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# Experimental Researches on Vegetable Assimilation and Respiration. No. II. On the Paths of Gaseous Exchange between Aerial Leaves and the Atmosphere

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# XI. *Experimental Researches on Vegetable Assimilation and Respiration.*—No. II. *On the Paths of Gaseous Exchange between Aerial Leaves and the Atmosphere.*

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## Section I.—*Introduction.*

THAT individual cells within the substance of all plants conduct the gaseous exchange necessary for their metabolism by diffusion through their proper cell-walls must be beyond doubt, and that the exchange of gases between a water plant, as a whole, and the surrounding liquid is of the same nature seems as indubitable. When, however, we consider land plants, it becomes less obvious in what way or ways the continuous exchange of gases between the plant and the atmosphere is effected.

The epidermis of such plants is covered with a more or less well-developed cuticle, through which specialized openings, stomata, occur with varying distribution and frequency. In maturer parts the epidermis and its cuticle may be replaced by suberised tissue, and the stomata by less specialized openings, the lenticels.

In such aerial plants, then, there appears to be a choice of two paths by which gases might pass between the air and the interior of the plant. Firstly, by diffusion across the substance of the external membranes; and, secondly, by movement through the small openings that interrupt them. The relative extent of these two processes has been much discussed, and much conflicting evidence, mostly of an indirect nature, brought up in support of the opposed views.

The gaseous exchange everywhere taking place between the plant and its surroundings reaches its climax in the process of assimilation in bright light. It is therefore the mechanism of exchange in green leaves that has excited most interest and attracted most investigation.

It is the object of this paper to deal somewhat exhaustively with this problem of gaseous exchange by combining a critical consideration of existing evidence with new direct experimental data obtained from living leaves under approximately natural conditions.

The differences of opinion on this question that exist among vegetable physiologists may be well illustrated by quotations from a few more important text-books.

On this subject, as on so many others, SACHS was the first to collect and critically weigh the previously scattered evidence; in his 'Handbuch,' 1865, he hesitatingly states that stomata take some part in gaseous exchange "aber doch so eng, dass sie selbst bei bedeutenderen Druckdifferenzen der inneren und äusseren Luft eine Ausgleichung nur *sehr langsam* gestatten" (1).

Later, in his 'Lehrbuch,' 1874, he deliberately assigns the chief part in assimilatory exchange to the stomata. "Layers of tissue in which a rapid interchange of gases is proceeding, are covered with an epidermis containing a greater number of stomata than those which require a less active interchange" (2), and "leaves owing to their thick cuticle require a large number of stomata in order rapidly to interchange large volumes of $CO_2$, with as large volumes of $O_2$ in sunshine." In his 'Vorlesungen,' 1882 (3), he divides the function of the stomata between discharging aqueous vapour, and at the same time facilitating the entrance and exit of carbon dioxide and oxygen.

Pfeffer, ‘Pflanzenphysiologie,’ 1881 (4), strikes a balance between the two opposed exchanges, cuticular and stomatic, and considers that both must play important parts.

Haberlandt regards the part played by the stomata in gaseous exchange as quite subsidiary. We find it stated in his ‘Physiologische Pflanzenanatomie,’ 1884, that “Eine subepidermale Assimilationszelle bezieht die Kohlensäure nicht blos aus den sie begrenzenden Intercellularräumen; sie empfängt diesen Nährstoff auch direkt von Aussen, indem die Kohlensäure mit ziemlicher Leichtigkeit durch die cuticularisirten Epidermiszellwandungen hindurchtritt” (5). He considers the specialized arrangement of intercellular spaces in assimilatory tissue as chiefly adapted to direct the outflow of plastic products from these cells, and confine it to the most direct routes.

Through these transitions we pass to the extreme view widely held in our own country, and thus expressed in Vines’ ‘Physiology of Plants,’ 1882. “It is often thought that the stomata are of primary importance in the absorption and exhalation of gases by leaves; Boussingault has found, however, that the upper surface of various leaves absorbed carbon dioxide more actively than the lower surface, although the upper surface had scarcely any stomata, whereas they were very numerous on the lower.”

“The stomata have evidently no effect upon the absorption of this gas ($CO_2$). The absorption of gases is effected then principally at least by the superficial cells of the leaves” (6).

Before proceeding to the experimental solution of this dilemma of contradictory opinion, we must consider in some detail the historical development of our knowledge on the subject.

## Section II.—*Historical Survey.*

The acquisition of knowledge on this subject has been much retarded by the difficulty of performing direct experiments by reason of the minuteness of the gaseous exchange that has to be localized.

Most of the direct experiments that have been recorded are either made by untrustworthy methods or fallaciously interpreted. The soundest work is indirect, and with this the difficulty lies in deciding how far it is applicable to the living leaf.

