

Observations on Stomata

Francis Darwin

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IX. *Observations on Stomata.**By* FRANCIS DARWIN, *F.R.S.*

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§ 1. METHOD.

THE most obvious method of investigating stomata is to examine them microscopically, and in this way a large number of researches into the conditions which open or close the stoma, and into the mechanism of the movements have been made. The majority of observers have either stripped off the epidermis or have cut surface sections. Neither method can be absolutely depended on as an index of the condition of the stomata in the uninjured leaf. LEITGEB* and others have shown that the stomata in

* LEITGEB (86). *The figures following the author's names in the footnotes, indicate the year of publication, e.g., in the present instance, 1886; the full reference is given in the list of works on p. 619.*

the periphery of such preparations are frequently more widely open than those in the centre—a fact which demonstrates the general untrustworthiness of the method. The stomata can only be well seen in stripped epidermis or thin surface sections, but such preparations are especially liable to the source of error above indicated.

In spite of the difficulty of accurately observing the stomata in thick sections, and in spite of the general objections to this class of work, there is no doubt that much valuable work has been done in this way. It is, however, significant, as STAHL has pointed out, that LEITGEB (86) and SCHELLENBERGER (97) working in this manner have arrived at diametrically opposite results in determining the fundamental question whether or no the majority of terrestrial plants close their stomata at night.*

A far better microscopic method is that employed by some of the old observers, and recently by KOHL,† viz., the examination of the uninjured leaf still attached to the plant. The difficulty of accurate observation is greater than in the case of surface sections, but this objection is amply compensated for by the leaf being in an absolutely normal condition. The method is excellent in many ways; unfortunately, it can only be applied to certain selected plants. KOHL uses *Trianea bogotensis*; I have employed *Caltha palustris*, *Ranunculus Ficaria*, *Potamogeton natans*, *Callitriche verna*.

Other methods are indirect in character, e.g., that of N. J. C. MÜLLER, who used the rate of flow of air through a leaf as an index of the degree to which the stomata are open. This method requires elaborate apparatus and must, I imagine, be slow in action and difficult to manage, but I have no personal experience of it.

Another group of methods depends on the fact that in adult leaves transpiration is stomatal rather than cuticular, so that, other things being equal, the yield of watery vapour will depend on the degree to which the stomata are open, and may be used as an index of their condition. A familiar demonstration is to place a leaf between two plates of glass, when the glass in contact with the stomatal surface becomes dewed with moisture, while the other plate remains dry. In 1878 MERGET‡ invented a refinement of this method; he used paper impregnated with certain salts of palladium and mercury, which assume a dark brown or black colour when moistened. In a hypostomatal§ leaf the paper in contact with the lower surface of leaf changes colour, while that on the astomatal upper surface retains its original tint or, at most, shows a change which is insignificant in comparison with the effect produced by the stomatal side.

* The fact that UNGER (62) (p. 335) did not find the stomata open at 5 a.m. in June is, according to my experience, clear evidence of the faultiness of the microscopic method.

† KOHL (86, 95).

‡ MERGET (78).

§ The term *hypostomatal* implies that all the stomata are on the lower surface; *amphistomatal* may be used for leaves having stomata on both sides, and *epistomatal* for the rarer cases in which all are above.

MERGET's method has never come into general use, and to STAHL* is due the credit of having introduced an easily applicable method of a similar sort, and of having shown that it may be employed as a valuable means of investigation. STAHL uses filter paper soaked in a solution of cobalt chloride (1 to 5 per cent.). Such papers are blue when thoroughly dried, and gradually assume a pink tint as they absorb the moisture of the air. The same change may be rapidly produced by placing the paper on the stomatal surface of a strongly transpiring leaf.

The difference between the two surfaces of a hypostomatal leaf is very striking, the paper on the lower surface shows a brilliant rose-coloured print of the leaf, while the other paper remains wholly blue. With proper precautions the paper on the upper side may, according to STAHL, remain blue for hours. Nothing can demonstrate better the insignificance of cuticular in comparison with stomatal transpiration.

In my laboratory at Cambridge a method of demonstrating stomatal transpiration has been for many years in use, and is described in the 'Practical Physiology of Plants.'† The index is the hygroscopic awn of *Stipa*, which, by untwisting when the instrument is fitted to the stomatal side of a leaf, gives evidence of transpiration on that side. The instrument which I have used in the present research is on the same principle, and will be referred to in these pages as the *hygroscope*, or as the *horn hygroscope* when it is desired to distinguish it from an instrument of another kind described at page 539. The instrument is shown in fig. 1.

Fig. 1.



Horn hygroscope.

C, cork block; T, tongue of horn; *b*, bristle; G, card quadrant. The tongue is shown curved, as though the instrument were resting on the stomatal surface of a leaf.

C is a block of cork ($5 \times 4 \times 4$ millims.), on the lower surface of which is cemented a strip about 3 millims. wide and 8 millims. long of hygroscopic material.‡ This *tongue* bears at its free end a bristle, *b*, to serve as an index; a cardboard quadrant, G, is cemented to the vertical side of the cork and bears graduations along its curved edge. If the hygroscope is placed on a dry substance the index remains at rest, but if on an evaporating surface, *e.g.*, the stomatal side of a leaf, the

* STAHL (94).

† F. DARWIN and ACTON (94), p. 94.

‡ My original stock of horn came from a Chinese or Japanese toy, and was called Chinese sensitive leaf. I now use shavings cut across the grain from pressed horn, heated between glass plates over a flame.

hygroscopic tongue immediately curves away from the source of moisture, and in so doing causes the index to travel over the graduations on the card.

The advantage of the instrument is that it gives the power of ascertaining in a few seconds whether the stomata are open or nearly closed.* When placed on the astomatal side of a leaf the index remains at zero; on the stomatal it rises in 5 or 10 seconds to a position of approximate rest,—a position which varies roughly with the degree of transpiration. In fig. 1 the index is at 2·2: in the records of my experiments such a reading would have been entered as 22. The same convention is followed in these pages, *e.g.*, a reading of 40 means that the index is at 4 in fig. 1.

The following experiments, however, give some idea of the meaning of the graduation of the hygroscope.

A leaf of *Ficus elastica* was weighed at short intervals of time and the loss of weight was calculated in milligs. per hour per 100 sq. centims. This loss is practically all due to stomatal transpiration, for control experiments show excessively small cuticular transpiration. As the stomata close and the transpiration diminishes it is possible to take readings of the hygroscope simultaneously with the weighings and in this way to compare the hygroscope readings with the actual transpiration.

AUGUST 27, 1897.—*Ficus elastica*.

Time.	Loss per hour per 100 sq. centims.	Hygroscope.	Average.
A.M.			
11.15 } 11.35 }	310	20-19	20
P.M.	265	19-15	17
12.6 } 12.36 }	169	15-15	15
	96	15- 9	12
2.3 } 2.33 }	72	9- 6	8
	60	6- 0	3
3.3 } 4.50 }	24	0- 0	0

Thus a moderate reading (20) on the hygroscope means about 300 milligs. per hour per 100 sq. centims., 24 milligs. per hour is imperceptible. The intermediate readings on the hygroscope are clearly not proportional to the transpiration amounts. Thus 169 milligs. is about halfway between 24 and 310. But the corresponding reading 15 on the hygroscope is not halfway between 3 and 20.

Weighings of a moist piece of filter paper confirm the value assigned to a reading of 20 on the hygroscope. Thus in one experiment a reading of 19 (hygroscope)

* The instrument does not distinguish between absolute and partial closure.

= 295 milligs. per 100 sq. centims., in the second case 20 (hygroscope) = 285 milligs. per 100 sq. centims.

Another series with *Ficus* gives comparable though considerably different results.

JANUARY, 1898.—*Ficus elastica*.

Time.	Loss per hour per 100 sq. centims.	Hygroscope.	Average.
A.M. 10.30	216	15-14	15
10.55			
11.40	116	14- 5	10
P.M. 12.13	61	5- 5	5
12.43	47	5- 3	4
2.20	23	3- 0	2
3.22	17	0- 0	0

January 21, 1898.—An *Ioichroma* leaf gave a hygroscope reading of 21. During this time it transpired 253 milligs. per 100 sq. centims. per hour.

January 22, 1898.—Another leaf of *Ioichroma* reads as follows:—

Time.	Loss per hour per 100 sq. centims.	Average of hygroscope.
A.M. 11.31	286	17
P.M. 12.3		
12.36	239	12
2.19	160	8

Sources of Error.

Heat.—If the hygroscope is placed on a warm dry surface, the index rises as though the surface were damp. Other hygroscopic substances are known to react to changes of temperature in the same way,* so that the fact itself is not surprising. In practice this property is a drawback only in experiments on the effect of considerable changes of temperature, and even here the difficulty is not insuperable. This is shown by the

* F. DARWIN (76); ENGELMANN (95).

following experiment. In order to show that stomata close when a leaf is killed, I have frequently scorched one half of a leaf by holding it over a gas flame. If readings are taken at once, the stomata appear to be open on the killed half, because the leaf is still warm; but if two minutes are allowed to elapse, the leaf assumes so nearly the temperature of the air that no heat effect is perceptible. The reading on the killed side is then 0, while the living half of the leaf gives a reading due to the openness of the stomata.

Moisture of the Air.—Since the movement of the index depends on the air being moister on one side of the hygroscopic tongue than on the other, it is clear that the condition of the air must affect the reading of the instrument. But it is also clear that this source of error is not in practice a serious one. In order to estimate the error I have taken hygroscope readings on substances such as a wet brick or the cut surface of a branch of *Taxus*, which give readings comparable to vigorously transpiring leaves. In both cases the evaporating body—brick or wood—was supplied with water below. The hygroscope in such experiments must be prevented from touching the wet surface by perforated zinc plate or fine wire gauze, &c. The following table gives readings of the hygroscope in various atmospheres:—

January 27, 1897.	Wet brick.	Yew wood.	Psychrometer.	Temperature.
			per cent.	° C.
Laboratory	42	65	54	16
Cactus house	42	65	70	15
Corridor	40	65	88	13
Stove	40	63	94	18

With another instrument the results were not so good as these.

June 15, 1897.	Wet brick.
Laboratory	32
Filmy fern house	24

The outcome of my experiments is that certain individual instruments give readings which do not vary through a great range of psychrometric conditions, but that, generally speaking, an atmosphere approaching saturation affects the readings considerably. In practice the fact is of no great importance, since I never make use of the hygroscope for absolute, but for comparative or relative readings.

Wind.—It is important that observations of any accuracy should be made in a place free from air-currents. In a wind, the air on the surface of the leaf is so rapidly renewed that the index of the hygroscope cannot react fairly. If the stomata are

widely open, a reading can be obtained out of doors, but it cannot be an accurate one. STAHL* points out that my hygroscopic index sinks to zero when the leaf on which it rests is fanned ; he ingeniously makes use of the fact in his discussion on *Desmodium gyrans*, in which he supposes the shaking of the terminal leaflets by the gyrating ones renews the air on the surface and leads to increased transpiration. It will be seen, therefore, that there is here a serious possibility of misinterpretation, since in a fanned leaf the conditions of increased transpiration accompany a fall in the index. This error is easily avoided if readings are not taken in a strong draught of air.

Some questions of manipulation may find a place here. It is generally easy to see when the index has approximately come to rest, but it is safer to leave the hygroscope on for a given time. I generally take readings after 10 to 20 seconds. *As soon as the reading has been taken the hygroscope must be removed until the next observation is made.*

The horn index often curves slightly with age or under changes in the dryness of the air ; in this way the zero or position of rest changes ; it is therefore necessary always to note the zero, and subtract it from the reading ; thus, if the zero is 5, and the index points to 30, I record the reading as 25.

Either owing to the curvation of the horn or the unevennesses of the leaf, the index often gives a sudden rise or "kick" as the instrument is put down. It is easy to distinguish a "kick" from a genuine rise, because, when the hygroscope is lifted, the index falls gradually after a true rise, suddenly after a kick. Occasionally it will be found necessary to raise the hygroscope very slightly above the surface by adding a scrap of thin paper under the cork, or a thicker object under the corner of the paper quadrant ; in this way the error from "kicking" is under control, though at the expense of some loss of sensitiveness.

The most serious fault in the method is that the horn index is constantly liable to deterioration, and it is necessary to have a good supply of new instruments.

Errors of Interpretation.

The hygroscope, strictly speaking, only localises transpiration ; the question how far the different degrees of transpiration found in different parts of a leaf depend on the distribution or on the aperture of the stomata must be considered.

Cases occur in which it is difficult to interpret the results—for instance, in variegated leaves the hygroscope gives a much higher reading on the green than on the white parts. Is this due to the stomata being fewer or less open on the white parts, or does it depend on the absence of chlorophyll apart from the behaviour of the stomata ? I do not propose to answer this question here, I only give it as an example of the kind of difficulty which meets us. In some leaves, particularly those of Monocotyledons, the hygroscope gives very different readings in different parts of

* STAHL (97).

the leaf, owing apparently to variations in the number of stomata. It is necessary to be aware of this fact, otherwise the observer might interpret his hygroscope readings as showing a variation in stomatal aperture in the different regions of the leaf. It is obviously necessary in all these cases to make all observations in one given region.

The use of the hygroscope as an index of the condition of the stomata depends on the assumption that in adult leaves the stomatal transpiration is so much greater than the cuticular transpiration that the latter may be practically neglected.

PFEFFER* discusses this question and states that if a small glass vessel is cemented on to the astomatal surface of *Ficus elastica* or *Ilex Aquifolium* the glass soon becomes dewed with moisture. He also quotes GARREAU's results to show that considerable amounts of water vapour pass through the astomatal surface.

On the other hand STAHL† shows that cobalt-paper placed beneath small vessels cemented to the astomatal surface of *Ficus elastica*, *Begonia manicata*, *Hedera Helix*, do not change colour for a long time. With *Ficus elastica* 24 hours were required, with *Hedera Helix* about 3 days.‡ STAHL justly remarks that when one remembers that on the lower surface of these leaves the cobalt-paper turns pink in a few minutes, it cannot be doubted that the cuticular transpiration is negligibly small in comparison with the stomatal loss of vapour. For my purpose the actual amount of cuticular transpiration is unimportant, because it is too small to affect the horn hygroscope. The instrument *always* remains at zero on the astomatal surface of even delicate leaves. This being the case, it is certain that the striking movement of the index on the stomatal side is not due to cuticular but to stomatal transpiration, and that consequently variations in this movement must, *other things equal*, depend on the aperture of the stomata. All the general results of my work agree with this statement. Thus a number of observers, using the microscopic method, agree that the stomata of typical land plants close as the leaves wither, and behave in the same way when the plant is put in the dark. Both these processes can be followed with the hygroscope, and it cannot be doubted that the gradual fall in the readings of the hygroscope corresponds to the shutting of the stomata which so many observers have recorded.

Direct evidence of this is obtainable by comparing hygroscopic and microscopic observations. The leaves of *Caltha palustris* and *Ranunculus Ficaria* are useful for this purpose, because the stomata are large enough to be examined under a moderately high power by simply placing the leaf on the stage of the microscope. In this way either cut leaves or leaves still attached to the plant can be examined. The stomata on the upper side of the leaf are more easily visible in both genera.

* PFEFFER, (79), p. 143.

† STAHL (94), p. 121.

‡ GARREAU states that no transpiration occurs from the astomatal surface of the Ivy.

MAY 4, 1897.—Leaf of *Caltha* cut and allowed to wither ; readings taken on upper surface.

Time.	Hygroscope.	Microscope (divisions of micrometer).
A.M.		
11.15	30	2
11.24	25	2
11.35	20	1-2
11.50	11	1
P.M.		
12.14	3	0

MAY 25, 1897.—*Caltha*, cut leaf withering.

Time.	Hygroscope.	Microscope.
P.M.		
2.15	30	$1\frac{1}{2}$
2.20	27	$1\frac{1}{3}$
2.25	20	$\frac{3}{4}$
2.30	7	0
2.35	0	0

MAY 25, 1897.—*Caltha*, rooted in water, leaf examined while attached to plant.

Time.	Hygroscope.	Microscope.
P.M.		
3.10	27	$1\frac{1}{2}$
Plant removed to dark room.		
3.17	24	$1\frac{1}{3}$
3.24	18	1
3.30	14	$\frac{1}{2}$
3.35	8	0
3.42	0	0

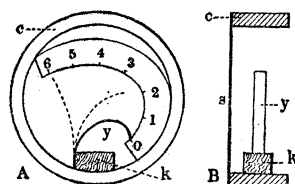
In all these experiments, and in others of the same class, it is observable (1) that a rough parallelism holds between hygroscope and microscope ; (2) that the hygroscope gives evidence of transpiration when the stomata appear to be shut, as seen under the microscope.

The Yucca Hygroscope.

STAHL has shown that, if the sensitive paper is placed under a glass dish cemented to the surface of a leaf, the cobalt method is capable of revealing, and, therefore,

localising very small amounts of transpiration. The horn hygroscope cannot be used to indicate accumulated products of transpiration. Leaving the instrument under a cover cemented to the leaf would diminish instead of increasing the readings. I have therefore made use of a hygroscope, which (like my *Stipa*

Fig. 2.



The Yucca Hygroscope.

A, seen from above; B, in section; c, the glass cell; s, cover slip forming the roof; k, cork block; y, hygrosopic tongue. The dotted lines, pointing to 2 and 6 on the scale, represent successive positions assumed as the tongue uncurls in damp air.

instrument) is affected by accumulation of water vapour. The material is the dried epidermis of *Yucca aloifolia*,* sometimes known as *leaf calico*; in dry air the material is strongly concave on one side, so that it looks like a rolled up sheet of paper. In moist air it instantly uncurls, becomes flat, and finally curls up in the opposite direction.

Fig. 2, B, shows the method of using the material in the Yucca hygroscope; c is a small glass cell (10 millims. \times 5 in height), such as is used for fungus cultivation, closed at one end by a cover slip (in fig. 2, B, the roof, s, is to the left, the open end, which would rest on the leaf, is to the right). On the vertical wall of the cell is fixed a bit of cork, which carries a strip or tongue of Yucca epidermis.

Fig. 2, A, shows the Yucca hygroscope from above, the membrane being here curled up, as in the dry position. A paper scale pasted on the glass cover of the cylinder serves to measure the change of form of the Yucca membrane. When placed on a leaf, transpiring even moderately, the membrane instantly uncurls, travelling from 0 to 2, or even 6, in a few seconds. The Yucca hygroscope can only be used in a dry room, in moist air the index is so much uncurled that the instrument is unfit for use. Since the position of the index does not depend on the air being more saturated on one side of the membrane than the other, but simply on the moisture of the air, it is clear that it can be used to detect slowly accumulating vapour. The sensitiveness of the Yucca instrument is, in one way, a drawback; it is easy to demonstrate with it the transpiration from most astomatal surfaces, and, therefore, when small amounts of transpiration are indicated by it, we cannot be sure how much is stomatal and how much cuticular. In the following instance the cuticular transpiration was practically *nil* and a very minute amount of stomatal transpiration was demonstrable.

* I owe this useful material to the kindness of Mr. THISELTON-DYER.

Two ivy leaves were gathered and allowed to wither for 19 hours, *Yucca* hygrosopes were then luted with wax on to the upper and lower surface, when movement of the index occurred only on the lower side and was doubtless a stomatal effect. This fact would probably have been demonstrated by the cobalt test or by weighing, but not by means of the horn hygroscope. It is worth pointing out that the cobalt test is practically applied by STAHL in two ways, *i.e.*, when the leaf is merely placed between glass plates, and an effect is looked for in one or two minutes, and secondly when the sensitive paper is covered by a small vessel luted on to the leaf. These two methods roughly correspond in effectiveness to the horn and *Yucca* hygrosopes; but the horn hygroscope is, I think, more sensitive than the cobalt method.* Thus a leaf of a garden *Chrysanthemum* gave on cobalt-paper a red print of part of the leaf while the rest remained blue; with the horn hygroscope the blue parts = 7, the pink 13, that is to say, the horn hygroscope shows transpiration in the blue part of the leaf where none is shown by the cobalt. All methods must finally be tested by weighing. Thus if a leaf is coated with wax on its stomatal surface, weighing will give the evaporation from the cuticular surface, and this may be applied as a correction to weighings of a leaf of which the astomatal surface is waxed. This method is clearly not absolutely accurate, but is the best that can be done.

The sensitiveness of the various methods I should class thus:—

1. Comparative weighing.
2. *Yucca* hygroscope and cobalt (long exposure).
3. Horn hygroscope.
4. Cobalt (short exposure).
5. Microscopic examination of uninjured leaf.

