1 B is the front view, all the leaves except *a* being cut off at their petioles. The petiole, thin in this view, is attached to the axis a little obliquely. The angle of insertion is small in a young whorl, and become larger as the leaf grows older.

The dark straight line which runs through each petiole at the middle is the conducting bundle, which extends to the midrib of the bilobed leaf-blade. The bristles stand roughly in a row, on the basal side of the leaf-blade. The leaf-blade is twisted to the left by more than 100 degrees, its midrib making an obtuse angle with the petiole both in the apical and in the lateral view of the whorl (Fig. 1 A, *a* and B, *a*). Thus its two lobes are situated not right and

Fig. 1 A. A whorl viewed from the apical side, five leaves being severed at the petioles. *a*, an open, *b*, a shut and *c*, a narrowed leaf ×3.5

B. Front view of the same whorl, leaves except *a* being cut off

left but rather up and down, and hence they are called by FENNER " die obere " and " die untere Spreitenhälfte." But as the dorsal side of the lamina is made to appear to be slightly ventral by the pronounced twisting, it is more convenient to name them *the free-side lobe* (die obere S.) and *the bristle-side lobe* (die untere S.), the designation being convenient especially when isolated individual leaves are treated.

The older internodes and leaf whorls die away successively as new ones are formed at the apex. In order to indicate each whorl according to its age, the author will use CzAJA's idea. He writes " ...., erhielt die jüngste, dessen Blätter schon funktionsfähig waren, und die sich durch ihre abstehende Haltung von der Knospe deutlich abhoben, die Nr. I, die folgende Wirtel zählten weiter." Rather frequently there are cases in which leaves are already capable of functioning but the internode between their whorl and the apical bud is not long enough to make the leaf stand apparently off the bud. In these cases, the whorl will be denoted No, O,
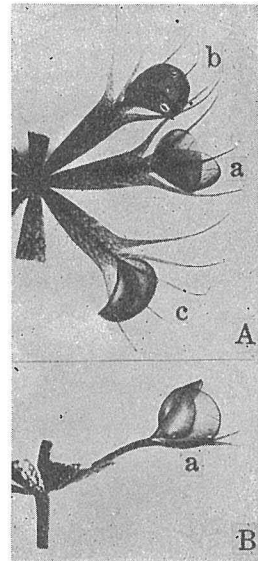
ii. The leaf-blade

A leaf-blade consists of a pair of lobes, which are convex out-wards and connected together by a midrib. Each lobe consists of two portions, the central and the marginal. The former is composed of three cell layers, the *inner* (morphologically the upper) and the *outer* (lower) *epidermis* and the *middle layer*, and may be called the *three-layered region*. The latter is thin, and notwithstanding that it is morphologically considered to be formed of two folded layers of epidermis, it looks as if it were one-layered, and will be called here-after *the one-layered region*. The boundary arc line between these two regions is called by FENNER "die Verschlussgrenze," viz. the *enclosure-boundary*, denoting the boundary line of the ultimate enclos-ure of the prey.

FENNER distinguishes five zones in each lobe, taking the inner and the outer surfaces separately. But it seems more convenient to divide the lobe into six zones, pertaining to the inner surface. Fig. 2 is a diagram of a lobe with the midrib viewed from the inside, and indicates the two regions and the six zones.

In the one-layered region, three zones are distinguished by FENNER, and they are adopted here too :

i. *The infolded rim*, which is the infolded portion along the margin of the lobe ("Randzone" or "Rand-saum ")[1], the bending being named *the marginal fold* ;
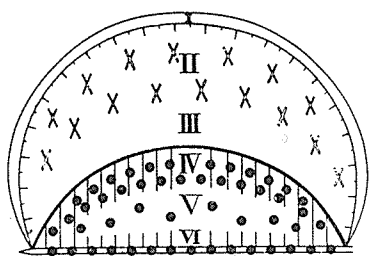
Fig. 2. A schematic diagram showing six zones of a lobe (For definitions, see text.)

ii. *the zone of quadrifid hairs,* which is studded with quadrifid hairs on the inside surface, and two-armed ones on the outside ("Zone der vierarmigen Drüsen der Innenseite" or "Zone der zweiarmigen Drüsen der Aussenseite ") ; and

iii. *the hairless zone,* which is normally bare on both surfaces ("drüsenlose Zone ").

The cells of the one-layered region have irregular rugged forms, as sketched by FENNER. They are elongated perpendicularly to the midrib in the hairless zone and in the proximal half of the zone of quadrifid hairs, while they are roughly isodiametric in the distal half of the latter. The two-armed hairs on the outside are distributed over

---

1) FENNER's designation is shown in brackets.

a wider area than the quadrifid ones on the inside surface, and hence a narrow distal portion of the hairless zone has a number of two-armed hairs on the outside.

As regards the three-layered region, the author follows his own classification, except

IV. *the densely-glanded zone*, which is densely covered with button-shaped glands inside, and with two-armed hairs outside ("Zone der dichtgedrängten, runden Drüsen" and "innere Hauptzone der zwei-armigen Drüsen" on the outside).

The writer discriminates from the last mentioned zone,

V. *the sparsely-glanded zone*, within which button-shaped glands are only sparsely distributed on the inside, and normally no hairs are found on the outer epidermis.

FENNER calls the midrib itself "die Gelenkzone." But the writer wants to mean by

VI. *the middle zone*, the narrow zonal portion of the lobe adjacent to the midrib, the midrib itself being excluded. In spite of the fact that the midrib is richly studded with button-shaped hairs on the inside and with two-armed hairs on the outside, this zone is generally bare on both sides.

Zones I to IV, and a part of the sparsely-gland-ed zone (v) are recogniz-ed in the lateral view of a shut leaf (Fig. 6 B), and the zones of the three-layered region, IV to VI, are observable in the back view of an open leaf (Fig. 7 A). The densely-gland-ed zone looks very dark, owing to the numerous glands and to intercellular spaces containing much air.

The cells in the three-layered region, from those adjoining the midrib to
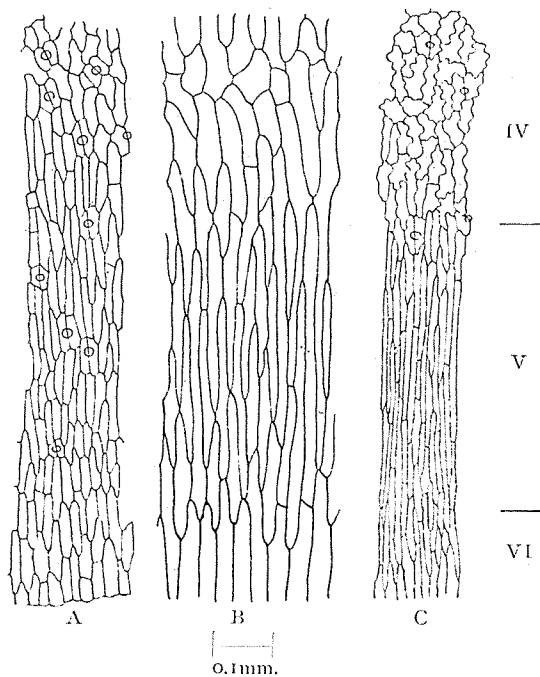

Fig. 3. Surface view of the three layered reigon. A: the inner epidermis; B: the middle layer; C: the outer epidermis

those contiguous to the enclosure-boundary, are shaped as shown in
Fig. 3, in which the midrib side is at the foot, the three zones being
denoted with the same symbols as in Fig. 2. In the outer epidermis
(c), the cells of the densely-glanded zone (IV) resemble those of the
one-layered region in their rugged shape, forming a striking contrast
with the cells of the sparsely-glanded zone. The cells of the middle
zone are somewhat broader than those of the last mentioned zone.
The small circular cells scattered in the densely-glanded zone are the
basal cells of two-armed hairs. In the inner epidermis (A), the densely-
glanded zone is not marked off in its cell forms so conspicuously as
in the above case. The circular marks in this layer represent the basal
cells of button-shaped hairs. The cells of the middle layer (B) are
much larger than those of both the inner and the outer epidermis.

As the leaf is transparent, the optical cross-section of the cells
can be observed in intact condition if a convex part of a lobe is placed
properly under the microscope (Fig. 1, Pl. IX)[1]. The middle-layer
cells are much thicker than the cells of the epidermis.

Both the outer and the inner walls of the middle layer unite
closely with the adjoining walls of both the outer and the inner
epidermis, so that there are formed four membrane layers as a whole
(cf. Fig. 4 or 13). As the extensibility of these walls comes into
question in considering the motor mechanism, it is convenient to
designate them beforehand:

the outermost wall = the outer (superficial) wall of the outer epidermis

the outer subepidermal wall = $\begin{cases} \text{the inner wall of the outer epidermis} \\ \text{the outer wall of the middle layer} \end{cases}$

the inner subepidermal wall = $\begin{cases} \text{the inner wall of the middle layer} \\ \text{the outer wall of the inner epidermis} \end{cases}$

the innermost wall = the inner (superficial) wall of the inner
epidermis.

In the three-layered region, chromatophores are most abundant
and large in size generally in the inner epidermis, while in the middle
layer and in the outer epidermis they are fewer in number and smaller
in size. The quantity of starch seems to correspond to that of chloro-
plast. The chromatophores in each epidermis lie along the wall
which borders the middle layer, quite analogously with the case of
Dionaea (cf. GUTTENBERG '25). There is more starch in the midrib
than in any part of the lobes.

---

1) In this case, the lobe is folded at the sparsely-glanded zone by the weight of the
cover glass in order to make the picture as clear as possible.

Some illustrations of the cross section of the midrib are given by CASPARY, GOEBEL and HABERLANDT, but as they are too schematic, a photograph is shown in Fig. 4.

In the infolded rim and in the distal portion of the zone of quadrifid hairs, the lateral walls are undulated. This structure may make the part resistant to tearing, as FENNER thinks, and the lobe margin is made stronger by folding.

When one slightly presses a marginal part of the one-layered

Fig. 4. Cross-section of a midrib ×160

region with a needle from the outside, it gives in concavely; as soon as the pressure is released, however, it springs back to its original curvature just as in the case of a halved rubber ball. This tendency is retained, though much reduced, even if the leaf is immersed in plasmolytic agents, while it is very flexible and yielding if cut into strips. Hence the elasticity of form may be attributed chiefly to the convex curvature itself of the region. When the margin of a lobe is pressed from the inside, it is more resistant to deformation, than when it is pressed from the outside as described above. This character may also be due to its convexity.

The densely-glanded zone is rigid in its resistance to both stretching and bending, owing to its thickness (it is the thickest in the lobe) and its well-developed cell walls. The midrib also serves as a main support, though not able to resist transverse bending.

The marginal fold hardly changes its shape in any stage of the movement of the leaf. This can be easily understood if the outline of the margin is sketched. The enclosure-boundary also changes little, and hence the circumference of the one-layered region is very little deformed when the movement is going on (cf. Fig. 20).

Phases in the Leaf Movement

i. The open stage

In the sensitive condition of the leaf, the free margins of the lobes stand apart from each other; it is in *the open stage* (Fig. 5 A). A leaf in that stage looks as in Fig. 6 A from the bristle-side, and as in

Fig. 7 A, from the underside (back) of it. The midrib is roughly straight in the lateral as well as in the back view.
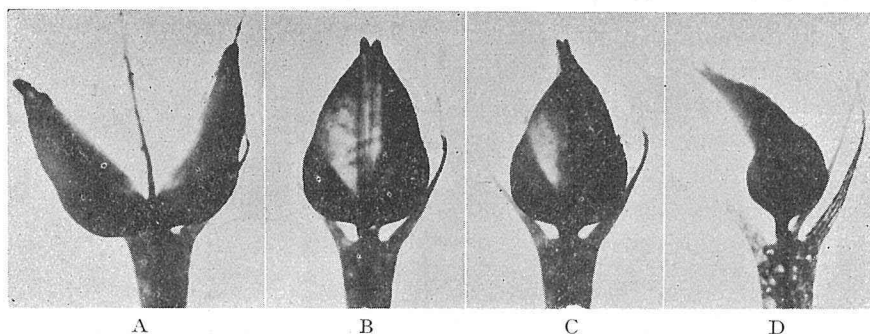
Fig. 5. Apical views of a leaf-blade, when it is open (A), shut (B), going to narrow (C) and quite narrowed (D) ×10
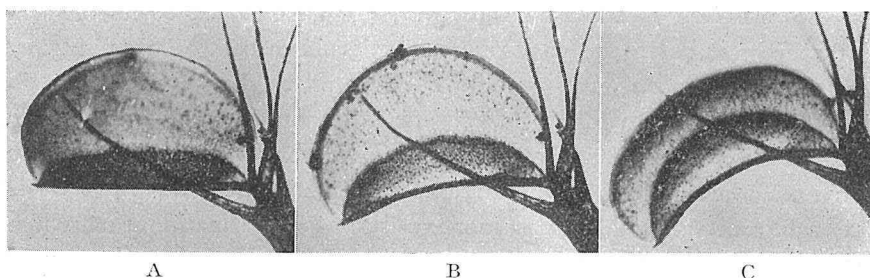
Fig. 6. Lateral views of a leaf-blade from its bristle-side, when it is open (A), shut (B) and narrowed (C) ×8.5
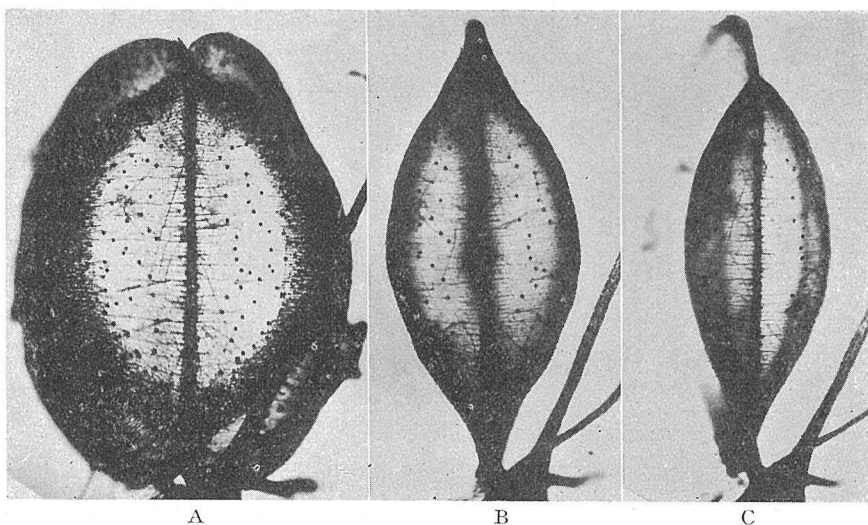
Fig. 7. Back views of a leaf-blade, when it is open (A), shut (B) and narrowed (C) ×14

To study the leaf movement, it is essential to observe the leaf from any desired direction. The leaf-blade is severed for that purpose from the plant under water by cutting through its petiole; carefully floated in a small glass spoon, it is then transferred into a suitable vessel containing water at a proper temperature[1]. A clip which is supported by a set of ball-and-socket joints is lowered into the vessel, and the petiole is held by it, and when this clip is moved appropriately, the leaf is set in any desired orientation. If the leaf is to be observed under the microscope, the base of the clip joints is attached to an arm of a micromanipulator, so that the position of the leaf may be smoothly adjusted.

ii. The shutting

Long sensory hairs project from the inner epidermis. They number 30-40 in a leaf, half of them on the middle zone, about 40% on the densely-glanded zone mostly along the enclosure-boundary, and only a few (10%) on the sparsely-glanded zone. A sudden closing of the leaf results from tactile stimuli, the rims of both lobes coming in contact with each other. This movement which brings an open leaf into *the shut stage,* may be called *the shutting movement.*

The shutting movement can be effected not only by small animals touching sensory hairs, but also artificially through mechanical, chemical, thermal or electrical stimuli acting upon various parts of the leaf, as CZAJA has stated. Figs. 5 B, 6 B and 7 B show the result of stimulation with induction shocks, a pair of electrodes being put into the vessel at the right and left sides of the sensitive open leaves, after the photographs shown in Figs. 5 A, 6 A and 7 A had been taken. The midrib, which is nearly straight in the open stage (Fig. 6 A) bends upwards when the leaf shuts (Fig. 6 B). Hence, when the apical view of a shut leaf is photographed, the inclination of the leaf must be adjusted a little in order to make the leaf axis stand just vertical to the field of the view.

If an open leaf is cross-sectioned optically at the middle, the marginal parts of the lobes show the curvature seen in Fig. 8 A, in which the free-side lobe is on the right side, and the bristle-side lobe on the left. When the

Fig. 8. Diagrams showing changes of marginal portion of the one-layered region and of the marginal fold, when the leaf is open (A), loosely-shut (B), closely shut (C), in the first step of the narrowing (D) and quite narrowed (E)

leaf shuts, the lobes in some cases come into contact as seen in Fig. 8 B, while in other cases, the shutting movement goes on further until

---

1) A sudden change of temperature should be avoided, because it gives rise to the shutting movement, as will be shown in Part II.

the lobes press stronger upon each other, taking a form like that shown in Fig. 8 c. Throughout this paper the leaf will be said to be in *the loosely-shut stage* in the former cases, and in *the closely-shut stage* in the latter cases. Intermediate stages between the two can naturally exist, the magnitude of the excitation, the rigidity of the marginal fold etc. determining such degrees of the shutting. Details, however, will be mentioned later.

iii. The narrowing

While the captured animal is swimming wildly in the hollow space between the lobes, the two lobes begin to press upon each other, and thus the leaf passes into a very closely shut stage. Then some minutes later, the free-side lobe bends sharply inwards near its margin, as is shown in Fig. 5 c and Fig. 8 D. The bending gradually travels on from the marginal part towards the middle, leaving the portion already recurved in an everted state and laid closely upon the inside surface of the opposite lobe, thus making the two lobes asymmetrical. This centripetal shifting of the bending ceases at the inner boundary of the one-layered region, where the epidermis tends to be two-layered. Fig.

9 shows a part of a cross section of a leaf near the enclosure-boundaries in this ultimate phase, the free-side lobe being shown on the right. The marginal part is, then, seen as in Fig. 8 E.

When this process is finished, the three-layered regions of the two lobes together make a narrow sickle-shaped space between themselves. The animal is driven into this small vesicle and cannot get out of the enclosure-boundary. The one-layered region of the free-side lobe is everted and applied closely to the

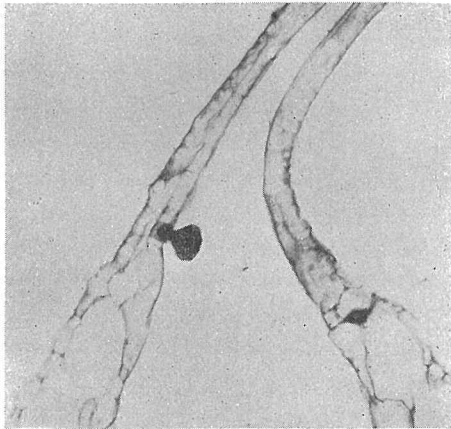Fig. 9. A part of a narrowed leaf in cross-section, near the enclosure-boundaries, the free-side lobe being on the right. A gland is seen dark. ×164

corresponding region of the opposite lobe in curvature, as if to prevent leakage of digested animal matter. Though this last phase of the responsive movement is described by CZAJA as " überkrümmt," it will be called *the narrowed stage*, hereafter, the process from the shut

stage to this ultimate stage being named the *narrowing movement*. The responsive movement of the leaf is, therefore, composed of two successive movements, the shutting and the narrowing, and the two together will be called *the closing movements*.[1]

The narrowed stage of *Aldrovanda* leaves may be analogous to that found in *Dionaea* by DARWIN, reached when the leaves are shut over nitrogenous matter. And hence the designation of the movement and of this stage with respect to *Aldrovanda* leaves, will be used also for *Dionaea* leaves. According to the writer's tests, the leaf of either of the two plants can be brought into the narrowed stage not only by repeated mechanical stimulation of the sensitive hairs, or by the chemical stimulus of nitrogenous matter, but also by strong stimuli of different kinds, electrical, traumatic etc.[2] Figs. 5 D, 6 c and 7 c show the narrowed stage, produced by a strong electrical shock, viewed from three directions. In this stage, the curvature of the midrib is much more pronounced than in the shut stage (cf. Fig. 6 c with 6 B). In Fig. 7 c, the one-layered regions lie on the left of the three-layered, but are almost out of focus. The three leaves, a, b and c, in Fig. 1 A represent an open, a shut and a narrowed leaf respectively, indicating the changes of angle between the midrib and the petiole with respect to the stages of the responsive movement.

iv. The recovery

If a leaf is excited with a weak stimulus, it will open again after remaining a few quarter hours in the shut stage, and this movement may be named *the reopening movement*. But if the stimulus given is strong, the leaf comes to the narrowed stage and then recovers, increasing the volume of the lobe cavity. This course, from the narrowed to the shut stage, will be called *the rebulging movement*. This reopening and rebulging are *the recovery movements*. Normally, the leaf begins to reopen as soon as it has returned to the shut stage after passing through the rebulging movement. The course of the reopening and the rebulging movements is a reversal of the shutting and the narrowing movements respectively, except for the time relation.

---

1) By "closing" has been meant, hitherto, the shutting movement only.

2) MACFARLANE (1892) has observed that there appears secretion when the leaf is stimulated with a glass rod or electricity, just as like when it is stimulated with nitrogenous matter.

Seat of the Movement

Fenner thinks that the motile mechanism of the leaf has its seat in the midrib and writes as follows (p. 369): The membranes of sheath cells which gird the central conducting bundle are very thin and elastic, and this makes the movement easy; and the case is also the same with cells of the outer epidermis which run parallel to the conducting bundle. These cells are vaulted when the leaf is open, while they are flat in the shut state. Czaja also believes that the hinge, "das Gelenk," which makes the two lobes movable, is situated in the midrib, and indeed if one compares the pictures in Fig. 5 with each other, one may form the opinion that the lobes are moved at the midrib.

On the other hand, every author who has worked upon the leaf movement of *Dionaea* has agreed with Darwin's assertion (p. 247) that "the chief seat of movement is near the midrib, but is not confined to this part; for, as the lobes come together, each curves inwards across its whole breadth." And Guttenberg believes that the case of *Aldrovanda* may be analogous with this. The analogy is highly probable, but there is no solid ground for refuting Fenner's and Czaja's idea. The present chapter deals with the investigation carried out to determine what part of the leaf is essential for its movement.

a. Preliminary Researches

In the first place, Fenner's observation about the change in the convexity of the epidermal cells on the midrib was tested with an ordinary and an "ultropak" microscope. But the facts are quite contrary to his description: the epidermal cells on the back of the midrib show no difference at all, whether the leaf is open or is closed by electrical stimulation.

One may be at first tempted to use the method of comparing the apical views of the leaf with respect to various stages in order to find the chief seat of the movement. This method necessarily assumes that the outline of the apical view of a leaf represents the transverse section of the leaf, as the leaf is broadest at that part (cf. Fig. 7 A, B and C). Though such an assumption is correct with an open leaf, the midrib being straight (Fig. 6 A), the case is quite different with a shut or a narrowed leaf, for the midrib curves in these stages (Fig. 6 B and C).

How the curved midrib alters the apical view may be explained
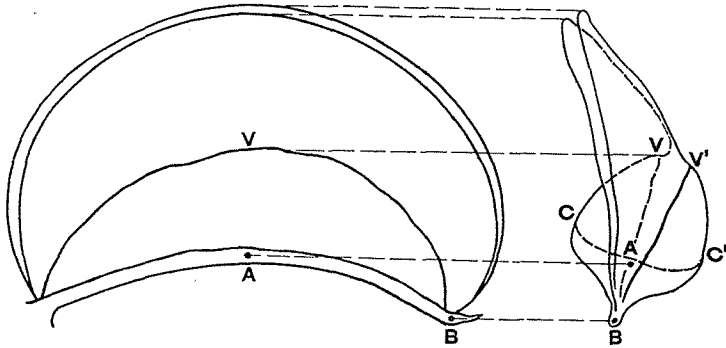
Fig. 10. A diagram showing the relation of the apical and the lateral views of a narrowed leaf, indicating how the median part of a leaf is invisible in the apical view

by Fig. 10, which contrasts the apical and the lateral views of a leaf in the narrowed stage. As a result of curving, the middle point, A, of the midrib is shifted far above the apical point, B. Thus, the cross section of the middle of the leaf is VCAC'V', instead of VCBC'V', viz. the outline of the apical view. The outlines of apical views of a leaf at different stages represent the forms of the leaf sectioned by different planes; by the plane perpendicular to the midrib in the case of an open leaf, but by an oblique and curved one in the case of a shut or a narrowed leaf. They are, therefore, not comparable with each other in their nature.

CZAJA was careful enough when he sketched the apical view of a shut leaf; for, by properly focussing the microscope, he may have caught the true curvature of the lobes at the middle, which was hidden behind. In spite of that, he was mistaken when he sketched the narrowed stage, and he could not get rid of the notion that the lobes were moved at the midrib.

b. Research with the Back and the Lateral Views

Many button-shaped hairs are recognized clearly in the back view, as in Fig. 7. Now if we follow the shifting of each of such glands when the leaf closes, we may be able to know at what portion the bending has occurred. Hence the back view may serve for the purpose.

The densely-glanded zone, which is perceptible in the open stage (Fig. 7 A), goes out of sight in the shut stage (B), and the marginal part of the sparsely-glanded zone also disappears in the narrowed stage (c). However, no fewer than thirty glands in each lobe are perceptible throughout the three stages. When the distance between the midrib

and each of these glands is measured with respect to the three stages, it is found that those glands which lie nearest to the midrib scarcely shift their position when an open leaf comes to the shut and the narrowed stages.

If the leaf movement were caused by the midrib itself acting as the hinge, those glands which are studded nearest to the midrib should shorten their distance from the midrib by ca. 20% when the leaf comes from the open to the shut stage, and by ca. 50% when it comes from the open to the narrowed stage. And as no shifting of such dimensions is recognized in Fig. 7, it is certain that the midrib is not the hinge. And as it is also known that the portion of the lobe lying between the midrib and the glands nearest to it scarcely moves, one can conclude that neither the midrib nor the middle zone is the seat of the responsive movement, or at least neither exhibits any conspicuous movements.

The lateral view also serves to support this conclusion. If the movement were performed by the midrib, or by the middle zone, the distal part of this zone should make its appearance in the lateral view as the movement proceeds. As a matter of fact, however, the middle zone never comes into sight throughout all the stages of the movement (Fig. 6). The zone must, therefore, remain always perpendicular to the plane of the pictures.

c. Research with the Cross Section

The method used above is quite free from artefacts. With it, however, nothing more is proved than that the midrib does not take a direct part in the leaf movement as the hinge. Hence some other method must be tried to ascertain the seat of the movement.

Above all, it is important to know the form of the median cross section of the leaf at various stages. But fixed materials can not be utilized for the present purpose, for, with them, difficulties are met with in attaining the natural curvature of the leaf, especially of that in the open stage. The reasons are as follows :—

a) According to the writer's experience, only concentrated formalin can fix the leaf in the open stage, while many other fixatives and various combinations of them are certain to make the leaf shut before they kill it. But even with formalin, some deformation of the leaf is recognized when it is dead.

b) Even if the change in the curvature due to formalin is admissible, the lobes will necessarily bend further or unbend, or even shrink in the course of dehydration and paraffin-infiltration, as their tissue is very delicate.

c) Even supposing some leaves have been imbedded quite in their natural form, the microtomed leaf is too flexible to retain its natural curvature rigidly when the paraffin-ribbon is warmed and stretched in order to be fixed on a slide glass.

d) Finally, assuming that all these difficulties have been overcome, it is still impossible with the fixed preparation to follow the successive stages of curvature with one and the same leaf.

However, the author has contrived a method which is satisfactory for the purpose—*the agar-imbedding method*.

Highly concentrated agar-sol is cooled down to a temperature of
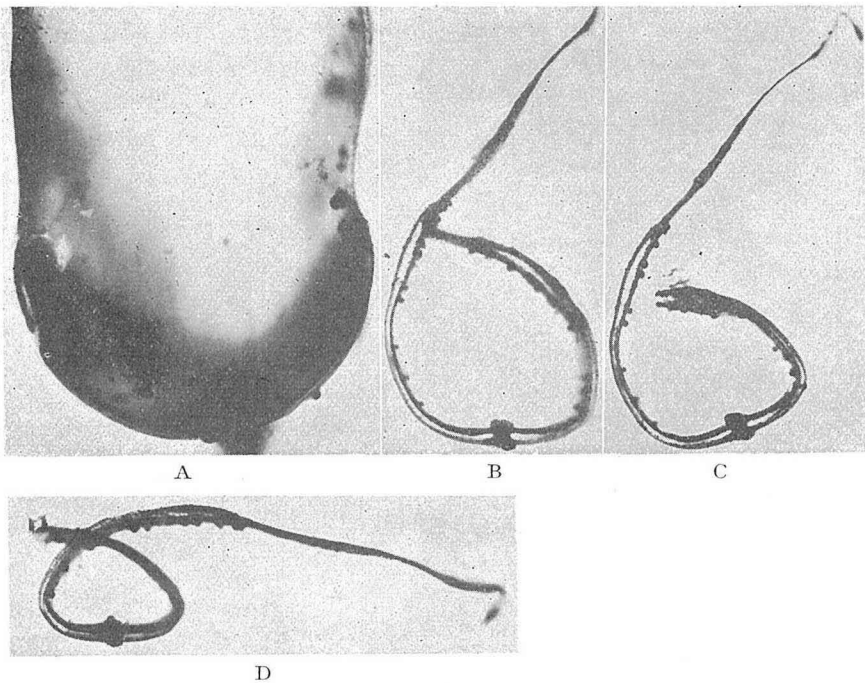
Fig. 11. A: Apical half of an open leaf, cut after imbedding in agar. This represents the median cross-section of an open leaf, the leaf being hindered from closing by the agar-gel held between the two lobes.

B-D: A thin section from the halved leaf, A. The agar being removed, the piece shows the narrowed stage (B). The one-layered region of the free-side lobe is severed. The slice of leaf, absorbing water, bends more and more remarkably (C and D). ×27

about 40° C.   On the other hand, the plant is transferred from the culture medium into warmer and warmer water gradually, until accomodated to a temperature of about 40°.   An open leaf is cut off from this plant at its petiole, and carried by means of a small glass spoon into the agar-sol, which is roughly as warm as the leaf itself. Thus, without receiving any stimulation in the agar, the open leaf is imbedded in the agar-gel as it is, when the agar cools.   The block of agar containing the leaf is then cut in two with a razor, so that the leaf in it is halved at the middle, perpendicularly to the midrib. The apical half of such a preparation is shown in Fig. 11 A, the free-side lobe being shown on the right.

As the cutting gives rise to strong excitation, this halved leaf strives to bend its lobes.   Hence the figure just mentioned shows a state of equilibrium between the force exerted by the leaf in its efforts to close and the elastic resistance of the agar-gel.[1]   However, as the leaf surface does not adhere to the agar, its closing tendency scarcely affects the outline of the agar outside the leaf.   Hence the normal curvature of the open leaf is left moulded upon the outside agar.   This relation of the curvature of the sectioned leaf to that of the agar is shown in Fig. 12.   Here, though the sparsely-glanded zone (upper right in the figure) has separated itself from the agar, the middle zone (lower left) has not, which suggests the position of the motile zone.[2]

When a hand-section is made from such material, the agar held between the lobes inevitably falls off, and the lobes immediately begin to press upon each other.   Hence, to investigate the open stage, we must be contented with a halved leaf as in Fig. 11 A, instead of a thin section.

When the obstruction by the agar is removed, the sectioned leaf comes directly to the narrowed stage, scarcely

0.1 mm.
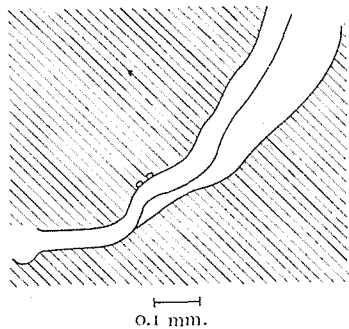
Fig. 12. A part of a leaf lobe as in Fig. 11 A, magnified near the motile zone to indicate how the lobe separates from the agar (hatched part) owing to the narrowing force

1)   As the lobes have bent a little, the curvature of the lobes in the figure may be slightly different from that in the natural case, the undulation on the motile zone (Fig. 13) disappearing.

2)   In this case, as the agar was a little softer than its maximal strength, the separation of the leaf was marked.

pausing at the shut stage, for the cutting is a very strong stimulus in itself. When the greater part of the one-layered region of the free-side lobe is cut off, the rest of the sectioned piece shows very nearly the curvature it has when unobstructed, the cut end of the free-side lobe pressing against the other lobe, as in Fig. 11 B.[1] The relative position of the two enclosure-boundaries in this figure is a little different from that in Fig. 9, but the natural curvature of the leaf in the narrowed stage can be conceived well enough. The form of the figure is very similar to that given by GOEBEL.

When about a half of the densely-glanded zone of the free-side lobe is cut off, the lobes are rendered freely movable again ; they bend slowly on, and after passing through such a stage as that shown in Fig. 11 C, they become sharply bent as shown in Fig. 11 D, after a few quarters of an hour or so. The lobes of the leaf of *Aldro-vanda* are, just like those of *Dionaea* (cf. DARWIN, p. 247, BATALIN, p. 108), capable of bending more sharply, if allowed, than in the natural case of the responsive movement. And this may also be analogous with the case of CHRISTALLE's experiment, in which one of a pair of stigmatic lobes of *Mimulus* bent so far that it touched the pistil, if the other lobe was cut off.

If the four pictures in Fig. 11 are compared with each other, it is clearly seen that the middle portion of the leaf, i. e. the midrib and the middle zones do not change their form throughout those stages. And it is proved that the motile zones lie on both sides of that portion, one in each of the lobes. The width of the immovable portion, including the midrib, is about 0.5 mm.

The densely-glanded zone never changes its curvature either, and the bending occurs only in the sparsely-glanded zone, being especially pronounced in a narrow portion nearer the middle zone. This portion will be called *the motile zone*.

Though it is not settled by the present method whether or not the motile zone is further differentiated for the shutting and the narrowing movements separately, it is highly probable that both the movements are performed by one and the same zone, for easily extensible and permeable membranes are located in the narrow zone (see p. 166 ff.).

In addition, the following facts are ascertained with agar sections :

1. The one-layered region does not take any part in the closing

---

1) In photographing this, the cover glass was supported by thin slices of agar in order not to press and deform the material. The piece used for Fig. 11 B was 0.23 mm, thick.

movements, just corresponding in this respect to the peripheral portion of the *Dionaea* leaf.[1)]

2.   The densely-glanded zone of the three-layered region is thicker than the rest, and easily distinguishable also in a cross section.

3.   The middle zone in a cross section of an open leaf is not so well delimited as in its back view.   But if the sectioned leaf is allowed to narrow, the extent of the zone can be sharply determined.

4.   The lobes are capable of bending in a far greater measure than they show in the natural responsive movement (cf. D).   Therefore, the lobes must be pressing upon each other with some strength, when they are normally in the narrowed stage.

5.   The free-side lobe bends to a greater extent than the opposite lobe.   The asymmetrical form of the leaf in the narrowed stage is caused by this differentiated movement in the pair of lobes, as will be discussed later.

6.   The outer epidermis of the free-side lobe is stretched generally by more than 10% as a whole, when an open leaf comes to the narrowed stage.   The percentage must be far greater if the length is measured only with the sparsely-glanded zone.   In the opposite lobe, however, the extension is far less.   The change in length of the inner epidermis is so small that some measurements prove expansion while others prove contraction; but in any case the magnitude of the change is insignificant.

d. Characteristics of the Motile Zone

It has been determined that in the lobe there is a zone which actually bends in case of the responsive movement.   This zone is also discriminable from the rest of the lobe by the character of its cell walls and of its cytoplasm, demonstrating anew that the zone is a special part in the leaf.

1. *Undulation on the Lower Surface*

When an open leaf is viewed under the microscope from its apex or from the back, and the direction of the incident light is changed variously, one may perceive that a zone in each lobe reflects light in quite a different way from the rest.   Fig. 2, Pl. IX represents one of such cases.   The open leaf is placed just as in Fig. 7 A, the magni-

---

1)   In *Dionaea* leaves, BATALIN has pointed out that the peripheral portion of the lobe, on the distal side of the vascular bundle anastomosis, is insensible to traumatic stimuli and does not take part in the leaf movement.

fication being also the same. Only the position of the light source is different, being above the leaf in the present case. The dark vertical band in the middle represents the midrib. On the right side of it, there are seen two bright arcs, with a dark one intervening, while in the corresponding portion on the left side the middle portion is shining. When the light source is moved, the zones on the right and left indicated with arrows in the plate, show a great deal of variation in the mode in which they reflect light, so that it is perceived that the surface and the intercellular spaces in these zones are undulated. And when this figure is compared with Fig. 7 A and Fig. 11, it is seen that the zone with the undulation forms the motile zone.

The undulation on the motile zone disappears when the leaf shuts, and does not appear again until this is quite reopened.

The undulation is stretched when a sensitive leaf is sectioned, as the leaf strives to narrow then (cf. p. 162 F. N., and 11 A). Hence in order to obtain a cross-section of a leaf with the undulation, open leaves are imbedded in agar-gel, left for several days and sectioned

0.1 mm.
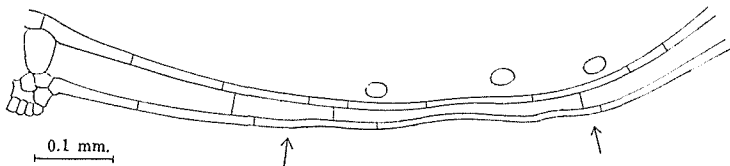
Fig. 13.   Cross section of the motile zone (the portion between the arrows), showing the undulation of the outer epidermis (Half schematic)

after death. Fig. 13 is a half-schematic sketch of a part of a cross-sectioned leaf treated in such a way. This figure shows that it is in the outer epidermis that conspicuous undulation occurs, whereas in the inner epidermis there is hardly any.

2. *Extensibility of Cell Walls*

i. Tests with acetone

If an *Aldrovanda* leaf is immersed in acetone, methyl or ethyl alcohol, many small blisters rise in a row on the outer surface of the motile zone. They grow larger and larger, until at last most of them burst. Fig. 4, Pl. IX shows an intermediate state of the vesiculation on the free-side lobe.[1]   Blisters on the bristle-side lobe reflect incident

---

1)   As the blisters are colourless and transparent, the shadow of the iris of the microscope was drawn very near them, in order to have them impressed in the photograph, and hence their contours are shown much thicker than they ought to be.

light coming from above, and make the bright spots arranged in a flat
arc in the photograph.

These blisters are due to swelling out of the outermost wall, torn
off from the lateral walls. If the magnification is increased, detached
scars of those lateral walls are recognized on the expanding wall (Fig.
5, Pl. IX). If a leaf with blisters is immersed in absolute ethyl alcohol,
the contents of the blisters turn dark brownish and optically hetero-
geneous, the rotation of the contents caused by the penetrating alcohol
being thus made perceptible. That may be due to flocculation of the
cytoplasm.

Such vesiculation never occurs on the rest of the three-layered
region[1] except the motile zone. The position of the blisters can be
understood from Fig. 3, Pl. IX. As the blisters are not easily recog-
nized in this view, they are differentiated by colouring :—An isolated
single lobe of a mature but young leaf is dipped in 80% aqueous
solution of acetone saturated with neutral red, for about 25 seconds.
After washing quickly in water, the lobe is laid on a slide glass with
its outside above, and is pressed by the weight of the cover glass.
Thus only the blisters are coloured by the dye.

Generally acetone or alcohol enters living cells very quickly and
raises their internal pressure so much as to make them burst when the
cell walls can not stand against it (cf. HOLDHEIDE '31). In the present
case, too, acetone may raise the internal pressure of all the cells in
the leaf, but only the cells in the motile zone vesicate outwards. It
is conceivable, therefore, that the outermost wall of the motile zone is
very easily extensible, in contrast with those of the rest of the leaf.
If a leaf is left in a concentrated acetone solution for several minutes,
the blisters grow larger, adjoining ones often uniting to make a larger
one, until they burst owing to too great extension of the wall. Gener-
ally, however, a few blisters are left without bursting on or near both
ends of the zone, indicating that the ductility diminishes towards both
ends of the zone.

If a leaf is returned to water from acetone when blisters are raised
to some extent, these become smaller and smaller, as the acetone in
them gets out. But the cell walls which were once stretched con-
tract little and become rumpled when the pressure inside vanishes.
The walls are, therefore, not elastically but plastically extended.

Not only the outer epidermis, but also several cells of the middle

---

1) In the one-layered region, several cells raise small blisters.

layer raises its outer wall outwards, when immersed in acetone or alcohol. Hence the outer subepidermal wall is also easily extensible, though not so much so as the outermost wall. The thickness of the middle layer is increased only by 1.5 times at most, even where over-lying epidermal cell have burst already. Vesiculation never occurs on either side of the inner epidermis, and the rise in the internal pressure of the middle layer never makes the inner subepidermal wall swell towards the inside, but necessarily causes the outer subepidermal wall to swell outwards. Thus it is concluded that the outermost wall is the most easily extensible, the outer subepidermal wall less easily, while the two inner walls are least extensible.

ii. Tests with ammonia

If an *Aldrovanda* leaf is immersed in a strong ammonia solution, maceration occurs in the outer subepidermal wall, and owing to the pressure suddenly liberated in that wall, the outer epidermis suddenly bulges outwards and the two inner layers inwards. Such bulging out takes place only on the motile zone,[1] and hence the outer subepidermal wall seems to be differentiated at the motile zone from the rest with respect to its chemical character, in that it is easily subject to maceration. The blisters raised by ammonia look, to the naked eye, just like those raised by acetone, except for the colour: the leaf is green when treated with acetone, while it is dark brown when treated with ammonia.

After that bulging, the outer epidermis is much more elongated than the two inner layers. This fact also demonstrates that the walls, the outer as well as the inner, of the outer epidermis are very easily extensible within the motile zone.

iii. Tests with mechanical tension

If the densely-glanded zones of both lobes of a leaf are nipped with small clips from opposite directions, and the clips are drawn gradually apart from each other, the leaf is in general torn in two when the tension reaches 10–20 gms., the break occurring at the motile zones. This indicates that the leaf has least mechanical strength at that zone (except the one-layered region), and this may be due to the high ductility of the two outer walls. And if the outermost wall is

---

1) Some cells in the one-layered region also swell out, just as in the case of acetone treatment.

observed microscopically, it behaves as if it were a paste at the instant
when it is broken by the tension, which suggests that that part of the
wall is highly swollen and is of the nature of a sol.

3. *Permeability of Cell Wall*

i. Tests with alcohol

Capillary-active substances such as alcohol, acetone etc., rapidly
enter the intercellular spaces, and drive out the air which filled them.
When an *Aldrovanda* leaf is immersed in such chemicals, the air in
the transverse intercellular spaces of the lobes is driven away, often
in such a way that the air which is present between the motile zone
and the midrib flows towards the midrib centripetally, while that be-
tween the motile zone and the thickly-glanded zone flows towards this
zone centrifugally. This fact indicates that alcohol or acetone enters
those intercellular spaces most rapidly through the cell walls of the
motile zone. The permeability of the cell walls, therefore, is different
in the motile zone from that in the rest of the three-layered region.

It is detected from the bubbling out of air that the broad inter-
cellular spaces in the midrib have their openings near its tip. Such
openings often serve for the entrance of alcohol, so that some lines
of intercellular air in the midrib are often shifted towards the petiole
before those in the lobe are transferred. In such cases, the latter are
often driven centrifugally towards the enclosure-boundary. But such
cases have nothing to do with the above reasoning, that various
substances, at least alcohol and acetone, pass through the wall in the
motile zone more easily than through the walls in the rest of the leaf.

ii. Tests with osmic acid

If an *Aldrovanda* leaf is dipped in 2% osmic acid for one minute,
the motile zone is coloured dark brown, turning blackish after ten
minutes. As no other part shows this change in colour, the motile
zone is differentiated from the rest (see Fig. 14). A detailed figure
of the inner epidermis in the most deeply blackened part is shown in
Fig. 6, Pl. IX[b]. The staining seems to be due to the cell contents.
The cells adjoining the motile zone are also stained, the stain being
deepest at the ends nearer to the zone, but fading towards the oppo-
site ends.

---

1) The outer epidermis also looks like this.
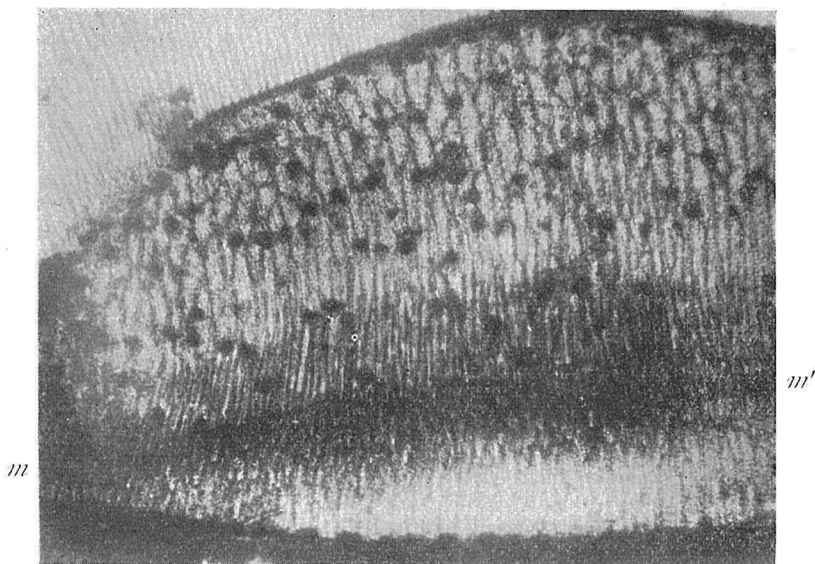
Fig. 14. A part of the three-layered region, immersed in 2% osmic acid for 10 minutes. The motile zone (*m-m'*) is darkest. ×67

It is ascertained by further tests[1] that the staining proceeds from the cells into which the osmic acid has penetrated rapidly, and in each cell, from the side through which the acid has entered. Hence, Text-fig. 14 and Fig. 6, Pl. IX, may demonstrate to some extent, the distribution of the permeability of the cell walls to osmic acid. Thus it may be stated that the cell walls in the motile zone are very permeable to osmic acid, as well as to alcohol and acetone.

4. *Intracellular Features*

i. The chromatophores are smaller in the motile zone than in the other parts, and hence the green shade is lighter there on the whole.

ii. The zone is very poor in starch. Hence the zone can be marked off from the rest of the lobe by adding iodine.

iii. If a leaf which has received a traumatic stimulus is immersed in 0.5 mol sucrose solution for several hours, the cells of the outer and the inner epidermis in the densely-glanded zone and in the one-layered region show the so-called " perfekte Systrophe " (cf. GERM '32), whereas the cells of either epidermis in the sparsely-glanded and

---

1) If a lobe is cut into pieces and dipped in an osmic acid solution, the cut cells on the edges blacken first, and then the inner cells, the side of them adjoining the cut cells blackening earliest.

the middle zone show " Vakuolenteilung " or " Vakuolenzerklüftung,"
similar to what GERM observed with *Drosera* tentacles.   In the motile
zone especially, the cleavage of the vacuoles is so pronounced that
no large vacuoles remain, whereas the vacuoles are cleft into few
parts in the other portions.

e. Motile Zone in the *Dionaea* Leaf

In order to confirm the widely accepted idea of the localization
of the seat of the movement in *Dionaea* leaves (cf. p. 158), optical
cross-sections of the leaf were made with respect to different stages
of its movement[1].   In the lateral view, the midrib of *Dionaea* leaf
is curved upwards through all the stages of the movement, so that the
simple apical view does not represent the shape of the median cross

section.   A fine white cotton thread
was therefore attached transversely
with paste to the under surface of
the leaf along its middle line.   The
outline of the thread, not being hidden
behind the apical part of the leaf,
roughly represents the transverse curv-
ature of the outer surface.   Figs. 15
A, B and C were thus drawn in three
successive stages, and overlapped into
one.

When a leaf is fully open (A),
both of the lobes are normally concave
towards the under side, though GUT-
TENBERG ('26) holds that such are
exceptional cases.   If the open leaf

Fig. 15.   Cross section of the
outer surface of a *Dionaea* leaf, when
it is open (A), shut (B) and nar-
rowed (C)

is excited, the two lobes become convex outwards, and the margins
coming very near to each other, the marginal spikes become inter-
crossed.   If the excitation is caused by touching the sensory hairs a
few times, the shutting movement goes most commonly so far that
the spikes intercross at their middle (B).[2]   This stage may correspond
to the shut stage of the *Aldrovanda* leaf.

But if such a shut leaf is still further stimulated, by touching it
several times with a glass rod inserted between the margins, it shuts
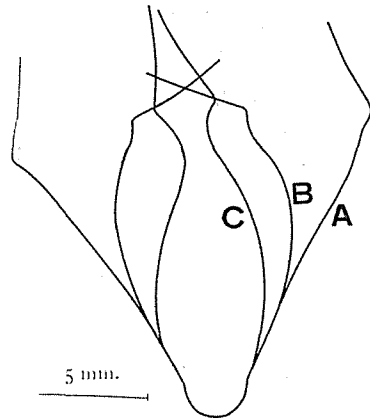
1) No sketches of cross sectioned *open* leaves are found in the literature, except
KURTZ's diagrammatical one.
2) The 5th frame of Fig. 3, Pl. XV in LLOYD's paper shows this stage.

up completely, the spikes intercrossing at their bases. And as the lobes press upon each other with increasing force, their marginal zones are forcibly everted, until at last the spikes become nearly parallel (c).[1] This may be called the narrowed stage.

If we compare the figures, we see that the curving of the lobes takes place at some distance from the midrib, the motile zones being much broader in proportion than those of *Aldrovanda* leaves. The everted portion of each lobe which corresponds to the one-layered region of *Aldrovanda* is narrow in proportion. In Fig. 15, as the leaf was viewed from its apex, the lobe on the left hand corresponds to the free-side lobe of *Aldrovanda*.[2] It is interesting also to note that this lobe bends more than the opposite lobe in many instances, though not so decisively as in *Aldrovanda*.

Mechanism of the Leaf Movement

A. Introductory Considerations

The responsive movement of *Aldrovanda* leaves can be divided into two, the shutting and the narrowing movements. The former is of a rapidity rarely found among active movements of plants, while the latter proceeds so slowly that it may take some scores of minutes to complete itself. These two steps can be clearly separated from each other (cf. Fig. 29 A, B and p. 223). Hence in considering the mechanism of the leaf movement, each should be treated by itself.

As *Aldrovanda* resembles *Dionaea* in its leaf structure and in its mode of movement, the two belonging to the same family, theories concerning the motor mechanism of the latter will be referred to, though most of them pertain to the shutting movement only. And after the motor mechanism of *Aldrovanda* has been elucidated, that of the *Dionaea* will also be discussed.

a. Possible Mechanisms Common to the Shutting and the Narrowing Movements

Both the types of movement are caused by sharper bending of the motile zones, as was proved in the foregoing chapter. As the motile zone consists of three layers of cells, and as quick movements of plant organs are, in general, caused by changes of turgidity, any of the following four cases can effect the shutting or narrowing move-

---

1) The extreme form of this stage, generally assumed by cut leaves, is shown by LLOYD, Fig. 6, Pl. X.

2) In LLOYD's Fig. 6, Pl. X, the right and left is reversed to the present case.

ment. Of them, the first two relate to an increase of turgor, and the latter two to a decrease.

As it has hitherto been assumed that the leaf movement of *Aldrovanda* is performed by the midrib, there is no need to mention here any of the statements made about this plant. But, with regard to *Dionaea*, we have many plausible theories, which may be cited in their proper category of possible mechanisms. In the motile zone of *Dionaea*, there is a considerable pile of cells, while in *Aldrovanda*, there are only three cell layers. The following correspondence should be, therefore, assumed when the motor mechanism of either one is deduced from that of the other.

Aldrovanda	*Dionaea*
the outer epidermis.	the outer epidermis only, the outer half of the parenchyma, or both of them.
the middle layer.	the parenchyma as a whole.
the inner epidermis.	the inner epidermis only, the inner half of the parenchyma, or both of them.

A) Increase of turgor

1) Active elongation of the outer epidermis

Brown ('16) thinks that the closure of *Dionaea* leaves " is largely due to the increase in volume of tissues lying near the lower leaf surface." Such tissues must mean the outer epidermis in the case of *Aldrovanda*.

2) Increase in turgidity of the middle layer

If the cell walls on the outside of the middle layer are, on the whole, more easily extensible than those on the inside, the outer epidermis will be elongated more than the inner when the turgor of the middle layer increases, thus making the leaf shut.

The outer two walls in the motile zone of *Aldrovanda* leaf have been proved, by means of acetone and ammonia, to be far more easily extensible than the inner two walls in that zone. Hence the mechanism in question is possible. Guttenberg attributes the shutting movement of *Dionaea* leaf to an increase of turgor in the swelling tissue, asserting that the leaf has a necessary construction for that mechanism.

B) Decrease of turgor

1) Decrease in the thickness of the lobes

If the cells of the motile zone contract in their thickness proportionately more than in their length when their turgidity diminishes,

the lobe will be bent more sharply than before. No one has as yet proposed this type of mechanism.