The subject is chiefly of importance in relation to what may be called the absolute zero of stomatal transpiration. The hermetical closure of the stomata is a term often used. Does such a state exist? †

It cannot be denied that the closure of the stomata may be very effective. This is shown in the following weighings of *Tropæolum majus* leaves.

The loss of weight is given in milligrams per hour per 100 sq. centims.; the first two readings on one set of leaves (April 22, 1898), the next two on another set (April 23, 1898).

* On the other hand, the cobalt method has a number of compensating advantages. Observations made with a given solution of cobalt chloride and with a given brand of filter-paper are comparable, whereas the readings made with two hygrosopes are only roughly comparable. Cobalt paper is easily prepared and does not lose its virtues, and is easily used.

† See LIDFORSS (96). I think that further research is needed before it can be right to assume, as the author does, that the closure of the stomata in evergreens in winter depresses respiration to such an extent that the sugar present does not lead to starch formation.

	Loss
1 hour after being cut	37
3 hours „ „	11
18½ „ „ „	6
22 „ „ „	4

The leaves, though flaccid, were not by any means dry, so that the small amount of transpiration can hardly be due to actual absence of water in the tissues. The third and fourth readings correspond in amount to cuticular transpiration.

Nevertheless, I am inclined to believe that the stomata when said to be shut allow more water-vapour to pass than the unbroken epidermis. According to this view, the word “closed” or “shut,” as applied to stomata, is essentially only relative. Whether this is finally exact or not, I wish to make perfectly clear that I use the word “closure” or “shutting” in this sense. *When, for instance, I say that stomata are closed by darkness, I mean that the reading has sunk to zero on the horn hygroscope scale, which indicates a great diminution in stomatal aperture, but has no relation to absolute closure.*

For convenience of discussion some simple word is wanted to express this, and closure seems the best for the purpose.*

* The use to which the hygroscope may be put is illustrated in the sections which follow the present one. It seems worth while, however, to mention a few observations which are interesting as demonstrations, and some of which will, I hope, prove to repay closer investigation.

The general distribution of the stomata on the two surfaces of leaves is easily demonstrable. In the case of floating leaves, the lower surface, though devoid of stomata, is well known to transpire. With a dry *Nymphaea* leaf the lower surface gave readings which, in a terrestrial leaf, would have indicated widely open stomata. The opposite state of things is found among succulents which, in spite of the presence of stomata, do not produce a rise in the hygroscope reading. Some other stomatal surfaces are in the same case. Thus the horn hygroscope remains at zero on all the numerous petals which I have examined, and also on the red leaves surrounding the flowers of *Poinsettia*. I have already mentioned the difference between the white and green parts of variegated leaves. A striking demonstration may be made if the hygroscope is pushed cautiously from the white to the green part of a leaf, when the index may be seen to rise suddenly as it passes the boundary into the green tissue. It is worth noting that the stomata on the white and on the yellow parts of at least some variegated leaves close in darkness and as the leaf withers.* The difference in the green and non-green parts seems to depend on the aperture rather than the number of the stomata, but on this point more observations are needed.

When leaves are attacked by fungi or by insects, the hygroscope readings are usually either at zero on the diseased parts or clearly lower than on the unaffected parts.

STAHL† has shown with the cobalt method that the stomata are shut on fallen autumn leaves and

* WEISS (78), p. 398, speaks of the stomata on non-green parts behaving like normal ones. KOHL (86), pp. 39, 66, found, however, that certain stomata, devoid of chlorophyll, were almost incapable of movement.

† STAHL (94), p. 127.

§ 2. CLOSURE DURING WITHERING.

The fact that in most plants the stomata close as the leaf withers has long been known.

The following figures show the nature of the process. In this case the horn hygroscope was placed on the leaf as soon as it was cut from the plant and left on continuously; this ensures that the readings are taken at absolutely the same part of the leaf throughout. It is, however, inadmissible as a regular method since it ultimately produces a permanent bend in the horn tongue of the machine.

JANUARY 31, 1896.—*Entelea arborescens*.

Time.	Horn hygroscope.	Time.	Horn hygroscope.	Time.	Horn hygroscope.
3.8	45	3.32	38	3.58	20
3.14	45	3.37	30	4.0	18
3.22	45	3.42	30	4.4	18
3.26	40	3.49	25	4.7	15
3.29	38	3.52	25	4.9	13
3.30	40	3.54	20	4.17	13

This is an example of a leaf in which the stomata re-act slowly (closure was only perceptible after 18 minutes), and in which the process continues for a considerable time, *i.e.*, more than an hour.

In the following experiment a much more rapid effect is seen, the hygroscope falling to zero in 20 minutes.

that the closure can be shown to appear as the autumnal colours come on. This fact is also well shown with the horn hygroscope.

Another fact noted by SCHWENDENER, LEITGEB, and STAHL, namely, the closure of the stomata of evergreens in winter, is easily verified. It is worth noting that in the warm winter of 1897-98 the stomata were found clearly open on January 20 (temperature 12°5), in the following:—

Prunus Laurocerasus and *lusitanica*, *Hedera Helix*, *Capsella Bursa-Pastoris*, *Cheiranthus Cheiri*; the hygroscope was at zero on *Buxus sempervirens* and *Ilex Aquifolium*.

In many leaves, especially those of Monocotyledons, the hygroscope readings vary in different parts of the same leaf. Thus in *Thalia dealbata* the readings increased from 5 at the base, through 12 in the middle up to 18 at the tip. In *Agapanthus umbellatus* four regions of the leaf from the base upwards gave 0, 2, 7, 10. These facts, as already stated, are of importance in relation to the use of the instrument, and show clearly the necessity of making no comparisons except between corresponding parts of leaves. The same thing is true with regard to leaves of different ages, the hygroscope readings on the stomatal side of young leaves are often considerably lower than on full-grown leaves.

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MAY 25, 1897.—*Caltha palustris*.

Time.	Hygroscope.
P.M. 2.15	30
2.20	27
2.25	20
2.30	7
2.35	0

The following experiments are taken at random to illustrate the kind of results obtained.

SEPTEMBER 7, 1897.—*Plumbago capensis*.

Time.	Hygroscope.
P.M. 2.0	24
2.5	Leaf cut and taken to laboratory; psychrometer 71 per cent.
2.15	20
2.25	9
2.45	0

SEPTEMBER 7, 1897.—*Cestrum elegans*.

Time.	Hygroscope.
P.M. 2.2	31
2.5	Cut and taken to laboratory; psychrometer 71 per cent.
2.18	16
2.47	0

SEPTEMBER 7, 1897.—*Lapageria rosea*.

Time.	Hygroscope.
P.M. 2.3	20
2.5	Cut and taken to laboratory; psychrometer 71 per cent.
2.27	8
3.0	0

When the *Yucca* hygroscope* is used it becomes clear that the closure of the stomata is a more prolonged process than appears from the above experiments.

DECEMBER 21, 1897.—*Iochroma Warscewiczii*. A Leaf gathered and placed in the Laboratory.

Time.	Horn hygroscope.	<i>Yucca</i> hygroscope.	Temperature.	Psychrometer.
P.M.				per cent.
12.5	0 † 20	10 ‡ 70	16	60
12.31	0 0	8 40		
12.46	0 0	8 42		
2.6	..	7 40		
3.6	..	7 28		
3.52	..	7 15	16.2	63
4.17	..	5 10		

At 4.17 the experiment was closed as the leaf began to look dry and brittle.

It will be seen that the *Yucca* readings on the stomatal (lower) surface continue to diminish for nearly four hours after the horn hygroscope has reached zero. We may suppose that at 4.17 the stomata are practically closed, since the stomatal reading is the same as the astomatal was at the beginning of the experiment. What may be the meaning of the diminution in the *Yucca* readings on the astomatal side is not clear. It might conceivably be due to the closure of stomata which are so rare that the leaf was believed to be astomatal above. But it is more probably due to the gradual loss of water from the leaf.

* In this experiment the edge of the *Yucca* hygroscope was slightly greased, and this is generally advisable.

† Here and elsewhere the numerator gives the reading on the upper surface, the denominator that on the lower surface of the leaf.

‡ The readings of the *Yucca* hygroscope give the amount of movement in one minute, except at 12.5, when the index of the instrument on the lower surface moved through 70 divisions in a few seconds.

DECEMBER 21, 1897.—*Datura suaveolens* leaf gathered and taken into the Laboratory.

Time.	Horn hygroscope.	Yucca hygroscope.	Temperature.	Psychrometer.
P.M.				per cent.
2.6	$\frac{0^*}{18}$	$\frac{10^*}{70}$	16°	61
2.41	$\frac{0}{4}$	$\frac{5}{35}$		
3.27	$\frac{0}{0}$	$\frac{5}{30}$		
4.1	$\frac{0}{0}$	$\frac{0}{22}$	16.3	63

DECEMBER 21, 1897.—*Cestrum aurantiacum*; a leaf gathered and taken into the Laboratory.

Time.	Horn hygroscope.	Yucca hygroscope.	Temperature.	Psychrometer.
P.M.				per cent.
2.0	$\frac{0}{11}$	$\frac{13}{62}$	16°	61
2.46	$\frac{0}{0}$	$\frac{7}{30}$		
3.22	..	$\frac{5}{20}$		
4.9	..	$\frac{5}{18}$	16.3	63

* For an explanation of the symbols $\frac{0}{18}$, $\frac{10}{70}$, &c., see note †, p. 545.

DECEMBER 22, 1897.—*Myoporum laetum*.

Time.	Horn hygroscope.	Yucca hygroscope.	Temperature.	Psychrometer.
A.M.				per cent.
10.31	$\frac{0}{20}$	$\frac{10^*}{70}$	$15\cdot5$	59
11.3	$\frac{0}{15}$	$\frac{9^\dagger}{70}$		
11.46	$\frac{0}{11}$	$\frac{8}{70}$		
12.36	$\frac{0}{10}$	$\frac{5}{60}$		
2.19	$\frac{0}{0}$	$\frac{0}{25}$	16	60

[In all this series the time given in column I. is the average ; thus, the last entry, 2.19, represents two readings taken at 2.17 and two at 2.20 P.M.]

It is clear that transpiration continues, though at a diminishing rate, long after the horn-hygroscope registers zero. The question as to how far the closure of the stomata is carried has already been briefly discussed, but certain weighing experiments bearing on the point are in place in the present section.

A leaf of *Ficus elastica* was cut and allowed to wither for 5 hours, during the subsequent 24 hours it transpired on an average at the rate of 12 milligs. per 100 sq. centims. per hour ; another experiment showed that the astomatal side loses less than 1 millig. per 100 sq. centims. per hour. The loss was therefore from the stomatal side chiefly ; the only doubt is whether the cuticular transpiration on the stomatal side is the same as that on the astomatal side, and this is a point which cannot be decided. The same doubt applies to the following observations.

* Instantly.

† After $\frac{1}{2}$ minute.

APRIL 23, 1898.—*Fuchsia serratifolia*. At 10.40 A.M. four leaves were cut, and allowed to wither; at noon two were coated with melted wax mixture on the upper surface, the other two were waxed below.

<i>Cuticular transpiration,</i> <i>i.e., leaves waxed on lower surface.</i>		<i>Stomatal transpiration,</i> <i>i.e., leaves waxed on upper surface.</i>	
Time.	Loss per hour per 100 sq. centims.	Time.	Loss per hour per 100 sq. centims.
12.22 P.M.	milligs. 10	12.31 P.M.	milligs. 69
2.47 „	10	2.53 „	38
3.59 „	7	4.5 „	16
7.20 A.M., April 24	9	7.28 A.M.	15
3.40 P.M.		3.46 P.M.	

Thus, after 4 to 5 hours' withering, the stomatal transpiration was nearly four times the loss from the cuticular surface; and during the final 8 hours (after 21 hours of withering) the stomatal loss still clearly preponderates.

§ 3. THE TEMPORARY OPENING OF THE STOMATA PRODUCED BY WITHERING.

The hygroscopic method has shown itself to be valuable in analysing the process of withering, especially in revealing the interesting fact that in many plants the closure of the stomata is preceded by a well-marked temporary opening. This fact illustrates the dependence of the stomata on the pressure of the epidermic cells. It has an important bearing on the general mechanism of the stomata, and is discussed in a later section.

The following experiment shows the result.

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APRIL 8, 1897.—A plant of *Campanula Vidalii*, which had been in the Laboratory since the previous day, was employed, one leaf being cut off and allowed to wither, while a similar leaf left on the plant served as a control.

Time.	Experiment.	Control.	Psychrometer.	Temperature.
A.M.			per cent.	° C.
5.15	20	22	62	14
Cut off				
5.17	29	22		
5.18	32	22		
5.20	33	22		
5.22	33			
5.25	33	22		
5.28	30			
5.35	20	22		
5.41	0	22	62	14

APRIL 8, 1897.—*Poinsettia*.

Time.	Experiment.	Control.	Psychrometer.	Temperature.
A.M.			per cent.	° C.
11.30	20	23	60	16
Leaf cut				
11.31	25	23		
11.32.5	32			
11.35	32			
11.37	33	23		
11.39	33			
11.41	28	23		
11.44	20			
11.50	0	23	60	16

APRIL 8, 1897.—*Campanula pyramidalis*.

Time.	Experiment.	Control.	Psychrometer.	Temperature.
noon.			per cent.	° C.
12.0	32	30	60	16
Leaf cut				
P.M.				
12.1.5	37			
12.3	40	30		
12.4.5	42			
12.7	42	30		
12.10	40			
12.13	28	30		
12.16	20			
12.21	20	30	60	16

These experiments serve to demonstrate the temporary opening of the stomata; the effect follows so rapidly on the cutting of the leaf that a control leaf is hardly necessary. A sudden rise in the hygroscope reading is never seen in a plant kept in uniform conditions, so that the following experiments in which no control was used are nevertheless quite trustworthy.

DECEMBER 31, 1896.—*Tropæolum majus*, lower surface. Temperature, 18°.
Psychrometer, 70 per cent.

Time.	Hygroscope.	Time.	Hygroscope.
P.M. 2.15	40	P.M. 2.45	58
2.30	40	2.48	55
2.34 cut		2.53	52
2.35	45	3.0	30
2.37	60	3.7	0
2.40	59		

AUGUST 14, 1897.—*Acacia Cyclops*.

Time.	Hygroscope.	Time.	Hygroscope.
A.M. 11.25	10	A.M. 11.35	17
11.27	11	11.40	15
11.27.5	cut.	11.50	9
11.28	14	12.0	5
11.31	16		

Besides the cases mentioned, I have seen the "temporary opening" in the following:

Araujia albens.

Chrysanthemum sinense (Garden
variety).

Entelea arborescens.

Euphorbia fulgens.

Euphorbia splendens.

Homalanthus populifolius.

Ricinus communis.

Sparmannia africana.

Thevetia nereifolia.

No doubt it will be found in many others, Section 5 shows that it occurs among aquatics. Plants with milky juice seem, however, to show the phenomenon especially well, probably the lactiferous vessels serve as a drainage system, so that the severance of the leaf instantaneously lowers the tension of the leaf.

§ 4. ON THE OCCURRENCE OF THE "TEMPORARY OPENING" AT DIFFERENT TIMES OF DAY.

A few experiments were made as to the occurrence of the "temporary opening" of the stomata at different times of day and under various conditions. It seems best to give these experiments in the present section, although their chief interest is in connection with the phenomena of periodicity and with the mechanism of the stoma, which are dealt with in later sections.

The chief result arrived at is that, if the stomata are naturally closed in the evening, the preliminary opening is absent when the leaf is cut, but that it occurs in the early morning before the stomata have naturally opened.

The following is a typical example of the latter half of the experiment.

JANUARY 12, 1898.—Two *Poinsettias*, in pots, in the corridor of the plant-houses.
(Temperature 13°. Psychrometer 78 per cent.)

Time.	Experiment.	Control.
A.M.		
6.55	0	0
6.56	Leaf cut	
6.57	0	
7.0	6	0
7.1	7	
7.5	3	
7.10	0	0

Altogether eight experiments on *Poinsettia* were made in the morning, and in all of these a preliminary opening occurred; six evening experiments were made, and in none of these was there a rise in the hygroscope readings.

With *Euphorbia splendens* three experiments were made on January 9, 1897 :—

7.0 A.M. a preliminary opening.
5.15 P.M. none.
5.36 P.M. none.

With *Campanula Vidalii*, January 7, 1897 :—

6.45 A.M. preliminary opening.
4.0 P.M. none.

These experiments are too few to be of any value, still as far as they go they confirm the results with *Poinsettia*.

When the stomata are closed during the day by artificial darkness, the results are

doubtful. Fifteen experiments were made, in five of which the preliminary opening occurred, in the other ten it was absent.* It would seem that artificial darkness is unfavourable to opening, though not to the same degree as nocturnal darkness.

§ 5. WITHERING IN AQUATIC AND MARSH PLANTS.

Some difference of opinion exists on the question how far the stomata of aquatics are affected by withering. Thus, SCHWENDENER† considered the stomata to be immovable. KOHL‡ took the opposite view that most water plants have completely movable stomata. More recently he§ has stated that *Salvinia* is the only aquatic plant with motionless stomata.

HABERLANDT|| finds a certain amount of closure, but he thinks KOHL's statement too sweeping. STAHL¶ describes certain plants as having non-shutting stomata. In face of a good deal of divergence of evidence it seems worth while giving an analysis of a few experiments on aquatic and marsh plants.

It will be seen that I find movement in many of the stomata, but that I confirm the main fact—that aquatics, as a class, do not close their stomata to the degree observable in average land plants.

The following tables give the change in the hygroscope readings after various "periods of withering," that is, after the leaves had been cut from the plants and allowed to lie on the laboratory table for the specified times.

Name.	Period of withering.		Change in reading.
	h.	m.	
<i>Hydrocleys Commersoni</i>	1	33	20 - 21
" "	3	2	53 - 55
" " **		27 - 22
<i>Limnocharis emarginata</i>	1	2	$\frac{24}{17} - \frac{0}{0}$
<i>Limnanthemum nymphoides</i>	2	20	60 - 62
" "	0	40	15 - 15
<i>Villarsia parnassifolia</i>	2	58	$\frac{43}{34} - \frac{0}{11}$

* With *Tropæolum*, in which the stomata have been closed by artificial darkness, cutting the leaf causes a preliminary rise so slight as to be perceptible only with the Yucca hygroscope.

† SCHWENDENER (81), p. 853.

‡ KOHL (86), p. 25.

§ KOHL (95).

|| HABERLANDT (87).

¶ STAHL (94), p. 123.

** In the third experiment on *Hydrocleys* the leaf was killed by heat.

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Name.	Period of withering.		Change in reading.
	h.	m.	
<i>Thalia dealbata</i>	3	32	14 - 14
" "	3	54	20 - 15
" "	2	41	$\frac{20}{22} - \frac{13}{15}$
<i>Eichhornia crassipes</i>	1	46	15 - 18
<i>Pontederia cordata</i>	0	37	10 - 13
<i>Sagittaria montevidensis</i>	1	35	22 - 23
" "	3	48	$\frac{25}{33} - \frac{10}{17}$
" "	3	8	19 - 17
" "	4	52	20 - 20
" "	1	35	$\frac{42}{38} - \frac{25}{35}$
<i>Sagittaria sagittifolia</i>	3	9	$\frac{20}{22} - \frac{10}{0}$
<i>Trianea bogotensis</i>	4	49	43 - 32
" "	4	44	45 - 48
<i>Myosotis palustris</i>	1	15	$\frac{10}{25} - \frac{0}{5}$
<i>Alisma Plantago</i>	2	2	$\frac{12}{25} - \frac{0}{4}$
" "	1	15	$\frac{10}{12} - \frac{0}{0}$
<i>Menyanthes trifoliata</i>	3	8	$\frac{22}{35} - \frac{0}{0}$
<i>Caltha palustris</i> *	1	0	30 - 3
<i>Veronica Beccabunga</i>	1	23	27 - 27
<i>Nelumbium speciosum</i>	2	45	$\frac{25}{28} - \frac{16}{19}$
<i>Nymphaea Martiacea chromatella</i> †	3	0	$\frac{35}{38} - \frac{8}{35}$
" " "	2	39	$\frac{35}{28} - \frac{9}{30}$
<i>Nymphaea pygmaea</i> var. <i>helvola</i>	3	0	$\frac{37}{45} - \frac{28}{45}$
" " "	3	34	$\frac{15}{27} - \frac{6}{21}$

* *Caltha* often closes more quickly than in this instance.

† In the experiments on *Nymphaea* the readings on the lower astomatal surface were taken by way of control, because, as the lower surface becomes dry and brittle, the readings on it drop considerably.