4) Decrease in turgidity of the inner epidermis

If the turgor of the inner epidermis were decreased or annulled by excitation, the bending of the lobe would be brought about through either of the following two kinds of mechanism:

a) Cell walls which had been stretched by turgor, would contract elastically, and this shortening would cause the movement. DARWIN's ('75) and DE CANDOLLE's ('76) views fall into this category. MACFAR-LANE ('92) has an allied notion, but guided by BURDON-SANDERSON's idea of "irrito-contractility" common to both the vegetable and the animal kingdom, he attributes the contraction of the cells in the inner parenchyma not to the elastic force of the cell walls, but to muscle-like contraction of the cytoplasm.

b) The force of the outer layers would be allowed to come into action and bend the lobe, owing to the vanishing of the antagonistic force of the inner epidermis. This possibility is proposed by BATALIN ('77), who writes (p. 139): "Der Zustand, in welchem sich das un-gereizte Blatt befindet, ist das Resultat des Gleichgewichts zwischen zwei Kräften: einer, die sich bemüht das Blatt zu schliessen—und einer andern, die sich bemüht dasselbe zu öffnen." "Wenn sich in Folge der Reizung aus den Zellen der obern Seite Wasser ausscheidet, so wird das Gleichgewicht gestört—."

ZIEGENSPECK ('28) holds a similar opinion, but he thinks the important factor is loss of turgidity in "die Schlossgewebe," the hinge tissue, which lies over the vascular bundle, and this can not be held in the case of *Aldrovanda*, for there is no corresponding tissue in action.

Combinations of certain two of the above four cases are also possible. For example, MUNK ('76) regards both the relaxing of the inner parenchyma and the active stretching of the outer one as the shutting mechanism. Though BROWN thinks the increase in size of the cells in the ventral or convex region plays an important rôle, as mentioned above, he is also inclined to believe that a contributory cause is "a decrease in the turgor of the tissues near the upper sur-face." BATALIN, however, in spite of his own measurements which prove an actual extension of the outer surface during the shutting, refuses to acknowledge that there is any active functioning of the outer tissues and regards such an extension as being of a passive nature.

b. Evidence against Decrease in Thickness as
the Mechanism of the Movement

As it is easily proved that a decrease in thickness of lobes causes neither shutting nor narrowing, this will be given in the first place as the counter evidence.

Let ABCD (Fig. 16) represent the cross section of the motile zone of a lobe, assuming that the two surfaces of the zone make concentric arcs. The length of the outer surface, o, and that of the inner surface, i, can be expressed with the radius of curvature of the inner surface, r, the thickness of the zone, d, and the angle, α, which AD and BC make at the centre of curvature, P :

$$\begin{cases} i = \alpha r \\ o = \alpha(r + d) \end{cases} \qquad (I)$$

Now, if α and r become α' and r' respectively, when d changes into d', o and i remaining constant,

$$\begin{cases} i = \alpha'r' \\ o = \alpha'(r' + d') \end{cases} \qquad (II)$$

Eliminating i, o, r and r' from the above four equations, we have

$$\frac{d'}{d} = \frac{\alpha}{\alpha'} \qquad (III)$$

Fig. 16. A diagram to show geometrical relation between the thickness and the curvature of the lobe. For explanation, see text.

In the open stage of *Aldrovanda* leaves, $\alpha = 23° \times \dfrac{\pi}{180} \doteqdot 0.4$ estimated large enough ; while in the shut stage, $\alpha' = 0.82$, at a rough estimation. Hence

$$\frac{d'}{d} \doteqdot 0.49 \ ;$$

that is to say, the thickness of the motile zone should be reduced to half, if an open leaf were shut owing to a decrease in the thickness of the lobes. But the thickness of the zone, viewed as in Fig. 1, Pl. IX, shows no pronounced changes as the reaction proceeds, not to speak of such a great diminution as one half.

The above calculation has been carried out upon the assumption that the outer and the inner surfaces do not contract, even if the turgor may be decreased so much as to make the lobes thinner. But they would also contract when the turgor diminishes. And hence the thickness of the motile zones should be reduced to less than a half when the leaf shuts. The case is similar when a shut leaf comes to be the narrowed stage.

In addition, the middle layer, which is the thickest of the three layers, is prevented from reducing its thickness by the special structure of its walls (cf. p. 193 and Fig. 18). Thus a decrease in thickness of the lobes can not be the cause either of the shutting or of the narrowing movement.

c. On Osmotic Values of Cells

Some authors mention the osmotic values of leaf cells of *Dionaea*. But as sectioned pieces of leaves are in the narrowing phase, the distribution of the suction pressures in the leaf in the open or the shut stage cannot be discussed with such sectioned material.
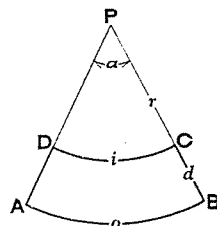
All the cells of an *Aldrovanda* leaf can be observed without sectioning, as it is transparent. But cells in the motile zone do not normally exhibit plasmolysis. They will show systrophic contraction of vacuoles if the leaf is immersed in sugar solutions etc., or if they are deformed, e. g. flattened under a cover glass, or if some cells in the three-layered region are wounded, or if electrical shocks are given previously. In such cases, the volume of the contracted vacuoles has no numerical connection with the concentration of the plasmolytic agent used, as there is no normal plasmolysis. Hence it is impossible, for the present, to find the osmotic suction pressures of the various cells of *Aldrovanda* leaves by the usual plasmolytic method.

B. Mechanism of the Shutting Movement

a. Decrease of Turgor as the Cause of the Movement

BÜNNING ('29) has proved that there is an excretion of cell sap from the sensitive cells of the filaments and pistils of various plants when they react upon stimulation; GUTTENBERG ('28) and his pupil WEIDLICH ('30) hold that the first effect of excitation in the pulvinus of *Mimosa* is loss of turgor on the under side; and lately CHRISTALLE ('32) has demonstrated an oozing out of a fluid from the cut surface of a pistil of *Mimulus* when it reacts. Thus, it is considered in general, that a quick movement of a plant organ can be attributed far more probably to a decrease of turgor in some tissue, than to an increase in another.[1]

In spite of all those examples, should the quick movement of *Dionaea*, and of *Aldrovanda*, be attributed to an increase of turgor, as GUTTENBERG asserts? Even he says (p. 674): "Die Deutung ZIE-GENSPECKS hat zweifellos den Vorzug, dass die Annahme eines Turges-cenzverlustes als Ursache einer raschen Bewegung plausibler ist als die einer Turgescenzerhöhung." The following experiment demonstrates that the shutting movement of *Aldrovanda* leaves is not caused by an increase of turgor in any tissue:

If an open leaf of the age of No. III, or No. IV is immersed in a 0.15 mol sucrose solution, and is stimulated one minute or two after that[2] with a finely pointed stick, it reacts and shuts. But if the

1) Such notion has prevailed since the time of PFEFFER (cf. PFEFFER pp. 451—3).

2) The leaf is moved about in the solution so as to be brought into thorough contact with the intended concentration as rapidly as possible.

stimulus is applied when the leaf has been kept in that solution for a slightly longer time, it cannot shut completely. The longer the immersion in the solution is prolonged, the smaller becomes the shutting movement called forth by the excitation, and at last, after being kept for 4 minutes in the solution, the leaves do not react at all.[1] Such behavior is not due to a diminution of excitability, but is due to a fall in the power of reaction ; for the threshold stimulus to induction shocks does not rise in degree during the course of that inactivation, just as in KOKETSU's case of *Mimosa* ('27). The case is also demonstrated by GUTTENBERG ('25), who covered the outer and the inner epidermis of a *Dionaea* leaf with glycerin, the sensory hairs being left free, and showed that touching of these did not cause the reaction.[2]

How is the reactivity lost in the sugar solution ? If the shutting movement were due to sucking in of water from the medium by a certain tissue, the immobility in a 0.15 mol solution should mean that the suction pressure of that tissue is lower than that of the solution. If so, the leaf should be incapable of shutting at the instant of contact with the solution, and should not need to be kept in the solution for several minutes. The shutting movement, therefore, is not caused by sucking in of medium water by any tissue. On the other hand, in respect to the narrowing movement which is caused by increase in the turgor of the outer epidermal cells, the leaf becomes hardly able to move as soon as it is immersed in a sugar solution (p. 189). And the latter fact suggests that in so far as the bending is effected by the increase in the turgor and the volume of the cells, water from the outside is required, the water out of other tissues being not sufficient for that. As, however, the shutting movement is not due to water in the medium, it is concluded with high probability that this movement is not due to an increase in the turgor of any tissue.[3]

b.   New Theory on the Shutting Mechanism

GUTTENBERG ('25) has tried to interpret the shutting of *Dionaea*

---

1) The leaves regain their motility if replaced in water, though they often respond to the stimulus of a sudden change of environmental osmotic pressure. The relation of such disappearance and recovery of motility with the concentration of the solution, or with the age of the leaf will be studied in Part II.

2) Besides, he describes that leaves covered with glycerin shut slowly when the midrib is wounded. But this case belongs to the narrowing process, contrary to his idea.

3) The 0.15 mol solution disturbs the shutting movement by decreasing the turgor of the cells which are to propel the movement (cf. p. 183). Hence the suction pressure of those cells seems to be lower than that of the 0.15 mol sucrose solution.

leaves as due to a loss of turgor, that being the most probable explanation; "Das Blatt ist offen, so lange alle Zellen der Spreitenflächen ihre volle Turgescenz besitzen. Je höher der Turgor der Innenepidermis ist, um so mehr verlängern sich ihre Zellen, um so mehr öffnet sich also das Blatt. Erlischt ihr Turgor, dann fällt tatsächlich ein Widerstand weg für den Fall." This view is convincing, being quite similar to that of BATALIN. But he has been forced to give up this provisional interpretation owing to three difficulties which will be mentioned later (p. 180).

The writer, however, has reconstructed the theory of the shutting movement being due to a decrease of turgor, and, by his theory, not only are the difficulties which GUTTENBERG met with solved, but also all the other features related to the shutting movement are accounted for. The new theory will be advanced with respect to *Aldrovanda*, and will be extended then by deduction to the case of *Dionaea*. The descriptions will deal only with the motile zone, unless otherwise stated.

As has already been shown in the case of the acetone treatment, the outermost wall is far more easily extensible than the outer subepidermal wall, and this wall, again, than the inner subepidermal wall (cf. p. 157). Owing to the walls being of such a character, the outer epidermis and the middle layer are endowed with an ability to bend the lobe with their turgor force. The inner two walls, however, are thick and the least extensible of all. Hence the deformation is only slight however strong the turgor may be; and the greater the turgor, the more inflexibly is the original curvature of the layer maintained. Thus when the three layers are all in a turgid condition, the outer two layers strive to bend the lobe, while the inner epidermis resists that. As the inner epidermal cells are thick-walled, they can withstand the bending moment of the outer two layers,[1] the lobe thus being put in the open stage of unstable equilibrium.

The outer epidermis is wound sinuously when the leaf is open, as shown in Fig. 13, which suggests that the layer is restrained from free elongation, namely from bending the lobe, by the rigidness of the inner epidermis.

Let it be assumed that when an open leaf is stimulated, the permeability of the cells of the inner epidermis is increased as the reaction. Then, sap from the cells may be pressed out by their own wall pressure and also by the force of the outer two layers. If the turgor of

---

1) A thick-walled toy-balloon withstands a bending moment with less flexibility than a thin-walled one. Verification of the theory of the open stage is found on p. 192.

the inner epidermis vanishes in such a way, the outer epidermis may be allowed to elongate itself, and hence to bend the lobe. This may be the shutting movement. BURDON-SANDERSON's finding that the electrical change which occurs when the *Dionaea* leaf is stimulated, has its seat at or near the upper surface of the lamina, is favourable to this assumption, for bioelectrical change (especially towards negativity, as in the cited case) is believed to be accompanied by increase of permeability. In the above it is assumed that the change in permeability occurs only in the cells of the inner epidermis, the outer two layers remaining quite turgid. That assumption is not far-fetched; it has been shown by BÜNNING ('30, p. 53) that the irritable cells of *Sparmannia* filaments occur only among the epidermal cells.

The writer's view deviates from ZIEGENSPECK's in that the latter thinks the hinge tissue plays the most important rôle, while the former attributes the mechanism to no other parts of the leaf than the motile zone. The writer's opinion differs from BATALIN's in two points: 1) The latter believes that when the leaf is open the inner side, which is intrinsically shorter than the outer, is elastically stretched so that it becomes longer by strong turgor. 2) He assumes an active contraction of tissue, which is not combined with loss of turgidity. The first point is also the important point in which the writer's interpretation diverges from GUTTENBERG's provisional one (quoted on p. 177). As the extensibility of the cell walls of the inner epidermis is very small, as demonstrated already, the assumption of a highly elastic elongation of it is of course improbable.

The special feature of the newly introduced view is that in it a conspicuous contraction of the inner epidermis is not indispensable to the shutting movement, and that only the relaxing of this epidermis forms the cause of the movement. It is the inner epidermis that reacts upon the stimulation, but it is the outer two layers that propel the shutting movement.

BATALIN thinks it necessary to assume active contraction of the upper parenchyma; he considers that, without this assumption, the lobe would not bend when immersed in water, because the upper parenchyma would absorb no less water than the outer one. But according to the writer's theory, the inner epidermis does not absorb water, for it has lost its semipermeability, and when any part of the parenchyma absorbs water and expands, the lobe should bend inwards, since the inner epidermis withstands the extension.

While a leaf is open, a component of the turgor force of the

outer epidermis is working in such a direction that it thickens the layer, and makes it meander sinuously (cf. Fig. 13). But as soon as the turgor of the inner epidermis vanishes, most of the turgor force is used to stretch the meandering and then to elongate the cells of the layer.[1] At last, however, the bending of a lobe is blocked by the opposite one, when their margins come in contact with each other, namely when the leaf reaches the shut stage.

The suction force of the outer epidermis, S_z, is conditioned by the formula,

$$S_z = S_i - (A + W),$$

where S_i denotes the suction pressure of the cell content, W, the wall pressure, and A, the pressure exerted by the inner epidermis to oppose the bending. The excitation reduces A to the minimum, and hence makes S_z larger. Therefore, when the leaf is shut, the outer epidermis sucks in water and increases its volume a little, notwithstanding that S_i remains constant. Circumstances as such, however, will be discussed in the next chapter.

c. Shutting Mechanism of the *Dionaea* Leaf

It is highly probable that the mechanism of the shutting of *Dionaea* leaves is analogous with that of *Aldrovanda* leaves. But the circumstances are different in the two plants, because the lower superficial wall of the *Dionaea* leaf is cutinized as in ordinary land plants, and is accordingly far less extensible than the corresponding wall of the *Aldrovanda* leaf; 1) it never puts out vesicles in acetone, and 2) when a surface section is made, the piece that consists of the lower epidermis and some piles of underlying parenchyma cells curves with the epidermis as the concave side, the epidermis being negative in tissue-tension to the parenchyma.

The cuticle of the lower leaf surface, however, is thinner than that of the upper surface, as is stated by MACFARLANE. And when the upper epidermis alone or with one more subepidermal layer is pared at the part of the lobe where the movement is most pronounced at the shutting, this part of the lobe curves. making the lower epidermis convex, to the same extent as an intact lobe shows in the shut stage (B in Fig. 15). When both the inner and the outer epidermis are pared off, the parenchyma mass left naturally shows more pro-

---

1) This view was first introduced by ZIEGENSPECK in discussion about the action of " die Schlossgewebe."

nounced curvature than a lobe from which the upper epidermis alone
is severed. This bending force of the parenchyma overcomes the
resistance of the lower epidermis (more properly, its cuticle). and bends
the lobe (without the upper epidermis) to the extent just mentioned.[1]

After a leaf has reached a certain maturity, the transverse growth
of its upper epidermal cells surpasses that on the lower side. Thus
the lobes are unbent, the bending force of the parenchyma being
overcome by the turgor force of the upper epidermis, until the lobes
become rather concave and are curved towards the outside—the open
stage of the leaf. But as soon as the cells of this epidermis lose their
turgor from excitation, the lobes are curved inwards by the paren-
chyma. Thus the *Dionaea* leaf shuts in a way analogous to that in
which the *Aldrovanda* leaf closes. While excited *Aldrovanda* leaves
normally perform a complete closure, of the all-or-nothing type, *Dionaea*
leaves normally show "partial closure," as MACFARLANE and BROWN
have already described and as is now shown in Fig. 15 B.[2]

The bending of *Aldrovanda* lobes proceeds until the opposite lobes
meet together, because the outermost wall in the motile zone is very
easily extensible; whereas *Dionaea* lobes cease to curve before such
external obstruction appears, as their bending is restrained by the
cutinized wall of the lower epidermis. This is the most important
difference between the shutting movements of the two plants.

d.   Criticism of GUTTENBERG's Interpretation

The three reasons why GUTTENBERG has abandoned the view
(p. 177) that the shutting movement is to be attributed to a decrease
of turgor in the inner epidermis are as follows :—

(a)   According to BROWN's measurements, the shortening of the
inner surface upon shutting is so small as to be doubtful; if the leaf
were shut by the assumed mechanism, the elastic contraction of it
would be considerable.

(b)   No movement takes place, when the upper surface is covered

---

1)   As will be discussed later, a wound given to the irritable part of a leaf-blade causes
the narrowing mechanism to work, which is an increase of turgor in the parenchyma cells,
different from the shutting mechanism. But this does not preclude the conclusion from the
above observation concerning the extensibility relation among the piles of cells.

2)   Partially closed *Dionaea* leaves may close completely, if the stimulation is continued
repeatedly. This, however, is a reaction to be accounted for by the narrowing mechanism.
Hence the "partial closure" is the final stage of the shutting movement, and the shutting
reaction of *Dionaea* is, properly speaking, of all-or-nothing nature, as well.

with a concentrated glycerin solution, that is to say, when the tissue near the upper surface is deprived of water through the surface.

(c) Sudden immersion of a *Dionaea* leaf in boiling water does not cause it to close, as DARWIN found by experiment, whereas filaments of *Berberis*, *Sparmannia* and *Helianthemum* which are believed to react with loss of turgor, show the responsive movement however quickly they are dipped into boiling water.

These three points can be answered with the writer's theory.

(a) Though the loss of turgidity in the upper epidermis induces the shutting movement, highly elastic contraction of it is not essential to the movement, for the movement is propelled by a mass of cells lying on the outside of that layer.

(b) Water must pass through a thickly cutinized wall incomparably more slowly than through an uncutinized one. Hence, when the upper surface is covered with a concentrated plasmolyticum, the water that are deprived slowly from the upper epidermal cells through the cutinized wall (if that should occur) must be readily supplied from the underlying parenchymatous cells successively. Therefore, if there is a sufficient supply of water in the conducting system, no cells in the leaf lose turgor, while if there is insufficient water, there occurs "eine Gesamtwasserabgabe," as GUTTENBERG himself says (p. 180), "nicht aber......(die) einseitigen Herabsetzung des Turgors."[1] In either case, the shutting movement cannot be caused.

(c) The reaction of *Berberis* etc. takes place when sensitive cells contract with the elasticity of their own walls (cf. BÜNNING '29). Hence the reaction may occur when those cells lose their turgor through being injured by heat, no matter whether other cells in the filament are turgid or not. On the contrary, as the movement of a *Dionaea* leaf is propelled by turgor of the parenchyma, and not by the force of the sensitive upper epidermis itself, the movement may not occur if cells of the latter are injured by heat at the same instant as the former. Such a difference is shown by the fact that the latter undergo no change in their reactivity even if deprived of so much water that plasmolysis occurs, as stated by BÜNNING ('30, p. 58), while the loss of the power of reaction in the former goes parallel with loss of turgor.

On the boiling water fixation of open *Dionaea* leaves, the writer can add a new observation: the leaf shuts when immersed in boiling

---

1) The discussion is essentially intended to dispute MUNK's view (as in p. 173).

water, if a sensitive hair is stimulated once or twice just before the immersion. In general, sensitive hairs must be stimulated twice, three times or more before the leaf reacts, as MACFARLANE, BROWN and SHARP, and BROWN have proved by experiment. The stimulation or stimulations preceding the last one may perhaps be raising the irritability of the leaf, that is, perhaps be bringing the inner epidermis into a condition in which it is ready to react. Hence, when a leaf stimulated beforehand is immersed in boiling water, the upper epidermis which has been brought into a highly sensitive condition may react sooner than the motor cells are injured by heat. On the contrary, in a leaf which is not previously stimulated, the less sensitive upper epidermis can not react so quickly as to precede the injury of the motor cells. The sensitized condition of the upper epidermis seems to vanish in a few minutes, for if leaves are immersed in boiling water several minutes after the preliminary stimulation, they do not react and hence die open. *Aldrovanda* leaves are more sensitive than *Dionaea* leaves, the latent period of the shutting movement being shorter, and the movement itself being quicker. Hence an *Aldrovanda* leaf shuts without fail, however quickly it may be immersed in boiling water, as the inner epidermis reacts to the thermal stimulus before the outer two layers are injured by heat. The fact that *Aldrovanda* and sensitized *Dionaea* leaves react upon sudden immersion in boiling water, just as filaments of *Berberis* etc., suggests that the shutting movement of the former has a common cause with the bending movement of the latter.

From what has been said above, it is clear that there are no grounds left for rejecting the theory that the shutting movement is to be attributed to the loss of turgor. In the next place, the most important four of the facts which GUTTENBERG considers as supporting his theory of swelling tissue (p. 172), will be mentioned :—

(a) The lower epidermis is more easily extensible than the upper one.

(b) The leaf becomes immovable in sugar solutions.

(c) An isolated single lobe of a leaf, or a cross-sectioned strip of it, bends stronger than ever if left in water; while it unbends if transferred into a sugar solution.

(d) A surface-sectioned lamella of a lobe curves itself invariably in such a way that the epidermis is on the concave side, whichever epidermis it may be, the upper or the lower. And the curving becomes so pronounced as to cause rolling in of the lamella, when it.

is immersed in water; while it unbends when transferred into an osmotically active solution.

GUTTENBERG's and the author's interpretation of these four facts will be given in the order in which they are stated above :—

(a) The easiness with which the lower epidermis is extended is essential for the writer's interpretation, as well as for GUTTENBERG's.

(b) GUTTENBERG holds that this fact proves that an increase of turgor is the cause of the movement. On the contrary, however, the allied experiment with *Aldrovanda* showed, by the time relation, that the shutting movement is not due to an increase of turgor (cf. p. 176). A lobe cannot bend however sensitively the upper epidermis may react, if the cells which are to propel the movement have been deprived of turgor by the sugar solution.

The latter two facts, (c) and (d), concerns material which is hurt or cut. A traumatic stimulus given to a three-layered region of an *Aldrovanda* leaf, causes a strong excitation in it. And the leaf thus wounded gets into the narrowing phase, passing through the shutting phase. The mechanism working in the former case differs from that in the latter, as will be proved later. The portion of a *Dionaea* lobe, corresponding to the three-layered region of an *Aldrovanda* lobe, namely that surrounded by the midrib and the anastomosis of vascular bundles near the margin, has a similar character.[1] Observations made with sectioned pieces of the leaf, therefore, have a bearing upon the narrowing mechanism, but not directly upon the shutting mechanism. No authors have discriminated these circumstances. Fact (c) is quite in accordance with the writer's interpretation of the narrowing mechanism of *Dionaea*, which is quite similar to what GUTTENBERG holds to be the shutting mechanism.

Though fact (d) concerns wounded material, it is important also in the discussion of the shutting mechanism, for it reveals the extensibility relation of cell walls in the leaf. The small extensibility of the wall of the lower epidermis (of *Dionaea*) seems, at first sight, to be very favourable to GUTTENBERG's theory, and not to the writer's. But, just like other facts, it is quite in accordance with the latter, as was discussed on pp. 179–180.

---

1) MUNK found that cuttings into the *marginal* region of a *Dionaea* leaf do not cause excitation in the leaf at all.

c.   Elongation of the Outer Epidermis

1.   *Irreversible Extension of the Outer Walls*

When a shut leaf of *Aldrovanda* is immersed in a sugar solution, it does not open wide, only the margins of the two lobes coming apart.   An analogous effect is seen in *Dionaea*.   Thus, leaves of either plant can not shut, and stay open even if stimulated, when the turgor is lost while they are open; while they do not return to the open stage if they lose turgor after they have once shut.   GUTTENBERG attributes this irreversibility to a fixation of the extension of the cell walls by growth, while ZIEGENSPECK maintains that it is due to over-extension of the cell walls.   The former bases his opinion on the fact that some minutes are needed for a shut *Dionaea* leaf to become irreversibly shut, while the latter holds that some duration of time is necessary even for a fixation of mere over-extension.

According to the writer's observations, leaves of either plant often do not reversibly open, even if they are immersed in sugar solutions at the same instant as the shutting takes place.   The irreversibility is perceived with fewer exceptions when *Aldrovanda* leaves are made to shut in ice water, and transferred into the sugar solution after being kept some minutes in the former.   Hence, over-extension may be the more probable interpretation.   And there seems to be no need to assume a real growth of the cell walls, since the walls of the outer epidermis are highly plastic, as mentioned before.   Experiments with acetone (cf. p. 192) show that the ductility of the outermost wall of the motile zone decreases as the leaf grows older.   Hence that wall is growing larger and thicker, perhaps undergoing physico-chemical changes at the same time, until the leaf becomes quite old.   Therefore, growth (intussusception and apposition) certainly exists.   Only, it should be distinguished from passive and plastic stretching of the wall[1] resulting from the shutting movement.

2.   *Contraction of the Outer Walls*

i.   Sugar solution

Sugar or glycerin solutions influence *Aldrovanda* and *Dionaea* leaves in two ways:

---

1)   This may, however, be the first step of the growth, according to HEYN.

1) Deprivation of motility by diminishing turgor (influence upon motor cells, see p. 183, b);

2) Stimulation by change of osmotic state (influence upon irritable cells, see Part II).

In case the first effect appears sooner than the second one, open leaves become immobile as they are, while in the reversed case, they shut. Which of these two cases actually takes place depends upon the concentration of the solution and the condition and character of the leaf. As *Dionaea* leaves are fairly cutinized and are massive, the first effect is apt to lag behind the second one, hence it is more common for open leaves to shut and shut leaves to shut closely. *Aldrovanda* leaves on the contrary, since they let water pass easily and are not massive, seldom shut in sugar solutions.

If leaves (of either plant) are immobilized in the open stage, they unfold a little wider, and, if they are made to shut or shut closely, their lobes come apart from each other afterwards. It takes several hours for shut *Dionaea* leaves to unfold markedly as they are slow to lose water, while several minutes to half an hour suffices for *Aldrovanda* leaves to do so. Besides, young leaves open quickly and wide, even to the extent of being half-opened, while the velocity and the extent of the unfolding decrease as they grow older. Cross-sectioned thin pieces of a *Dionaea* leaf spread in sugar solution quickly—in several minutes—as water is very easily lost in this case.

Such unfolding of leaves in sugar solution is naturally due to disappearance of the turgor which has been striving to bend the lobes, resulting in a contraction of the walls, chiefly on the outer (lower) side.

ii. Boiling water

When *Dionaea* leaves are suddenly dipped into boiling water, open ones (not preliminarily excited as in p. 182) unfold wider at the instant of the immersion, and narrowed ones relax the tightness with which the lobes are pressed together. The unbending of the lobes is most clearly seen when cross-sectioned pieces of leaf are dipped in boiling water. The cause may be the same as that of the unfolding produced by the sugar solution, for the cells lose turgor by dying. Only with shut leaves the effect is different. They seem to be in a very sensitive condition, ready to narrow, for they shut more closely when

immersed in boiling water.[1]   The effect of contraction of the outer wall
is so small that it is not recognized in this case.

When *Aldrovanda* leaves, open, just shut or left shut for some
minutes, are immersed in boiling water, they come, before dying, to
the closely-shut stage (Fig. 8 c), or even to stages a little more
narrowed (D), if they are old.   If they are very young, on the contrary,
their lobes come apart from each other after the close shutting-up,
sometimes even becoming half opened (if immature).   Thus, the
younger the leaf, the more the outer wall contracts in boil-
ing water, just as in sugar solutions.

iii.   Alcohol

If a shut *Dionaea* leaf is transferred into more and more concent-
rated alcohol and then at last into xylol, it becomes open.   Though
this phenomenon was falsely explained by BROWN by the osmotic
relations in the dead leaves, it may be accounted for by dehydration
of plastically extended cell walls at and near the lower surface of the
motile zone, for the distance between pairs of dots such as BROWN
marked on a leaf decreases as the leaf is transferred from water to
alcohol, and from this to xylol (cf. Table VI in BROWN '16).   Shut
Aldrovanda leaves also open, though only partially, when dehydrated
in the same manner.

As to the unfolding of leaves in sugar solutions and boiling water,
it is not decided whether that is due to elasticity, in addition to the
plasticity, of the walls on the outer (lower) side, or to a decrease of
swelling, i. e. dehydration, caused by such treatment.   However, judging
from the alcohol-xylol treatment, the latter cause seems to play a more
important rôle than the former.

C.   Mechanism of the Narrowig Movement

Notwithstanding that DARWIN noted the intense pressing together
of the halves of a narrowed *Dionaea* leaf, some authors after him
have paid attention only to the flattening of the lobes in that stage:
BATALIN attributes the narrowing movement to shortening or contrac-
tion of the outer surface of the leaf; and GUTTENBERG considers an

---

1)   Leaves which have been in the shut stage for more than a quarter of an hour scarcely
reacts on immersion, perhaps owing to depression of the sensitivity.   The phenomena of
the sensitization of open leaves by preliminary stimulation, and the disappearance of such
sensitized condition with time (p. 182) may be analogous to the present ones.

epinastic growth to be the cause, when the leaf is prevented from reopening by an ever renewed chemical stimulus. It has also been widely believed that the narrowing movement is effected only by chemical stimuli. This idea is presented typically by MUNK, who named this movement "die Resorptionsbewegung," discriminating it from "die Reizbewegung" which is our shutting movement, and he even thought that the former was conditioned by chemical changes on the exterior of the leaf, while the latter was caused by forces in the leaf itself. Pronounced bending of an isolated single lobe has been supposed to be due to the shutting mechanism (cf. GUTTENBERG etc.).

Observations upon *Aldrovanda*, however, have proved that the narrowing movement is effected by a sharper bending of the lobes, just as in the case of the shutting movement, only the velocity and extent of the bending being different. And sectioning of the leaf as in the case of Fig. 11 has shown that not only a chemical but also a traumatic stimulus causes the narrowing movement.

That the case of *Dionaea* is analogous is demonstrated by the following experiment: A *Dionaea* leaf was cut into two halves, through the midrib longitudinally, and when both the halves had bent markedly in water, an attempt was made to make the cut surfaces come into complete contact with each other as before. Some force was needed for that procedure, as the marginal portions of the lobes resist. When the cut surfaces were restored to their original contact, the lobes were flattened, their marginal portions recurving outwards, as in Fig. 15 c. Thus, it is shown not only that a *Dionaea* leaf is brought into the narrowed stage by a traumatic stimulus, but also that the lobes are flattened and are recurved along their margins not by a force causing them to unbend, but by a force that makes them bend themselves more strongly. *Dionaea* leaves are narrowed also with induction shocks. Thus the circumstances are analogous in *Aldrovanda* and *Dionaea*, and the mechanism of the narrowing movement may, therefore, be analogous in the two plants.

No theories have been put forward with respect to the narrowing mechanism of *Aldrovanda* leaves, nor have any precise studies been made concerning that of *Dionaea* leaves. The writer is going to demonstrate a narrowing mechanism which is almost the same as what GUTTENBERG holds to be the shutting mechanism. GUTTENBERG's two items, (c) and (d) on p. 182, are, indeed, concerned with the narrowing mechanism, though he took them for the shutting phase. Other facts

which support his theory of swelling tissue uphold, as a matter of
course, the present theory of the narrowing mechanism.

The original proofs will be given with *Aldrovanda* :

a.   Observation with Sugar Solution

In an earlier chapter (p. 176), it was noted that leaves become
less and less movable, the longer they are left in a sugar solution,
becoming at last quite unresponsive to stimulation, and that fact was
used to prove that the shutting movement is not due to suctioning of
water.   In what manner, then, is the narrowing movement conditioned
by the sugar solution?   As this movement starts from the shut stage
of the leaf, sugar solution of a certain concentration can be applied to
either the outside or the inside alone, or to both sides.

In order to fill the leaf cavity with sugar solution, the finely pointed
spout of a pipette was inserted between the lobes of an open leaf, and
when the leaf shut upon the spout, the solution was poured into the
leaf cavity.   As the sensory hairs must have been stimulated by the
flowing in of the solution, water was also poured in, even in the case
of the control which contained water inside, so as to make the seismon-
ic stimuli given as much alike as possible.   The leaves were thus
filled with a sugar solution or water, and immersed in a solution or
water, so as to make four kinds of combination :
  (A)   water on both sides (normal condition) ;
  (B)   sugar solution inside, water outside ;
  (C)   water inside, sugar solution outside ;
  (D)   sugar solution on both sides.

It did not take 10 seconds to pour the solution or water into the
leaf cavity and then to transfer that leaf into the next medium, picking
the petiole with a pincette.   Then a strong induction shock was ap-
plied as soon as the leaf was immersed in the second medium, followed
by two more shocks with an interval of 20 seconds between each.

The progress of the narrowing movement was expressed by the
diminution of the distance between the enclosure-boundaries of oppo-
site lobes, the distance in the shut stage being assumed to be 10.
Each experiment was made with leaves belonging to the same whorl,
one or two leaves usually being put in each of the four conditions.

Figs. 17 A, B, C and D represent the narrowing process of four
leaves belonging to a whorl of the age of No. II, each in one of the
above-mentioned conditions, A, B, C and D respectively.   The suc-
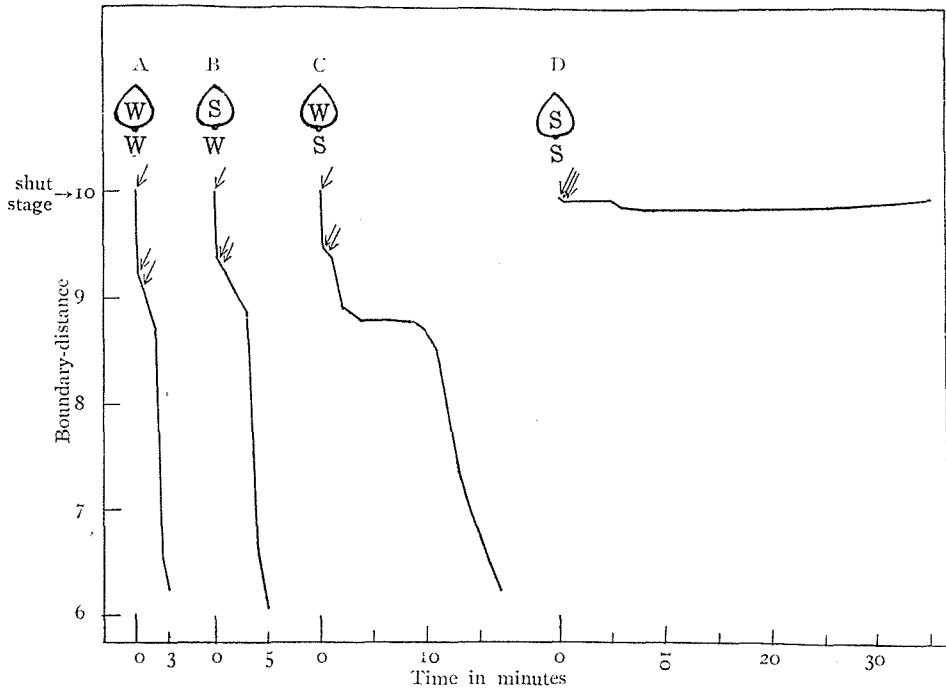rose solution was 0.2 mol in concentration.   The cases given are

Fig. 17. Modes of progress of the narrowing movement represented by boundary-distance (shut stage = 10), when, A: water is present on both sides of the leaf; B: water outside, 0.2 mol sucrose solution inside; C: water inside, sucrose outside; and D: sucrose on both sides. Each arrow indicates the instant of application of a strong break shock.

representative, and there were often cases in which less apparent distinctions could be perceived.

It is obvious from the figure that a rather conspicuous movement occurs soon after the first stimulation, if any one side of the leaf is in contact with water, while only a negligible movement can follow the strong excitation, if the sugar solution is present on both sides of it. This forms a striking contrast with the case of the shutting movement, in which an open leaf can shut almost completely if stimulated within a few minutes after immersion in the sugar solution. The narrowing movement must be due, therefore, to the sucking up of medium water by some leaf tissue, whereas the shutting is not. In a 0.15 mol sucrose solution the narrowing movement can proceed, though only gradually, whereas as described before, the shutting movement cannot, if the immersion is prolonged.

As the narrowing movement is caused by the sucking in of environmental water by the cells which propel the movement, progress

of the movement in 0.15 mol solution necessarily means that those cells have a stronger suction pressure than that solution. In the shutting phase, on the contrary, as those cells are deprived of the turgor and at last become incapable of causing the movement, they must have a lower suction pressure than that solution. Thus, it is deduced that when the leaf is in the narrowing phase, the osmotic suction pressure of the propelling cells is raised from a lower to a higher value than that of the 0.15 mol sucrose solution. But the suction pressure is not raised as high as that of the 0.2 mol solution, for the movement does not proceed in this solution.

b. Observation with Acetone

Acetone, or alcohol penetrates into living cells of *Aldrovanda* leaves very quickly and raises their internal pressure so much as to produce vesicles in the walls of the outer epidermis and of the middle layer in the motile zone, as shown in Figs. 4 and 5, Pl. IX. It is more interesting, however, to notice the quickly proceeding narrowing movement in acetone, and the connection of it with the progress of the vesiculation.

As soon as a shut leaf is immersed in an acetone solution, the margins of its lobes begin to press upon each other, and it finishes the narrowing movement within 10 seconds, or within a few scores of seconds in the slowest case. Such a quick narrowing movement never occurs when the leaf is ordinarily stimulated, however intensely.

As acetone is capillary-active, it might be able to enter into the cavity which is enclosed by the lobes, through the rims of their margins. But hardly any quantity of it can get in, as water is constantly flowing out through there while the narrowing movement proceeds. Therefore, it is only the outer surface of the leaf that is in contact with the acetone, and acetone must penetrate first into the outer epidermis, then into the intercellular spaces and the middle layer. Hence the internal pressure of the outer epidermis is raised markedly first, and then that of the middle layer. Thus, the above experiment demonstrates that the narrowing movement can occur if the turgor pressure of the outer epidermis increases much. The rôle of the middle layer is also indisputable, for its internal pressure must be raised to some extent, side by side with that in the outer epidermis.

Generally, slight vesiculation can be readily seen when the narrowing movement is finished. But if the vesiculation proceeds so

quickly as to cover most of the motile zone before the narrowing movement comes to its end, this goes on no further, and the leaf dies injured by acetone at some intermediate stage between the shut and the narrowed. This fact shows that the narrowing movement can progress no further if the cells in the outer epidermis, or those in the middle layer, too, do not increase their transversal tension as a result of the bulging out of the outer walls.

When the internal pressure of the motor cells is raised quickly by immersing the leaf in a concentrated acetone solution, both the narrowing movement and the vesiculation proceed quickly. But the former cannot be accelerated so much as the latter when the acetone is highly concentrated, for the motor cells must overcome various hindrances when they are promoting the movement, as will be mentioned later (p. 229). Therefore, when the acetone is too highly concentrated, the vesiculation proceeds faster than the movement, thus making the leaf narrow no more than to an intermediate stage. Even in concentrated acetone, however, no vesiculation occurs, if the leaf is so old that its membrane has lost its high extensibility. In such cases, the narrowing movement can be completed.

In order to see the effect of the concentration of acetone and the age of the leaf, every open leaf from No. I to VIII in age, of an individual plant, was immersed in one of four different solutions (20, 40, 60 and 80%). The time required to finish the narrowing movement in the solution, and the time elapsed before the formation of small blisters, were measured, the result being shown in Table 1. The duration of the movement was denoted as infinity if it stopped before reaching the narrowed stage, whether the movement was stopped at the closely-shut or an almost narrowed stage.

Table 1

Time is in seconds.

% of acetone		No. of the whorls							
		I	II	III	IV	V	VI	VII	VIII
80	movement	∞	∞	∞	∞	∞	∞	∞	∞
	vesiculation	5	7	10	20	40	35	∞	30
60	movt.	∞	∞	∞	∞	45	45	60	55
	vesn.	7	15	10	10	45	65	∞	∞
40	movt.	20	20	75	75	60	30	95	45
	vesn.	20	20	90	70	80	40	∞	∞
20	movt.	55	65	120	150	80	—	—	60
	vesn.	25	90	∞	150	150	—	—	∞

It may be seen from the table that there is a tendency for both the movement and the vesiculation to occur faster, the younger the leaf. The movement of young leaves proceeds faster than that of older ones, because the various factors which oppose the movement are weaker in the former; and blisters rise quicker in the former, because the cell walls are more easily extensible.

When a leaf of the age of No. I was dipped into 20% acetone, the vesiculation appeared much sooner than the movement (cf. the table). But as the blisters grew and extended slowly, most of the motile zone was free from vesiculation before the leaf narrowed. Hence the movement could be finished.

If leaves are left in an acetone solution for several minutes, all of them become widely open, whether they have narrowed completely or have stopped narrowing at some intermediate stage. This may be due to penetration of acetone into the inner epidermis, after it has penetrated the outer two layers, and the writer's interpretation of the open stage, that the inner epidermis strives against the bending of the lobe when it is turgid, is confirmed here again. In the above case, as the outer two layers have lost the force which enables them to bend the lobe on account of the vesiculation, the leaf opens wider than the normal widely-open stage, each of the lobes presenting the characteristic curvature of the turgid inner epidermis alone. The inner epidermal cells lose the semipermeability when the leaf shuts, but acetone is able to raise the hydrostatic pressure of those cells by its quick penetration through the cell wall.

Immature leaves open in acetone very quickly, perhaps owing to quick penetration. For example, when a leaf of the age of No. I was immersed in 80% acetone solution, it instantly became closely-shut, the vesiculation began in 5 seconds, and a sudden separation of the lobe margins took place 50 seconds after the immersion. The leaf went on gradually opening, and became fully open 80 seconds after the immersion.

Of the above two observations with sugar (a) and acetone solutions (b), the former has demonstrated that the narrowing movement is due to taking in of surrounding water by some cells of the leaf, and also that it is accompanied by an increase in the suction pressure of the cells; while the latter has proved that the narrowing movement can take place if the internal pressure of the outer epidermal cells, and perhaps also of the middle layer, is raised, and also that the movement can not proceed if those cells lose that pressure. It may be

certain, therefore, that the narrowing movement is caused by an increase of osmotic value and turgor pressure in the cells of the outer epidermis or of the outer two layers. Then, in what a relation does the middle layer stand to the outer epidermis in its action of propelling the movement? The osmotic values of the cells in *Aldrovanda* leaves cannot be measured by plasmolytic tests, as was noted before, and hence we are obliged to adopt indirect methods :—

c. Observation with Agar-Sections

When an open leaf imbedded in agar-gel is cross-sectioned, the lobes, striving to narrow, come to the state shown in Fig. 12. If the motile zone of one of such lobes is examined with high magnification, figures such as those seen in Fig. 18 A can be often observed.
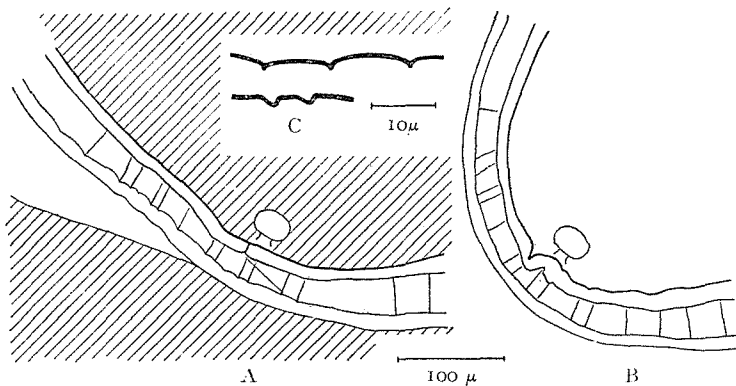

Fig. 18. Cross section of the motile zone, A: when the bending of the lobe is hindered by the agar (hatched), and B: when it is allowed by removing this. C: The profile of thickenings and furrows on the side wall of the middle layer seen through the outer epidermis, the lobe being pressed flat

In the lateral walls of the middle layer, there are numerous transverse lines, really thickenings and furrows which may be also clearly seen in the flat position of the lobe highly magnified, as shown in Fig. 18 c. They can support, without doubt, the thickness of the middle layer unaltered, so that the outer subepidermal wall is restrained from approaching towards the inner epidermis.

If the agar held between the lobes is removed, the lobe in Fig. 18 A is allowed to bend freely, reaching the state shown in 18 B: the effort of the outer epidermis to bend is accomplished, and the swelling out and wrinkles of its inner wall vanish. Such behavior of

the outer epidermis reminds us that the turgor expansion of the layer
plays a major rôle in the narrowing movement.   The walls of the inner
epidermis are puckered forcibly in Fig. 18 B, proving that that layer
does not participate actively in the narrowing movement.

d.   Thickness of the Layers

Fig. 1, Pl. IX was taken under pressure of the cover glass.   But
similar figures are observed along the most curved motile zone, with
leaves which are supported in water quite freely.   Hence the thickness
of the cells in this zone can be measured at the open, the shut and
the narrowed stages of a leaf.

Though the change is very small, the outer epidermal cells are seen
thickened while the leaf is just narrowing, approaching then roughly
to the original thickness as the stage draws nearer to the ultimate
narrowing.   To our regret, however, the change in the thickness of
the middle layer cannot be measured accurately, because the inner
subepidermal wall is not well focussed.

e.   Miscellaneous Remarks on the Narrowing Movement

i.   Rôle of the middle layer

The observations made with agar-sections (C), seem to prove that
the middle layer plays a less active rôle in the narrowing movement,
rise of turgor in the narrowing phase being doubtful.   But even if it
may not work actively, it has statical functions : 1) it maintains the
rigidity of the lobe, 2) makes a cushion between the inner and the
outer epidermis which stretch or unstretch in different manners, and
3) may help to bend the lobe with its usual turgor, as its outer wall
is more easily extensible than the inner wall (p. 167).

ii.   Water supply to motor cells

According to Fig. 17, the narrowing movement proceeds faster
when water is present on the outside of the leaf (B), than when the
water is on the inside (C), suggesting that the motor cells take in
necessary water chiefly through the outer surface.   The circumstance
may be effected by two conditions ; a) that the motor cells are in the
outer epidermis, and perhaps also in the middle layer, but not in the
inner epidermis ; and b) that the walls of the outer epidermis may be
very permeable to water, just as to acetone, alcohol, neutral red and
osmic acid.

When the inner surface is in contact with a sugar solution (B), the movement proceeds more slowly than when both surfaces are touched by water (A). But as the difference is very small, it is deduced that the water which is necessary for the narrowing movement is hardly supplied through the inner surface of the leaf. And as the narrowing movement is not retarded even if the petiole is lifted above the surface of the water, the water must be supplied chiefly through the outer surface of the lobes.

iii. Analogy with *Drosera* tentacles

HOOKER ('17) has demonstrated that the inflexion of *Drosera* tentacles "is produced by an acceleration of the rate of growth on the convex side and the median section." This work is mentioned by GUTTENBERG ('25) to confirm his view of the shutting mechanism. But as the bending movement of *Drosera* tentacles is to be regarded as corresponding to the narrowing movement of *Aldrovanda* and *Dionaea* leaves from the slow rate of the movement, HOOKER's observations corroborate the writer's interpretation of the narrowing mechanism.

If a group of *Drosera* tentacles is immersed in a diluted peptone solution containing sucrose dissolved in it so as to make a 0.4 mol solution, and another group into the same peptone solution containing no sugar, the former tentacles do not react at all while the latter bend perfectly. Such a fact is analogous to the case of the narrowing movement of *Aldrovanda* and *Dionaea*.

iv. Plastic extension of outer cell walls

By immersion of the leaf in a concentrated sugar solution, its narrowing movement may be sustained, but the stage which the leaf showed at the instant of immersion cannot be reversed markedly by such deprivation of turgor, for the outer walls are extended plastically, just similar to the case of the shutting movement. Inflected *Drosera* tentacles also do not unbend in sugar solution, and though HOOKER attributes this irreversibility to growth, it may be more proper to refer it to plastic extension of the cell walls, just as in the case of the shutting and narrowing movement of *Aldrovanda* and *Dionaea* leaves.

v. Mechanism of the eversion of the free-side lobe

Almost all *Aldrovanda* leaves have their free-side lobe everted when they narrow. Why does not the bristle-side lobe evert? When shut leaves are pressed chiefly upon the bristle-side lobe with a pincette,

the conversely narrowed stage is not seldom reached, the bristle-side lobe being everted. Hence a gradual progress of the movement, as in the normal case, seems to be rather important to cause the eversion exclusively in the free-side lobe.

The force of the motile zone causing it to bend itself can be dissolved into two components, one making the lobe margin press against the opposite lobe, and the other drawing the lobe margin nearer to the midrib. Hence, as the free-side lobe has a tendency to bend more markedly than the other lobe (p. 164), it may strive to fold itself nearer to the midrib than the opposite one, both of them at the same time pushing against each other. But as the margins of them thus pressing do not slip, the free-side lobe bends when the bending force is greatly increased, leaving the marginal part attached to the corresponding part of the opposite lobe—it is everted at the margin. After it is once everted, the eversion is easily spread towards the middle of the lobe, as the motile zone continues to bend on.

Besides the above mentioned differentiation in the bending force of the two lobes, the physical character of the one-layered region in them may also differ inherently, for young leaves growing from the bud at one time take a shape corresponding to the narrowed stage, before they attain the open stage for the first time. And when *Aldrovanda* leaves are immersed in strong plasmolytic or fixing agents, the free-side lobe often everts of itself.

The distance between the motile zone and the midrib is a little larger in the free-side lobe than in the bristle-side lobe. That may also help to produce eversion of the free-side lobe instead of that of the other.

In the narrowed stage of a *Dionaea* leaf, both the lobes recurve at the margins. But cases in which the lobes that correspond to the free-side lobes of *Aldrovanda* leaves[1] recurve more markedly than the other lobes, are met more frequently than the reversed cases, or cases in which paired lobes recurve to the same extent. This may be due to the stronger tendency of the former lobes to bend themselves, analogously with the case of *Aldrovanda*. When lobes of a *Dionaea* leaf are severed separately, the lobe in question bends more strongly than the other lobe.

f. Narrowing Mechanism of *Dionaea* Leaves

The narrowing movement of the *Aldrovanda* leaf is due to an

---

1) The lobes which are on the left hand when leaves are viewed from their apices.

increase in turgor of the outer epidermis and the middle layer, the former being supposed to work far more actively than the latter. But in the *Dionaea* leaf, the lower epidermis seems not to play an important part, but rather to hinder the narrowing ; for the lower epidermis has a negative tension against the underlying parenchyma cells (cf. p. 179). Hence, an increase of turgor in the swelling tissue may be the cause of the narrowing movement. It is not decided, however, whether the turgor is increased more in the lower portion of the tissue analogously to the outer epidermis of *Aldrovanda* leaf, or whether the turgor is raised without such differentiation. But the former case seems to be the more probable, as only the lower parenchyma works as a swelling tissue in the midrib (cf. p. 199).

If a drop of peptone solution is spread over the inner surface of a leaf which has been severed from the plant at the petiole, the petiole being dipped in water, the lobes are recurved along the margins, but the eversion hardly proceeds any further, or if at all, incomparably more slowly than in the normal case. If a leaf is cut off from the petiole at the junction, stimulated in the same way, and laid in a moist cell, it narrows in a similar way, though rather more slightly. Thus the water necessary for the narrowing movement seems to be supplied from the root system, perhaps helped by the root pressure. But when severed leaf-blades are soaked with water, the movement proceeds more quickly than in normal ones.

D. Curvature of the Midrib

a. Midrib of the *Aldrovanda* Leaf

It has been often noticed that the midrib of the *Aldrovanda* leaf curves upwards more and more pronouncedly as the responsive movement proceeds (cf. Fig. 6). If both of the lobes are cut off from the midrib when the leaf is in the shut or in the narrowed stage, the midrib retains roughly the same curvature as it has shown before the lobes are severed. From this, it may be judged that the midrib has been curved passively, and is retaining the given curvature plastically. If the curvature of the midrib in the narrowed stage of a leaf were due to an active mechanism of the midrib itself, the midrib would curve more when cut off from the shut leaf, for the traumatic stimulus thus given would bring its cells into the narrowing phase.

When an isolated midrib is immersed in a sugar solution, the curvature becomes much more pronounced than it would be in the

narrowed leaf. Hence the mild curvature of a turgid midrib must be due to the turgor force of the parenchymatous cells lying under the conducting bundle, and it may also be judged that the cell walls of the under epidermis are more extensible than the conducting bundle.

A midrib uncurves when it is returned from a sugar solution into pure water, and passing through the original curvature it becomes at last roughly straight, as if it lay in an open leaf. On being touched with a pincette such an isolated midrib bends and unbends within certain limits, as if it were a piece of lead wire. These observations demonstrate that, though the midrib has a weak tendency to straighten itself, it can not persist in that tendency, and yields plastically to external forces. Of course, the midrib is quite unable to promote the responsive movement by active backward curving (cf. p. 200).

How the midrib is curved upwards when a leaf shuts and narrows can be understood easily from the morphological features. As the motile zone forms an arc, it is farthest from the midrib at the middle (cf. Text-fig. 14 and Fig. 2, Pl. IX). Hence at the middle part of a leaf, a rather wide portion of the lobes on both sides of the midrib is left flat even when the leaf is narrowed, as seen in Fig. 11 B, while near both ends, the lobes are bent as in Fig. 19. On the other hand, the enclosure-boundary hardly changes its shape with progress of the responsive movement, as it can be seen projected upon a parallel plane (Fig. 20). Therefore when the leaf narrows, the distance between the motile zone and the enclosure-boundary is relatively more shortened at the middle than towards both ends of it. Thus the midrib is curved upwards in the manner shown with a broken line in Fig. 20.