It will be seen that in *Hydrocleys Commersoni*, *Limnanthemum nymphoides*, *Thalia dealbata* (1 case), *Eichhornia crassipes*, *Pontederia cordata*, *Sagittaria montevidensis* (3 cases), *Trianea bogotensis*, *Veronica Beccabunga* the readings of the hygroscope did not diminish by any amount worth considering.

Limnocharis emarginata, *Sagittaria sagittifolia*, *Myosotis palustris*, *Alisma Plantago*, *Menyanthes trifoliata*, *Caltha palustris*, *Nelumbium* and *Nymphaea mariacea* showed clear tendency to closure. It may be a chance, but it is worth noting that the species which partly close in withering are more largely European than those in which the stomata remain widely open.

To the above cases I have added observations on species which STAHL classes with marsh plants as not closing the stomata on withering.

Name.	Period of withering.		Change in reading.
	h.	m.	
<i>Alnus glutinosa</i>	1	7	14-0
<i>Betula alba</i>	1	30	5-0
<i>Salix purpurea</i>	0	12	12-0
<i>Salix amygdalina</i>	0	52	21-0
<i>Klugia Notoniana</i>	2	50	27-0
<i>Osmunda regalis</i>	0	45	23-0

In these and similar cases where my results differ from STAHL'S*, I hardly see how the difference is to be explained. The horn hygroscope shows an undoubted partial closure of the stomata, but this does not necessarily mean that the stomata were absolutely shut. STAHL shows that the plants in question do not cease to give off water by the stomata, while the point I wish especially to insist on is that a large number of aquatics (including marsh plants) show a marked tendency to close their stomata on withering.

From my experience I should hesitate to believe that the stomata are in any case immobile. I have not, however, investigated *Salvinia*, KOHL'S one instance of immobility. The mobility of the stomata is evident, even in some plants in which the stomata are usually open, after many hours withering. Thus *Sagittaria montevidensis* exhibits extremely well the phenomenon observed by LEITGEB, viz., the opening of the stomata when relieved of epidermal pressure. I have observed this microscopically, and also with the hygroscope, as shown in the following table.

* There is clearly some variability in behaviour in any given species. Thus in two cases *S. montevidensis*, in two cases *Thalia*, and in one case *Hydrocleys* (when killed), showed clear diminution in the hygroscope readings. *Trianea* in one case also partially closed.

JANUARY 18, 1897.—*Sagittaria montevidensis*.

Time.	Hygroscope.	Time.	Hygroscope.
A.M.		A.M.	
9.20	20	9.32	25
9.28	20	9.41	27
		10.21	25
Leaf cut and taken to the Laboratory.		11.5	23
(Psychrometer, 55 per cent.)		2.10	20

The same thing was seen with *Eichhornia*, and it will be noted in the table given above that with this species the terminal reading was higher than the original reading.

§ 6. ON THE EFFECT OF COMPRESSING THE STEM.

A diminished water supply may be readily produced by compressing a branch in a vice. In this way it has been shown* that the transpiration current diminishes, increasing again when the compression is removed. The experiments that have been made on this point have been directed to the amount of water absorbed by cut stems, not, as far as I know, to the actual amount of transpiration. The fact that the negative pressure increases during compression would seem to show, as might be expected, that the loss of water is not diminished in proportion to the diminished transpiration current.

My present experiments tend to show that transpiration is not immediately or even rapidly diminished.

Indeed, in some cases the first obvious effect is increased transpiration, which I assume to be due to opening of the stomata.

DECEMBER 9, 1896.—*Dadalacanthus nervosus*.

Time.	Hygroscope.	Psychrometer.	Temperature.
P.M.		per cent.	°
2.0	20	79	14
2.5	20		
Vice put on.			
2.7	35		
2.14	31		
2.20	30		
2.30	25		
3.0	20		
3.15	20		

* F. DARWIN and PHILLIPS (86).

It is probable that the rise in the hygroscope reading is of the same character as the "temporary opening" described for cut off leaves. It is probably due to the severing of a good deal of tissue, which necessarily occurs on strong compression being applied, and need not be connected with the reduced water supply. This view is supported by the behaviour of the plant next morning.

DECEMBER 10, 1896.—*Dædalacanthus nervosus*.

Time.	Hygroscope.	Time.	Hygroscope.
A.M.		A.M.	
9.15	0	9.40 (about)	Vice tightened.
9.30	0	10.40	0
Vice unscrewed.		10.42	Vice unscrewed.
9.31	15	10.42½	21
9.35	15	10.43	22
9.38	10		

The immediate increase in transpiration, which follows the loosening of the vice, is difficult to understand; it may be the simple physical result of increased water supply, but it may, of course, be due to the stomata opening. The converse action, *i.e.*, the tightening the vice, produces no *immediate* check, and this points to the diminution of transpiration ultimately produced being due to closure of the stomata rather than to its being a simple physical consequence of diminished water supply. The fact that the leaves of aquatic plants (whose stomata do not readily close) continue to transpire vigorously after being severed from the plant, shows that absolute cessation of water supply does not necessarily produce any rapid diminution in transpiration. The following experiments show the gradual effect of compression.

JANUARY 26, 1898.—*Dædalacanthus nervosus*.

At 12.6 vice applied and produced a clear increase in the hygroscope reading. The vice was left on, and in the afternoon the following readings were taken.

Time.	Hygroscope.	Time.	Hygroscope.
A.M.		A.M.	
2.19	15 (average)	3.29	15
2.26	Vice loosened.	3.30	16
2.27	19 (average)	3.31	Vice loosened.
2.32	18	3.32	19
2.37	18	3.36	21
Vice tightened.		3.47	20
2.37.7	19	3.51	22
2.38.2	18	3.57	Vice tightened.
2.53	16	3.58	20
3.11	17	4.15	16

DECEMBER 10, 1896.—*Poinsettia*.

Time.	Hygroscope.	Psychrometer.	Temperature.
A.M.		per cent.	°
10.45	20	80	14
11.0	20		
11.2	Vice put on.		
11.3	20		
11.5	29		
11.7	30		
11.10	20		
11.30	10		
11.45	10		
11.46	Vice loosened.		
11.47	10		
11.48	20		
11.55	25		
P.M.			
12.30	20		
2.0	20		

The chief interest of these experiments is to show that variations in water supply, apart from any change in the psychrometric condition of the air, apparently lead to changes in the aperture of the stomata.

§ 7. ON THE EFFECT OF DRY AIR.

The fact that many plants close their stomata when brought from a greenhouse to a laboratory cannot be doubted by anyone who investigates the subject, nevertheless it does not seem to be widely recognised that this is the case, and that it occurs in cases where there is not the slightest visible flaccidity.

The following figures illustrate the fact.

The plants were brought from the greenhouse to the laboratory (where the thermometer was 15; the psychrometer 61 per cent.). The hygroscope was read at once, and again after half an hour.

	At once.	After half hour.
<i>Cineraria</i>	30	15
<i>Poinsettia</i>	20	5
<i>Pelargonium zonale</i>	30	20
<i>Salvia sp.</i>	35	10
<i>Tropæolum majus</i>	25	10

In another similar experiment, the result was supplemented by replacing the plants in damp air :—

DECEMBER 14, 1896.

	When brought from greenhouse to laboratory.	After one hour in laboratory, psychrometer 64 per cent.	After two hours in greenhouse.
	Hygroscope.	Hygroscope.	Hygroscope.
<i>Cineraria</i>	25	10	20
<i>Poinsettia</i>	25	3	13
<i>Tropæolum</i>	30	10	20
<i>Pelargonium zonale</i>	35	20	25
<i>Salvia sp.</i>	35	8	25

In the above experiments it might be objected that the illumination in the laboratory was less bright than in the greenhouse, and that the closure was due to the dull light, not the dry air of the room. In the following cases this objection does not hold:—Two pots of *Narcissus* were brought into the laboratory where the air was fairly dry (70 per cent.), and after a time one plant was covered with a bell jar. Here the temperatures were equal, and the dry-air plant was, if anything, the better lighted of the two.

JANUARY 1, 1898.—*Narcissus*.

Time.	Experimental.	Control.
A.M. 9.50	19	20
	Covered with bell jar.	
10.10	15	7
10.30	15	0
10.41	13	0

JANUARY 4, 1898.—*Nicotiana affinis*. Two plants in pots brought into the laboratory. (Temperature 17°. Psychrometer 65 per cent.)

Time.	Experiment.	Control.
P.M. 2.16	22	23
	Covered with bell jar.	
2.36	20	10
2.51	18	3
3.15	17	0

In the next experiment the conditions were alternated.

JANUARY 3, 1898.—*Poinsettia*. Two plants in pots brought into the laboratory.
(Temperature 16°.5. Psychrometer 62 per cent.)

Time.	Experiment.	Control.
A.M. 11.45	20	17
P.M. 12.25	0	0
2.10	Covered with bell jar. 16	Left in dry air. 0
2.43	Removed bell jar. 4	Covered with bell jar. 0
3.10	0	6
3.40	0	13

JANUARY 4, 1898.—*Poinsettia*. Two plants in pots brought into the laboratory.
(Psychrometer 66 per cent.)

Time.	Experiment.	Control.
A.M. 10.31	15	19
10.45	Bell jar on. 16	13
11.6	14	0
11.10 (about)	Bell jar removed.	Bell jar on.
11.30	5	3
11.45	3	4
P.M. 12.10	0	10

Similar results may be obtained with *Tropæolum*.

MAY 7, 1897.—*Tropæolum majus*.

Time.		Hygroscope.
P.M. 2.0	Dry air	27
2.52	Damp air	35
3.30	Dry air	24
4.7	Damp air	34

Thus the hygroscope reading rises after each period of dampness, and falls after each dry period.

§ 8. ON THE EFFECT OF EXTREME DRYNESS OF AIR.

It has been shown, in the case of leaves cut off and allowed to wither, that two opposite effects are produced, *i.e.*, that the stomata first open and then close. The present section shows that a similar state of things holds good for dry air.

The method employed was to attach by means of wax-mixture* the stomatal surface of a leaf to the mouth of a bottle half filled with sulphuric acid. After a certain number of hours the leaf was detached, and readings were at once taken with the hygroscope.

NOVEMBER 30, 1896.—*Dædalacanthus nervosus*.

The plant stood in the "intermediate pit" (temperature $14^{\circ}5$), and a leaf still attached to plant was waxed on to the acid bottle at 11.45 A.M., after it had been ascertained that the leaves all gave a hygroscope reading of about 20.

At 2.45 the part exposed to acid was 40, the rest of the leaf 30.

JANUARY 12, 1897.—*Dædalacanthus*.

Time.	Part over H_2SO_4 .	Other parts.	Other leaves.
A.M.			
9.0	7		
9.5 (about)	Waxed over H_2SO_4 .		
P.M.			
12.10	15	10	10
3.15	20	10	10
A.M.			
Jan. 13, 9.30	0	0	8

* Beeswax 3 parts, resin 15 parts, vaseline 35 parts.

JANUARY 14, 1897.—*Dædalacanthus*. Two plants (experiment and control) removed from the corridor (temperature 11° , psychrometer 90 per cent.) to the laboratory (temperature 15° , psychrometer 56 per cent.).

Time.	Part over H_2SO_4 .	Other parts of experimental leaf.	Other leaves on experimental plant.	Control plant.
A.M. 9.45	30	30
10.15	30	30
	Now exposed to H_2SO_4 .			
10.45	25	25
11.10	20	20
P.M. 2.0	28	10	15	15
4.0	30	7	10	10
A.M. Jan. 15, 9.0	0	..	15	

In this experiment the control plant and the control leaves on the experimental plant are gradually closing, owing no doubt to the dryness of the laboratory air, while the part of the leaf exposed to H_2SO_4 is, at 2 P.M. and 4 P.M., wide open. On the following day the leaf gave a reading of 0.

DECEMBER 1, 1896.—*Poinsettia*. 9.45 A.M., several leaves were tested and found to be well open, *i.e.*, 30. One leaf waxed down to a bottle of H_2SO_4 .

Time.	Part over H_2SO_4 .	Other parts of experiment leaf.	Other leaves.
noon 12.0	50	30	30
P.M. 2.20	45	25	25
3.50	45	15	15
A.M. Dec. 2, 9.30	0	0	15

The exposure to H_2SO_4 clearly has a great effect, since, on the following day, not only the part exposed to acid but the whole leaf was at 0.

Another *Poinsettia* leaf exposed to H_2SO_4 on December 2 (and which showed widely open stomata in the part so exposed) was noted as appearing to be dead on December 3.

DECEMBER 16, 1896.—*Tropæolum*.

Time.	H ₂ SO ₄ leaf.	Control leaves.
A.M.		
9.45	$\frac{15^*}{30}$	
	Lower surface exposed to H ₂ SO ₄ .	
11.30	$\frac{25}{35}$	$\frac{15}{30}$
12.0	$\frac{25}{40}$	$\frac{15}{30}$
P.M.		
2.0	$\frac{30}{45}$	$\frac{15}{30}$
3.30	$\frac{30}{45}$	$\frac{10}{25}$
A.M.		
Dec. 17, 9.30	$\frac{0}{0}$	$\frac{15}{20}$

The remarkable thing about this experiment is that the upper surface of the leaf, *i.e.*, the surface which was exposed to ordinary air, showed the opening of the stomata even more clearly than the lower surface. Here, as before, the leaf was at 0 on the following day, and subsequently died.

These results are not easy to understand, the only explanation seems to be that the epidermic cells suffer more from excessive transpiration than the guard cells, and thus produce a prolonged "preliminary opening." But I am not satisfied with this view of the matter, which clearly requires further research.

§ 9. THE EFFECT OF SHAKING.

BARANETZKY,† as is well known, showed that very slight degrees of disturbance affected transpiration. A sudden shake increases the loss of water, as shown in the following figures taken from his paper:—

50,
52,
68,
47,
55,
54.

* The symbol $\frac{15^*}{30}$ expresses the readings on the upper and lower surfaces of the leaf.

† BARANETZKY (72).

Each line represents the loss of weight per half hour expressed in centigrams; the rise from 52 to 68 is the result of shaking, after which the loss (47) falls below the normal and finally recovers.

KOHL* and EBERDT† observed the increase of transpiration, while WIESNER‡ failed to observe it. KOHL did not find that the transpiration fell below the normal after the shaking ceased. Thus one of his experiments gives:—

31·0,
31·0,
31·0,
35·5,
31·1,
31·0.

Each line represents the loss during 15 minutes, the underlined figures (35·5) being for the period of shaking.

Finally, it should be noted that BARANETZKY and KOHL record that *prolonged* shaking depresses the rate of transpiration. The cause of the phenomena in question is obscure, and various explanations seem admissible.

The term “shaking” is a somewhat vague one; it might be applied to a rapid movement of the plant through the air, which would be equivalent to a current of air blowing on the leaves, and would obviously increase the transpiration. But this, I imagine, is not the form of shaking employed by KOHL and BARANETZKY.§

The latter author assumes that changes in the turgor of the mesophyll cells, induced by the shock, compress the air in the intercellular spaces and force saturated air out by the stomata, which is then replaced by dry air. BARANETZKY’S explanation is, however, not quite clear to me.

Assuming that his view is correct, that the result of shock is to temporarily fill the intercellular spaces with drier air, it is clear that the application of my hygroscope to a leaf which has been shaken ought to give a lower reading of the instrument, since, until the air in the intercellulars is saturated again, the horn index is exposed to air unusually dry. In order to test such a possibility, I have made a few experiments on inanimate objects. A piece of moderately stale bread supplies good material, since it has “intercellular spaces,” and its surface is sufficiently dry to allow the hygroscope being placed on it without the interposition of wire gauze, &c. If a piece of bread is tied to a string and whirled round in the dry air of a room, it

* KOHL (86), p. 87.

† EBERDT (89), p. 68.

‡ WIESNER (76), p. 486.

§ EBERDT (89), p. 177, however, puts down the results obtained by himself and others to the renewal of the air on the leaf surfaces. In the same way STAHL (97), p. 99, considers the shaking of the terminal by the rudimentary leaflets in *Desmodium gyrans* to be an adaptation to increase transpiration.

will be found that immediately after the operation the hygroscope gives a low reading, which rises in a few minutes to a constant, to be again depressed by whirling the bread through the air. The principle is the same as that already mentioned in relation to one of the precautions necessary in using the hygroscope, *i.e.*, that the air of the laboratory must be still, because the draught from a window or other aerial disturbance will tend to remove the layer of saturated air on the leaf surface, replacing it by drier air.*

The following is a sample of the results obtained with bread in a very dry room. (Psychrometer 55 per cent.)

Time.	Hygroscope.
P.M.	
1.56	41
1.58	40
Bread swung in the air.	} still.
2.2	38
2.3	35
2.4	40
2.7	41
2.10	40
Bread swung.	} still.
2.13	30
2.23	40

Thus the readings are lower after disturbance, and rise when the disturbance ceases.

In my experiments on living plants I have never got results from gentle shaking, nor indeed from the considerable disturbance caused by attaching a leaf by a thread to the pendulum of a metronome so that periodic shocks are applied.

In all the experiments in which a decided effect was produced the shaking was of a violent character, so that in many cases obvious flaccidity ensued. The hygroscope gave diminished readings directly after the shaking, and this is probably due to the partial closure of the stomata, not to the expulsion of air from the intercellulars and its replacement by drier air.

* See the observation of STAHL, already quoted (p. 537).

JUNE 16, 1897.—*Impatiens sp.* Two plants in pots.

Experiment.		Control.	
Time.	Hygroscope.	Time.	Hygroscope.
P.M. 3.40	20	P.M. 3.45	20
3.43	20		
3.46			
Shaken violently for 5 minutes.			
3.51	10		
3.53	9	3.54	20
4.5	11	4.7	19
4.20	14		
4.30	13	4.32	15

JUNE 16, 1897.—*Eupatorium (Weinmannianum?)*. Two plants in pots.

Experiment.		Control.
Time.	Hygroscope.	Hygroscope.
A.M. 11.21	28	29
11.23.5 } Shaken		
11.28.5 } violently.		
11.30	15	30
11.34	10	
noon.		
12.0	15	27

JULY 26, 1897.—*Cucurbita sp.* Two plants in pots.

Experiment.		Control.
Time.	Hygroscope.	Hygroscope.
A.M. 11.0	23	25
11.5 } Shaken		
11.10 } violently.		
11.11	7	23
11.17	5	
P.M. 12.25	9	25
3.15	18	22

Similar results were obtained with a *Lonicera*, *Pelargonium zonale*, *Commelina Sellowiana*, *Cineraria*, *Tropaeolum*, *Vicia Faba*.

With marsh plant no closure of the stomata was produced by shaking, and this is what might be expected in view of the small response such plants make to withering. On the other hand, a distinct increase in transpiration was produced in some cases.

FEBRUARY 10, 1898.—*Sagittaria montevidensis*. A leaf was cut and brought into the Laboratory.

Time.	Hygroscope.	Time.	Hygroscope.
P.M. 3.26	23	P.M. 3.31	40
3.27	25	3.32	40
Shaken violently for 2 minutes.		Shaken violently.	
3.29	21	3.34	35
3.29.5	25	3.43	30

The experiment was then repeated, the result being checked by a control leaf.

FEBRUARY 10, 1898.—*Sagittaria montevidensis*. Two cut leaves brought into the Laboratory.

Experiment.		Control.	
Time.	Hygroscope.	Time.	Hygroscope.
P.M. 3.41	35	P.M. 3.41	30
Violently shaken for 2 minutes.			
3.44.5	30		
3.45	45		
3.46	40		
3.46.5	42	3.52	30
..	..	Shaken violently for 2 minutes.	
..	..	3.58	38
..	..	3.59	40
..	..	4.2	35

Similar effects were seen on shaking the leaves of *Thalia dealbata*.

There can be no doubt that these results are due to the deturgescence of the epidermic cells relieving the pressure and allowing the guard cells to open.* The fact that the stomata of terrestrial leaves do not open when the leaf is shaken is no doubt due to the violence of the treatment causing the deturgescence of the guard cells, and so quickly that the preliminary rise is absent. It is possible, however, that BARANETZKY'S and KOHL'S results may be due to temporary opening of the stomata.

* See §3, p. 548.

It will be noticed that a slight fall in the hygroscope readings follows the shaking in *Sagittaria*; whether this corresponds to the physical effect produced with pieces of bread I cannot say. The question seems hardly worth the labour which an answer would require.

§ 10. ELECTRIC STIMULATION.