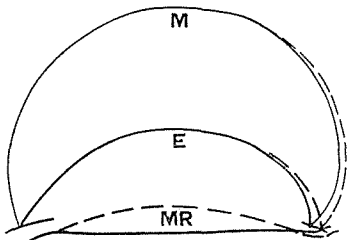Fig. 19. A cross-section of a narrowed leaf near its basal end

Fig. 20. A leaf in the open (unbroken lines) and the narrowed (broken lines) stage, projected on a vertical plane lying between the enclosure-boundary (E) and the margin (M). The inflexion of the midrib (MR) in the narrowed stage is shown.

Besides the curvature of the motile zone, the distribution of the bending capacity in that zone makes the midrib more curved; the zone bends most

sharply at the middle part and much less towards both extremities. The shut stage differs from the narrowed stage only in degree (slight in all respects).

b.   Midrib of the *Dionaca* Leaf

The midrib of the *Dionaca* leaf is different from that of *Aldrovanda* chiefly in two points : (1) it is more massive, and (2) the transition from it to the lobes is less distinct.   It is large enough to be cut longitudinally, and the cut pieces can retain their own curvature hardly influenced by external forces.   Hence in order to investigate the midrib, it is more convenient to use *Dionaca* than *Aldrovanda*.

MUNK ('76) is inclined to believe that the curvature of the midrib diminishes when the leaf closes ; while BATALIN ('77) states that the curvature becomes more pronounced in that case, asserting, at the same time, that its upward curving has a promoting effect upon the leaf closure.   The writer's own observation accords with the former author's : if the curvature of the underside of a midrib is sketched with a camera lucida, with respect to the successive stages of the responsive movement, the curvature shows hardly any changes, except in the narrowed stage, in which the curvature is, if any thing, rather diminished.

When both the lobes are cut off from the midrib, this takes on a curvature the same as, or a little less curved than, that in the narrowed stage, uncurving a little more when left in water.   When isolated midribs are immersed in a sugar solution, their curvature is increased strikingly, as in the case of *Aldrovanda* ; and when they are put into water again, they unbend, though not so much as to become straight.   Hence it can be concluded that the midrib is stretched by the turgor of the parenchyma which lies on the underside of the conducting bundle, just like that of an *Aldrovanda* leaf.

Even if the upper epidermis is pared off, or a portion or the entire depth of the parenchyma overlying the conducting bundle is cut off with it, the curvature changes little.   This also confirms the view that the curvature of an isolated midrib is maintained by the turgidity of the parenchyma *underlying* the conducting bundle, giving tension to the bundle and the lower epidermis, and that the conducting bundle must be less extensible than the lower epidermis.   In spite of GUTTENBERG's notion that the parenchyma of the midrib overlying the conducting bundle is a continuation of the swelling tissue of the lobes, it has become apparent that it is the lower parenchyma that forms the continuation. And that fact is favourable also to the assumption that the paren-

chyma in the lobe is differentiated in such a way that the cells under-
lying the conducting bundle swell more actively than those overlying
it, just as in the case of *Aldrovanda*, in which the outer epidermis
swells more than the middle layer. A similar feature, that paring off
of the upper epidermis hardly affects the curvature, was also found
in the motile zones of lobes (cf. p. 179). It may be duly supposed,
therefore, that the motor mechanism is extended into the midrib,
though much weakened, and the midrib has a tendency to uncurve
itself in the narrowing phase. Accordingly BATALIN's idea of the
inflexion of the midrib promoting the leaf closure should
now be abandoned.

When *Dionaea* leaves shut and narrow, their midribs do not curve
upwards as in the case of *Aldrovanda*, for (1) the motile zones are
broad and do not bend so sharply as the latter's, (2) the lobes and
the midribs are massive and are not so sharply delimited from each
other, and (3) the midribs themselves have a strong tendency to un-
curve, not being so yielding as those of *Aldrovanda* leaves.

E. Mechanism of the Recovery Movements

a. Reopening

Each author's interpretation of the mechanism of the recovery
movement in *Dionaea* naturally depends on his idea of the mechanism
of the closing movement. On the assumption that " the several layers
of cells forming the lower surface of the leaf are always in a state
of tension," DARWIN believes that " the lobes begin to separate or ex-
pand as soon as the contraction of the upper surface diminishes ; "
MUNK took the reopening mechanism to be the reverse of the shut-
ting mechanism, and hence due to an elongation of the upper surface
and a contraction of the lower surface.

However, since BATALIN proved that the lower surface elongates
when the leaf shuts but hardly contracts when it opens, every author has
attributed the reopening movement to " epinasty ; " BATALIN, BROWN
and GUTTENBERG all do so. BROWN's measurement, demonstrating that
the transverse enlargement of the under surface of the lobe is much
greater during the period of shutting and reopening than in normal
growth, is the most cogent evidence in support of the theory.

The present writer, too, endorses this view, with respect to both
Dionaea and *Aldrovanda* : the upper epidermis, which lost its semi-
permeability and turgor on excitation, recovers its normal permeability

and accordingly the turgor. The wall undergoing appropriate alteration (increase of plasticity, after HEYN), this epidermis grows chiefly transversely—namely longitudinally with respect to each cell. The reopening movement is very slow because it is caused by real growth.

The lower surface on the motile zone of the *Aldrovanda* leaf has a simple curvature when the leaf is shut, but it becomes wavy (as in Fig. 13) when the leaf has reopened wider than a certain degree. Such an undulation of the lower epidermis is due to the unbending of the lobe, for by this, the noncontractile lower cell walls are made a little too long for the attained curvature.

The leaf does not open very widely, but stays in a half opened stage, if it is older than No. IV or so, or if the temperature is lower than about 25°. A young leaf at a very high temperature opens fully. These facts make one conjecture that a strong force is needed to unbend the lobe over a certain limit, against the bending moment of the outer two layers. The reopening velocity is also differentiated into two speeds, fast from the shut stage to the half opened and very slow from this phase to the widely opened, as will be shown later.

b. Rebulging

As the walls of the lower epidermis are irreversibly stretched also in the narrowed stage, the rebulging movement may be due to a growth of the upper epidermis, in the same way as the reopening. If the distance between the enclosure-boundaries of the two lobes of a leaf is measured when the leaf is just shut, and then when it has rebulged after narrowing, the leaf proves to have grown a larger amount in width than would be expected from the normal rate of growth in the width (cf. Fig. 35). This fact confirms the above idea. In the rebulging phase, the turgor of the lower epidermal cells which is raised at the narrowing, may have returned to the value which is characteristic in the open and the shut stages.

The case of *Dionaea* may probably be analogous. HOOKER has proved that the unbending of *Drosera* tentacles is also "caused by an increase in the rate of growth on the concave side and in the median section, accompanied by compression of the convex side."

Process of the Leaf Movement

A. General Method

1. How to Register the Leaf Movements

As the reaction of *Aldrovanda* leaves comprises both very quick and slow movements, the method of recording them must be properly selected for each of them, or even for a portion of one. However, any phase in the whole leaf movement can be registered by the change in the distance between some two points, one on each of the pair of lobes.

The opening of a leaf is represented in the most magnified form by the distance between the margins of its lobes at the middle, and this measurement will be used very often, being denoted the *margin-distance*. This distance is greatest when a leaf is open, and converges to minimum when it shuts. Hence it is best suited for registering the shutting and the reopening movements, but it can never be used for the study of the narrowing and rebulging movements. In these cases, therefore, the distance between the enclosure-boundaries, *the boundary-distance*, will be recorded.[1] To measure the margin- or the boundary-distance, the apical view of leaves should be given. Hence a device for getting desired views of leaves by using ball-and-socket joints (see p. 155) must be adopted before the recording is commenced.

2. Method of Stimulation

As application of seismonic stimuli to sensory hairs would often cause some shifting of the position of the leaf, it is most convenient for observations and autographic recordings to use electrical stimulation. In the experiments now to be described, single break shocks from an induction coil of Du Bois-Reymond's type were chiefly used, though a direct electric current was employed in one case. In all the cases, the electrodes were a pair of aluminium plates, 3×5 cm^2, dipped in the medium (water) parallel to each other, with the leaf to be stimulated midway between them. Czaja's method of stimulating with small-surfaced metal electrodes is not only inconvenient for the present purpose, but is less preferable, as will be discussed

1) The margin-distance does not necessarily mean the distance between the points which lie at the very margins of the lobes and just in the middle, but may also include that between appropriate points selected for the purpose in the neighbourhood of the defined spots, and similarly with the boundary-distance.

in Part II. When large metal plates are used as the electrodes, a leaf can be put in as uniform an electric field as possible, though the uniformity of the current would necessarily be disturbed by the leaf itself.

Of innumerable orientations of a leaf relative to the direction of the electric current, three characteristic ones are shown in Fig. 21, the direction of the current flow being indicated with arrows. Even if each of the three leaves, A, B and C, is rotated with an axis parallel to the direction of the current flow, the relation of the leaf to the electric current does not change. Therefore, A, B
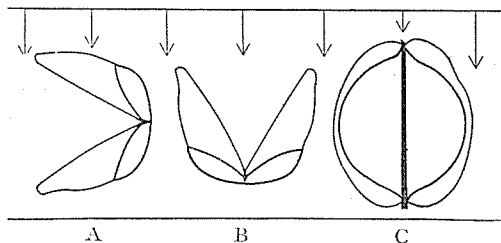

Fig. 21. Three characteristic positions of the leaf relative to the stimulating electric current, lateral (A), dorsal (B) and longitudinal (C)

and C in Fig. 21, which may be designated as lateral, dorsal and longitudinal respectively, are representative of three modes of orienting the leaf to the electric current. The threshold stimulus of a break shock is found to be much higher when the leaf is in the longitudinal position (C), than when it is in the lateral (A) or the dorsal (B) position.[1] In stimulating a leaf with electricity, therefore, the direction of the current in connection with the leaf should be noticed. In all of the following observations, the lateral position was adopted, except in one case (p. 205) in which the dorsal one was used.

B. Quick Phase of the Shutting Movement

The shutting movement is very quick in the earliest period, though is retarded afterwards. It was possible to record the process of that quickest movement on rotating photographic film, free from artefacts. Descriptions of the method and the results, and discussions on recorded types of the movement follow.

a. Apparatus

The diagrammatic plan of the apparatus is shown in Fig. 22. The whole is set up in a dark room. In a large box (B), is placed a 100-C. P. Ediswan projection lamp (P) as a light source on one side. The wall of the box on the opposite side of this has a hole,

1) These facts will be discussed in greater detail in Part II.

into which a horizontal microscope (M) is inserted, the objective lens being pointed towards the light source. Between the lamp and the microscope is placed a glass case (C) filled with water. Two metal electrodes are placed in this case, 7.5 cms. apart from each other.
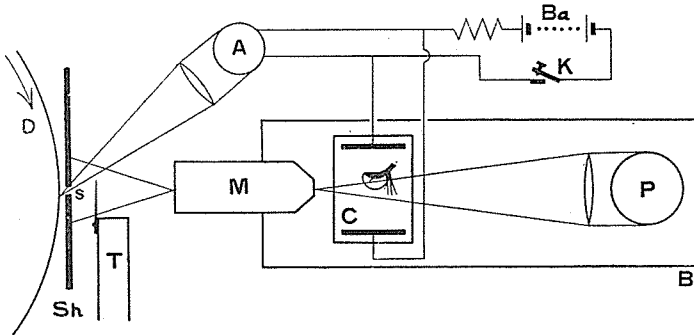

Fig. 22. Diagrammatic plan of the apparatus for recording the quick phase of the shutting movement. Explanation in text

Care is taken to prevent the light inside the box escape out, except through the microscope (M). The light coming through this microscope is projected on a screen (Sh), which has a slit (S), 6 cms. long and about 0.7 mm. wide. And as this slit stands vertically, the light beam which is permitted to come through the slit marks a vertical bright line on the drum of a myograph (D). A pen projects from a tip of a tuning-fork (T) and makes a dark point on that bright line.

An argon light tube (A) is fixed upon the box (B), over the microscope, the light from this tube being focused by a lens upon the upper end of the slit. (As A overlaps M in the plan, it is drawn to the side of its due position in Fig. 22). The argon tube is connected electrically in parallel with the electrodes in the case (C). Hence, at the very moment when the stimulating circuit is closed by a key (K), the tube is lighted without any loss of time,[1] because its lighting is caused by a discharge in the gas, and the moment of stimulation is exactly impressed on the film which is wound round the drum (D) to record the leaf movement.

b. Method

A severed leaf-blade is transferred into the glass case (C), and is

---

1) Theoretically, the electrical capacity of the tube and of the electrodes in the case (C), together with the inductance of the connecting wire, may delay the lighting of the tube. However, this need not be considered for the present, the lag being perhaps of the order of 10^{-6} secs.

fixed in the position as seen in Fig. 22, i. e. in the dorsal position. The apical view of the leaf is projected on the screen (Sh) and the images of the tips of the two lobes are made to fall just upon the slit (S). The ocular lens of the microscope (M) being shaded, a sheet of photographic film is wound round the drum (D). The tuning-fork is set vibrating, and the screen on the microscope being taken off, the myograph is started revolving, followed by a closure of the key (K), a moment after. By means of the image on the screen, the leaf is seen to have shut. Measurement of the temperature of the water in the glass case (C), and development of the photographic film completes the procedure.

Electricity used for stimulation.—The battery (Ba) was of 110 volts. There being a resistance in series and the argon tube in parallel, the potential between the electrodes for the stimulation was 96 volts, and hence the potential gradient was 1.3 volts per cm. The current passed through was about 24 m. amperes at the instant of the closure of the key, and as the area of each electrode was 15 sq. cms., the mean current density was about 1.6 mA./cm².

c. Record

By the method described above, the shutting movement of leaves of various ages was recorded at various temperatures. Five of the myograms are shown in Figs. 1–5, Pl. X. The record starts at the left end of each print. The two black lines, which are apart from each other at the left end of the print and come in contact or become nearer to each other at the right end, are impressed by the tips of the two lobes of a leaf, the bristle-side lobe being put over the other. The sine curve is the trace of the shadow of the pen point jutting out from the tuning-fork, which vibrates 50 times a second. The parallel dark straight lines are impressed by bristles. The argon tube marks a bright band along the lower margin of each of the prints. Hence the left end of this band indicates the instant at which the stimulating current was started, and in each of the myograms this instant is marked with an arrow. Near the left end of each print, a white line is drawn transversally to indicate the inclination of the slit, the inclination at each recording being impressed by over-exposure upon an end of the film. Hence all the points lying on any one line parallel to that white line represent the same instant.

i. The latent stage

The normal process of the shutting movement is shown in Fig. 1, Pl. X, which is a myogram made with a leaf of whorl No. II at 30°. The reaction of the bristle-side lobe begins at *a*, i. e. about 0.09 seconds after the stimulation, and the opposite lobe starts to move almost at the same instant. The latent period of the shutting movement is largely conditioned by the temperature, the intensity of the stimulus, and the age of the leaf, as will be explained in detail in Part III. At a low temperature, for example, the latent period is much prolonged, as is seen in Fig. 3, Pl. X, which was printed with a leaf from No. VI at 10°. But, however greatly the latent period itself may be altered, the two lobes of a leaf start to move almost at the same time, notwithstanding that each lobe has its own motile zone. Thus, it can be concluded that the two lobes of a leaf show the same reaction time when they receive a stimulus of the same intensity, under conditions common to both. And this may also mean that the two lobes of a leaf have always the same irritability.

An electric current stimulates both lobes of a leaf at the same instant. In order to stimulate a single lobe only, a tactile stimulus was applied to sensory hairs of a lobe with a micromanipulator. But no reaction lag of the unstimulated opposite lobe could be perceived by mere observation. Excitation must therefore be conducted from one lobe to the opposite one very quickly, as in the case of *Dionaea*.

ii. The quick phase

After the latent period is over, the lobes move very quickly, and pass through about four fifths of the shutting course within about one fiftieth of a second, in the case of Fig. 1. In this case the shutting velocity measured at the margin of the free-side lobe is about 9.4 cm./sec. at the quickest instant of the shutting movement. But at *b*, the movement is retarded. The period *a–b* may be designated *the quick phase* of the shutting movement and the one following it *the slow phase*. Most of the curves pass very smoothly from the latent stage to the quick phase, and from this to the slow phase, as is seen in Fig. 2 or 3, Pl. X, the former being printed from a leaf from whorl No. II at 27°. These curves, however, will show a similar form to that in Fig. 1, if they are condensed in length. The maximal velocity of the lobe margin is 8.3 and 1.6 cm./sec. in the cases of Figs. 2 and 3 respectively. The quickest record the writer has measured is 16.1 cm./sec.

The two lobes of a leaf show a slight difference in the progress of the quick phase; not only does the free-side lobe travel a longer way than the opposite lobe, as can be seen by comparing Text-fig. 5 B with A, but also the movement goes on in a different time relation. In Fig. 2, Pl. X, for example, the bristle-side lobe is moving a little more quickly than the other one at one instant, and at another instant this relation is reversed. Roughly speaking, however, the movement of the two lobes proceeds rather symmetrically.

As the movement passes gradually from the quick to the slow phase, it becomes more difficult to determine the duration of the quick phase than that of the latent period. Nevertheless, rough estimation shows that the duration of the quick phase is short at high temperatures, and long at low temperatures—about 1/100 sec. at 35°, 1/100 to 1/50 sec. at 25°, 1/50 to 1/20 sec. at 17°, and 1/50 to 1/5 sec. or still longer at 10°.

The shutting movement must be retarded by water, because the lobes have to move against the friction of it, pressing out a certain amount of the water which is present between them while the leaf is open. If an electrical stimulus is applied to a single isolated leaf floating freely in the water, it moves suddenly backwards, as it shuts and presses out the water to its front.

iii. Pause in the quick phase

Usually one out of three myograms shows that the quick shutting movement pauses temporarily on its way, as is shown in Figs. 4 and 5, Pl. X. The former was recorded with a leaf of No. II at 27°, and the latter with one from No. I at 27°. In Fig. 4, the movement which started at *a* pauses at *c* an instant, starting again with greater velocity than ever. In Fig. 5, the movement stops at *c* and does not start again till *c'*.

As these myograms show only the movement of the margins of the lobes, such two-stepped curves would be produced if the pliant one-layered regions were to swing once while they are shutting, even if the movement of the motile zones were continuous and one-phased. But observations of the movement under the microscope prove that this is not the case; the one-layered regions do not swing, and yield little to the water pressure,[1] and hence the two-stepped curves of

---

[1] The one-layered region has due rigidity owing to its convexity and to the infolded rim (p. 153).

the myograms represent a type of shutting movement which pauses on its way. Perhaps FENNER may have observed this type of shutting movement when he stated (p. 374) that the leaf movement is not continuous, but " sich zitternd und ruckweise vollzieht."

The pause in the shutting movement may perhaps be due to some resistance of the cell walls that have to be stretched when the lobes shut. So far as the writer's observations go, this phenomenon takes place at various temperatures, but not at high ones ; in leaves of various ages, though most frequently in very young or old ones ; and after electrical as well as mechanical stimuli, provided they are very weak.

If the mechanical structure of the stalk which connects the leaf-blade to the petiole is weak, the leaf-blade yields to the reaction of the water which is forced out by the lobes. The undulation of the curves at the beginning of the slow phase as seen in Fig. 4, Pl. x, may be due to swaying caused in such a way.

C. Slow Phase of the Shutting Movement

After the quick phase every shutting leaf passes into the slow phase before the margins of its lobes come in contact, the movement going on more slowly than ever. In each of the myograms, Figs. 1-5, Pl. x, only an early portion of this phase is shown, from b on to the right end of the print.

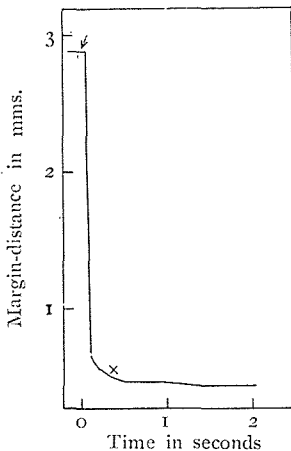As the movement in this phase is too slow to be recorded with a myograph, cinematography was adopted, with average exposures of 22.2 a second. The margin-distance plotted with respect to every frame of film is shown graphically in Fig. 23. The movement is rather quick at the beginning of this phase, but becomes very slow after about half a second.

As this slow movement continues further for some minutes, its velocity ever decreasing, the change in the margin-distance was observed under the microscope, with an ocular micrometer. Fig. 24 A is a record thus obtained, with a leaf of No. V stimulated with a break shock, the coil distance being 8 cms. As the scale of the ordinate is magnified, only the lower part of the graph is shown, the open stage and most of the quick phase

Fig. 23. The shutting movement represented by the margin-distance. The arrow indicates the instant of stimulation. At ×, the margins of the infolded rims of the lobes touch each other.
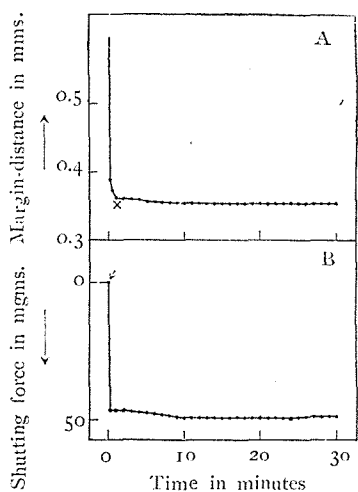
Fig. 24 A. The slow phase of the shutting movement of a leaf from No. V, stimulated by a weak shock (8 cms.) at 25°. The open stage and most of the quick phase is omitted. × : the loosely-shut stage

B. The shutting force exerted by a leaf from the same whorl, in the same conditions as A. In the abscissa, the distance between the silica rods of a dynamometer is taken upwards and hence the sum of the forces exerted by the two lobes is taken downwards.

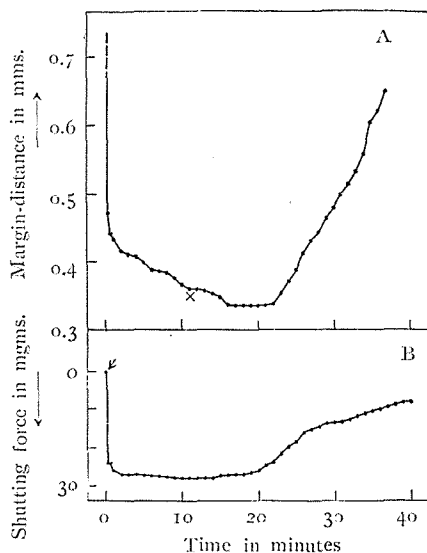being omitted. The first point in the figure represents the tenth second after the stimulation.

Fig. 25 A-B. Just analogous to Fig. 24 A and B, only the age of the leaf differs, i. e. No. O. × : the loosely-shut stage. Upward curves mean reopening (in A) and decrease of the force (in B).

The movement is comparatively quick within the first one minute, but after that becomes so slow as to be hardly distinguishable. The leaf is then in the loosely-shut stage. Some leaves stop moving in this stage, while others do not, as in the case of Fig. 24 A, moving quite a little more. Thus receiving the same stimulus, some leaves reach the loosely-shut stage, some others the closely-shut, while others reach some intermediate stages between the two. The cause of such differences will be shown later. Also, the shutting reaction may be said to be subject to the all-or-nothing principle, if the closeness of the shutting is neglected.

A case of the slow phase is shown more precisely in Fig. 25 A, which was obtained with a very young leaf (No. O) of the same individual as used for Fig. 24, stimulated in the same manner. Its slow phase takes fifteen minutes, while a mature leaf completes it within a minute or so, which shows that the movement in the slow phase is not uniform in its velocity.

The absolute value of the margin-distance must vary with the

size of the leaf and the points with which the measurement is made.
But Figs. 24 A and 25 A may be compared with each other, though
without legitimate accuracy, if the values of the margin-distances in
the loosely-shut stage are assumed to correspond.   If the two graphs
are compared with each other in such a way, the following is under-
stood :   The quick phase of the former ceases long before the lobe
margins touch each other.   (Note the position of the mark ×.)   It
takes only 22 minutes for the former to reopen, but much longer for
the latter.

Though many young leaves behave somewhat alike, such slow-
ness of the movement as in Fig. 25 A, is a rare case.   In general,
the leaves of No. II and some of No. III shut closer in the first
moment, finish the rather quick movement within 4 minutes, and then
the indistinguishably slow movement continues for several minutes.
They begin to reopen 25 to 30 minutes after the stimulation if the
coil distance for the stimulating shock is 8 cms.

D. Progress of the Working Mechanism

In the above, the whole process of the shutting movement is
elucidated.   However, the force that is acting cannot be analysed from
that alone, for various forms of mechanical resistance must come into
play in the movement.   In order to find out about the original force
exerted, and at the same time the intervening resistance, the writer
measured the force exerted by the leaf when it strives to shut, the
changes in that force with time being followed.

a.   Apparatus and Method

The apparatus is a simple one, as shown in Fig. 26. Two fine
silica rods (S, S), ca. 0.2 mm. in diameter, are inserted into a glass tube
(G) and fixed there with sealing wax (hatched), parallel to and at a
suitable distance from each other, their free ends being out of the
glass tube.   Ink marks are put near the tips of the rods, one on each,
the distance between which (d) is that to be measured.   The glass
tube is fixed upon an arm of a micromanipulator, a set of ball-
and-socket joints holding the leaf to be experimented on being fixed
upon the other arm of it, and the two placed together in water in a
petri-dish.   The silica rods and the leaf are set in the position shown
in the figure, so that the middle part of each margin will touch one
of the silica rods when it reacts to a stimulus.   Thus the lobes will
push the silica rods nearer together when they strive to shut.

The distance between the spots marked on the silica rods is measured before and after the leaf is stimulated. And as the connection between strain and stress in each of the silica rods is previously calibrated with a torsion balance, it is possible to deduce from
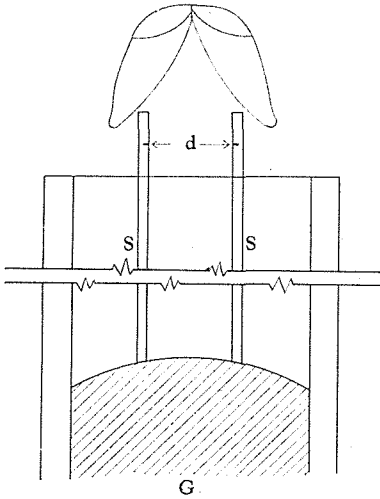

Fig. 26. A diagram of the silica-dynamometer with a leaf in its proper position. Explanation in text
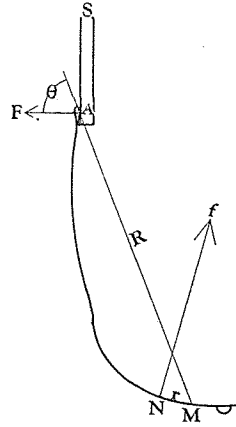
Fig. 27. Explanation in text

the distance between the rods the strength of the force exerted by two lobes of the leaf at every instant. Such a set of silica rods for measuring the force of reacting leaves will be called, for the present, *the silica dynamometer*.