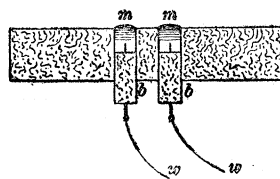
N. J. C. MÜLLER* showed that induction shocks produce closure of the stomata. It is easy to control the fact on the leaves of *Callitriche*, or the stripped epidermis of *Ranunculus Ficaria*.† The further question as to the mode of action of the shock is not so easy.

LEITGEB‡ assumes that the effect is simply due to death or injury of the stoma. Electric shocks which visibly affected the circulation in the guard cells did not close them. If the stomata were made to close, they never opened again. But MÜLLER observed reopening after partial closure of the stomata.

In the following experiments it will be shown that a weak shock produces an opening of the stomata, while a stronger one closes them. LEITGEB (*loc. cit.*, p. 144) suggests that such an effect might be produced by an electric shock diminishing the turgor of the surrounding epidermis, and thus lessening the pressure on the guard cells, but it does not appear that he observed any such effect.

In my experiments I used a Leclanché cell and Ruhmkoff coil, and electrodes of which the structure will be understood from fig. 3.

Fig. 3. Electrodes.



b, b, cork plugs partly filling two holes in a cork plate; through them a pair of needles enter the mercury *m, m*, on which the leaf rests; *w, w*, wires through which the current passes.

In a cork plate two holes, 7 millims. in diameter and 8 millims. apart, are cut with a cork borer; the holes are half filled with corks pushed in from below, *b, b*, and the depressions or wells§ thus formed are filled with mercury, *m, m*. The wires,

* MÜLLER (72).

† F. DARWIN and ACTON (94), p. 99.

‡ LEITGEB (86), p. 143.

§ The wells in the cork plate were in some cases filled with gelatine, to which the leaf slightly adheres and makes a good contact.

w, w, coming from the key which controls the current, end in needle points, which are pushed from below through the corks into the mercury. The leaf which is to be investigated is, while still attached to the plant, allowed to rest on the projecting mercury menisci, and can be held in place by cork or cardboard strips (as in "setting" butterflies), so that it can be kept in contact with the mercury without being itself pierced by the pins. I usually investigated leaves having the stomata on the lower surface, in which case the leaf was inverted, the astomatal surface being in contact with the electrodes, while the stomatal surface was exposed and could be tested with the hygroscope.

In the following tables the column headed "control" gives readings on a part of the leaf not subjected to stimulation. All the leaves used were attached to plants growing in pots.

JUNE 4, 1897.—*Centaurea sp.*

Experiment.		Control.
Time.	Hygroscope.	Hygroscope.
P.M.		
12.19	26	
12.21	25	25
12.22.5	} Stimulated.	
12.24		
12.24.5		29
12.26	28	
12.29	29	25
12.32	} Stronger stimulus	
12.37		..
12.39	25	
12.44	24	30
12.49	21	30

JUNE 5, 1897.—*Hymenocallis (Harrisi?)*.

Experiment.		Control.
Time.	Hygroscope.	Hygroscope.
A.M.		
11.9	0	0
11.10	} Stimulated.	
11.12		
11.14		6
11.17	6	0
11.20	3	
11.35	3	
11.35.5	} Stronger stimulus.	
11.37		
11.38	0	0

The last experiment is worth giving, since it shows that the opening may occur on stimulation, even when the stomata are very slightly open (0 with hygroscope).

When the electric stimulation is repeated after the effect of the first stimulus has passed off, I have never observed any obvious rise in the hygroscope readings; either the reading remains unchanged or begins to sink. The following experiment illustrates this:—

JUNE 3, 1897.—*Campanula pyramidalis*.

Experiment.		Control.
Time.	Hygroscope.	Hygroscope.
A.M.		
10.12	27	28
10.20	28	28
10.23 } Stimulus.		
10.25 }		
10.26 }	33	29
10.28 }	40	
10.30 }	39	28
10.35 }	35	
10.43 }	31	29
10.45 }	31	
10.47 { Stimulated }		
10.49 { again }	..	28
10.49.5 }	30	
10.53 }	30	
10.55 }	28	28
10.56 { Stimulated }		
10.59 { again. }		
11.0	20	28
11.5	20	

Altogether a large number of experiments were made; a rise in the hygroscope reading was observed in:—

Tropæolum, *Cineraria*, *Lonicera*, *Eucalyptus* sp., *Commelina Sellowiana*, *Polygonatum*, *Fuchsia*, *Amaryllis*, *Narcissus*, *Arisæma ringens*, *Eryngium planum*, *Oxalis*.

With the following plants no effect was produced: *Nerium Oleander*, *Ricinus*, *Teedia lucida*.

There were, of course, also cases in which no effect was produced with some of the above-mentioned plants, e.g., *Tropæolum*, *Narcissus*, *Hymenocallis*.

My attention was not especially directed to the above-mentioned question as to whether, when the stomata are closed by a strong current, they ever recover and re-open. I am inclined to think that they do not re-open.

The following experiment shows that the *opening* of the stomata may be produced without permanent injury.

May 29, 1897, an *Amaryllis* leaf has been stimulated (12.0 P.M.—12.2 P.M.), the

line of stimulation running up the centre of the leaf. At 12.10 the central stimulated area gave readings at 25 and 27, the edges gave readings of 10. When the hygroscope was placed on the leaf and pushed cautiously across the stimulated area, the index suddenly rose, and fell again as the other edge of the leaf was reached. Later on, at 12.30, this difference disappeared. The leaf was again stimulated twice over in the same region at 12.31 and 12.34; nevertheless, next day, the stimulated part was 20, and showed practically no difference from the rest of the leaf which gave 19 with the hygroscope.

§ 11. THE ACTION OF POISONS.

A few experiments were made on the action of chloroform and of pure CO₂.

Chloroform.

The experiments were made by placing the plant under a bell-jar with a small quantity of chloroform in a watch-glass. In most cases the bell-jar was removed to take the hygroscope readings, which were made as rapidly as possible, so as to avoid weakening the experimental atmosphere. It is, however, possible to take readings without removing the bell-jar. If a tubulated jar is used, a glass rod can be fitted into a hole in the rubber cork, and can be pushed vertically up and down, so that the hygroscope, suspended to the lower end of the rod, can be placed on the leaf (which lies horizontally in the middle of the enclosed space), and can be removed in the same way. The drawback is that the zero of the hygroscope is liable to change.

The chief results obtained are that the stomata are partially closed by chloroform or ether without the leaf being killed, as is proved by the subsequent rise in the hygroscopic reading. The action of these narcotics is usually slow.

A few typical experiments are given.

APRIL 1, 1897.—*Tropæolum*. Leaves cut and placed with their stalks in water.

Time.	Hygroscope.	Time.	Hygroscope.
P.M. 2.15	12 upper 30 lower	P.M. 2.55 (about) chloroform removed.	
Chloroform immediately added.		4.30	$\frac{0}{0}$
2.40	$\frac{0}{20}$	A.M. April 2, 1897, 9.35	$\frac{8}{15}$
2.51	$\frac{0}{0}$		

The closure in this case occurred unusually quickly. The recovery was, as usual, slow.

APRIL 5, 1897.—*Convallaria majalis*. The upper surface of a cut leaf which had been in water since April 3.

Time.	Hygroscope.	Time.	Hygroscope.
A.M. 10.25	30	P.M. 1.0	10
Chloroform added.		2.0	7
10.30	30	3.30	4
11.5	22	Chloroform removed.	
11.45	15	A.M. April 6, 1897 9.0	15

Salvia sp.—Cut leaves in water since April 2, 1897.

Time.	Hygroscope.	Time.	Hygroscope.
April 3, 1897, A.M. 10.0	20	Chloroform removed.	
Chloroform added.		P.M. 1.0	0
10.15	12	A.M. April 5, 1897, 10.0	
10.25	6		10
10.40	0		

Here again the action was fairly quick. Leaves of the same *Salvia* were (April 3, 1897) treated with ether with a similar effect. The hygroscope readings fell to zero in about 40 minutes, and the stomata were fairly open the following day.

Two experiments were made with *Nymphæa*, and one with *Sagittaria (montevidensis?)*, but the hygroscope readings did not fall more than sometimes occurs on leaves of these plants placed in dry air. In one case indeed the control leaf (in dry air) showed a lower reading than the etherised specimen.

Carbonic Anhydride.

The stomata are partially closed by CO₂, but the effect is not a rapid one.

The experimental plant was in a small flower pot, which could be introduced into a jar through which a current of CO₂, previously washed in water, flowed. The experimental and the control plant were kept in a greenhouse so as to be sufficiently illuminated, and to supply damp air for the control plant.

Time.	Experiment.	Control.
April 6, 1897, ^{A.M.} 10.15	30	30
	Placed in CO ₂ apparatus.	
^{NOON.} 12.0	20	32
^{P.M.} 2.15	15	30
5.30	10	28
5.0	10	25
April 7, 1897,* ^{A.M.} 10.0	0	20

The experiment was repeated on two other occasions with *Campanula pyramidalis* with a similar result, except that in one the hygroscope reached zero much more rapidly.

In the following experiment the conditions were the same as above described.

Tropæolum.

Time.	Experiment.	Control.
April 29, 1897, ^{P.M.} 3.45	12 30	13 32
April 30, 1897, ^{A.M.} 11.15	0 0	10 25

A few experiments were made with marsh and aquatic plants, but with inconclusive results.

§ 12. LIGHT (PRELIMINARY).

A great deal has been written on the opening and closure of the stomata produced by light and darkness, but much remains to be done. For instance, but little is known of the effect of heat as distinguished from light. Again, there is much contradictory evidence as to whether or no the stomata of the majority of plants close at night. There is similar divergence of opinion as to the mechanism of the nocturnal closure, and an almost complete absence of evidence as to the biological meaning of the phenomenon. Again, hardly anything is known as to the diurnal course of the stomatal movements or as to its inherent periodicity. On some of these points I hope to throw light.

The fact that stomata are widely open in bright sunshine is well known. The following notes illustrate the difference between bright and less bright diffused light.

* The CO₂ was allowed to run during the night.

JULY 30, 1897. Two *Cucurbita* plants were in the corridor with a bright top light (temperature 26°). One plant was shaded by being covered with thick netting which diminished the light without affecting the other conditions.

Experiment.		Control.	
Time.	Hygroscope.	Time.	Hygroscope.
P.M. 3.10	31	P.M. 3.13	29
3.15	<i>Shaded.</i>		
3.27	22	3.29	30
4.10	15	4.12	30

That they are often closed or nearly so on dark days even in summer is not so familiar,* although some of the older writers speak of the stomata shutting on damp days.

The following observations were made on a very dark wet day (August 20, 1897). The plants observed were necessarily in the glasshouses, since the wet leaves from outdoor plants cannot be used with the hygroscope. The temperature in which the observations were made varied between 18°·2 and 21°·6 at 12.20 P.M., so that effect could hardly be due to a low temperature. Nor, I think, could it be due to the moisture of the air, since most plants under bell-jars over water, if sufficiently illuminated, have widely open stomata. Nor can the apparent closure of the stomata be due to the effect of the saturation of the air on the hygroscope, since moistened filter paper gave as usual good readings with the instrument.

The hygroscope was at zero on both surfaces of the following: *Acacia Cyclops*, *Ruscus androgynus*, *Canna indica*, *Iris Robinsoniana*.

It was at zero on the lower surface of the following in the hothouse (temperature 21°·6): *Ficus elastica*, *Pandanus Veitchii*, *Zinziber officinale*, *Phyllanthus nivosus*, *Begonia sanguinea*, *Hoya globulosa*, *Pentadesma butyracea*, *Cola acuminata*.

In the temperate house (temperature 18°·3) the following gave 0 as a reading on the lower surface: *Myoporum latum*, *Amicia Zygozeris*, *Lochroma fuchsioides*, *Citrus Aurantium*, *Cissus oblonga*, *Pleroma macranthum*.

In the corridor (temperature 18°·2) the following were at zero: *Rosa gigantea*, *Melianthus major*, *Dolichos Lablab*, *Lonicera sempervirens (minor)*, *Cestrum elegans*, *Fuchsia sp.*, *Kennedyia nigricans*, *Passiflora cœrulea*, *Cobæa scandens*.

The only plants that gave readings above zero were the following (from all three houses):—

* LEITGEB (86), p. 149, remarks with surprise that UNGER (62) noted no differences on dark stormy days, though he examined his plants three times a day.

	Hygroscope.
<i>Plumbago capensis</i>	5
<i>Malva sp.</i>	6
<i>Buddleia madagascariensis</i>	4
<i>Peperomia magnoliaefolia</i>	5
<i>Smilax discolor</i>	6
<i>Cissus antarctica</i>	5
<i>Cassia laevigata</i>	4
<i>Cassine Maurocenia</i>	6

On similar dark days in winter the same sort of thing was often observed.

The effect of different degrees of illumination is well shown in the following observations on amphistomatous leaves, in which it is shown that the stomata on the surface facing the window are well open, while those on the surface facing the laboratory are much less open.

AUGUST 12, 1897.—*Acacia Cyclops* (Phyllodes).

The plant in a pot was placed in the window of the laboratory (Psychrometer 69 per cent.), the hygroscope reading on the two surfaces of the phyllode being equal (13). In the tables the *light* and *dark* sides mean those facing the window and the room.

P.M.	
2.7	Placed near window.
2.33	$\frac{7}{0}$ light side. dark side.
2.35	Plant reversed.
3.10	$\frac{9}{0}$ light. dark.
3.12	Plant reversed.
3.30	$\frac{5}{0}$ light. dark.

AUGUST 13, 1897.—*Moraea iridioides*.

The plant was in the temperate house, and was better illuminated on one side than the other.

The hygroscope gave $\frac{10}{5}$ light dark at 2.0 P.M.

Time.	Hygroscope.
P.M. 2.4	Plant reversed and placed in the Laboratory window.
2.40	$\frac{10}{2}$ light. dark.
2.42	Reversed plant.
3.14	$\frac{11}{0}$ light. dark.
3.16	Reversed plant.
3.46	$\frac{7}{0}$ light. dark.

Similar results were obtained with cut leaves of *Agapanthus*, *Narcissus*, and *Iris*.

A large number of experiments were made on dorsiventral hypostomatous leaves by fixing them horizontally in an inverse position, so that the stomatal surface was directly illuminated.* In most cases the inverted leaves showed no difference in hygroscope-reading from the normal leaves, and where a slight effect was visible, it was not constant in character.

With regard to the relation between the upper and lower stomata on dorsiventral amphistomatous leaves, I have also made many observations with uncertain results. In my preliminary paper† I spoke of the greater sensitiveness of the upper stomata to external conditions generally. I still think that this is a fair statement, but I prefer to put it more generally: the upper stomata will sometimes be found shut or half shut when the lower ones are open, whereas (in dicotyledons at least) the reverse condition does not occur. I am unable to define the conditions under which the upper stomata behave differently from the lower ones. I hope to follow out the question of different idiosyncrasies of the two sets of stomata, a point which has not received attention hitherto.

STAHL‡ has shown that sunshine closes the stomata in some cases, and this I can confirm.

* MEISSNER (94) found that dorsi-ventral leaves, fixed in the inverse position, assimilated clearly less than normal leaves. He ascribes the result in part to the closure of the stomata, which he says occurs partially or completely if the stomatal surface is exposed to sunshine.

† F. DARWIN (97).

‡ STAHL (97), p. 92.

The following observations were made on various plants in bright sunshine :—

July 7, 1897.—*Oxalis incarnata*,* in sunshine the leaves being in the paraheliotropic position, hygroscope 0. One hour after being put in the shade the hygroscope reading was 15.

July 30, 1897.—*Epimedium* in bright sun, 0.

July 31, 1897.— „ „ in dull weather, 18.

These, which are typical shade-loving plants, show closure of the stomata in bright sun. Marsh plants, on the other hand, are not shut in sunshine. The following readings were all taken in bright sun :—

<i>Sagittaria sagittifolia</i>	$\frac{30}{38}$	<i>Osmunda regalis</i>	$\frac{27}{38}$
<i>Alisma Plantago</i>	$\frac{25}{25}$	<i>Caltha palustris</i>	$\frac{20}{38}$
<i>Nasturtium officinale</i>	$\frac{42}{45}$		

Leguminosæ show paraheliotropic positions, and it might have been expected that these would show closed stomata in sunshine. This, however, is at least not always the case.

	Leaves in sun.	Leaves in shade.
<i>Gleditschia sinensis</i>	16	5
<i>Robinia Pseud-acacia</i>	27	15
<i>Amicia Zygomeris</i>	38	

§ 13. THE EFFECT OF DARKNESS.

The following series is given as illustrating the manner and rate at which the stomata close in darkness.

NOVEMBER 5, 1897.—*Pelargonium zonale*.

Time.	Hygroscope.	Time.	Hygroscope.
A.M.		A.M.	
10.0	45	10.38	20
10.20	In dark room.	10.47	15
	Psychrometer,	10.54	5
	80 per cent.	11.2	0
10.30	35		

* On February 16, 1898, *Oxalis Ortgiesi*, in the paraheliotropic position, had its stomata well open. This was the species in which STAHL found closure. No doubt here, as elsewhere, the stomata are uncertain in behaviour.

MARCH 16, 1897.—*Narcissus*.

Time.	Hygroscope.	Time.	Hygroscope.
A.M.		A.M.	
11.6	30 27	11.26	$\frac{10}{8}$
Put at once in dark.		11.33	$\frac{3}{2}$
11.13	27 23	11.38	$\frac{0}{0}$
11.18	15 14		

These examples show clearly enough the gradual character of the "closure." A similar but reversed series of readings is obtained when a plant is brought from darkness to light.

It must once more be insisted on that, although the horn hygroscope gives zero in the dark, the leaves still transpire. *Melianthus*, *Eupatorium*, *Chrysanthemum* were placed in the dark room for about one hour when the horn hygroscope gave 0. The *Yucca* hygroscope was placed on the leaves and showed clearly that transpiration was continuing, though slowly.

The above examples give a fair idea of the period required to bring the horn hygroscope to zero. Further details will be given under the heading *Periodicity*, where it will be shown that the times required for closure and opening of the stomata depend on the time of day at which the experiment is made.

§ 14. DISTRIBUTION OF NOCTURNAL CLOSURE IN THE VEGETABLE KINGDOM.

This part of the subject has been studied both by artificially darkening plants in the day and by investigating leaves at night. The natural closure of plants growing out of doors cannot be studied with the hygroscope, since the smallest amount of moisture, *e.g.*, dew, on the leaves would simulate open stomata.

In the following discussion I have placed together the results of plants observed at night in greenhouses, and of plants artificially darkened in the day.

It is true that certain plants may be found in which artificial darkness does not usually close the stomata, though they are closed at night. In such cases (*e.g.*, *Sparmannia africana*, *Humea elegans*, and some British Orchids) I reckon the plants with those in which closure of the stomata occurs at night. I do not know cases of the opposite phenomena, *i.e.*, where artificial darkness produces closure in a plant whose stomata are not nocturnally closed.

The only clear cases of open stomata among ordinary terrestrial plants were the following* :—

* It must not be imagined that these plants show no reaction of any kind to light. A *Cyclamen* in
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Cyclamen persicum.
Eranthis hyemalis.
Erythronium Dens-canis.
Helleborus viridis.
Ranunculus Ficaria.

Richardia africana.
Tradescantia sp.
Turnera elegans.
Urceolina aurea.
Viola (odorata?).

Altogether 75 species were examined, so that we may estimate that about 13 per cent. of ordinary terrestrial plants do not close their stomata at night.* As above explained, *Humea elegans* and *Sparmannia africana* are counted as closing their stomata, though in artificial darkness they remain open. The same is true of three species of British Orchids examined, namely *O. maculata*, *Morio*, and *latifolia*.

Nyctitropic Plants.

These are placed in a different class, because STAHL, in his interesting paper,† found that the stomata of sleeping plants are very commonly open at night.

The difficulty of the question is shown by the following facts:—In writing out the subject in the winter of 1897–98, I concluded that there was practically no difference between nyctitropic and other terrestrial plants in the matter of nocturnal closure.‡ The balance was, however, rather in favour of the non-sleeping plants, among which I found about 12 per cent. *clearly* open at night, among the sleepers only one species out of eighteen observed was *clearly* open, but about 20 per cent. were just perceptibly open. In the spring of the present year I determined to make more observations, and these showed my former calculations to be incorrect. When observations are made on plants at a fairly high temperature, the difference between sleepers and non-sleepers comes out clearly.

bright light (not sunshine) gave with the hygroscope 25 to 30, while a control plant in duller light was at 12 to 10. Similar differences occur in *R. Ficaria*, and doubtless in other species.

* This percentage is probably too high, since I was guided to some of the above plants by the wish to test LEITGEB's facts.

† STAHL (97).