Notwithstanding that every portion of the motile zones is engaged in the bending of the lobes, only the marginal points of the lobes at the middle are resisted by the silica rods. Hence the portions of the lobes beside the middle are drawn nearer to each other. The lobes are greatly deformed and a complicated relation of strain and stress must exist in them. But as the acting force of the leaf is as a whole in equilibrium with the elastic force of the silica rods, the leaf can be regarded as a rigid body in this state.

Therefore, the entire force produced by the leaf at every moment is expressed by the silica dynamometer. Fig. 27 represents the cross section of a leaf lobe pressing against a silica rod (S). Let the motile zone of this lobe be MN, its width being r, and let this lobe touch s at a point A, and the distance AM be R. If the magnitude of the force produced by MN be f, the leaf is pressing against s with a

moment of force fr. Now if s be reacting to the leaf with a force F, which is perpendicular to s itself and makes an angle θ with AM, the leaf is receiving a moment of force $R \cdot F \cdot \sin \theta$.

Therefore the relation $f \cdot r = F \cdot R \cdot \sin \theta$ holds for one lobe, and $f' \cdot r' = F' \cdot R' \cdot \sin \theta'$, for the opposite lobe of the leaf.

$(F + F')$ can be measured with the silica dynamometer, and assuming that $r = r'$, $R = R'$ and $\theta = \theta'$, $(f + f')$ can be calculated. But as those three constants are only roughly measurable, the reacting forces of leaves will be given in the form $(F + F')$ in the following, and not converted into $(f + f')$.

Thus, by observing the changes in the distance between the silica rods, the process of the entire force exerted by the leaf to move the lobes can be directly measured, the effects of hindrances, normal or accidental, to the leaf movement being rejected.

b.   Result

1.   Old Leaves

Fig. 24 B represents the process of the reacting force of a leaf measured by the above-mentioned method. The leaf used belongs to the same whorl of the same individual as that used for Fig. 24 A, stimulated in the same way. Hence this figure may serve to give information about how the force works when the leaf is moving in the slow phase.

The free length of the silica rods of the dynamometer used was 15 mms. The distance between the marks on the rods was 1.2 mm. when unacted on by external forces, and became 1.0 mm. when each was acted on by a force of 25 mgms.

Fig. 24 B shows that the leaf exerts a force of about 46.5 mgms. at the first instant of the reaction. After remaining constant for a minute or two, the force increases gradually, until it amounts to a little more than 49.3 mgms. The process of the exertion of the force forms a striking contrast with that of the movement, for the movement proceeds conspicuously farther in the interval between the 10th second and the 1st minute (cf. Fig. 24 A); but afterwards, both the movement and the force show a slight and gradual progress.

2.   Forms of Resistance to the Movement

As the movement does not proceed parallel with the force causing it, some factors must be impeding it. The various obstacles which

the lobes have to contend with in their movement will be mentioned here.

(A) Water.

1) One form of water resistance is that due to the rotatory motion of each lobe—chiefly the form resistance (cf. KAUFMANN, p. 218). This comes into question only when the velocity of the movement is very great.

2) Another form is that which comes into play when the water is pressed out through the opening between the lobe margins—the frictional resistance. This grows very large when the leaf is on the point of being quite closed, as the friction near the boundary layer rapidly increases (cf. KAUFMANN, p. 210 ff.).

(B) Resistance by parts of the leaf.

3) Elasticity of the marginal fold: The form of the marginal fold of an open leaf is shown in Fig. 8 A. It becomes B, C or some intermediate form between them, when the leaf shuts. In general, old leaves show the loosely-shut stage (B) and young ones the closely-shut stage (C). This is represented also in Fig. 24 A and Fig. 25 A, for the movement after the loosely-shut stage (marked ×) is continued further in the latter than in the former case. Such a difference with the age of the leaf is conditioned by the elasticity of the marginal fold, for the force acting to make the leaf shut is weaker in a young leaf than in an old one, the cell walls of young leaves being more yielding than old ones. The elasticity of the marginal fold varies with individual plants, and perhaps from leaf to leaf in a whorl, the form of the shut leaf being conditioned greatly by that character. It can thus be understood that the marginal fold resists the movement after the margins of the lobes are in touch with each other.

4) Firmness of the lobe: If a marginal portion of a lobe is cut off, that leaf shuts, when stimulated, so far as to make the cut end of the lobe touch the opposite lobe. This shows that the shut stage is only an inhibition of the shutting movement, in which the lobes meet together. Each lobe must withstand the pressure of the opposite lobe which does not yield. And such firmness of the one-layered region is supplied chiefly by its convex curvature, as already stated (p. 153). This firmness of the lobe is the last and an unsurmountable obstacle to the *shutting* movement. When, however, the bending force of the lobes becomes so strong as to overcome it, the movement enters the next stage, the narrowing. The last mentioned resistance (3) is an

expression of the present one, but as it directly conditions the form of the shut stage, it is put in a separate category.

In the apical view, the rim of an open leaf is not straight but slightly convex near the midrib (Fig. 5 A). Hence for the margins of the lobes wholly to meet each other, they must become flat. Such a deformation, as well as the upward curving of the midrib, needs little energy, and will play no effective rôle in impeding the movement.

3.   *Relation among the Motile Mechanism, the Resistance and the Movement*

If an open leaf is stimulated, the turgidity of the inner epidermis vanishes suddenly, setting free the force of the other two layers which causes them to bend inwards. Each lobe strives to bend itself with an average force of about 23 mgms. (in the case of Fig. 24 B). But the 1st and 2nd forms of resistance (cf. p. 213) do not permit the shutting to be accomplished in an instant. The movement is rather quick at first—the quick phase, but it is much retarded when the margins of the lobes are drawn near and the 2nd form of resistance grows large—commencement of the slow phase. The 1st form of resistance becomes negligibly small when the movement is greatly retarded. And after the margins of the infolded zones have touched each other ($\times$ in Fig. 23), the 3rd form of resistance begins to act, the 2nd growing greater than ever. The movement is held up temporarily 1 minute after the beginning of the quick phase ($\times$ in Fig. 24 A), the force of 50 mgms. and the 3rd and 4th forms of resistance being equilibrated. This is the loosely-shut stage, and the movement up to this stage has been advanced by no other force than that exerted at the first instant of the quick phase. Hence that phase, an early part of the slow phase, may be called *the residual quick phase*. The outer epidermis (and the middle layer) in the motile zone of an open leaf has a suction force S_z, conditioned by the formula,

$$S_z = S_i - (A + W) \qquad\qquad \text{(cf. p. 179).}$$

The pressure in the inner epidermis, A, vanishes when the latent period is over. But as it is replaced by the resistance of water which impedes the movement, that factor is not nullified in an instant, but decreases gradually, to become minimum when the leaf is loosely-shut. Then S_z is maximum, and the two layers of cells suck water in, thus strengthening the shutting force again. And this gradual increase of

the force effects the slow secondary shutting movement following the residual quick phase. The phase may be called *the slow phase proper.*[1]

The progress of the force as measured by a silica dynamometer must be somewhat different from the normal, for the lobes do not bend to a normal extent when the dynamometer is used. In this case, a fraction of A is replaced by the elastic force of the silica rods, the

Table 2

	Open stage & latent period	Shutting movement				Shut stage & latent period of nar. movt[2]
		Quick phase	Slow phase			
			Residual quick phase		Slow phase proper	
			Margins not yet touching	Margins touching		
1) Form res. of wate	o	[diagram]	small	negl.	negl.	o
2) Res. by pressing out of water	o	[diagram]	[diagram]	[diagram]	[diagram]	o
3) Res. by marginal fold	o	o	o	[diagram]	[diagram]	max.
4) Res. by the opposite lobes	o	o	o	[diagram]	[diagram]	max.
Turgesc. in the inner epid.	max.	o	o	o	o	o
Mechanism	equil. betw. inner epid. & outer two layers	turgidity of outer two layers			recovery in turgor of outer two layers by renewed suction of water	equil. betw. outer two layers & 3rd & 4th resistance

maximal increase of S_z being hindered. However, the result obtained with the dynamometer may not deviate so much from the normal case as to prevent the presumption that the force follows its normal course; for, as each lobe is supported by a silica rod at a single point, the portions of the lobe on both sides of that part bend to an extent only a little less than in the normal case, and the forces ex-

---

1) This phase is discriminated from the narrowing phase (see later) by the constancy of S_i, S_z being increased only by disappearance of A. In the narrowing phase, however, S_i increases much (cf. p. 193).

2) As to resistance in the narrowing movement, see Fig. 32.

erted by these portions secondarily owing to a decrease of A and the suction of water, are applied to the silica rod.

The movement in the slow phase proper continues for several minutes, striving against the 2nd and the 3rd forms of resistance, and ends when the secondarily increased force of 49.3 mgms. comes into equilibrium with the 3rd and 4th forms of resistance. Pressure by the opposite lobe (A') takes the place of A in the suction force equation of the outer two layers, which, therefore, suck in water until the equation $S_z = S_i - (A' + W)$ is fulfilled. The equilibrium will be maintained until the shutting force is reduced by the reopening mechanism (namely by an increase of A), or increased by the narrowing mechanism (namely by an increase of S_i).

Though the magnitude of the force and the velocity relation of the phases vary with the leaf, they can be represented by Figs. 24 A and 24 B. And the above-mentioned connection between the motile mechanism, the various forms of resistance, and the resulting movement holds in general, and can be summarized as in Table 2.

4. *Young Leaves*

Fig. 25 B was obtained with a leaf belonging to the same whorl as the one used for Fig. 25 A, and hence represents the course of the shutting force in very young leaves. The leaf exerts a shutting force of about 35 mgms. at the instant of the quick movement, and about 41 mgms. at the maximum. The force is apparently weaker than in the case of the old leaf. Young leaves have much narrower three-layered regions than old ones, the immaturity being much pronounced in the motile zone, notwithstanding that the one-layered regions have nearly the maximal area already. Therefore, the moment of the force exerted upon the margins of the lobes; i. e. upon the silica dynamometer, is naturally smaller. The weakness of the shutting force, however, may be chiefly due to some immaturity of the motile mechanism itself.

As the shutting force is weaker, the young leaf enters the residual quick phase when the opening is still wider than in the case of the old leaf, as is seen in Fig. 25 A, and the slow phase proper begins while the movement is still proceeding in the foregoing phase, as the suction of water by the outer two layers reveals its effect during this phase. The resistance by the marginal fold is very small in the young leaf, for the membranes are still very flexible. Hence the very closely shut stage can be easily reached in young leaves in spite of their weak shutting force.

Fig. 25 is a case of an extremely young leaf, as was noticed before, and hence most young leaves react in some ways intermediate between those shown in Figs. 24 and 25.

Young leaves begin to reopen very soon, and the measurements show that a decrease of shutting force occurs parallel with this (cf. Figs. 25 A and 25 B). The reopening begins sooner in Fig. 25 B than in 25 A. This may perhaps be owing to an elastic force in the silica rods helping the growth of the inner epidermis. An analogous example was observed by BOSE (p. 56) with *Mimosa*, the pulvinus of which recovers from the reaction when helped by an external force.

5. *Age of the Leaf and the Shutting Force*

The shutting force varies with the age of the leaf, as may be understood by comparing Figs. 24 B and 25 B. Table 3 gives the mean shutting force of every two leaves with respect to whorls of one and the same individual, measured 1 minute after the stimulation. Each pair of leaves showed quite similar values. The stimulus was a single break shock, the coil distance being 8 cms., not much over the threshold intensity.

Table 3

Whorl No.	VIII	VII	VI	V	IV	III	II	I	O
$F+F'$ in mgms.	36.5	51.0	54.3	45.6	47.8	45.6	39.8	38.7	21.8

The shutting force becomes stronger as the leaf grows older, the leaves of No. IV, V and VI having roughly the same value. But very old leaves exert a weaker force, e. g. No. IX and such leaves often do not shut even if the silica dynamometer is taken away after the measurement. This may be due to a decrease in the extensibility of the outer cell walls.

6. *Margin-Distance and the Shutting Force*

The lobes can move little if the distance between the silica rods of the dynamometer is large, while they shut half-way or even more, if that distance is small. In the former case the dynamometer indicates the shutting force of the leaf in a widely open state, while in the latter case, it shows that in a half open state or so. Measurements made in such two cases are given in Table 4, the force being

determined 1 minute after stimulation. The distance between the points on the outside of the silica rods is 2.27 mm. in the large dynamometer, and 1.2 mm. in the small one, when they are unacted

Table 4

Whorl No.		X	IX	VIII	VII	VI	V	IV	III	II	I	Sum	Ratio
$F+F'$	Large dynam.	55	70	67	76	78	67	70	61	58	32	634	100
in mgms.	Small dynam.	47	43	54	47	54	45	47	32	36	22	427	67.4

on by any forces. They diminish to 2.11 mm. and 1.0 mm. respectively when a force of 25 mgms. is applied to each of the rods to bend it inwards. The result with the latter dynamometer is naturally smaller than with the former, for the turgor of the outer two layers must be lessened when the lobe is considerably bent as in this case. Though the dynamometer gives $(F+F')$, and not $(f+f')$, the difference in the results with the two dynamometers shows that $(f+f')$ varies to a corresponding extent, for sin θ changes only about 3% while R hardly varies, in the equation,

$$(f+f')r = R \cdot \sin \theta \ (F+F'). \qquad \text{(cf. p. 212)}$$

Figs. 24 B and 25 B and Table 3 were obtained with the smaller dynamometer. When the large one is used, the secondary increase in the exertion of force is scarcely recognized, as the outer two layers
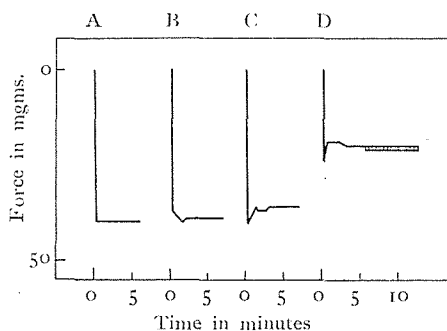

Fig. 28 A, B, C: types of the exertion of force in the shutting process measured with the large dynamometer; B, C, D: those with the small one, B & C being common to both of the cases

suck in less water. The type seen in Fig. 28 A is common in such a case, and also there are some similar to Fig. 28 B or 28 C. When the small dynamometer is used, the types of Fig. 28 B or 28 C are also seen, as well as those of Fig. 24 B and 25 B, though the secondary increase of force is recognized in most cases, thus changing the type of Fig. 28 C in that of Fig. 28 D.

7. *Temperature and the Shutting Force*

When a leaf left at 10° for a few hours is stimulated, it will shut slowly after a long reaction time, as is recorded in Fig. 3, Pl. X. The movement stops when the lobe margins just touch each other, and sometimes even before that.

These facts are clearly explained by dynamometer measurements, for the force exerted is very weak at low temperatures; there was even a case in which a leaf of No. II exerted a force of only 12 mgms. upon the small dynamometer at 10°. Such weakness may be due to incomplete change of permeability and turgidity in the inner epidermis. The shutting movement is greatly retarded at low temperatures, as a weak force cannot easily overcome the 1st and 2nd forms of resistance, and it is stopped by even a slight action of the 3rd and 4th forms.

8. *Intensity of the Stimulus and the Shutting Force*

All the experiments given above are on the reaction of a leaf to a stimulus hardly over the threshold value, the coil distance of a Du Bois-Reymond coil being 8—9 cms. If strong stimuli, say coil distance = 0 cm., are applied, many more leaves reach the closely-shut stage than when the weak stimuli are used, though some leaves exceptionally stop in the loosely-shut stage, showing no difference from those in the latter case. Such a difference in the ultimate shut stage is caused by a difference in the strength of the shutting force.

Leaves of various ages, from No. O to No. VI, were stimulated with break shocks, the coil distance being 0 cm. in one case, and 8 cms. in the other, and their shutting force was measured with the large or the small dynamometer, at 1 minute after the stimulation. The mean strength of the force with three individuals is shown in Table 5.

Within the limit of the intensity of stimulus used, the stronger the stimulus, the stronger was the shutting force of a leaf, no matter which of the dynamometers was used.

Table 5

Coil dist.	$F+F'$ in mgms.	
	Large dynam.	Small dynam.
0 cm.	64.7	49.0
8 cms.	61.0	46.7

There may be leaves the marginal folds of which

are resistant enough to shutting forces called forth by weak stimuli, but not to those of strong stimuli. Such leaves may come to the loosely-shut stage if stimulated weakly, and reach the closely-shut stage if stimulated strongly. Difference in the shutting forces caused by strong and weak stimuli may perhaps be due to the degree to which turgor is lost in the inner epidermal cells.

The progress of the movement and its mechanism with strong stimuli, will be shown in the following section.

E.   Narrowing Movement

1.   *Process of the Movement*

It has been stated above that a leaf which is excited by a strong stimulus passes through the same course in shutting as one weakly stimulated, except that it is inclined to shut more closely. The stronger the initial stimulus, the longer is the commencement of the reopening delayed ; and when this exceeds a certain intensity, the leaf narrows instead of reopening. The stronger the stimulus, the earlier the narrowing movement begins.
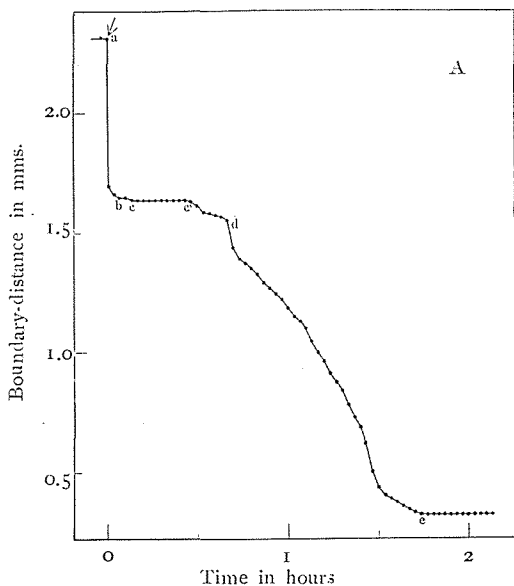
Fig. 29 A represents the change in the boundary-distance of a leaf of No. III at $23°$, stimulated by a break shock, coil distance o cm. The open leaf, which is st imulated at a, shuts and finishes the residual quick phase at b. This is a more closely-shut state than in the case of weak stimuli. The slow phase proper ends 10 mins. after the stimulation (c) and no further movement is perceived. Between the 26th and the 28th min. (c'), however, the narrowing of the boundary-distance begins, the lobe margins being more tightly pressed together than in the case of

Fig. 29 A.   A representative narrowing process; a : the open stage, the stimulus being given at the arrow ; b : the loosely-shut stage ; c : the closely-shut stage ; c′: beginning of the narrowing movement; d : eversion at the margin of the free-side lobe ; e : the narrowed stage

Fig. 8 C.   Then the movement is stopped temporarily and starts again at *d*, the free-side lobe being bent along its margin and coming into a state like that in Fig. 8 D.   The movement is accelerated
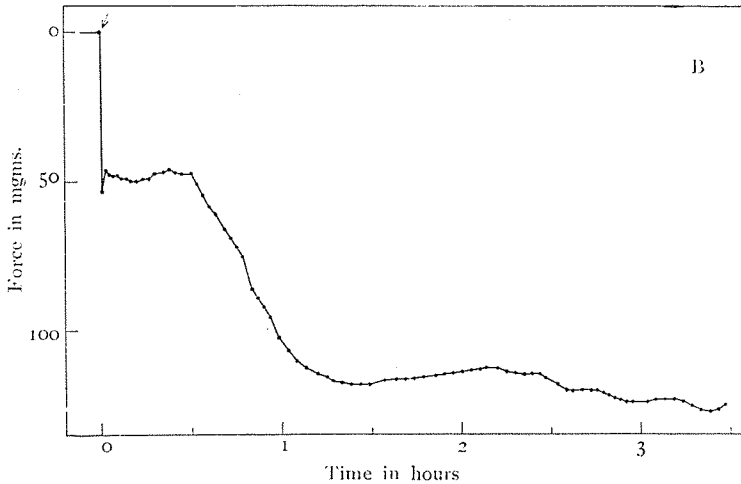

Fig. 29 B.   A representative process of the exertion of force in the narrowing phase

for a time as soon as the free-side lobe is bent, and then it goes on rather uniformly, though not quite evenly.   The bending of the free-side lobe is gradually shifted towards the enclosure-boundary, as the boundary-distance narrows, and the portion where the recurving has passed on is left lying closely upon the opposite lobe.   The movement becomes inconspicuous at *e*, though the leaf is found to be a little more narrowed a few hours later.   At *e*, the leaf is in the narrowed stage as seen in Fig. 5 D, the free-side lobe being bent as in Fig. 9, and the one-layered regions of the two lobes lie upon each other as in Fig. 8 E.

   In the course of the narrowing movement, the enclosure-boundary of the free-side lobe is hidden behind the one-layered region in the apical view.   Hence while observing an early portion of the narrowing movement, one should select another point as the mark, say a two-armed hair on the densely-glanded zone of that lobe, in order to make use of it after the enclosure-boundary is hidden.   The distance measured with the newly adopted mark can be easily transformed by calculation into the boundary-distance, if both are measured in parallel while the latter is still measurable.

   Fig. 29 A represents the change in the boundary-distance, and hence the sum of the movement which each of the lobes displays.

It was, however, proved (by Fig. 11) that the free-side lobe bends more than the opposite one.

It must be noted that an apparent pause exists between the shutting and the narrowing movement (Fig. 29 A, $c-c'$), though the relation of the duration of such a pause to the velocity of the narrowing movement following it is not determined. For example, the leaf used in Fig. 35 showed a quicker narrowing movement (duration ca. 30 mins.) after a longer pause (ca. 30 mins.), while another leaf of the same whorl showed a still slower movement (ca. 100 mins.) after a pause of the same order, under the same conditions. Such relations are complicated by many factors which will be discussed later. In general, however, the following tendencies are noticed : the stronger the stimulus, the shorter is the pause before the narrowing, and also the greater is the narrowing velocity ; among the leaves of Nos. II—V, the younger the leaf, the more quickly the movement proceeds ; the lower the temperature, the greater is the duration of every phase. These tendencies cannot be shown here numerically, as individual deviations are so large. Leaves show varied types of the narrowing process if observed in various conditions, but the critical phases are all represented in Fig. 29 A.

2. Water in the Leaf Cavity

The volume of the cavity enclosed by the lobes is smaller when the leaf is narrowed than when it is shut. Therefore, some of the water in that cavity must be pressed out during the narrowing movement. In order to find out how water flows out, a concentrated solution of neutral red was enclosed in the cavity of a shut leaf, and this leaf being made to narrow with a strong stimulus in pure water, the way in which the dye solution flowed out was observed.

At the beginning of the narrowing movement, the red solution gets out from the whole margin, but the path is gradually restricted after the free-side lobe margin is everted. When the water surrounding the leaf circulates slowly, the pressed out dye solution is constantly taken away by the stream. Hence the marginal parts where the dye solution is stopped are seen clearly contrasted with the remaining parts, where the dye solution is still coming out continuously.

While the bend of the recurving free-side lobe is passing over the zone of four-armed hairs, the leaf is prevented from sealing itself closely, so that the water can get out in a wide range. But after the hairless zones have come in close contact with each other, the water

inside can flow out through only one outlet or two, a red line or two being noticed at the margin of the leaf.[1] If such a red line is observed with the microscope, small particles suspended in the outer medium are seen repelled vigorously at the outlet, which indicates that the coloured water is flowing out of the leaf cavity with some rapidity, and also suggests that the pressure in that cavity is raised considerably.

In *Utricularia* bladders the water is believed to be pumped out through living cells (cf. KRUCK '31, or LLOYD '31). But such is out of question in the case of *Aldrovanda,* for the water can flow far more easily between the lobes than through living cells.

3. *Process of the Narrowing Force*

While a leaf stimulated with an 8 cm. break shock reopens from the shut stage, one to which a o cm. break shock is given narrows further. The shutting force measured 1 min. after the stimulation is stronger in the latter case than in the former (Table 5, p. 219). A representative result of dynamometer measurements when leaves are stimulated by a o cm. break shock is given in Fig. 29 B. The leaf used belongs to the same whorl as that used in Fig. 29 A. The conditions are also the same, except that a dynamometer is inserted. The dynamometer was the large one used before.

After the maximal shutting force is produced, the force is weakened a little, and varying irregularly for 30 mins., it begins to increase steadily. If Fig. 29 B is compared with 29 A, this steady increase of force is understood to be the cause of the narrowing movement. And the pause in the movement before the narrowing begins is proved not to be due to external hindrance, but to latency of the narrowing mechanism itself. The separation of the forces in two steps as illustrated here, suggests that the narrowing movement may be caused by a different mechanism from the shutting movement.

The process of the exertion of force in the interval between the shutting and the narrowing differs according to the leaf, but is not much differed from that shown in Fig. 29 B. In many leaves the decrease of force after the maximal shutting occurs later than in the present example. The variation of the force in this period is much

---

1) The site of the outflow is not definite; it seems therefore to be determined by mechanical conditions that parts of the one-layered region may happen to be in.

more pronounced when a strong stimulus is applied (Fig. 29 B), than when a weak stimulus is given (Figs. 24 B, 25 B, and 28).

The force continues increasing with the narrowing, then it decreases a little, and afterwards increases to the maximum. The maximal strength of the force differs with different leaves, but generally does not deviate much from 130 mgms. In Fig. 29 B, the increase of force looks as if it were uniform because the points were taken generally every 2 minutes. But continuous microscopical observation proves that the force increases little in one small interval, and very much in another. This may perhaps be due to irregular action of the mechanism.

While the narrowing movement stops temporarily some minutes after starting ($c' - d$ in Fig. 29 A), there is no hesitation in the increase of force corresponding to that. Therefore, it is obvious that the unevenness of the movement in that phase is conditioned not by the causative mechanism, but by external hindrances.