‡ The hygroscope was at 0 in the following:—

<i>Albizzia Saman.</i>	<i>Coronilla sp.</i>	<i>Psoralea bitumenosa.</i>
<i>Amicia Zygomeris.</i>	<i>Dolichos Lablab.</i>	<i>Phaseolus multiflorus.</i>
<i>Arachis hypogæa.</i>	<i>Homalanthus populifolius.</i>	<i>Oxalis lasiandra.</i>
<i>Cassia corymbosa.</i>	<i>Kennedya Marryattæ.</i>	<i>Trifolium sp.</i>
„ <i>floribunda.</i>	<i>Maranta sp.</i>	

The following were just perceptibly open:—

<i>Dolichos Lablab.</i>	<i>Oxalis incarnata.</i>	<i>Phyllanthus glaucescens.</i>
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Cassia lævigata was the only species which was clearly open, and with this species the reading was zero on another occasion.

On March 14, 1898, observations were made at night† on plants from the "Stove," where the temperature was between 19° and 20° C. Not one nyctitropic plant was among those with shut stomata, the list being—

<i>Acalypha</i> sp.	<i>Drimiopsis Kirkii</i> .
<i>Begonia</i> sp.	<i>Dracæna Cooperi</i> .
<i>Castanospermum australe</i> .	„ <i>Sanderiana</i> .
<i>Centradenia grandiflora</i> .	<i>Schismatoglottis neoguineensis</i> .

Of the plants found open, on the other hand, 70 per cent. were nyctitropic, as the following list‡ shows, in which the sleepers are marked with an asterisk—

* <i>Amicia Zygozeris</i> .	* <i>Oxalis scandens</i> .
<i>Arisæma fimbriata</i> .	* <i>Psoralea sericea</i> .
* <i>Averrhoa Carambola</i> .	* <i>Phyllanthus nivosus</i> .
* <i>Albizzia Saman</i> .	<i>Tradescantia (fluminensis?)</i> .
* <i>Cassia lævigata</i> .	<i>Turnera elegans</i> .

It is not justifiable to draw conclusions from so small a number of observations, but the result is sufficiently striking to make it probable that it will be confirmed.

I am supported in the belief that a high temperature is of importance for nocturnal closure in the case of sleeping plants, by observing on March 9 that *Amicia Zygozeris*, *Cassia lævigata*, and *Psoralea sericea* were shut at night in the corridor at a temperature of 13° C., whereas these plants when moved into the Stove (19° to 20° C.) were open, as recorded in the above list.

Marsh and Aquatic Plants.

These form the only biological division in which I have uniformly found a large proportion of plants which do not close their stomata at night. The fact is not new,§ nevertheless it seems worth while to give the observations.

The stomata were widely open in the following, 10 P.M., February 17, 1898 :—

<i>Eichhornia speciosa</i> .	<i>Nymphæa</i> sp.
„ <i>azurea</i> .	<i>Pontederia cordata</i> .
<i>Heteranthera reniformis</i> .	<i>Sagittaria montevidensis</i> .
<i>Hydrocleys Commersoni</i> .	<i>Thalia dealbata</i> .
<i>Nelumbium speciosum</i> .	<i>Villarsia parnassifolia</i> .

† From 9 P.M. onwards.

‡ *Arisæma* and *Albizzia* are not counted with the species mentioned on p. 578 as having stomata clearly open at night, as they hardly came up to the standard.

§ SCHWENDENER (81), p. 853; LEITGEB (86), p. 171.

They were closed (*i.e.*, the hygroscope was at zero) on—

Callitriche verna.*

Caltha palustris.

Colocasia metallica.

Saccharum officinarum.

Trianea bogotensis.†

Succulents.—I have not been able to ascertain whether or not succulents as a class show any closure of their stomata at night, because even in bright sunshine the transpiration is so small that they very rarely give any reading with the hygroscope nor, indeed, with the more sensitive *Yucca* hygroscope.‡

Summary of Observations on Nocturnal Closure.

The history of this question is briefly as follows:—Up to comparatively recent date it was generally assumed that, roughly speaking, all plants had closed stomata at night. In 1886 LEITGEB pointed to a number of exceptions, and concluded that almost as many plants have open as shut stomata at night. This view has been supported by STAHL to the extent that he finds many plants with open stomata at night. Lastly, it has been opposed by SCHELLENBERGER, who concludes that a vast majority of plants shut their stomata at night. My own conclusion is that in all plants, except aquatic, marsh, and nyctitropic plants, there is a great preponderance of nocturnal closure—such closure being not necessarily complete.

The microscopic method employed by LEITGEB has disadvantages which have been already referred to. Thus, if the stomata are examined in surface section, the method of preparation is an obvious source of error. While, if the leaf as a whole is examined, I believe that it is not easy to distinguish partial from complete closure. These considerations suggest that the divergence between the conclusions arrived at by LEITGEB and SCHELLENBERGER depends in part on the difficulties of the microscopic method. On the other hand, certain facts tend to show that LEITGEB'S observations are correct. Thus among the common plants which LEITGEB found to have open stomata at night are *Cyclamen persicum*, *Viola odorata*, *Eranthis hyemalis*, *Erythronium*, *R. Ficaria*, all of which occur among those which I have found clearly open at night. This being so I have little right to throw doubt on LEITGEB'S results, or on the microscopic method generally.

If we turn to observations made by the hygroscopic method we again meet with contradictions, for STAHL is in general agreement with LEITGEB, while my observations are opposed to that author.

* *Callitriche* was observed with the microscope.

† KOHL found that *Trianea* shut its stomata in artificial darkness; this, however, is not always the case according to my observations.

‡ I found, however, *Æonium tabuliforme* and *Crassula sp.* with open stomata during the day.

LEITGEB has shown conclusively that the behaviour of the stomata is variable, depending on the conditions in which the plant has been kept. Thus the occurrence of nocturnal closure in *Lilium*, *Impatiens*, and *Saponaria* seemed in LEITGEB'S results* to depend on the hygroscopic condition of the air.

The facts that I have given on nyctitropic plants bear out this view, and it is one which renders the whole problem so difficult of solution that only general conclusions can be drawn.

My own view is as follows:—

- (1) That the nocturnal closure of the stomata is not so complete as to prevent transpiration, but that it does greatly diminish it.
- (2) That this degree of closure is extremely common in unspecialised terrestrial plants.
- (3) That nyctitropic plants have stomata more open than other plants.
- (4) That the majority of aquatic plants have open stomata at night.

§ 15. BIOLOGY OF NOCTURNAL CLOSURE.

The two functions of the stomata are (i) the regulation of gaseous interchange and (ii) the regulation of the loss of water by transpiration. It is, therefore, reasonable to seek the biological meaning of nocturnal closure under these headings.†

We have seen that the closure of the stomata is associated with dry air or with diminished water supply, and it is not, therefore, clear why the stomata should be closed at night when the air is damp and cool, and there is absolutely no risk of excessive transpiration.

BLACKMAN‡ has pointed out that the passage of CO₂ into the leaf is a more difficult process than the entrance of oxygen, because the difference of tension on which it depends is so small. It follows from this that the absorption of CO₂, which is the first stage in the process of assimilation is physically at a disadvantage in comparison with respiration. It may well be that assimilation requires a wider stomatal aperture than is necessary for respiration. And since economy in the use of water is in most cases desirable, the partial closure at night would effect this economy at the most advantageous time. The fact that aquatic plants with a constant water supply and an extremely moist atmosphere have open stomata at night may be adduced to support this view. The theory has much in its favour, but it does not exhaust the subject.

* LEITGEB (86), p. 168.

† LEITGEB (86), p. 183, is of opinion that no special biological meaning can be given to the nocturnal closure. He considers the stomata chiefly important only as regulators of transpiration, and thus looks on their closure as superfluous in the moist air of night, or even hurtful as checking respiration—for he clearly recognizes the aerating function of the stomata.

‡ BLACKMAN (95).

In the *Power of Movement in Plants* it was suggested that nyctitropic movements are advantageous by protecting the leaves from the chilling effect of radiation. Some experimental proof of the injury so caused to leaves was adduced, at the same time it was allowed that the experimental results (which were obtained on clear cold nights in autumn) were but partially applicable to tropical plants. But, as my father wrote* “in every country, and at all seasons, leaves must be exposed to nocturnal chills through radiation, which might be in some degree injurious to them and which they would escape by assuming a vertical position.” With our present knowledge, this sentence might have been more strongly expressed, for we know now for certain that a temperature which is not low enough to kill or actually injure leaves may be detrimental by preventing the translocation of starch. This has been clearly shown by SACHS to be the case, and I have myself seen instances of the kind.

SACHS says† that on August 8, after a cool night, when the temperature at sunrise was 9° C., the leaves of *Phaseolus*, *Ampelopsis*, and *Aristolochia* were not completely cleared of starch. On the morning of August 3 (temperature at 5 A.M., 8° C.), the leaves of *Dioscorea Batatas*, *Catalpa*, and *Morus* were quite full of starch. In August (morning temperature, 9° C.) *Helianthus*, *Solanum*, *Datura*, *Atropa* were completely cleared of starch by sunrise. In October (morning temperature, 6° C.), there was no translocation in *Nicotiana* and very imperfect destarching in *Datura* and *Atropa*. Thus the fall of temperature from 9° to 6° prevented destarching in the last-named plants. It must be remembered that leaves which are full of starch do not assimilate, so that many hours of assimilation would be lost at the lower temperature (6°). I cannot doubt that nyctitropic plants must be at an advantage in this respect, and that it is an advantage in which tropical plants may well share; especially if, as seems probable, the check to translocation depends on a relatively, not an absolutely, low temperature.

It must be allowed that if nyctitropism is an advantage by preventing chilling by radiation, then other arrangements for preventing the lowering of the plant's temperature at night must also be advantageous. And this I believe to be one advantage of the closure of the stomata at night. A leaf which transpires actively is, like a wet-bulb thermometer, considerably cooler than the surrounding air. Therefore, by checking nocturnal transpiration, loss of heat is prevented. It is easy to demonstrate this by direct experiment. I have used the leaves of *Tropæolum majus*: a leaf, still attached to the plant, is wrapped round the bulb of a thermometer like a cigarette paper, and kept in place by a ligature. Another thermometer is wrapped in a *Tropæolum* leaf severed from the plant, and in which the stomata are shut ‡

* DARWIN (80), p. 286.

† SACHS (84), p. 8.

‡ *Tropæolum* is good for the purpose because the closure of the stomata is fairly complete in a cut leaf; the experiment requires a leaf of which the stomata close at night, and this I find to be the case

The following tables illustrate what has been said; I hope to follow out the subject with more accuracy. Two thermometers graduated in $\frac{1}{5}^{\circ}$ C., and having small bulbs, were wrapped, one in a withered *Tropæolum majus* leaf, the other in a leaf attached to the plant. The wet and dry bulb thermometers graduated in $\frac{1}{10}^{\circ}$ C. were placed in the greenhouse close to the pot of *Tropæolum*. The sun was shining intermittently on the plants, &c.

APRIL 19, 1898.

Time.	Withered leaf.	Attached leaf.	Dry bulb.	Wet bulb.
P.M.				
12.28	18.8	15.8	18.2	13.2
12.31	19.2	16.6	18.3	13.3
12.52	20.6	18.0	19.1	14.6
4.45	16.0	14.8	16.1	11.2
9.4	9.4	9.4	9.5	7.4
The door was now opened to produce a draught.				
9.21	8.0	7.9	8.4	6.2
9.43	7.4	7.4	7.8	5.8

These tables show a considerable difference of temperature between the withered attached leaf during the day, which disappears at night, even when the wet and dry bulbs show a considerable difference. There is, however, I think, usually some difference between the two *Tropæolum* leaves at night. Thus, at 9.40 P.M., April 18, the door of the greenhouse being open:—

Time.	Withered leaf.	Attached leaf.	Dry bulb.	Wet bulb.	<i>Pontederia.</i>
P.M.					
9.40	8.0	7.8	8.0	6.3	6.8

The above table also gives the reading of a thermometer wrapped in a piece of a *Pontederia* leaf, whose stomata remain open at night and do not close readily on withering. It will be seen that it was lower than the dry bulb thermometer and lower than the *Tropæolum* leaves, whose stomata were closed; a similar result was obtained on the following evening.

The greatest difference between the withered and attached leaves is seen in sunshine; thus, in one of the preliminary trials, I got with *Tropæolum*—

with *Tropæolum* except at high temperatures. STAHL, on the other hand, found the stomata open at night.

MARCH 2, 1898.

Time.	Withered.	Attached.
A.M. 10.15	22.3	18.4

On wet dark days the difference is small :—

MARCH 1, 1898.

Time.	Withered.	Attached.
P.M. 12.38	13.9	13.2

The following figures illustrate the relative rise in the attached leaf as the stomata shut—

APRIL 15, 1898.

Time.	Withered.	Attached.	Dry.	Wet.
P.M. 3.40	17.4	16.4	17.3	11.6
4.27	15.8	15.3	15.8	10.7
4.45	15.8	15.2	15.8	10.8
5.8	15.6	15.2	15.6	11.0
5.51	15.4	15.1	15.4	10.8
6.21	14.6	14.3	14.6	10.6

It is not easy to find evidence bearing on my theory which I desire to put forward in a tentative spirit.

The fact that aquatic plants do not, as a rule, close their stomata at night, is in favour of the hypothesis, for they live in a more or less saturated atmosphere, where transpiration, with its accompanying loss of heat, is always small, and where, therefore, the stomata may safely remain open. This applies especially to floating leaves, but must to some extent be applicable to aquatics with aerial leaves. Another consideration is only applicable to floating leaves, namely, that since their temperature must be decided by that of the water, the closure of the stomata would be insignificant in effect. In order to test the point practically, I placed leaves of Ivy (*Hedera*), Laurel (*P. Laurocerasus*), Turnip (*B. Rapa*), &c., floating on a small pond, while corresponding leaves were placed close to the edge of the pond on a wicker table 60 centims.

in height. After a clear still night the leaves examined at 5.45 A.M. (April 17), when the temperature was 2° C. and a good deal of hoar frost was on the ground. All the leaves on the table were dripping with dew, while those on the pond were perfectly dry on their exposed surfaces. It was clear that the floating leaves had been kept above the dew point by the water, which was 8° C.

Thus the absence of nocturnal closure in certain floating leaves is to some extent favourable to the theory. There remains to be explained the cases in which this class of leaf does close its stomata at night, and also the aerial aquatic type, which last forms a difficulty, since it is hard to suppose that it is to be placed in a totally different biological class to the floating aquatics. Further research is necessary on the whole subject. One thing is certain: whether or no the saving of heat is the chief advantage of nocturnal closure, such a saving is one result of that act.*

It will naturally be asked what is the relation of my theory to STAHL'S interesting contribution to the nocturnal physiology of plants.† It is a question which is not easy to answer. His view is, so far, the same as that given in the *Power of Movement*, namely, that nyctitropism is an adaptation to avoid the chilling effect of radiation. He holds, however, that the lowered temperature is not injurious, *per se*, but as leading to depression of the transpiration stream, and, therefore, to diminished supply of mineral food. He points out,‡ that since many nyctitropic leaves closed their stomata at night, the supposed increase of transpiration cannot take place during the night itself, but rather in the early morning. His view is that nyctitropism is especially an adaptation against dew. It was shown in the *Power of Movement* that leaves fixed horizontally are found in the morning covered with dew,§ while similar leaves, which had been allowed to sleep, were comparatively dry. The fact was only used as an argument to prove the chilling of the horizontally fixed leaves. STAHL, however, finds in it the actual danger which the sleeping plant avoids. He supposes that in the early morning the dew-covered leaves transpire but slightly, while those which have been kept relatively dry by nyctitropism, transpire normally. He gives experimental evidence for this view, and adds that dew may remain on leaves for several hours, so that a considerable fraction of the available daylight may be lost.

If STAHL'S view is right, then the nocturnal closure of the stomata may be classed with nyctitropism as an adaptation for avoiding a nocturnal lowering of the temperature. If, further, we accept STAHL'S theory in its entirety, we may believe that the nocturnal closure of stomata is useful by helping to prevent the deposition of

* The closure of the stomata of evergreens in the winter (as observed by STAHL) suggests itself as a kindred fact, but it seems more probable that this is an adaptation for water economy.

† STAHL (97).

‡ STAHL in speaking, p. 73, of nyctitropic leaves whose stomata are open at night, remarks that they are nevertheless less open than by day.

§ DARWIN (80), p. 294.

dew. But we must avoid the paradox that the stomata close at night to increase transpiration.*

Finally, I confess that I think a certain caution must be exercised in regard to STAHL's most interesting theory. It seems to me rash to assume that the wetting of a leaf is simply and entirely injurious in relation to transpiration.

Water may be injurious in other ways; for instance, by washing mineral food out of the leaf. I have unpublished notes which show that this may be considerable with leaves still attached to the plant, and cut crops are known to waste their salts in long continued rain.

There is, too, the danger long ago pointed out by SACHS† that the stomata may be blocked by water, and thus the gaseous interchange hindered. It was from this point of view that I investigated the relation of stomata to bloom,‡ and showed that stomata tend to occur where they have the best chance of avoiding wetting. Without specially directed research I would not press this theory, but it is worthy of note that HABERLANDT§ seems to have no doubt as to the reality of the necessity of guarding against blocking of stomata, and he seeks to show that the form of stoma in floating leaves is an adaptation to this end. On the other hand STAHL|| gives some reason to believe that wetting a leaf does not block the stomata sufficiently to interfere with gaseous interchange.

These considerations are chiefly brought forward to show that, granting STAHL's contention that nyctitropism is an adaptation to avoid wetting by dew, and granting further that wetting a leaf will interfere with its activity as a transpiring organ, it does not follow that wetting produces no other hurtful effects.

To summarise the above discussion :—

I. The simplest theory of nocturnal closure is that the gaseous interchange by day requires wide open stomata, which implies a high degree of transpiration. The gaseous interchange at night does not require the stomata to be so widely open; it is, therefore, advantageous that they should be partly closed at night to economise water. Aquatic plants living in a damp atmosphere and abundant water supply do not need this economy, and have in many cases open stomata at night. According to STAHL's views aquatic plants would, from living in a damp atmosphere, suffer

* It is characteristic of the nyctitropic position that the leaves are folded close against one another, so that the whole amount of surface exposed is much less at night. This arrangement must not only hinder loss by radiation, but must also check transpiration, as STAHL (97), shows is the case; so that the sleeping position, in addition to lessening the effects of radiation, must tend to prevent the chilling effect of evaporation, just as I believe to be the case with the nocturnal closure of the stomata.

† SACHS (68), p. 178.

‡ F. DARWIN (86).

§ HABERLANDT (87), p. 97.

|| STAHL (97), p. 82.

from depression of the transpiration stream, and this would be an additional reason for not closing at night.

II. Transpiration has a cooling effect on leaves; it is therefore probable that nocturnal closure is advantageous by keeping up the temperature at night, just as the same effect is produced by nyctitropism, which prevents cooling by radiation.

Cooling which does not kill leaves is hurtful by preventing nocturnal translocation; therefore, anything which prevents even a slight lowering of temperature at night must be advantageous.

III. STAHL has shown that nocturnal cooling is hurtful by leading to deposition of dew, and therefore to depression of transpiration in the early morning. It is at present impossible to say whether the saving of heat due to nocturnal closure of stomata has any appreciable effect in preventing dew formation, but it must clearly tend in that direction.

§ 16. THE DAILY PERIOD.

The following tables give the diurnal variation in the readings of the hygroscope, and give therefore a rough idea of the changes that occur in the stomata throughout the day.

The first series of observations were made on a plant of *Narcissus* grown in a pot, and kept in the cool corridor of the plant houses, where it had a good top light and where the sunlight could fall on the leaves. The readings were taken on a marked part of a leaf, and this is a necessary precaution, since in Monocotyledons the hygroscope readings vary considerably at different distances from the base :

MARCH 12, 1897. Sunrise, 6.23 A.M.; Sunset, 5.59 P.M.

Time.	Hygroscope.	Remarks.	Temperature.
A.M.			°
5.33	$\frac{0}{0}$ upper. $\frac{0}{0}$ lower.	Dark.	11
5.48	$\frac{0}{0}$	Faint light.	
5.58	$\frac{0}{0}$	Much lighter.	
6.10	$\frac{0}{0}$	Light.	11.5
6.20	$\frac{0}{5}$	Sunrise, 6.23.	
6.43	$\frac{3}{5}$		
7.0	$\frac{7}{12}$	Sun.	13
7.18	$\frac{12}{19}$		
7.40	$\frac{18}{22}$		
8.15	$\frac{18}{25}$	Sun.	14
9.5	$\frac{22}{28}$..	17
9.55	$\frac{25}{28}$		
10.55	$\frac{33}{37}$	Clouds.	16.2
P.M.			
12.10	$\frac{35}{35}$	Sun, but plant not in direct sun.	
1.0	$\frac{35}{35}$		
2.0	$\frac{35}{38}$..	20
3.0	$\frac{33}{35}$	Sun.	
3.51	$\frac{31}{35}$		

The closure in the evening was observed on the following day.