4. Elimination of the Resistance due to Pressing Out of the Water

The narrowing movement must necessarily be accompanied, and hence hindered, by the pressing out of water through the shutting up of the lobes, as explained above. If a pore is made by a needle in the one-layered region of the bristle-side lobe this resistance will be nullified, and the water in the lobe cavity allowed to flow out freely. Hence only the effect of the other resistance, namely that
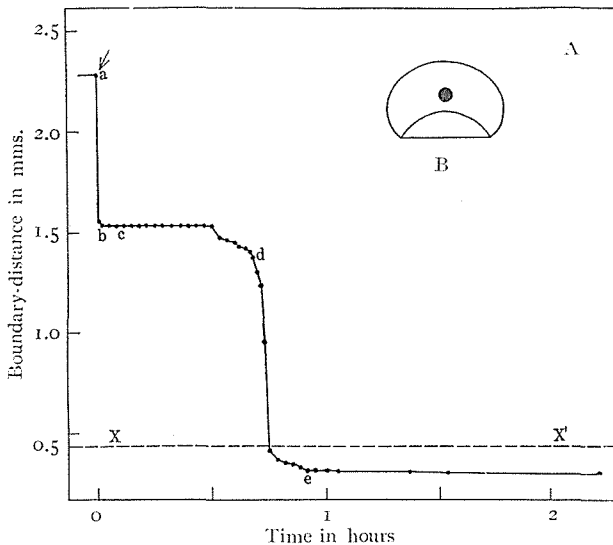

Fig. 30 A. The narrowing movement of a leaf, the bristle-side lobe of which is bored as shown in B. a, b, c, d and e as in Fig. 28 A. Below XX', the pore in the lobe no longer serves as the direct outlet for water from the leaf cavity

of eversion of the free-side lobe will be manifested in the process of
the movement, for (1) the irritability of a leaf is not altered by a wound
in its one-layered regions, quite unlike the case of the three-layered
regions, and (2) boring the bristle-side lobe has naturally nothing to
do with the physical character of the opposite lobe which alone is
everted when the narrowing takes place.   Fig. 30 A shows the process
of the narrowing movement of a leaf, on the bristle-side lobe of which
a pore has been made as shown in B.   It is interesting to compare
Figs. 29 A, B and 30 A with each other, for the first represents the
case in which the movement is affected by the two forms of resistance,
the pressing out of water and the everting of the lobe, the second,
by neither of them, and the third, by the latter form only, the ex-
ternal conditions being the same in all cases.

The narrowing movement does not occur until the 30th minute
after the stimulation, just as in the case of the normal unbored leaves,
in spite of the fact that the resistance due to the outflow is nullified
in the present case.   A leaf, therefore, is kept in the shut stage only
by the resistance of lobes, withstanding a shutting force of about 50
mgms.   The resistance due to water is proved to have nothing
to do with the maintenance of the shut stage.

The narrowing movement is retarded soon after its starting, just
as in the normal unbored case.   Here, it is proved again that the
pause in the movement in the early stage of the narrowing
movement is due to difficulty in everting the free-side
lobe margin, and not to the resistance of water at all.   As the
resistance of the lobes does not depend upon the velocity of the
movement, the delay of the movement indicates insufficient strength of
the force.   Hence the duration of that temporary retardation denotes
the period during which the force has gathered strength so as to be
sufficient to overcome the hindrance.

The movement is accelerated as soon as the lobe margin is everted
(*d*), just as in the case of a normal leaf.   But the velocity after that
is incomparably greater in the present case.   The line *XX'* indicates
the critical point, below which the pore no longer serves as a direct
outlet of the cavity water, as the eversion of the free-side lobe has
passed over the pore on the opposite lobe.   The movement is retarded
as soon as the lobe cavity is deprived of its artificial water outlet.

The quickest narrowing movement is not uniform in its velocity;
in certain cases, 1/6 to 1/7 of the whole narrowing movement is
performed within only 3 seconds; a slower movement follows, then

after about 1 minute, a very quick one again etc. One minute plot-
ting shows nothing about such minute changes. Such an alternation
of quick and slow movements indicates lack of homogenity in the
mechanical structure of the one-layered region of the free-side lobe,
for example accidental thickenings of cell walls (see also p. 227), as
well as irregular action of the mechanism (p. 224).

The above experiment proves that the velocity of the nar-
rowing movement of a normal leaf, after the margin of the free-
side lobe is once everted, is limited by the velocity with which
the water is pushed out. The circumstances are also confirmed
by the following observation.

If the dynamometer is removed from a leaf when the leaf is
exerting the maximal force (ca. 120 mgms.) upon it, the two lobes
suddenly come in close contact and press upon each other. However,
a quarter of a minute is necessary for the leaf to come into the state
shown in Fig. 8 D, and a minute or more, for the narrowing movement
to advance half way on its course. This indicates that even if an
open leaf should suddenly exert a strong force quite sufficient to
bring it into the ultimate narrowed stage, the leaf would not narrow
in an instant, being retarded by various hindrances. The dominant
resistance in this case must be that of water, for the eversion of the
lobe can be completed in an instant as the acting force is strong
enough.

5. *Physical Characters of the Lobe*

When the bristle-side lobe is cut off along the midrib, the free-
side lobe, attached to the midrib, shows the
form seen in Fig. 31 A, the middle cross
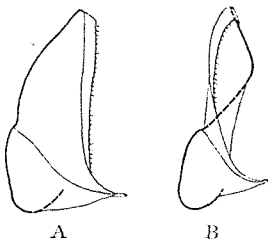section of the lobe being denoted by a thick
line. This lobe has bent as far as possible,
for the cutting is a strong stimulus and
obstruction by the opposite lobe is absent.
When this lobe is everted carefully with a
pincette and fingers, it gets into a state of
unstable equilibrium, showing a form like
that in Fig. 31 B. The convex dome of the
one-layered region in A becomes concave
in B, the convexity of the three-layered
region being preserved. The thick line in
B corresponds to that in A.

Fig. 31. Apical view of
the free-side lobe attached to
the midrib, the other lobe
being cut off. The cross-sec-
tion is shown with a thick line.
A: natural (over-narrowed)
state; B: an equilibrated state,
when the one-layered region
is everted

It is probably no easy task for a leaf which has just started narrowing, to cause eversion along the whole margin of the lobe, and hence the movement is suspended a while before the eversion occurs. But once it has taken place, much less force is required to move curving inwards, for the lobe is coming nearer to an equilibrated state (Fig. 31 B); hence the narrowing movement can go on very quickly, provided water in the lobe cavity can get out freely as in the case of Fig. 30 A.

If a thin-walled rubber ball is cut into two halves, and one of them is everted half way, just to correspond to the case of Fig. 31 B, the equilibrium in such a state is quite unstable and the rubber wall will either wholly evert itself or will spring back to its original state. In the case of the lobe, the three-layered region and the midrib prevent the entire eversion of the lobe, and hence the lobe would only return to its original state, A, through its own elastic force. Hence a force is needed to shift the eversion inwards, though quite a weak force suffices for it. And more force is required when the bending comes nearer to the enclosure-boundary, namely when the eversion of the one-layered region is about to be completed; for the cells become thicker and then, at last, two-layered (cf. Fig. 9). The resistance of the lobe comes to its maximum, and the narrowing movement is stopped in the state shown in Fig. 9.

In the narrowed stage, the lobes are constantly pressing upon each other, to keep the one-layered region of the free-side lobe wholly everted. And when the force of the lobes diminishes, the leaf going to rebulge, the eversion of the one-layered region is recovered centrifugally through its own elasticity. In some leaves, however, the free-side lobe remains unaltered in the wholly everted state, even after reopening. These leaves are found generally to be quite immature or abnormally formed.

In an early phase of the narrowing movement, the eversion does not occur along the whole margin of the free-side lobe, or only at the middle part, and then some folds are formed at the boundaries between the parts everted and those not everted. These folds hinder the narrowing movement greatly. Accidental thickenings of cell walls in the one-layered region of the free-side lobe which may be detected with the microscope, also hinder the centripetal progress of the eversion from time to time. The progress of the narrowing movement is conditioned not only by the usual resistance of the water and the lobe, as mentioned before, but also by those accidental impediments.

6. *Stimulation of the Narrowing Movement*

There is a latent period of about 30 minutes before this movement starts. The whole process of the stimulation is, however, not yet known. Briefly considered, the shutting movement may seem to give rise to the narrowing movement. But when the stimulation, either seismonic or electrical, is weak, this later movement does not follow, so that the shutting movement itself may not account it, that is to say the narrowing movement must depend upon its own stimulation. In the natural case this is realized by repeated tactile stimuli coming from the prey that must be transmitted through the inner epidermis, and the movement goes on quickly if the stimuli are given very often (p. 230). It seems therefore probable that repeated shocks from the inside may cause a physiological change in the outer layers which effects the narrowing movement. Whether the reaction in the case of electrical shocks follows the same procedure or whether these are perceived directly from the outside, is also a question, but from the results obtained with tetanic electrical stimulation (cf. p. 231 and Part II), the latter case seems to be the more probable.

7. *Recapitulation of the Factors Conditioning the Narrowing Movement*

The narrowing movement was demonstrated to be caused by active elongation of the outer epidermal and the middle layer, though chiefly by the former (p. 193). The turgor of the outer epidermal cells begins to increase after the latent stage, and the narrowing movement starts and goes on, overcoming various hindrances. The relations among the chief factors in the narrowing movement, namely boundary-distance (A), velocity of the movement (B), force exerted by the lobes (C), the resistance offered by the free-side lobe (D) and the resistance of water (E) are shown diagrammatically in Fig. 32. In this figure the abscissa indicates, not the time relation, but critical points in the movement, and the values of the ordinates are only imaginary.

Through the secondary increase of bending force (c) at *b*, the narrowing movement (A) begins. But this is stopped at *c* by increase of D, in spite of the continuous increase of C. And as soon as the margin of the free-side lobe is everted by the increased strength of c at *d*, D decreases abruptly, and the narrowing movement proceeds

markedly. Though E might progress parallel with B in general, it grows comparatively greater as the narrowing movement goes on, and especially so after the shutting up of the leaf cavity has come to the hairless zone. Accidental hindrances are omitted in the diagram.

In Fig. 29 B, the force diminishes after the leaf has once exerted nearly its maximal strength, growing stronger again in the 2nd hour. Such a decrease of force may be due to the deformation in the outer two layers of cells as shown in Fig. 33. This figure is sketched with a cross-sectioned leaf, when the space between the three-layered regions was not filled with agar; the motile zone has bent to a certain degree, but as the one-layered regions of the lobes are fixed by the agar in their original position in the open state, the distal part of the motile zone is forced to be concave. The condition may be the same when a dynamometer is opposing the approach of the one-layered region while the motile zone is striving to bend itself sharper. After the force grows strong enough, cells in this part may be subjected to such deformations as those shown in Fig. 33, the force exerted upon the dynamometer being thus decreased. The force of the motile zone, however, is still growing greater, and as the deformed part of the
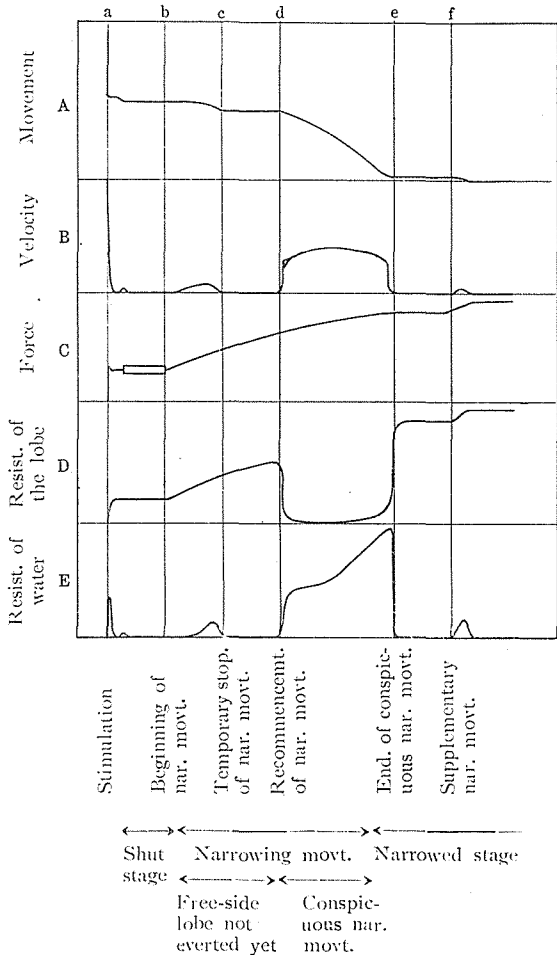

Fig. 32. Diagram showing the relation of progress of the movement (A), velocity (B), force exertion (C), resistance of the lobe (D) and that of the water (E)

lobe does not lose its rigidity, the dynamometer reading increases
again.   A leaf which has once exerted the maximal force upon a
dynamometer does not reopen.   This
may be due to the injurious effect of
such deformations in the cells.   From
the above reasoning, it is to be supposed
that in a normally narrowing leaf, the
decrease of force before the maximum
may not appear, and the process of the
force may be as shown in Fig. 32 c.

Usually a very slight narrowing
occurs after the conspicuous movement
has once finished, as seen at the 5th
hour in Fig. 35 or at f in Figs. 32 A
and 32 B.   In spite of the smallness of
this additional movement, the causing

0.1 mm.

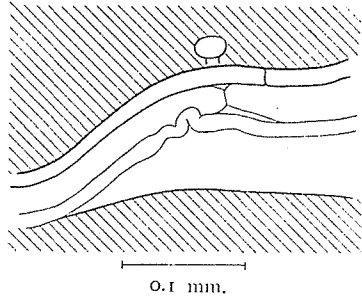Fig. 33.   A deformation of
the cells of the outer epidermis
and the middle layer, when the
narrowing movement is hindered
by the agar

force (c) must be very strong, for both of the resistances (D & E)
are very large then.

8.   In the Case of Capturing the Prey

All the observations above mentioned were made with leaves to
each of which no more than a single electrical shock had been given.
They served for analysing the phases and factors in the shutting and
the narrowing movements.   But the case must be different when a
leaf captures a small animal, for 1) the leaf is stimulated mechanically,
not electrically and 2) the stimuli are given repeatedly, instead of
once.   Hence the movement of a leaf when it captures an animal will
be described here.

An open leaf is fixed under the microscope so as to show its
apical view, and many individuals of *Bosmina*, which are most com-
monly found captured by *Aldrovanda* in our culture, are put in that
vessel.   They swim about quite at random, and seem not to be at-
tracted by the leaf; some even swim into the space between the
pair of lobes and get out of it again unconcerndly.   But when one of
them, swimming among the sensory hairs, touches a few of these by
chance, the leaf reacts and shuts the animal in.

The captured *Bosmina* swims wildly in the space which is sur-
rounded by the whole inside surface of the leaf.   But when the narrow-
ing movement begins and goes on, the free space becomes smaller
and smaller, until at last, when the leaf is quite narrowed, it can swim

only within the enclosure-boundary. The process of the movement when an individual of *Bosmina* is captured is shown in Fig. 34. The animal was ca. 0.47 mm. in length. It stopped swimming after about 3 hours' imprisonment, though still moving its appendages, and then died a good many hours after that. It is not determined whether such inanimation and killing of the prey is due simply to its suffocation, or to some special poison as KRUCK ('31) has proved in *Utricularia*.

The process in the present case differs from that in Fig. 29 A in the respects that 1) the interval between the shutting and the narrowing movement is very short, 2) the narrowing movement goes on more rapidly, and 3) the movement is

Fig. 34. The movement of a leaf when it captures a living prey (at the arrow). a, b, c, d and e as in Fig. 29 A

quick in its early phase but much retarded in the later half of the process. But the temporary cessation of the movement (*d*) when the margin of the free-side lobe is about to be everted is also apparent in the present case.

The narrowing movement can be called forth by chemical stimulation by some dissolved nitrogenous matter. But when the leaf has captured a small animal, substances from this animal have not enough time to effect the narrowing, for this movement is finished within half an hour or so. Hence it is very conceivable that the movement is conditioned only by seismonic stimulation of the sensory hairs, while, as will be stated in Part II, a few touches applied to sensory hairs never cause narrowing but only shutting. Therefore, it is to be concluded that the captured animal gives weak but tetanic stimuli to the leaf. Swimming amuck in the leaf cavity, it strikes sensory hairs one by one, and again and again, thus causing irritation in the leaf repeatedly, as soon as any of the hairs recovers its sensitivity.

The above conclusion is verified by imitation of the case with weak and tetanic electrical stimuli: When weak break shocks (coil distance 8 cm.) are applied to a leaf every minute or more frequently, the narrowing movement occurring shows the above mentioned three characteristics. Application of strong and tetanic stimuli causes much faster movement, and finishes the narrowing within 10 minutes or so,
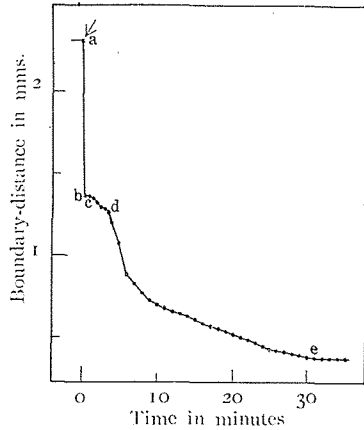
F. Recovery Process

CZAJA has described that if the stimulus applied to the leaf of *Aldrovanda* be weak, the leaf reopens after six hours, whereas if it be strong, it does not open till after twelve hours. He has also stated that the leaf can open again and again, if its preys are small, while it never reopens after it has preyed upon a comparatively large organism, for too much refuse is left inside of it. However, no description has yet been given of the phases through which the leaf passes when it opens again from the narrowed stage, or from the shut stage.

a. Rebulging Movement

1. *Process*

The course of the rebulging movement is a reversal of that of the narrowing, except in the time relation; the boundary-distance grows larger, and the bending of the everted free-side lobe is shifted back centrifugally, and when the lobe has regained its original convexity, the leaf is in the shut stage and the rebulging movement is over, the
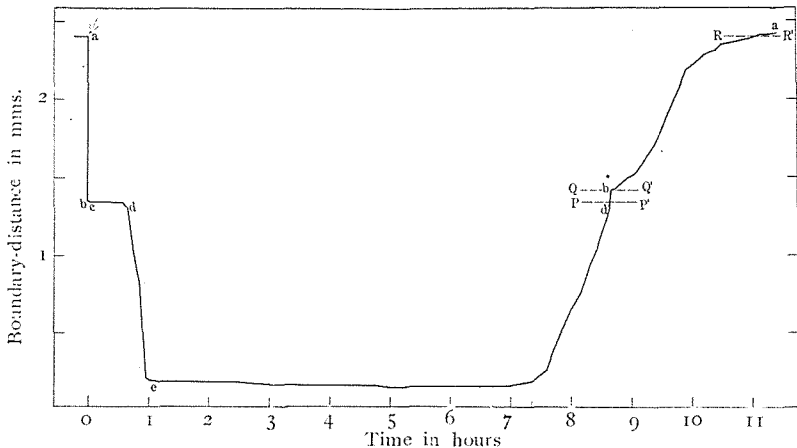

Fig. 35. The whole process of the leaf movement, shutting (a-b-c), narrowing (d-e-), rebulging (-d-b), and reopening (b-a).   a, b, c, d, e and the arrow, as in Fig. 29 A.   PP': the boundary-distance of the shut stage *before* the narrowing as in c; QQ': that *after* the narrowing; RR': that of the open stage before the stimulation

movement, then, entering the reopening phase. Fig. 35 shows the changes in the boundary-distance of a leaf when it is shut, narrowed,

rebulged and then reopened. In order to have the course completed quickly, a young leaf belonging to No. II was used and the water was kept at a temperature as high as $32°$ C. The stimulating electricity was a momentary flow of a constant stream of 30 volts, the electrodes being 8.5 cms. apart from each other.

The narrowing proceeds very slowly until the 5th hour after the stimulation, but the movement is reversed in the 6th hour, conspicuous rebulging appearing in the 8th.

The eversion of the lobe margin exerts great resistance against the progress of the narrowing movement in its earliest phase, as has often been stated above. Making an interesting contrast with this, the rebulging movement is accelerated by the elasticity of the lobe in its latest phase. The free-side lobe has a force in itself which enables it to regain its original convexity, as soon as the leaf recovers from the equilibrated state (as in Fig. 31 B).

Such elasticity can be also recognized under the microscope by touching the lobe with a glass rod attached to a micromanipulator; the margin of each of the lobes can be sharply bent by the glass rod either to the outside or to the inside, but it springs back again to its original form without leaving any traces of such bending. This character must be very convenient in the rebulging movement, for the leaf has only to unbend the motile zone, the one-layered region regaining its convexity by itself.

The rebulging movement is caused by active growth of the inner epidermis (p. 201). But its latest phase must be accelerated by the elasticity of the free-side lobe, thus causing passive elongation of the inner epidermis (and also compression of the outer epidermis). Hence, when the rebulging is finished and the stress of the lobes has disappeared owing to their coming apart, the recovery movement may naturally cease or be retarded until the full mechanism of the real growth overtakes the passive elongation of the cells (cf. Fig. 35). The boundary-distance at the shut stage is greater after the leaf has rebulged (QQ') than before it narrowed ($PP' = c$). This proves that the leaf has grown in breadth during the course of narrowing and rebulging, though the numerical difference does not represent the amount of the growth, for the rebulged leaf is more loosely shut, than before the narrowing.

2. *Entrance of Water into the Leaf Cavity*

As the volume of the cavity between the lobes increases when

the leaf rebulges, water must get into the cavity as the movement
progresses.   In order to determine through what route the water
enters, a narrowed leaf was put in a solution of phenol red and was
allowed to rebulge in it.   The dye solution had entered the cavity
when a half rebulged leaf was examined in pure water.   This dye
never enters living cells, even the two-armed hair cells which would
take up various dyes and substances very easily.   Hence the dye
solution must have come into the leaf cavity not through
living cells, but necessarily through the narrow space between
the one-layered regions, which is analogous with what happens
in the narrowing movement, in which the water is pressed out by
the same route.

3.   *Elimination of Water Resistance by Boring*

The velocity of the narrowing movement is limited by that of
the pressing out of the cavity water.   In order to determine if what
happens in the rebulging movement is analogous with this or not,
the recovery process of the leaf (Fig. 30) was observed, the result
being plotted in Fig. 36.   The abscissa indicates the time after the
initial stimulation, 10 hours being omitted between Fig. 30 and the
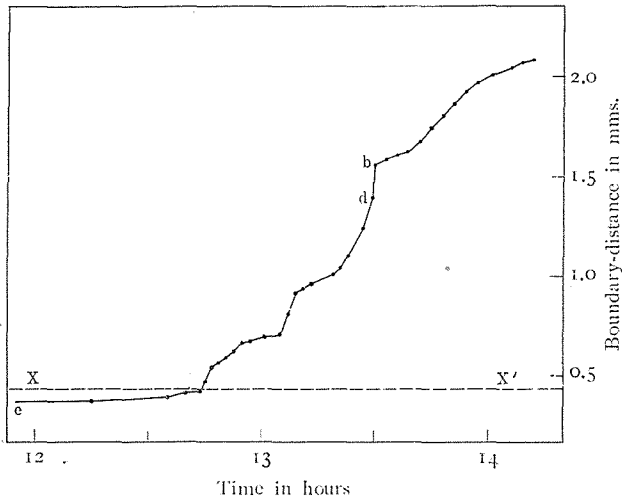present figure, because the process in this interval is similar to that of
the 2nd-7th hour
in Fig. 35.   The
broken line, XX',
has the same
meaning as in
Fig. 30.

The move-
ment is very slow,
until the bending
of the free-side
lobe reaches the
pore on the op-
posite lobe.   But
as soon as water
is able to flow
freely into the
leaf cavity (Fig.
36, XX'), the re-

Fig. 36.   Continuation of Fig. 30 A, representing the re-
bulging movement of a perforated leaf. e, d, b and XX',
as before

bulging is accelerated suddenly. This indicates that the leaf has been striving to rebulge, but has been limited by slow inflow of water. The movement is, however, retarded again. The rebulging mechanism, i. e. an active growth of the inner epidermis, follows gradually and hence the movement is slow, limited by the progress of the mechanism itself, even if sufficient water is supplied. Perhaps the second acceleration of the movement may be due to an accelerated active growth, slightly helped by the elasticity of the everted free-side lobe. After a retardation, the rebulging goes on quickly, accelerated again by the elasticity of the free-side lobe.

4. *Summary of the Rebulging Movement*

The various forms of resistance to the rebulging movement are shown in Table 6, in comparison with the case of the narrowing movement. While the narrowing movement takes place when the thin-

Table 6

		Narrowing	Rebulging
Internal resistances	I. Inextensibility of walls of active cells	small	large
	II. Antagonistic force of non-active cells (by turgor)	small	large
External resistances	III. Resistance of water flow	large	large
	IV. Resistance due to elasticity of the one-layered region	small (large at the start & the end)	negative i.e. helping

walled very extensible cells of the outer epidermis stretch themselves, the rebulging is carried out by active growth of the inner epidermis which has thick and less extensible walls. And while the stretching of the outer epidermis, in the former, is hardly resisted by the inner epidermis, the growth of the inner epidermis, in the latter, is opposed by the outer two layers. The "internal resistances" in the table indicate these features. The "external resistances" are two in number, one due to the pressing out or sucking in of water from or into the leaf cavity, and one due to the elasticity of the one-layered region of the free-side lobe. The rebulging movement may be summarized as follows :—The movement is caused by an active growth of the inner epidermis, and is helped by the elasticity of the free-side lobe to some extent, the extent being increased as the movement draws nearer to

its end (the shut stage). Its velocity is limited by the inflow of water through the gap between the pair of lobes. Hence the pressure in the leaf cavity is negative while the movement is going on, just the reverse of the case of the narrowing movement. But even if the resistance of the water flow is nullified, the movement is not accelerated so much as the narrowing movement is, for, as can be understood from Table 6, the rebulging movement must still overcome two other kinds of resistance (I and II), while in the narrowing movement there is hardly any more opposition.

b.   Reopening Movement

If a leaf is made to shut by a weak stimulus, it will reopen directly from the shut stage, while if excited by a strong stimulus, it reopens after narrowing and rebulging. However, according to the writer's experience, the reopening process does not differ in those two cases, except in the earliest phase of the movement. Fig. 25 A shows an early phase of the former case, and Fig. 35, the latter case, the former being represented by the margin-distance, and the latter by the boundary-distance. These graphs do not, as a matter of course, indicate the movement of each of the lobes separately. In this respect, photographic recording of the movement is superior to measurement of the margin-distance.

In the following paragraphs are described the method and results of recording on kymograms the reopening movement from the shut stage which is brought forth by a weak stimulus.

1.   *Apparatus and Method*

The apparatus used in recording the quick phase of the shutting movement (Fig. 22) is again used, modified in some respects; the projection lamp (P) is replaced by an ordinary 32-watt electric lamp, and the myograph (D) with a kymograph, while the tuning-fork (T) and the electrodes in the glass case (c) are removed. To mark the time, the argon-tube (A) is connected electrically in series with a clock-work mechanism which closes the circuit every quarter or half hour.

An open leaf is held in the glass case (c) just in the same manner as in the previous case. The image of the tips of the two lobes are focused upon the drum of the kymograph, projected through the slit (s).

In order to bring the leaf into the shut stage, a sort of stimulus

which CZAJA called " Stossreiz " is used : if the lobes of a leaf are pressed together gently from both sides with a pair of pincers, they react to the stimulus by becoming deformed, when they attain a certain nearness.[1] With this method of stimulation, there is little danger of giving a leaf more than the minimal excitation, for the lobes can jerk themselves away spontaneously from the pincette as soon as they receive the threshold stimulus, even though the threshold value may differ largely with the leaf.

No sooner is the leaf stimulated than the clock-work mechanism is started, so as to cause the argon-tube to be lighted every quarter or half an hour after the shutting of the leaf. The lens of the microscope is covered, and a photographic film is wound round the drum of the kymograph. Then, the cover of the lens being taken off again, the kymograph is started to revolve.

2.   *Results*

Two of the kymograms are shown in Figs. 7 and 8, Pl. IX. The record starts on the left of the print. The white marks impressed along the lower margin show every quarter of an hour.[2]

In the case shown in Fig. 8, the leaf begins to reopen about 15 minutes after the shutting, and it takes then 30 minutes more to end the rapid reopening. However, the leaf is not fully open at this time, and the reopening is continued very slowly. Even at the end of the kymogram, i. e. 5.5 hours after the stimulation, the process is still going on. In the case of Fig. 7, the leaf begins to open about 25 minutes after the stimulation. The quickly reopening phase takes an hour or more, the following movement going on more rapidly than in the case of Fig. 8. Most of the reopening leaves show a process like the one seen in this figure. Both of the kymograms remind us that 70% to 80% of the reopening process is finished in a short time, but a very long time is required for the leaf to open fully. The reopening movement plotted in Fig. 35 represents only the quickly reopening phase.

---

1)   This form of stimulus will be studied more closely in Part II.

2)   In Fig. 7, the intervals which are twice as long as the rest represent half hours. As the gears of the kymograph were not well-made, the rotation was somewhat irregular and hence innumerable parallel lines were impressed across the print. When Fig. 8 was taken, an end of the thread of the screw was jutting out at the lower extremity of the axis of the drum, and hence the drum showed a slight up and down movement as it revolved, printing undesirable notches along the curves.

These kymograms were taken at about 35°. If the temperature is lower, the movement in the quickly reopening phase is slower, and the slowly reopening phase does not progress well. Leaves never become fully open at 25° or below. The case is analogous when a leaf grows older, and hence older leaves open less widely than younger ones. These circumstances make an interesting contrast with those observed in the shutting movement, in which case any leaf shuts completely under various conditions. The contrast is due largely to the difference in the antagonistic force of non-active cells, just as the rebulging movement differs from the narrowing (II in Table 6, p. 235). The opposition of the outer two layers to the stretching of the inner epidermis may grow much stronger when the leaf opens very widely. Hence the reopening movement is much retarded after the leaf has become 70%-80% open. And if in that case the temperature is not high enough, or if the leaf is old, the force exerted by the growth of the inner epidermis cannot overcome that strong opposing force, and the leaf will stay in a partially open stage. Only very young leaves open fully at high temperatures.
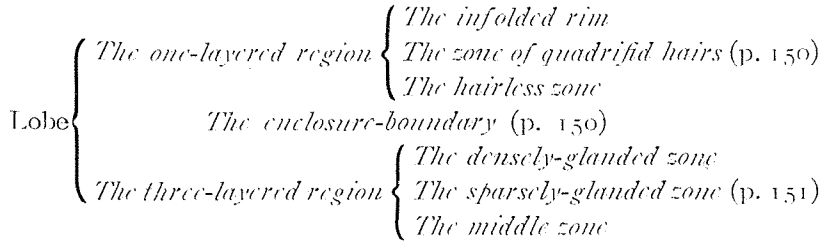
In Fig. 8, Pl. IX and Text-fig. 35, the movement is a little accelerated just after its starting. This may be due to the elasticity of the marginal fold, because a very young leaf, as in Fig. 25 A, does not show such a feature. This is interesting, as the same force hinders the slow phase of the shutting movement of a mature leaf, while it is too weak to exhibit the hindering effect in very young leaves (cf. p. 213).

In the myograms, the upper curves represent the movement of the free-side lobes. The two lobes move nearly symmetrically, though the free-side lobe moves in a greater measure, as would naturally be expected from the mode of the shutting movement.
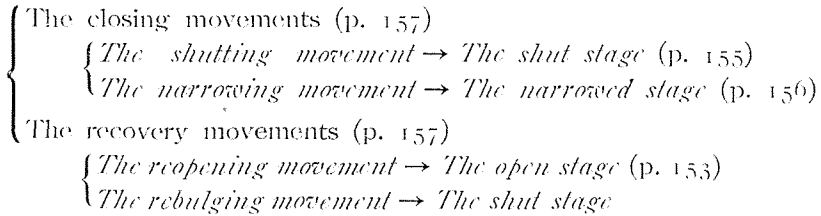
Summary

1.   An infusion of dead leaves and stems of some helophytes is a very suitable medium for the cultivation of *Aldrovanda* (p. 147).

2.   Some definitions are given for various parts of the leaf and phases of the movement :

Leaf $\begin{cases} \textit{The free-side lobe} \\ \textit{The bristle-side lobe} \end{cases}$ (p. 149)

Lobe
{
    The one-layered region { *The infolded rim* / *The zone of quadrifid hairs* (p. 150) / *The hairless zone*

           The enclosure-boundary (p. 150)

    The three-layered region { *The densely-glanded zone* / *The sparsely-glanded zone* (p. 151) / *The middle zone*
}

The leaf movement:
{
  The closing movements (p. 157)

    { *The shutting movement* → *The shut stage* (p. 155)

    { *The narrowing movement* → *The narrowed stage* (p. 156)

  The recovery movements (p. 157)

    { *The reopening movement* → *The open stage* (p. 153)

    { *The rebulging movement* → *The shut stage*
}

I. The motile zone

3. Both the shutting and the narrowing movement are caused by bending of the lobes, at zones ca. 0.15–0.25 mm. from the midrib on both sides of it (p. 163).

4. The motile zone is characterized by the undulation of its outer surface (p. 165), and the extensibility (pp. 166–7) and permeability (pp. 168–9) of the cell walls.

5. In the motile zone, the outermost wall is the most easily, the outer subepidermal wall less, and the walls of the inner epidermis the least extensible, as determined by the acetone treatment (p. 167).

6. The lobes are able to bend to a far greater extent than they show in the natural case of the narrowed stage (p. 163), the free-side lobe having the power to bend more than the other lobe (p. 164 and Fig. 11).

7. The motile zone of *Dionaea* leaves is also determined (Fig. 15).

II. The shutting mechanism

8. The shutting movement is caused neither by decrease in the thickness of the lobes (p. 174), nor by increase in the turgor of any cells (p. 176).

9. A theory of the shutting mechanism is proposed (pp. 176–8): When a leaf is stimulated, the inner epidermal cells react and lose their turgor, and allow the outer two layers to bend the lobe (to shut the leaf).

10.   In the *Dionaea* leaf, the parenchyma takes the rôle of the outer two layers of the *Aldrovanda* leaf, the outer epidermis of the former working rather to hinder the movement (p. 180).

11.   Guttenberg's notion is criticized (p. 180 ff.).

12.   When the *Aldrovanda* (and *Dionaea*) leaf shuts or narrows, its outer (lower) surface is irreversibly stretched (pp. 184 and 195). The outer (lower) cell walls of young leaves, however, contract to some extent if dehydrated (p. 185).

III.   The narrowing mechanism

13.   The narrowing movement of *Aldrovanda* leaves is caused by an increase of turgor, and hence an active elongation of the outer epidermal cells (p. 193). Four evidences (*a, b, c* and *d*, pp. 188–194) are given.

14.   The water needed for the narrowing movement is supplied mostly through the outer surface (p. 195).

15.   When *Aldrovanda* leaves narrow, only the free-side lobes are everted. The cause is shown (p. 195).

16.   The narrowing movement of *Dionaea* leaves is caused by an increase in turgor of the swelling tissue in the lobes (p. 197).

17.   The inflexion of *Drosera* tentacles is analogous to the narrowing (not the shutting) movement of *Aldrovanda* and *Dionaea* leaves (p. 195).

18.   The midrib of an *Aldrovanda* leaf has a very weak force enabling it to straighten itself in the turgescent condition (p. 198), but it is easily curved by the lobes when the leaf moves.

19.   The midrib of a *Dionaea* leaf is destined to be uncurved by a turgor force of the parenchyma which lies under the conducting bundle (p. 199), and hence it does not promote the leaf movement as Batalin maintains (p. 200).

IV.   Mechanism of the recovery movements

20.   The reopening and the rebulging movement of *Aldrovanda* and *Dionaea* leaves are caused by a transverse growth of the inner epidermis (pp. 200–1).

V.   The shutting movement

21.   The shutting movement of *Aldrovanda* leaves is composed of the quick phase (Fig. 1–5, Pl. x) and the slow phase (Text-figs.

23, 24 A and 25 A). At normal temperatures, the quick phase lasts for 1/100-1/50 sec., the movement of the lobe margin having a velocity of 6-16 cm./sec. at the quickest instant of the shutting (pp. 206–7).

22. The two lobes of a leaf show almost the same latent period and shutting process (pp. 206 and 207).

23. The shutting force is measured with silica dynamometers (p. 212).

24. Shutting force of a certain strength is produced at the instant when the latent period is over, and does not change until it is increased secondarily a little by the sucking in of water by the outer epidermis (Figs. 24 B and 25 B).

25. Rises and falls in the various forms of resistance in the shutting movement are given in Table 2.

26. The magnitude of the shutting force varies with the age of the leaf (Table 3), the margin distance (Table 4), the temperature (p. 219), and the intensity of the stimulus given (Table 5).

VI. The narrowing movement

27. The relations among the working force (measured with silica dynamometer), the resulting movement and the resistances intervening between them are summarized in Fig. 32.

28. When the narrowing movement proceeds, the water in the leaf cavity is pressed out through the contact surface of the one-layered regions of the lobes, overcoming the strong resistance (pp. 222 and 226).

VII. The recovery process

29. The course of the rebulging movement is a reversal of that of the narrowing, though it proceeds more slowly (Fig. 35). The movement is limited by the velocity with which water is sucked into the leaf cavity (p. 235) through the line of contact of the one-layered regions of the lobes (p. 234), but the mechanism itself, too, proceeds slowly (p. 235).

30. The reopening movement proceeds roughly as slowly as the rebulging movement, but is much retarded when it comes near its end (Fig. 8, Pl. IX and p. 237). Leaves cannot reopen fully if they are old, or if the temperature is not high enough (p. 238).

Literature cited

ARBER, A. 1920. Water plants. Cambridge.

AUGÉ DE LASSUS, M. 1861. Analyse du mémoire du GAETAN MONTI sur l'*Aldrovandia*, suivie de quelques observations sur l'irritabilité des Follicules de cette plante. Bull. soc. bot. de France, 8, p. 522.

BATALIN, A. 1877. Mechanik der Bewegungen der insektenfressenden Pflanzen. 2. Die Fliegenfalle. Flora, 35 (N. F.), pp. 105, 129 & 145.

BOSE, J. C. 1913. Researches on irritability of plants. London.

BROWN, WM. H. 1916. The mechanism of movement and the duration of the effect of stimulation in the leaves of *Dionaea*. Amer. Jour. Bot., 3, p. 68.

BROWN, WM. H. and L. M. SHARP. 1910. The closing response in *Dionaea*. Bot. Gaz., 49, p. 290.

BÜNNING, E. (*a*) 1929. Untersuchungen über die Seismoreaktion von Staubgefässen und Narben. Zeitschr. f. Bot., 21, p. 465.

——— (*b*) 1930. Die Reizbewegungen der Staubblätter von *Sparmannia africana*. Protopl., 11, p. 49.

——— (*c*) 1931. Ueber die Erregungsvorgänge bei seismonastischen Bewegungen. Planta, 12, p. 545.

——— (*d*) 1933. Weitere Untersuchungen über die Erregungsvorgänge bei seismonastischen Bewegungen. Flora, 27, p. 119.

BURDON-SANDERSON, J. (*a*) 1882. On the electromotive properties of the leaf of *Dionaea* in the excited and unexcited states. Phil. trans., p. 1.

——— (*b*) 1888. Ibid., p. 413.

CASPARY, R. (*a*) 1859. *Aldrovandia vesiculosa*, MONTI. Bot. Zeitg., pp. 117, 125, 133 and 141.

——— (*b*) 1862. *Aldrovandia vesiculosa*. Bot. Zeitg., pp. 185 and 201.

CHRISTALLE, W. 1932. Ueber die Reizerscheinung der Narben von *Mimulus luteus*. Bot. Arch., 34, p. 115.

COHN, F. (*a*) 1850. Ueber *Aldrovandia vesiculosa*, MONTI. Flora, 43, p. 673.

——— (*b*) 1875. Ueber die Funktion der Blase von *Aldrovandia* und *Utricularia*. COHN's Beiträge, 1, III, p. 71.

CZAJA, A. TH. 1924. Physiologische Untersuchungen an *Aldrovandia vesiculosa* L. PFLÜGERS Arch., 206, p. 635.

DARWIN, C. 1875. Insectivorous plants. (Revised by F. DARWIN, Cheap edition, London, 1908.)

DE CANDOLLE, C. 1876. Sur la structure et les mouvements des feuilles du *Dionaea muscipula*. Arch. Sci. Phys. Nat., 55, p. 400 (through BROWN and through GUTTENBERG).

DIJKMAN, M. J. 1931. The movement of the filaments of *Sparmannia africana* in seismonastic reactions. Kon. Akad. v. Wet. Amsterdam, 34, p. 1051.

FENNER, H. v. 1904. Beiträge zur Kenntnis der Anatomie, Entwicklungsgeschichte und Biologie der Laubblätter und Drüsen einiger Insektivoren. Flora, 93, p. 335.

GERM, H. 1932. Untersuchungen über die systrophische Inhaltsverlagerung in Pflanzenzellen nach Plasmolyse. I. Protopl., 14, p. 566.

GOEBEL, K. 1891. Pflanzenbiologische Schilderungen. 2. Teil, p. 57. Marburg.

GUTTENBERG, H. v. (*a*) 1925. Die Bewegungsmechanik des Laubblattes von *Dionaea muscipula* ELL. Flora, 118-9, p. 165.

——— (*b*) 1926. Zur Kenntnis lebender Bewegungsmechanismen. Planta, 1, p. 666.

——— (*c*) 1928. H. WEIDLICHs Versuche über die Bewegungsmechanik der Variationsgelenke. Planta, 6, p. 790.

—— (d) 1932. Wachstum und Bewegungserscheinungen. Fortschritte d. Bot., 1, p. 202.

HABERLANDT, G. 1906. Sinnesorgane im Pflanzenreich. Leipzig.

HAUSLEUTNER (a) 1850. (Nachtrag zu *Aldrovanda*.) Cultur der *Aldrovanda*. Bot. Zeitg., p. 831.

—— (b) 1851. Ueber die *Aldrovanda* in Schlesien. Bot. Zeitg., p. 301.

HEGI, G. 1906. Illustrierte Flora von Mittel-Europa. IV, Bd. 2, p. 506.

HEYN, A. N. J. 1931. Der Mechanismus der Zellstreckung. Rec. trav. bot. néerl., 28, p. 113.

HÖBER, R. 1933. Ueber den Einfluss des elektrischen Stroms auf die Permeabilität von Pflanzenzelle. Protopl., 19, p. 26.

HOLDHEIDE, W. 1931. Ueber Plasmoptyse bei *Hydrodictyon utriculatum*. Planta, 15, p. 244.

HOOKER, H. D. 1916. Physiological observations on *Drosera rotundifolia*. Bull. Torrey Bot. Club, 43, p. 1.

KAUFMANN, W. 1931. Hydromechanik, Vol. I. Berlin.

KOKETSU, R. 1927. Ueber die Erregbarkeit der Blattgelenke der welkenden *Mimosa pudica*. Bot. Mag. Tokyo, 41, p. 78.

KORZCHINSKY, S. 1886. Ueber die Samen der *Aldrovandia vesiculosa* L. Bot. Centrabl., 27, p. 334.

KRUCK, M. 1931. Physiologische und cytologische Studien über die Utriculariablase. Bot. Arch., 33, p. 257.

LLOYD, F. E. (a) 1931. The range of structural and functional variation in the traps of *Utricularia*. Flora, 25, p. 260.

—— (b) 1933. The carnivorous plants—A review with contributions. Trans. Roy. Soc. Canada, III, 27, p. 3.

MACFARLANE, J. M. 1892. Contribution to the history of *Dionaea muscipula* ELLIS. Contr. Bot. Lab. Univ. Pennsylvania, 1, p. 7.

MUNK, H. 1876. Die elektrischen und Bewegungserscheinungen am Blatte der *Dionaea muscipula*. Arch. f. Anat. Physiol. u. wiss. Med., pp. 30 and 167.

NIKITIN, P. A. 1927. Interglacial occurrence of *Aldrovanda vesiculosa* L. New Phytol., 26, p. 58.

PFEFFER, W. 1904. Pflanzen Physiologie. 2. Aufl. Leipzig.

PRINGSHEIM, E. G. 1924. Algenkultur. ABDERHALDEN, Handb. d. biol. Arbeitsmeth., XI, 2, p. 377.

SEYBOLD, FR. 1852. Einige neue Pflanzen der Flora Tyrols. Flora, 10, p. 403.

STUTZER, W. J. 1926. Zur Biologie der *Utricularia vulgaris*. Arch. f. Hydrobiol., 17, p. 730.

WEIDLICH, H. 1930. Die Bewegungsmechanik der Variationsgelenke. Bot. Arch., 28, p. 218.

ZIEGENSPECK, H. 1928. Zur Theorie der Bewegungs- und Wachstumserscheinungen bei Pflanzen. Bot. Arch., 21, p. 449.

Explanation of Plates

Plate IX

Fig. 1. Optical cross-section of cells in the motile zone, the lobe being folded sharply under the cover glass, the outer epidermis outside. ×160

Fig. 2. The back view of an open leaf (cf. Text-fig. 7 A) in a dark field, the air containing intercellular spaces reflecting light. The marginal bright arcs, and the dark ones on

the midrib sides of them, indicate that the surface of the leaf is undulated within those zonal portions, and represent the localization of the motile zones in the lobes. ×19

Fig. 3.   Arrangement of the blisters raised by acetone, indicating the seat of the motile zone. ×12

Fig. 4.   Blisters raised by acetone upon the motile zone of the free-side lobe. ×35

Fig. 5.   A blister raised by acetone magnified. ×160

Fig. 6.   The motile zone in Text-fig. 14 magnified, indicating that the cells are coloured most darkly at the ends nearer to the darkest part. The midrib side is on the right. ×330

Fig. 7.   A kymogram of the reopening movement of a leaf (from whorl No. IV) at 35°.   The white marks along the lower margin show quarter or half (×) hours, the hours being indicated below.

Fig. 8.   A kymogram of the reopening movement of a leaf (from whorl No. II) at 35°. (The notches on the curves are due to a defect in the pedestal of the kymograph used.)

Plate X

Figs. 1–5.   Myograms recording the shutting movement of *Aldrovanda* leaves, the lower curve of each representing the free-side lobe.   The pitch of the sine curves corresponds to 1/50 sec.
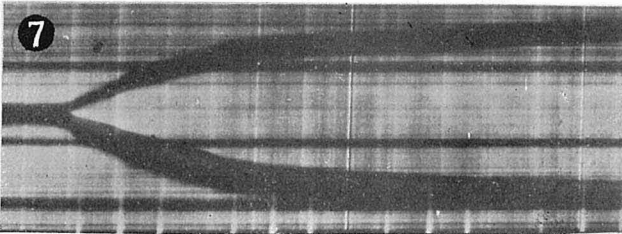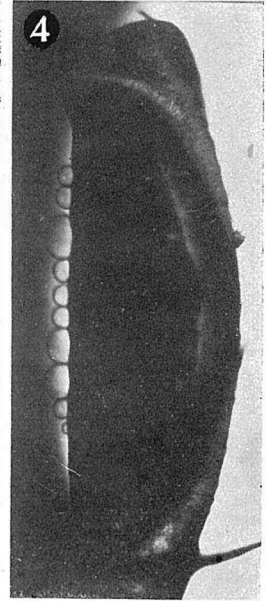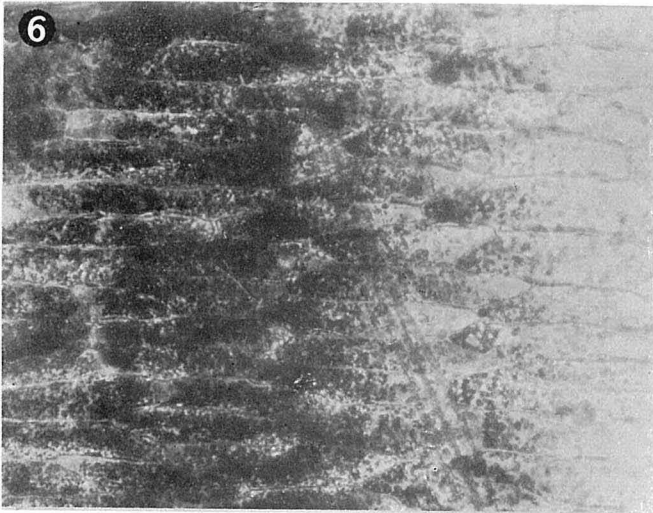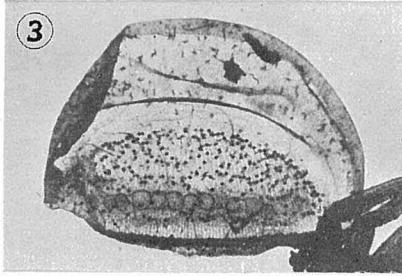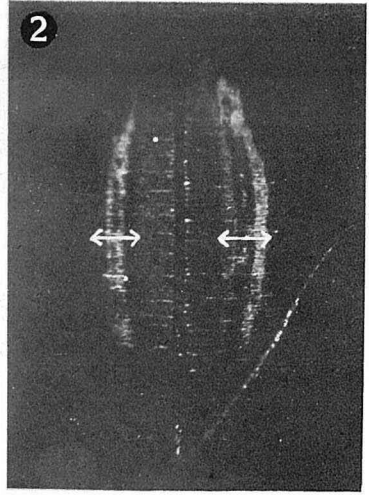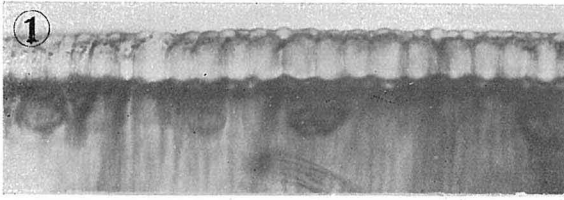
Fig. 1.   Whorl No. II, at 30°, showing the normal movement.   The quick phase begins at *a*, and passes to the slow phase at *b*.
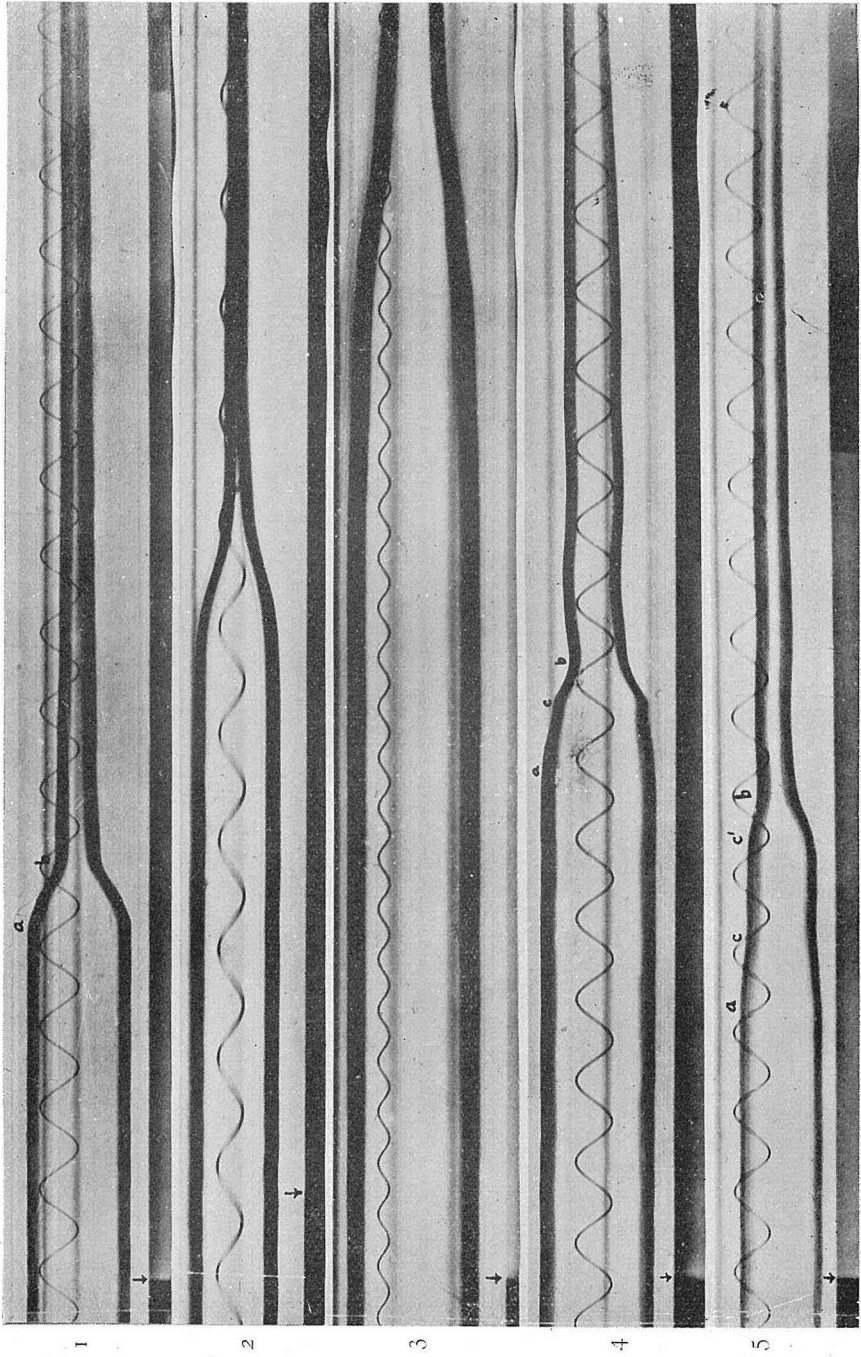
Fig. 2.   Whorl No. II, at 27°.   The critical points *a* and *b* in Fig. 1 are not strictly determined.   The two lobes do not move quite symmetrically.

Fig. 3.   Whorl No. VI, at 10°.

Fig. 4.   Whorl No. II, at 27°, showing a stepwise movement (*a–c–b*).   The undulation of the leaf in the slow phase is caused by an asymmetrical pressing out of water by the lobes.

Fig. 5.   Whorl No. I, at 27°, showing a long pause (*c–c′*) in the quick phase.

×   I ×   2 ×   3 ×

I   2   3   4   5

J. ASHIDA. Photo.

Pl. X