MARCH 13. Sunset, 5.59 P.M.—*Narcissus*.

Time.	Hygroscope.	Remarks.	Temperature.
P.M.			°
3.5	$\frac{36}{30}$	Clouds.	17
4.2	$\frac{25}{8}$..	17
4.17	$\frac{22}{4}$		
4.32	$\frac{20}{0}$..	16.5
4.55	$\frac{10}{0}$		
5.10	$\frac{4}{0}$		
5.21	$\frac{0}{0}$..	15.5

In this instance the upper stomata open later and shut earlier than those on the opposite surface of the leaf. But this is not always the case. With the same plant (April 1, 1897) the upper stomata opened first and closed simultaneously with the lower ones. It is probable that the differences depend on the illumination rather than on any inherent distinction between the stomata.

MARCH 12, 1897.—*Cineraria*. Fig. 4, p. 595.

The following observations were made on the lower stomata of two *Cineraria* leaves under the condition described for *Narcissus* :—

	Time.					
	5.30 A.M.	5.45 A.M.	5.55 A.M.	6.5 A.M.	6.15 A.M.	6.25 A.M.
Leaf w.	0	0	0	2	5	7
„ b.	0	0	0	0	5	5
Remarks	dark.	faint light.	much lighter.	lighter.	quite light.	bright.
Temperature. . .	11°	11°.5	..
	Time.					
	6.40 A.M.	6.55 A.M.	7.15 A.M.	7.35 A.M.	8.12 A.M.	9.0 A.M.
Leaf w.	8	10	10	10	12	17
„ b.	7	8	10	10	10	14
Remarks	sun.	sun.
Temperature.	13°	14°	17°
	Time.					
	9.50 A.M.	12.13 P.M.	1.5 P.M.	2.5 P.M.	3.5 P.M.	3.55 P.M.
Leaf w.	18	22	25	28	30	29
„ b.	16	30	29	30	30	30
Remarks	plant not in sun.	..	sun.	..
Temperature.	20°

Graphic representations of the above observations (leaf w.) and of some others are given at p. 595. The curve giving the variation in the hygroscope is marked *h*, the temperature curve is marked *t*. The notes from which fig. 6 was constructed are not given.

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As with *Narcissus* the closure was observed on the following day :

	Time.						
	3.0 P.M.	4.0 P.M.	5.0 P.M.	5.15 P.M.	5.30 P.M.	5.50 P.M.	6.9 P.M.
Leaf w.	32	25	14	10	10	5	0
„ b.	29	25	10	9	7	3	0
Remarks	no sun.	duller.					
Temperature	17°	17°	16°·5	16°	15°·5	15°	14°·5

The next two sets of observations are given so as to show the same species *Cineraria* at two other later dates :

APRIL 29, 1897. Sunrise, 4.38 A.M. ; Sunset, 7.24 P.M.—*Cineraria*.

	Time.						
	3.3 A.M.	3.17 A.M.	3.32 A.M.	3.42 A.M.	4.2 A.M.	4.23 A.M.	4.35 A.M.
Hygroscope .	0	0	2	4	5	10	15
Remarks . .	dark.	faint light.	lighter.	much lighter.	..	bright.	quite bright.
Temperature.	12°	..	12°	..	12°	12°	

	Time.							
	5.10 A.M.	6.10 A.M.	7.10 A.M.	9.15 A.M.	2.10 P.M.	2.49 P.M.	3.45 P.M.	4.32 P.M.
Hygroscope .	17	20	20	22	30	30	29	25
Remarks							
Temperature.	..	12°·5	12°·5	15°	17°	..	18°	18°

	Time.							
	5.0 P.M.	6.7 P.M.	7.3 P.M.	7.33 P.M.	7.46 P.M.	7.59 P.M.	8.8 P.M.	8.19 P.M.
Hygroscope .	22	20	15	13	10	6	3	0
Remarks . .								
Temperature.	18°	17°	16°	16°				

MAY 25, 1897. Sunrise, 3.57 A.M. ; Sunset, 7.57 P.M.—*Cineraria*.

Not the same plant as in Experiment, April 29.

	Time.						
	3.0 A.M.	3.15 A.M.	3.30 A.M.	3.45 A.M.	4.0 A.M.	4.30 A.M.	5.0 A.M.
Hygroscope .	0	0	2	4	7	17	23
Remarks . .	faint light.	lighter.	much lighter.	light.	bright.	..	bright.
Temperature .	12°	12°	12°	12°·5	13°	..	14°
	Time.						
	6.0 A.M.	7.0 A.M.	9.15 A.M.	10.15 A.M.	2.0 P.M.	3.0 P.M.	3.55 P.M.
Hygroscope .	24	26	25	24	27	28	25
Remarks	sun.	sun.	sun.	dull.	dull.	very dull, rain.
Temperature .	14°	15°	15°	17°	20°	19°	17°·5
	Time.						
	4.50 P.M.	6.0 P.M.	7.0 P.M.	8.0 P.M.	8.30 P.M.	9.0 P.M.	
Hygroscope .	25	25	25	17	10	0	
Remarks . .	bright.	bright.	dull.	very dull.	nearly dark.	dark.	
Temperature .	17°	17°	15°	14°	14°	13°·5	

In the next series the opening and closing of the stomata are given, but the middle of the day is omitted :

APRIL 29, 1897. Sunrise, 4.38 A.M.—*Pelargonium zonale*.

Time.	Hygroscope.	Temperature.
A.M.		o
3.0 dark.	0	12
3.19 faint light.	0	12
4.5	2	12
4.20 bright.	5	12

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APRIL 29, 1897. Sunrise, 4.38 A.M.—*Ornithogalum arabicum*.

Time.	Hygroscope.	Temperature.	Time.	Hygroscope.	Temperature.
A.M. 3.0 dark.	$\frac{0}{0}$	° 12	A.M. 3.40 much lighter.	$\frac{3}{1}$	°
3.15 faint.	$\frac{0}{0}$		4.0	$\frac{6}{3}$	12
3.20	$\frac{1}{0}$		4.15 bright.	$\frac{10}{7}$	
3.30	$\frac{2}{0}$				

MAY 3, 1897. Sunset, 7.24 P.M.—*Pelargonium*.

Time.	Hygroscope.	Temperature.	Time.	Hygroscope.	Temperature.
P.M. 4.57	25	18	P.M. 7.47	10	
6.6	22	17	7.59	8	
7.5	20	16	8.10	4	
7.35	15	16	8.21	0	15

MAY 3, 1897. Sunset, 7.24 P.M.—*Ornithogalum arabicum*.

Time.	Hygroscope.	Temperature.	Time.	Hygroscope.	Temperature.
P.M. 5.1	$\frac{18}{18}$	° 18	P.M. 7.45	$\frac{10}{7}$	°
6.5	$\frac{17}{15}$	17	8.0	$\frac{7}{4}$	
7.0	$\frac{15}{13}$	16	8.15	$\frac{0}{0}$	15
7.30	$\frac{12}{10}$	16			

MAY 25, 1897. Sunrise, 3.57 A.M.; Sunset, 7.57 P.M.—*Tropæolum*.

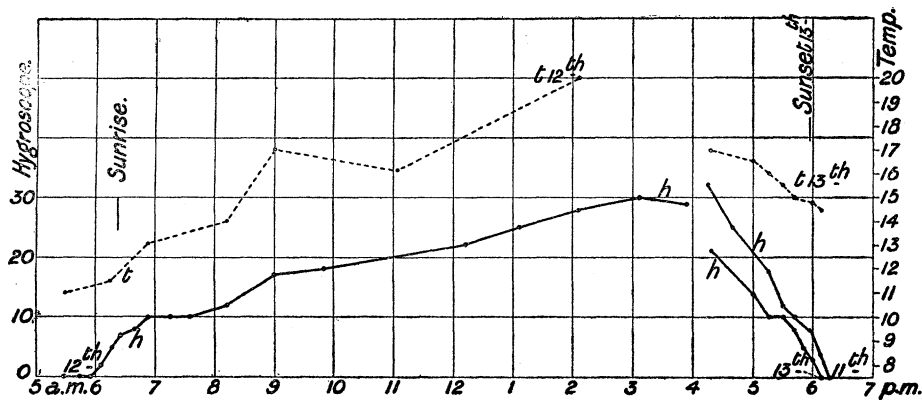
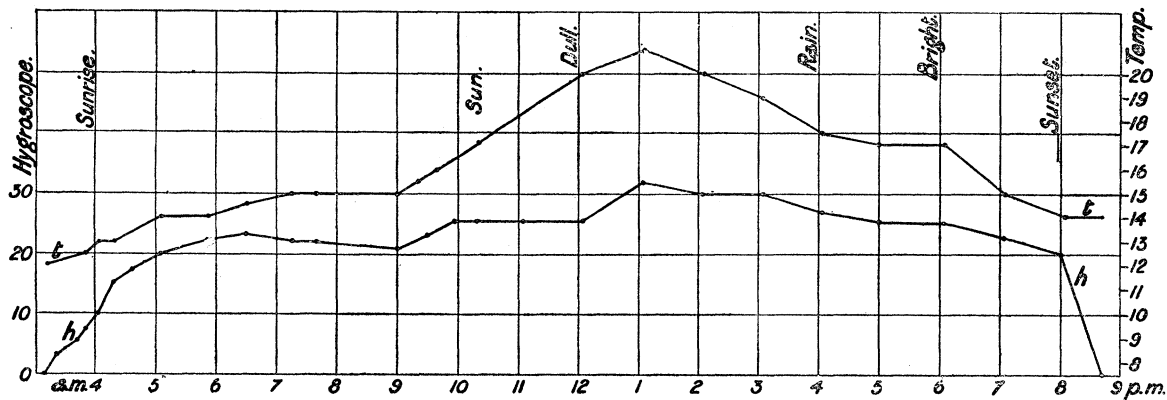
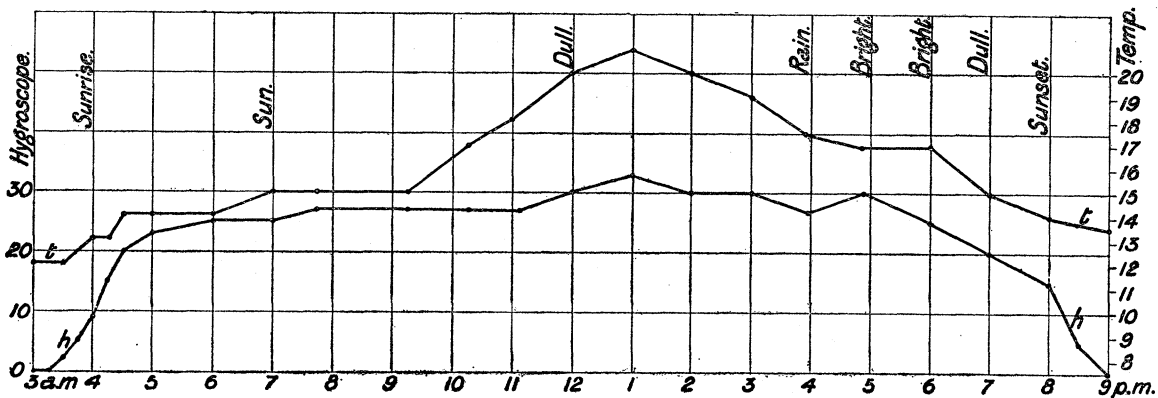
Time.	Hygroscope.	Temperature (C).	Time.	Hygroscope.	Temperature (C).
A.M.		°	P.M.		°
3.10 faint	$\frac{0}{0}$	12	1.5 dull	$\frac{13}{32}$	21
3.18	$\frac{0}{3}$		4.5 rain	$\frac{12}{27}$	17.5
3.40 light	$\frac{0}{5}$		5.0 bright	$\frac{12}{25}$	17
3.50	$\frac{2}{7}$	12.5	6.5 bright	$\frac{10}{25}$	17
4.5	$\frac{5}{10}$	13	7.5 dull	$\frac{8}{23}$	15
5.5	$\frac{10}{20}$	14	8.5 very dull	$\frac{7}{20}$	14
6.30	$\frac{12}{23}$	14.5	8.45 dark	$\frac{0}{0}$	14

[Given full in fig. 5.]

I have not enough data to form trustworthy generalisation, but it seems worth while to summarise the features of the eight cases of which I have good notes, viz., *Narcissus*, March 12; *Cineraria*, March 12, April 29, May 25; *Pelargonium*, April 29, May 25; *Ornithogalum arabicum*, April 29; *Tropæolum*, May 25. The stomata never open before it is light; the first effect perceptible with the horn hygroscope at from 8 to 35 minutes after the first faint light appears, or, which is the same thing, at from 3 to 68 minutes before sunrise.

The maximum opening of the stomata is not reached till the day is well advanced, i.e., at times between 11 A.M. and 3 P.M. But the stomata reach a fair aperture, i.e., two-thirds of the maximum reading at from half-an-hour to two and a-half hours after sunrise. The time of closure is usually from three-quarters of an hour to one hour after sunset, but *Narcissus* was shut on March 13 more than half-an-hour before sunset, and on the same day *Cineraria* was shut ten minutes after sunset.

Figs. 4, 5, and 6 give graphic representations of the hygroscope readings (line *h*) of *Cineraria*, *Tropæolum majus*, and *Pelargonium zonale*. It will be seen that in all three the rise in the morning and the fall at night are fairly steep. In figs. 5 and 6 the curve is more nearly horizontal during the day than in fig. 4, in which the hygroscope readings rise gradually from 7 A.M. to 3 P.M. In all three curves the maximum reading of the hygroscope corresponds roughly with the maximum temperature. In fig. 6 the hygroscope curve rises at 5 P.M., when the sky brightened after the rain, although the temperature was slowly falling.

Fig. 4.—*Cineraria*. March 12, 1897. (See p. 590.)Fig. 5.—*Tropaeolum majus*. May 25, 1897. (See p. 594.)Fig. 6.—*Pelargonium*. May 25, 1897. (See p. 590.)

§ 17. PERIODICITY.

The question whether there is an inherent periodicity in the diurnal changes of the stomata is one that has not received attention as far as I know.*

First Method.—The most successful results were obtained by the method employed

* The periodicity of transpiration has been often discussed: see EBERDT (89), p. 91, where, too, EBERDT's own conclusions are given. He believes himself to have shown the existence of periodicity.

by PFEFFER* in his studies on the periodicity in the "sleep" of flowers. He showed that a given change of temperature does not produce the same effect in the morning that it does towards night. Thus in the case of the Daisy (*Bellis*) a rise in temperature in the morning makes the ray florets open, while in the evening no such opening can be produced.

The converse experiment may also be made with falling temperatures.

In the case of stomata I have compared the period of darkness necessary to close them in the morning and evening hours, and conversely the length of illumination required to re-open them at these times. The result is most easily seen late in the afternoon, when darkness rapidly closes the stomata, but illumination has small effect, or even none whatever; whereas in the morning the shutting is slow and the re-opening comparatively rapid. These results are only explicable by the assumption that there is in the phenomena an inherent periodicity such as exists in nyctitropism.

In the case of nyctitropism the periodicity is so strong that the leaves continue to execute rhythmic movements in continuous darkness. Thus, for instance, a nyctitropic plant placed in a dark room at night will be found to have its leaves spread out in the day-position next morning. This does not seem to be the rule with stomata,† but I should not be surprised to find cases of its occurrence, since I am convinced that the stomata are influenced by inherent periodicity.

The following tables give a summary of the experiments. The first gives the effect of darkness in the morning as compared with the afternoon: the second table gives the contrast between the effects of illumination at these times. In each table the result will be appreciated by comparing the readings for a given species on those two sides, and not by comparing the readings of one species with those of others in the same column. It will be seen that in Table I. the times in the left-hand column are uniformly greater than those on the right. In Table II. the reverse is the case. The results are necessarily rough; nevertheless, I think they clearly point to a certain amount of inherent periodicity.

TABLE I.

	Darkened in morning.	Darkened in afternoon.
<i>Lonicera sempervirens</i> , June 16, 1897 . . .	$\frac{1}{4}$ shut‡ in $3\frac{1}{3}$ hours	$\frac{2}{3}$ shut in 22 minutes
<i>Convallaria</i> , March 19, 1897	shut 74 minutes	shut 45 "
<i>Narcissus</i> , March 16, 1897	" 54 "	" 36 "
<i>Phaseolus</i> , May 13, 1897	" 48 "	" 31 "
<i>Canna indica</i> , May 13, 1897	" 45 "	" 30 "
<i>Melianthus major</i> , June 17, 1897	$\frac{2}{3}$ shut in 77 "	" shut in 54 "
<i>Commelina Sellowiana</i> , June 17, 1897 . . .	$\frac{3}{4}$ " 84 "	{ quite " 21 "
<i>Veronica imperialis</i> , June 16, 1897	$\frac{3}{4}$ " 47 "	" " 34 "
<i>Eupatorium Weinmannianum</i> , June 15, 1897	$\frac{1}{10}$ " $3\frac{1}{2}$ hours	$\frac{3}{4}$ " 21 "
		$\frac{1}{4}$ " 23 "

* PFEFFER (73).

† It is conceivable that the opening of the stomata in continuous darkness (p. 606) corresponds to this phenomenon.

‡ $\frac{1}{4}$ shut means that the reading diminished by $\frac{1}{4}$.

TABLE II.

	Illuminated in morning.	Illuminated in afternoon.
<i>Lonicera sempervirens</i> , June 16, 1897	Well open in 32 minutes	Partly open in 39 minutes
<i>Convallaria</i> , March 19, 1897	Half " 19 "	Hardly opened at all
<i>Phaseolus</i>	Half " 24 "	Half open in 67 minutes
<i>Canna indica</i> , May 13, 1897	Fairly " 18 "	Fairly " 60 "
<i>Veronica imperialis</i> , June 15, 1897	Quite " 36 "	Not half " 62 "

Second Method.—The second method of testing the inherent periodicity of the stomata gave discordant results, and is only worth mention in connection with the results of the first method.

The plan was to take a pair of plants grown in pots and of the same age, one to serve as the "experimental," the other as the "control" plant. During the day they were kept in a good light and under identical conditions. At a fixed hour in the afternoon (*e.g.*, in the experiments made in March, 1897, at 4 P.M.) the experimental plant was darkened by being placed under a light-tight cylinder, which was removed at night. Thus the experimental plant was exposed to normal conditions in every respect except that its stomata were made to close every day at an abnormally early hour. In the experiments which succeeded it was found, after a fortnight or three weeks of this treatment, that the experimental plant closed its stomata before the control plant when the process of darkening was omitted. In other words, the experimental plant had acquired an artificial periodicity.

This result would be of great interest if it could be thoroughly relied on, but many of my experiments failed to show any trace of periodicity. It is possible that in these cases, like the etiolated *Mimosa* leaves in Jost's* interesting experiments, the stomata are guided by temperature changes so as to keep their normal rhythm in the absence of normal illumination. Or there may be some other unknown factors in the problem. In any case, the result is the same—namely, that I do not feel confidence in the experiments, and without the results of the first method I should not venture to state that the movements of the stomata are periodic.

§ 18. ON THE EFFECT OF DARK HEAT RAYS AND OF VARIOUS PARTS OF THE VISIBLE SPECTRUM.

The effect of heat is a subject which has been a good deal discussed, but without very satisfactory result, nor am I able to contribute much to the subject, principally because the index of the horn hygroscope reacts to dry heat as it does to moisture, *i.e.*, by curving away from a warm surface; and the experimental treatment of the point

* JOST (97).

is therefore difficult. LEITGEB* discusses the question, quoting N. J. MÜLLER's views as to heat causing the stomata to open, and SCHWENDENER's opposed opinion; LEITGEB also gives his own experiments. With the perianth of *Galtonia* he got clear opening of the stomata at a temperature of 33°, but with the leaves of *Galtonia*† he agrees with SCHWENDENER's results in certain leaves, *i.e.*, that dark heat does not open the stomata. LEITGEB explains the different behaviour of the perianth and the leaf, by the differences in the coercion exercised by the surrounding epidermis in the two cases. More recently KOHL‡ has shown by the microscopic method that heat from a blackened metal-plate, or a current of warm vapour rapidly open the stomata of *Trianea*; he also used light which had passed through a solution of iodine in carbon bisulphide. EBERT§ got similar results.

This last fact I have confirmed. By means of a heliostat the sun's rays were passed through the bisulphide and kept the stomata of *Campanula pyramidalis* open for two hours (March 30, temperature 17°). On March 29 the same species was kept open for three hours at 15°, and the stomata shut on being darkened. Another day a *Tropæolum* leaf was kept open at 13° for two hours, and shut in one hour by darkness.

And speaking from general experience, I cannot doubt that a high temperature has at least some effect in keeping the stomata open. Indeed, this was one of my difficulties when using sunshine and colour-screens.

The following may be worth quoting as showing a direct effect :

A *Tropæolum* was kept in the light at a temperature of 20°–24° C., and compared with a control plant at 13°·5–16°·5 C. At 5.45 P.M. the experimental plant gave a reading of 20 (hygroscope); the control plant gave 18. At 9.20 P.M. the effect of the heat was clear: the experimental plant was open (10 hygroscope), while the control was shut. The experiment was repeated on the following day, when at 10 P.M. the experimental plant, at 23° C., was open (12 hygroscope); the control, at 15° C., was shut.

The effect of raising the temperature on the nocturnal closure is also shown in the following experiments :

* LEITGEB (86), p. 126, 136, &c.

† *Loc. cit.*, p. 136.

‡ KOHL (95).

§ EBERT (89).

APRIL 1, 1898.—Seedlings of *Ipomœa purpurea* (cotyledons observed).

Time.	Experimental.		Control.	
	Hygroscope.	Temperature.	Hygroscope.	Temperature.
P.M. 3.15 4.0	19 16	° C. 20·5 18·7	18 17	° C. 20·5 18·7
	Placed in stove.		Left in corridor.	
4.30 5.15 6.0 6.20	17 15 12 9	22·3 21·9 21·0 20·6	12 9 3 0	18·1 17·6 16·8 15·9

APRIL 1, 1898.—*Cucurbita* seedlings in pots (cotyledons).

Time.	Experimental.		Control.	
	Hygroscope.	Temperature.	Hygroscope.	Temperature.
P.M. 3.0 3.50	24 22	° C. 20·7 18·7	27 22	° C. 20·7 18·7
	Placed in stove.		Left in corridor.	
4.36 5.20 6.7 6.28	24 20 14 11	22·3 21·8 21·0 20·6	20 14 7 2	18·1 17·6 16·8 15·9

In these cases it is clear that warmth delays the nocturnal closure.

Similar experiments were made by putting experimental plants into a dark box heated to 24°–30°, while control plants were placed in a cool, dark room. Thus cut leaves of *Salvia*, open to about 30 of the hygroscope, were at 9.30 A.M., April 9, 1897, placed in the dark at 30° C. and at 15° C. with the following result :—

Time.	Experimental.		Control.	
	Hygroscope.	Temperature.	Hygroscope.	Temperature.
9.30 A.M. 12 noon 3.0 P.M.	28 20 15	° C. .. 30 ..	28 0 0	° C. .. 15 ..

Similar results were obtained with *Trifolium subterraneum* and *Tropæolum*. With a *Pelargonium* and a *Narcissus* the plants in the hot-dark box closed as quickly as those in the cool-dark room.

My observations on *Trianea* were not, like KOHL'S, made in the dark, but in the laboratory close to a window. The plants were heated by bringing a warm glass plate close to one of the leaves for a few minutes, and allowing them to cool for four or five minutes before the hygroscope was applied. It will be seen that the high reading of the hygroscope lasted so long that it is impossible to suppose it due to the physical effect of the warm leaf on the index of the hygroscope.

NOVEMBER 27, 1896.—*Trianea*.

Time.	Hygroscope.
P.M. 3.0	40
heated	
3.8	55
3.20	50

NOVEMBER 28, 1896.

Time.	Hygroscope.
A.M. 10.30	40
heated	
10.38	60
10.45	58
11.0	50

On the part of the visible spectrum which opens the stomata.

There can be no doubt that light as distinguished from heat opens the stomata. When a plant is closed by covering it with an opaque cylinder, the change is essentially one of illumination, not of temperature.

KOHL (95) is the first, so far as I know, to investigate the effect of different parts of the spectrum on the stomata. He used incandescent gas and Reinke's spectrophore; the experimental plant was *Trianea*, and was examined microscopically. He makes out the interesting fact that the red light between B and C and the blue from F to the beginning of the violet are the only efficient parts of the spectrum. The

conclusions which KOHL draws will be discussed in the section on the mechanism of the stoma.*

My own results agree so far with KOHL's, that I find the red end of the spectrum the efficient part; I cannot, however, discover that the blue end has any decided effect. I began by illuminating my plants with sunshine passed through colour screens, and as I had Landolt's solutions, I used them, although they are not intended for use with sunshine and do not give the wave-lengths obtainable with them when incandescent gas is used. The results are further vitiated by the use in some of them of 15 millims. instead of 20 millims. troughs. The solutions were examined spectroscopically with the following results:—

Red.—Crystal-Violet, 5 BC† and K. Chromate.

15 millims. troughs	700–595, very brilliant.
	595–485, very faint.
	Beyond 485, cut off.
20 millims. troughs	700–597, very brilliant.
	595–505, very faint.
	Beyond 505, cut off.

Green.—Copper Chloride and K. Chromate.

15 millims. troughs	485–580	} let through.
20 millims. troughs	490–570	

Blue.—Crystal-Violet 5 BO† and CuSO₄.

20 millims.	A rather bright red band, 610–625; at the blue end, 485–410, let through.
15 millims.	The red band is rather wider, and at the blue end more green, <i>i.e.</i> , up to 500, comes through.

Owing to the omission in my notes of the width of trough used, it is necessary to strike a rough average between the 15 millims. and 20 millims. results. When this is done I think it is clear that the Red light is fairly satisfactory, since the only brilliant part is 700 to 595 or 597. What is called Green, and which contains both blue and yellow, is at any rate a useful piece out of the middle of the spectrum.

The Blue light is the worst; but it must be noted that the red light (610–630) which passes the blue screen is not in the important region between B and C.

* These results agree with those of WIESNER (76, p. 518) on the effect of red and blue light on transpiration.

† Supplied by the "Actien-Gesellschaft für Anilinfabrikation."

Red Light.

Out of 24 experiments with red light (sunshine and a screen of K. Chromate and Violet 5 BC), only three failed. That is to say, in the other 21 the stomata either remained wide open, or, being shut to begin with, they opened. I give the summarised details of a few experiments :—

APRIL 1, 1897.—*Narcissus* in pot.

Time.	Hygroscope.	Temperature.
A.M.		°
9.30	$\frac{20}{18}$	13
	red light	
11.45	$\frac{20}{14}$	
A leaf in a dark corner of the box = $\frac{0}{0}$		

APRIL 9, 1897.—*Tulip.*

Time.	Hygroscope.	Temperature.
A.M.		°
10.0	$\frac{25}{10}$	13
P.M.		
12.10	$\frac{22}{12}$	
A control leaf wrapped in black paper = $\frac{0}{0}$		
2.0	$\frac{0}{0}$ (after being placed in the dark)	
Exposed to red light in sun.		
3.30	$\frac{10}{5}$	
Control wrapped in black paper = $\frac{0}{0}$		

Green Light.

Twenty observations were made with sunshine passed through CuCl_2 and K. Chromate. In half the experiments the stomata were either "shut" or were nearly shut, *i.e.*, $\frac{2}{3}$. This means that in half the cases the green light opened the

stomata of shut leaves even when the light was concentrated by a lens.* I had, in fact, concluded that the experiments with colour-screens and artificial light must be made with the above water-plants when I happened to find, in the cotyledons of seedling *Ipomœas* and *Cucurbitas*, material sensitive enough for my purpose. The light of the incandescent gas was concentrated on the experimental plant by passing through a large globular flask before passing through the colour-screens into the experimental box. In this way it was found possible to cast a brilliant light on the plant, and at the same time to keep it cool.

The preponderant effect of red light is clear in the observations on seedlings, principally of *Cucurbita maxima*, a few of *Ipomœa purpurea*, and one of *Cobœa macrostemma*.

Out of twelve observations on red light (718–639) there was only one failure to open or keep open the stomata, and this occurred at 4.14 P.M. on March 8, 1898, almost too late in the day to expect the stomata to open after being closed by a period of darkness.

The observations on green light (540–505) were fewer, but gave constant results. In all six experiments the stomata were shut or $\frac{5}{6}$ shut. This agrees with KOHL'S observations, and differs from my results with *sunshine*, in which the "green" light, containing some yellow and blue rays, had some opening effect.

Blue Light.

Six observations were made with Landolt's Violet 5 BO and CuSO_4 screens (478–410), and three with ammoniacal CuSO_4 . In several experiments with Landolt's solutions the Violet 5 BO was diluted, the CuSO_4 screen remaining unchanged. The ammoniacal CuSO_4 screen was also variously diluted. Taking all the experiments together, the result is as follows :—The stomata were shut, or nearly shut, in eight cases ; the one case in which the stomata were open was with ammoniacal CuSO_4 highly diluted, and which allowed a good deal of red light to pass.

A few experiments made with the microscope on *Callitriche*, using Landolt's screens and incandescent gas, agree with the above. That is to say, red alone was efficient, the stomata shutting in green and blue light. If the blue was sufficiently diluted the stomata were made to open. Here follow the details of some experiments :—

* *Tulipa* and *Convallaria* gave some fair results, but they seem to be too easily affected by the heat to be trustworthy experimental plants.

MARCH 10, 1898.—*Cucurbita maxima* Seedling: Upper side of cotyledon illuminated by incandescent light passing through globe of water and Landolt's CuCl_2 and K. Chromate Screens.

	Time.	Hygroscope.	Temperature.
	A.M.		°
Green light	9.15	$\frac{21}{27}$	13.5
Red light	11.14	$\frac{0}{7}$	14.3
Blue light*	12.45	$\frac{16}{14}$	
	P.M.		
Red light	2.55	$\frac{0}{7}$	16.0
	4.33	$\frac{7}{11}$	17.1

MARCH 11, 1898.—*Cucurbita* Seedling; conditions as before. 9.15 A.M. plant placed in dark room.

	Time.	Hygroscope.	Temperature.
	A.M.		°
Red light	10.30	$\frac{3}{6}$	14.0
Blue light †	11.11	$\frac{11}{25}$	14.2
	P.M.		
Green light	12.59	$\frac{1}{4}$	14.6
Red light	2.0	$\frac{0}{0}$	15.1
	3.30	$\frac{7}{10}$	15.6

* Landolt's Violet 5 BO solution diluted with 7 vols. of water; Landolt's CuSO_4 not diluted.

† Diluted solution as above, 1-7.

MARCH 12, 1898.—*Cucurbita* Seedling; Conditions as above.

	Time.	Hygroscope.	Temperature.
	A.M.		°
Blue light [Violet 5 BO diluted]	9.0	$\frac{16}{22}$	15.5
	9.55	$\frac{8}{11}$	15.9
	10.40	$\frac{0}{5}$	16.1
Red light	11.30	$\frac{7}{11}$	16.4
	P.M. 1.0	$\frac{14}{16}$	16.7
Green light	2.30	$\frac{0}{5}$	
	3.18	$\frac{0}{0}$	16.9

§ 19. (A.) ON THE EFFECT OF PROLONGED DARKNESS; (B.) ON THE EFFECT OF AN ATMOSPHERE FREED FROM CO₂.

(A.) LEITGEB* has made a few observations on prolonged darkness, a fact which I had overlooked when writing my paper in the 'Cambridge Phil. Soc. Proceedings.'†

In my experiments the plants were placed in a dark room which was kept moderately damp by watering the floor or by placing on it large vessels of water.

The following examples will serve to illustrate the facts. The plants were placed on January 22, 10 A.M., in the dark room :

	January 22, 12.7 P.M.	January 22, 4.35 P.M.	January 26, 10 A.M.	January 28, 12 noon.	January 29, 2.15 P.M.
	Hygroscope.	Hygroscope.	Hygroscope.	Hygroscope.	Hygroscope.
<i>Pelargonium zonale</i>	30	0	0	3	12
<i>Richardia</i>	27	0	9	13	19
<i>Dædalacanthus</i> , sp.	35	0	30	35	37
<i>Sparmannia</i>	20	0	0	0	0

There is a good deal of variety in the behaviour: *Sparmannia* never opened, *Pelargonium* opened slowly, *Richardia* and *Dædalacanthus* still more quickly. It is

* LEITGEB (86), p. 167.

† F. DARWIN (97), p. 308.

worth noting that *Dædalacanthus* remained widely open, and became more open during January 26, 28, and 29.

In the next experiment the plants were darkened at 10 A.M., January 30, 1897.

	January 30, 10 A.M.	January 30, 5 P.M.	January 31, 11.15 A.M.	February 1, 12 noon.	February 2, 12.45 P.M.	February 3, 11 A.M.	February 4, 11 A.M.
	Hygroscope.	Hygroscope.	Hygroscope.	Hygroscope.	Hygroscope.	Hygroscope.	Hygroscope.
<i>Poinsettia</i>	35	0	0	8	13	15	18
<i>Salvia</i> , sp.	32	0	0	4	10	18	20
<i>Cineraria</i> .	40	0	0	10	25	25	25

It is clear that there is a tendency to a gradual opening of the stomata in continued darkness, but more work is needed on the subject before any more detailed conclusions can be drawn. There is clearly the same irregularity of behaviour here that we have noted in regard to nocturnal closure of the stomata. Thus, *Sparmannia** did not open between January 22 and January 29, whereas, in November, 1896, it was well open after 29 hours' darkness; this was also true of *Cineraria* which in January, 1897, had only begun to open after 48 hours; similar differences occur with *Salvia* in a less definite degree.

What the meaning may be of the facts here given is not easy to say. The most obvious analogy is that of nyctitropic plants, in which continuous darkness brings the leaves into a fixed position which resembles rather the day than the night position. The existence of some degree of periodicity in the diurnal variation of the stomata lends probability to this view. In itself it is not an explanation of the fact, since we do not know what is the cause of the analogous behaviour of sleeping leaves. It is, however, a classification of the fact that seems to me more satisfactory than the quasi-mechanical treatment which is often applied to the stoma problem.

It may, however, be better to look at the problem in a more simple and direct manner, as LEITGEB has done. He† has called attention to the variable result which follows prolonged darkness. The case of *Viola lutea*, described by him, seems to resemble my results, inasmuch as the stomata gradually opened during several days. LEITGEB is inclined to explain the result (without much reason, I think) to the continued effect of a moist atmosphere rather than of darkness. I should be much more inclined to believe that the stomata open in darkness owing to the epidermic cells being less resistant, and losing their turgor before the guard cells do so. This explanation is mentioned by LEITGEB only to be set aside, but it agrees well with his own general conclusions, and with his observations on the vitality of the guard cells; it also fits in with my results in Section 3.

The chief interest of the fact is in relation with the mechanism of the stoma, which is dealt with in a later section (§ 20).

* In some experiments already quoted *Sparmannia* could not be shut by darkness.

† LEITGEB (86), p. 167.

(B.) THE EFFECT OF AN ATMOSPHERE FREE FROM CO₂.

The interest of this question is principally in relation to the mechanism of the stoma. SCHELLENBERGER* states that in the absence of CO₂ the stomata shut, and concludes that they do so for the same reason that darkness is supposed to close them, *i.e.*, owing to the check to assimilation causing a loss of assimilative material, and, therefore, loss of turgor. This view will be discussed later on.

The plants used by SCHELLENBERGER were *Iris germanica*, *Helleborus sp.*, *Aconitum Lycoctonum*, *Alchemilla vulgaris*, *Viola odorata*, *Galega officinalis*; they were placed under a large bell-jar through which was drawn a current of air freed from CO₂ by passing it through 10 per cent. KHO. After two days he found all the plants with shut stomata, while control plants in a CO₂-containing atmosphere were open.

I have not been able to confirm SCHELLENBERGER, nor can I suppose that the difference in our results depends on the difference in the species of plants employed.

In some preliminary experiments I convinced myself that it is not difficult to get a result depending on differences in the dryness of the air. I took two beakers containing cut leaves of *Tropaeolum* and placed each set under a tabulated bell jar luted to a plate of glass. Under jar K was included a vessel containing KHO solution, while the control jar C contained a vessel of water. Jar K communicated with the outer air by a tube containing soda-lime, which, in the case of jar C, was represented by sawdust.

MARCH 11, 1897.

	Jar C.		Jar K.		
	Leaf I.	Leaf II.		Leaf III.	Leaf IV.
	Hygroscope.	Hygroscope.		Hygroscope.	Hygroscope.
10 A.M. . . .	$\frac{8}{17}$	$\frac{9}{18}$	10.8 A.M. . .	$\frac{10}{20}$	$\frac{8}{19}$
4.20 P.M. . . .	$\frac{5}{22}$	$\frac{10}{25}$	4.28 P.M. . .	$\frac{3}{10}$	$\frac{0}{8}$

In this case I have no doubt that the difference between the stomata in the two jars depended on the dryness of the air in jar K. Here it was perceptible to the eye that the air in C was damper than in K, but cases might easily occur in which the observer might be deceived unless he used a psychrometer.

In my later experiments care was taken that the air was sufficiently moist in the experimental chamber. A current of air was drawn slowly through the vessel, which was only big enough to contain the plant; the air passed through soda-lime, KHO,

* SCHELLENBERGER (96), p. 175.

and then through water to supply moisture; after passing through the experimental chamber it was usually made to bubble through baryta-water to test its freedom from CO₂.

In a test case a wet- and dry-bulb thermometer were included, and showed almost complete saturation in the experimental chamber. The apparatus was either in the laboratory, close to the window, or out of doors.

	Date.	Time.	Hygroscope.	Temperature.
<i>Narcissus</i> —a cut leaf . . .	March 17, 1897	3.30 P.M.	$\frac{25}{18}$	°
	„ 18 „	9.30 A.M.	$\frac{20}{20}$	
<i>Narcissus</i> —a cut leaf . . .	March 18, 1897	12 noon	$\frac{25}{20}$	
	„ 19 „	10.50 A.M.	$\frac{30}{29}$	
	„ 19 „	4.10 P.M.	$\frac{30}{35}$	
	„ 20 „	12.27 P.M.	$\frac{40}{40}$	
<i>Campanula pyramidalis</i> in pot	March 22, 1897	3.30 P.M.	30	
	„ 23 „	12.36 P.M.	30	
<i>Tropæolum majus</i> in pot . .	April 5, 1897	10.15 A.M.	$\frac{8}{20}$	
		2.30 P.M.	$\frac{10}{20}$	15.5
	„ 6 „	9.30 A.M.	$\frac{10}{15}$	17.2
„ 7 „	10 A.M.	$\frac{10}{18}$		
<i>Tropæolum majus</i>	May 14, 1897	2.20 P.M.	$\frac{15}{25}$	
	„ 15 „	9.30 A.M.	$\frac{15}{25}$	
	„ 16 „	3.50 P.M.	$\frac{19}{30}$	
<i>Teedia lucida</i>	May 17, 1897	12 noon	15	
	„ 18 „	12 noon	..	30
	„ 18 „	5 P.M.	..	23
„ 19 „	12.15 P.M.	15		17

In all these cases the baryta was, as might be expected, cloudy in the morning, owing to the respiration of the plant during the night, but during the day it remained clear.

The selection of plants is in one way unfortunate, inasmuch as *Tropæolum*, according to some authors, does not close at night, but, according to my own experience, the stomata close unless the temperature be unusually high.

Conclusion.

The conclusion to be drawn from the above series of experiments is that *when illuminated, stomata remain open in the absence of CO₂.*

§ 20. MECHANISM.

The mechanism of the stoma is a question which remains in much the same condition as were the other problems of movement, before the reform in plant physiology of about the years 1879–80. With the appearance of PFEFFER'S 'Physiology,'* SACHS' paper in his 'Arbeiten,'† and of the 'Power of Movement in Plants,'‡ a new era began, in which it was recognised that the reaction of plants to their environment is a phenomenon of irritability. There was, as PFEFFER points out, a revival of DUTROCHET'S conception of plants as "spontaneous" beings guided in their movements by the changes in external circumstances. This point of view gave the death-blow to such theories as that of DE CANDOLLE, who explained heliotropism as due to the direct action of the difference of light on the two surfaces of the organ leading to greater growth on the darker side. The problem of the stoma is still in the mechanical rather than in the physiological stage of development. I am far from hoping that I can do much to give the problem a position among "Reizerscheinungen," but to my thinking it is better to place it in that class even at the expense of leaving much unexplained.

Our knowledge of the anatomy of the stoma (as far as it concerns movement) remains very much where MOHL left it in 1856.§ That is, we still believe that the movements are principally brought about by the changes in turgor of the guard cells; increased turgor leading to increased curvature of the guard cells, and, therefore, to an increase in the aperture, and, *vice versâ*, the loss of turgor causing the slit of the stoma to close. The other principal factor, namely, the pressure of the surrounding epidermis, was also known to MOHL.||

* PFEFFER (81).

† SACHS (79), p. 282.

‡ DARWIN (80).

§ MOHL (56).

|| The chief advances in positive knowledge are due to SCHWENDENER (81), who pointed out certain

Two questions may be taken for discussion: (A) The nocturnal closure. (B) The closure of withering.

(A) Two opposite theories are held: (1) That of the school of SCHWENDENER, who holds that the guard cell is practically autonomous. He does not deny that the pressure of the epidermis is a factor, but he holds that the final decision of the matter rests with the guard cell. (2) The opposite view is that of LEITGEB, who holds that in those cases in which the stomata shut at night, the closure is due to the increased turgor of the surrounding epidermis; he attaches no biological meaning to nocturnal closure, and it comes to be pretty much a matter of chance whether it occurs or not. The logical advantage which LEITGEB (86, p. 151) claims for his view is that it requires no special hypothesis as to the action of darkness on turgor; our knowledge would lead us to expect that both the guard cells and the epidermis would increase in turgor at night, and that sometimes one, sometimes the other, would produce a dominant effect. Whereas SCHWENDENER and his school have to assume a special effect of darkness on guard cells, so that, while ordinary tissues increase in turgor at night, guard cells diminish.

In both the above views there seems to me something wanting which I may illustrate by the movements of sleeping plants. A nyctitropic pulvinus increases in turgor at night, but owing to one half increasing more than the other a definite curvature is the result. Here we do not seek for mechanical reasons why, for instance, the upper should increase more than the lower half in turgor. We think of the pulvinus as a whole, and believe that changes in illumination act as signals to it, in response to which, combined changes occur leading to adaptive movements.

In the same way I believe that we ought to look on the stomatal mechanism as a whole, and think of the opening or shutting of the stoma as a movement in which the guard cell and the general cell pressure in the leaf take a regulated share; and that such regulation occurs adaptively in response to environmental signals.

Nor do I see that there is any difficulty in believing that the guard cells may diminish in turgor in darkness, in spite of the fact that turgor is ordinarily increased by these circumstances. Darkness usually increases growth, but in the case of etiolated leaves (or in an etiolated *Coprinus*)* growth begins when the lamina (or cap in the fungus) are illuminated, while the growth of the petiole (or stipe) is checked under the same circumstances. In the same way it is perfectly conceivable

features in the guard cell which facilitate curvature during increased turgor; SCHWENDENER also showed that a change of shape in the guard cells occurring at right angles to the surface of the leaf influences the width of the opening. This last-named movement I shall not especially discuss; it may for my purpose be classed with the fundamental movement, since in both cases diminished turgor of the guard cells means closure of the stoma. The next most important work was that of LEITGEB (86), who brought into strong prominence the importance of the pressure of the surrounding epidermis on the guard cells.

* BREFELD (77).

that darkness may increase the turgor of the epidermis and diminish that of the guard cells. If this were so the increased epidermic pressure and the falling turgor of the guard cells would act together. Such a method of closure would, however, not be of the type formulated by LEITGEB, since it would be due to concerted action of the parts, not to the victory of the epidermis over the guard cells.

We have next to enquire what evidence there is as to the interaction of the two parts of the machine at night. SCHÄFER* has sought to show that the guard cells are autonomous by proving that stomata, freed by incision from surrounding pressure, do not open if they have been previously closed by darkness. I think this argument is just, although the facts do not seem to me precisely as SCHÄFER finds. Thus I have found slight opening in *Tropæolum*† when the darkened leaf is severed from the plant; the amount of opening is not perceptible with the horn hygroscope, but clearly affects the Yucca instrument. This is of no particular importance, since it is certainly true that the stomata open less easily in the dark. What is of importance is that in the early morning the stomata open when the leaf is cut, whereas they do not do so in the evening. If LEITGEB'S were the true explanation they ought to open in the evening; for if the nocturnal closure were due to epidermal pressure, the relief of pressure should allow the guard cells to recover their diurnal positions. Again, LEITGEB would necessarily assume that the morning opening is due to diminished epidermal pressure, and this should, therefore, *not* be the time for an effect to be produced by severing the leaf.

These considerations make it impossible for me to believe (with LEITGEB) that nocturnal closure is due to simple predominance of epidermal pressure.

It follows, therefore, that I ascribe importance to active changes of form in the guard cells, *i.e.*, movements *originating* in the guard cells. But it does not follow that I should agree with those who believe in the omnipotence of the guard cells. In the case of muscular action movements occur, in which one set of muscles is preponderant, but we do not therefore neglect their correlation with other muscles. Just as there is co-ordination in muscular action, there must be co-ordination in the action of stomata.

Some of those who, like SCHWENDENER, SCHÄFER, and SCHELLENBERGER, insists on the autonomy of the guard cells, seem to me not quite logical, since they do not deny the effect of epidermal pressure. Thus, SCHÄFER‡ says: "The above investigations make it indubitable that those are right who ascribe to the stomatal apparatus an autonomous function, independent of all antagonism of the epidermis, and who affirm that this function is carried out simply by the changes in the turgescence of the guard cells." It is difficult to understand how this can be stated when in the following sentence he allows that the turgor of the epidermic cells hinder the free action of the guard cells.

* SCHÄFER (88), p. 194.

† SCHÄFER mentions a minimal opening in one case.

‡ SCHÄFER (88), p. 204.

It seems worth while to point out how clear is the evidence of the existence of interaction between guard cells and the epidermis. I have shown in § 3 that when a leaf is severed from the plant the first effect may be to open the stomata. The mechanical effect of severance must be to diminish turgor, but diminished turgor of the guard cells can only lead to closure; therefore the result must depend on diminished pressure in the rest of the leaf. Exactly analogous evidence is given by the effect of wetting leaves or surface sections of leaves. Many of these experiments date from MOHL's time; LEITGEB has also shown that, for instance, the stomata of *Potamogeton natans* can be closed by wetting,* or even by damp air. This treatment must undoubtedly increase the turgor both of epidermis and guard cells; if, therefore, wetting a leaf closes the stomata, the fact proves clearly that the epidermic pressure may conquer the guard cells. It is precisely the converse of the numerous experiments given above, in which cutting a leaf from the plant causes a temporary opening of the stoma. It is true that in these cases we seem to have direct victory of the epidermis over the guard cells, but this need not make us doubt the existence of correlated interaction in the everyday life of the leaf. More recently STAHL† has conclusively shown that the closure of the stomata produced by watering plants with salt solution is due to the compressing action of the epidermic cells.

To sum up the preceding discussion—

(1) LEITGEB believes that nocturnal closure is due to the pressure of the epidermis.

(2) SCHWENDENER believes that it is due to loss of turgor of the guard cells.

(3) The view which I suggest is that in all cases the stomatal mechanism depends on correlation between guard cells and epidermal pressure. This view agrees more closely with No. 2 than with No. 1, since it ascribes an important part of the phenomenon to the variation in turgor of the guard cells. It will be seen, in the sequel, that it differs in one important particular, since I do not believe that the movement in the guard cells can be explained by the *direct* effect of light or darkness, but rather that these changes act as stimuli.

The immediate cause of the variation of turgor either in guard cells or epidermis is not known. MOHL connected the turgor of the guard cells with the assimilative function of their chloroplasts, and this view is still prevalent, though, as far as I know, no reason has ever been given for the guard cells behaving differently to other chlorophyllous tissues. Since the time of MOHL our knowledge of the causes

* LEITGEB (86), p. 154. The fact is easily confirmed.

† STAHL (94), p. 135.

which alter cell-turgor has been greatly increased.* We have cases in which the production of osmotic material is regulated in a manner of which no direct or nutritive explanation can be given. Knowledge of this sort may justify us in expecting that the turgor of the guard cells will be found to be regulated indirectly like other phenomena of irritability, but this does not permit us to pass over MOHL's theory. The idea is simple enough, *i.e.*, that increased assimilation leads to increase of osmotic material, and, therefore, to opening of the stoma, and, *vice versa*, darkness lowers the turgor by the cessation of osmotic supply. This view is theoretically correct, if we consider it merely qualitatively; but quantitatively looked at, it is to me inconceivable that half-an-hour's darkness should suffice to destroy the osmotic material, more especially as we know that the starch in the guard cells is almost indestructible by darkness.† LEITGEB pointed out that in etiolated petioles of *Crambe* the guard cells contain starch, and are open. The experiments given in § 19 on the effect of prolonged darkness seem to me quite inconsistent with the belief in the direct action of darkness. The gradual opening of the stomata under these circumstances may be due to the diminution of epidermic pressure, as in LEITGEB's experiments on *Galtonia*, but this implies persistent turgor in the guard cells. But darkness continued for three or four days should enormously lower the guard-cell-turgor, if half-an-hour's darkness depresses it enough to close the stomata. As already stated, I do not pretend to explain the opening in prolonged darkness, but it is clearly inconsistent with the prevalent view as to the direct connection between light and osmotic material.

SCHELLENBERGER has sought to uphold the theory in question by experiments on the effect of an atmosphere free from CO₂. He finds the stomata closed under these circumstances. My own experiments on this head convince me of the inadmissibility of his conclusion. I saw no signs of closure. If half-an-hour's darkness induces closure by starving the guard cells, the much longer periods of starvation produced by withdrawal of CO₂, as in my experiments, must have done so, if the theory were correct.

KOHL‡ points out that his results with *Trianea*, *viz.*, that red and blue rays open the stomata, agrees with the prevalent view, since these are the parts of the spectrum which are of assimilative importance.§ My own experiments confirm the most important part of KOHL's work, *viz.*, that the red rays are efficient in opening the

* Owing to the labours of PFEFFER and his pupils: see PFEFFER (97), pp. 121-122, &c., for references.

† See, however, LIDFORSS (96) on the starchless guard-cells of evergreens in winter.

‡ KOHL (95).

§ KOHL (95) has been unable to ascertain the nature of the osmotic substance: the guard cells seem neither to contain sugar nor acid, though his opening of the stomata with diastase point to sugar. KOHL is inclined to believe that the different effect of different parts of the spectrum on diastase comes into the problem, but into this I do not propose to enter.

stomata. The fact that I failed to get a secondary maximum at the blue end of the spectrum is hardly an argument against the correctness of KOHL'S assumption that the assimilative rays are effective in opening the stomata, since it is well-known to be difficult to demonstrate the blue maximum except by ENGELMANN'S bacterial method. That the rays of low refrangibility should be effective in producing opening is certainly a striking exception to our knowledge of the action of light on movement, and I agree with KOHL that his result is the best argument which we possess in favour of what may be called the nutritive theory of stomatal machinery.

The following explanation of the action of the different parts of the spectrum is very tentatively put forward. The change from light to dark, or *vice versa*, must have some immediate effect on the nutrition of a chlorophyllous cell ; and though I have given reasons for believing that the nutritive change is not enough to produce a result by direct action, I see no reason why such nutritive changes should not be the stimulus by which the guard cells discriminate between light and darkness.

But for this view there is no evidence, and there are obvious difficulties, *e.g.*, the behaviour of illuminated stomata in air freed from CO₂.

The action of heat in opening the stomata is a problem not quite parallel to the action of light. The chloroplasts in the guard cells differentiate them from the epidermis in relation to light, but not in relation to heat. There is no special reason why raised temperature should increase the turgor of the guard cells rather than the epidermis.*

PFEFFER,† in his admirable work on the "sleep" of flowers, has shown how the movements are influenced both by heat and light ; in other words, they are guided in the acts of closure by either of two changes, which in a state of nature run a parallel course. I should tentatively place the stomata in the same class, as an organism principally influenced by light, but in which the movements, associated with light and darkness, are "adherent to" or correlated with the parallel changes in temperature.

(B) CLOSURE DUE TO WITHERING.

SCHWENDENER has sought to establish the view that differences in illumination are the only normal cause of movement in the guard cells. He does not, of course, deny that the stomata close when the leaf withers, but he denies that in the normal life of the plant loss of water occurs to an amount necessary to close the stomata.

I find it difficult to understand this point of view : when we remember the innumerable adaptations which serve to economise water, it is inconceivable that the closure of the stomata should not cooperate. Those who deny that the stomata close adaptively as a precaution against too great evaporation must assume, I imagine,

* It is true that assimilation and metabolism are increased by heat, so that the guard cells might be specially affected.

† PFEFFER (73).

that the closure on withering is a chance outcome or bye-product of the machine. They cannot deny, however, that this closure does effect an economy, and this makes it even more difficult to understand their position.

In horticulture it is surely a matter of every-day experience that a plant withers slightly on a hot dry day and recovers in the evening or on being watered. And from this obvious degree of deturgescence up to full turgor, there are all possible intermediate stages. Anyone occupied with plants must constantly be struck by this, and it is often difficult to decide whether a plant does or does not show signs of want of water.

The stomata are not the only parts of a plant in which changes in turgescence occur. The changes in volume observed by KRAUS* (and which may, as I have shown, be especially well studied in the fruit of *Cucurbita*) are good evidence of turgescence-effects depending on normal amounts of psychometric changes in the air. GODLEWSKI† has shown that moist air produces a remarkable temporary acceleration of growth, while dry air has the opposite effect.

The experiments which have been given in § 7, on the effect of the dry air of a laboratory in closing the stomata, seem to me conclusive evidence that the stomata can shut under conditions which do not produce any change in the outward aspect of the plant. This being so there is no reason why the same thing should not occur in wild plants. Unfortunately, this is a question to which I have paid little attention. In the summer of 1896 I did, however, notice a number of instances in which the hygroscope gave either zero or a very low reading, and I connected the fact with the prolonged drought of the season. Until further observations of the sort can be made we must rely on experimental evidence and on horticultural experience. Some interesting questions arise as to the mode of action of the guard cells in dry air. Can we look at guard cells as sense-organs which, when the leaf is threatened by want of water, perceive the coming danger before the rest of the leaf? This idea is not wholly fanciful: STAHL§ has given some reason to believe that the transpiration of the guard cells is especially active; in dry air, or with a diminished water-supply, they should therefore be the first to suffer. It is conceivable that they might in this way act as "tell-tales"—just as fusible metals are used on the construction of automatic fire-doors.

These considerations are suggested by an experiment of STAHL's.|| Two *Tropæolum* leaves, one freshly gathered (F), the other (W) slightly withered and having its stomata closed, were placed between glass plates and exposed to the sun. The result was that W remained nearly as it was, whereas F became considerably more flaccid.

* KRAUS, G. (79).

† F. DARWIN (93).

‡ GODLEWSKI (90).

§ STAHL (94), p. 136.

|| STAHL (94), p. 121.

STAHL remarks that his experiment shows "that in saturated air the stomata do not close, even when evaporation from the leaf had led to complete flaccidity. Mere want of water in the leaf does not suffice to close the stomata if the guard cells have not so far lost turgor that they come into contact with each other."* The experiment is to me very difficult to understand: if the leaf as a whole lost water, why were the guard cells exempt? Especially if, as STAHL believes, the guard cells transpire to an especial degree. The only possible explanation seems to be that the guard cells robbed the rest of the leaf of water and thus retained their turgescence. But if so, the guard cells are practically less sensitive than the rest of the leaf, since they fail to close when the leaf is actually suffering.

The "preliminary opening," which has been described in § 3, seems to show that the guard cells are not the first to wither; it may also mean that they rob the rest of the leaf of water, as above suggested. But the fact that they soon wither and close shows that this source of water can only help the guard cells in cases where the general transpiration is slight, as in very moist air. If the guard cells had this power to any great extent, it would, as above pointed out, be a bad arrangement, and the facts given in § 7, showing that the stomata close before the leaf withers perceptibly, prove that the slowness in reaction of the guard cells, if it exists at all, has under ordinary conditions no marked effect.

The outcome of the above discussion of this difficult subject is, that we have no evidence of a specialised sensitiveness in the guard cells. The only alternative seems to be that they close or open according as the leaf *as a whole* is badly or well supplied with water, either owing to variation in the psychometric conditions or in the water-supply.

But there are great difficulties in this view. In the dry air of a room the stomata close rapidly, without the leaf becoming perceptibly flaccid; that is to say, the guard cells are more flaccid than the rest of the leaf. And this is a direct contradiction of the view to which we seemed to be driven—that the guard cells share in the general turgor of the leaf.

There is another possibility in which I am inclined to believe, although I can give no positive evidence for it. The guard cells may lose turgor "spontaneously," *i.e.*, not by simple evaporation, but in response to a stimulus. And this stimulus may be the slight flaccidity of the rest of the leaf.

§ 21. SUMMARY.

§ 1. The horn-hygroscope stands at zero on astomatal surfaces (with the exception of the lower side of floating leaves); variations in the readings of the instru-

* I have obtained a result which is not quite the same as STAHL'S. Two *Tropæolum* leaves, open to 35 of the hygroscope, were put into a crystallising glass nearly full of wet sand and covered with a glass plate; there was only just room for the leaves, and the air in the glass was very damp, and dewed the cover heavily. The stomata remained open for 2 hours, but after 5 hrs. 40 mins. they were shut.

ment, therefore, presumably indicate variations in the aperture of the stomata. This conclusion is confirmed by weighings and by microscopic observations. The zero of the hygroscope does not necessarily indicate absolute closure of the stomata—merely a great reduction in the aperture. The word “closure” is used in this sense.

- § 2. When a leaf is cut off and allowed to wither the hygroscope readings gradually fall, indicating a “closure” of the stomata.
- § 3. In many plants the fall in the hygroscope readings is preceded by a well-marked rise, indicating a temporary opening of the stomata due to diminution of epidermal pressure on the guard cells.
- § 4. The temporary opening of the stomata occurs much more readily in the early morning than in the evening.
- § 5. The stomata of marsh and aquatic plants do not so readily “close” on withering as do those of terrestrial plants. But many aquatics show clear diminution of stomatal aperture in these circumstances.
- § 6. The stomata can apparently be made to “close” by compressing the stem in a vice, and to open on loosening the vice.
- § 7. The stomata “close” in the dry air of a room, although the leaves show no signs of flaccidity.
- § 8. In excessively dry air the “closure” is preceded by a prolonged period of increased aperture.
- § 9. Gentle shaking produces no effect on the hygroscope readings. Shaking which is sufficiently violent to produce flaccidity “closes” the stomata; in aquatics, an opening similar to that described in § 3 is produced.
- § 10. Weak electric stimulation opens the stomata, stronger shocks “close” them.
- § 11. In chloroform or ether vapour the stomata “close” slowly and open again. In CO_2 they also “close” slowly.
- § 12. With some exceptions the stomata are more widely open in sunshine than in diffused light. Bright diffused light is distinguishable in effect from duller light. The stomata are “closed” on dark, stormy days, even in summer.
- § 13. The hygroscope shows a gradual “closure” of the stomata in artificial darkness and as evening comes on.
- § 14. The great majority of unspecialised terrestrial plants show at least partial closure at night. Nocturnal “closure” is less common in nyctitropic plants, and in aquatic plants.
- § 15. The biology of nocturnal “closure” is obscure. It is suggested (i.) that assimilation requires the stomata to be more widely open than is necessary for respiration, and that, therefore, economy in regard to water may be more safely carried on at night; (ii.) it may be shown experimentally that when the stomata are open the leaf is cooled by transpiration, therefore the nocturnal “closure” of the stomata effects a saving of heat,

- § 16. The stomata open as soon as it is light, and the hygroscope readings rise rapidly at first. The maximum readings occur between 11 A.M. and 3 P.M. The evening fall in the readings is rapid, and the zero is reached between sunset and an hour later.
- § 17. The diurnal opening and "closing" is a periodic phenomenon, but the periodicity is much less marked than (for instance) in nyctitropism.
- § 18. Heat tends to open the stomata: the red rays are the most effective part of the visible spectrum.
- § 19. In prolonged darkness the stomata tend to open again: they remain open in the light in an atmosphere free from CO₂.
- § 20. With regard to the general mechanism of the stoma, it is suggested that the pressure of the guard cells and that of the surrounding epidermis should be looked at as correlated, not as opposed and independent factors. It is argued that the closure of the stomata in darkness is not due to the starvation of the chloroplasts in the guard cells, but is an adaptive action of the same type as the other phenomena of irritability in plants. A somewhat similar view is tentatively suggested for the closure which occurs during withering.

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