The earliest paper that must be noticed is by Dutrochet (7), 1832. He draws attention to the extensive system of intercellular spaces to be found in leaves, especially in the lower strata, and demonstrates by injection the connection with the stomata and the interruption by the lesser branches of the veins, so that the spaces are marked off into small polygonal areas. From determinations of the composition of the gas normally contained within these spaces he concludes that this internal air has a real physiological significance, and that the leaf of the plant has a lung-like function, handing on to the outer air the carbonic acid formed within the bulk of the plant, and returning oxygen in exchange: a similar but much less concrete idea had been previously put forward by BRONGNIART.

Some few years later the nature of cuticule was first made clear, and the possibility of isolating it as a histological entity proved by BRONGNIART.

Interest in the rôle played by this limiting membrane in absorption and exhalation by the aerial parts of the plant, stimulated GARREAU (8) in 1850, to carry out a series of researches on these points.

Among other results he arrived at the well-known one that transpiration of water-vapour takes place to a much greater degree through areas provided with stomata than through uninterrupted cuticle, so that more water is given off from the lower than from the upper surface in the great majority of leaves.

In the same paper in which these often-quoted experiments are detailed, there is a usually neglected section which bears immediately on the subject now under consideration. In this GARREAU gives the results of the extension of his method to the comparison of the amounts of carbon dioxide given off by the two surfaces of leaves respectively.

He attached a glass chamber containing a small shallow capsule of lime water to corresponding areas on each surface of a leaf. The carbon dioxide liberated by the leaf produced a film of calcium carbonate, of varying thickness, on the surface of the lime water; this was too fragile for direct weighing, so he collected it on pieces of tared filter-paper, and after drying determined the gain in weight.

This method is of course decidedly inadequate, but the results tended strongly to show some relation between the distribution of stomata and the evolution of carbon dioxide.

Of the twelve experiments carried out in darkness seven are tabulated below, excluding only duplicates and those in which the amounts of transpiration and the number of the stomata were not determined.*

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* It may be of interest to point out here an error in the statement of one of GARREAU'S results on transpiration which is reproduced in most text-books. He states that with the leaf of *Syringa vulgaris* the stomata are in the proportion $\frac{100}{130}$ and the transpiration $\frac{30}{60}$. Now, as correctly given by WEISS (25), the leaf of this plant has no stomata on the upper surface. Unless GARREAU misnamed the plant that he employed, the correction entirely destroys the value of this result.

TABLE I.

| Name of plant. | Absolute amounts of $CO_2$ for 20 sq. centims. and 12 hours given by the two surfaces. | Ratios of these amounts. | Ratios of amounts of water transpired by the two surfaces. | Ratios of the numbers of stomata. |
|---|---|---|---|---|
| | cub. centims. | | | |
| 1. *Tilia europæa* | $\frac{0{\cdot}24}{3{\cdot}4}$ | $\frac{1}{14}$ | $\frac{2}{5}$ | $\frac{0}{60}$ |
| 2. *Plumbago europæa* | $\frac{1{\cdot}0}{7{\cdot}0}$ | $\frac{1}{7}$ | $\frac{1}{2}$ | $\frac{3}{5}$ |
| 3. *Tropæolum majus* | $\frac{1{\cdot}15}{4{\cdot}6}$ | $\frac{1}{4}$ | $\frac{1}{2}$ | $\frac{1}{8}$ |
| 4. *Aralia racemosa* | $\frac{0{\cdot}55}{1{\cdot}70}$ | $\frac{1}{3}$ | $\frac{3}{5}$ | $\frac{0}{65}$ |
| 5. *Polygonum orientale* | $\frac{0{\cdot}5}{1{\cdot}2}$ | $\frac{1}{2\frac{1}{2}}$ | $\frac{1}{2}$ | $\frac{1}{3{\cdot}5}$ |
| 6. *Althea officinalis* | $\frac{0{\cdot}35}{0{\cdot}70}$ | $\frac{1}{2}$ | $\frac{1}{1}$ | $\frac{1}{5}$ |
| 7. *Nicotiana rustica* (young) | $\frac{1{\cdot}7}{1{\cdot}7}$ | $\frac{1}{1}$ | $\frac{5}{7}$ | $\frac{3}{4}$ |

The numbers here given are reduced from GARREAU's numbers to equal areas and times. We shall show later that they are very inaccurate, and all too large, no doubt increased by atmospheric $CO_2$. The transpiratory and stomatic ratios are taken from his previous experiments.

In four experiments carried out in diffuse light, no film of calcium carbonate was formed in connection with either surface. The concurrence of assimilation with respiration would naturally be expected to produce this result, but in all five experiments carried out in direct sunshine a certain amount of carbon dioxide escaped from each surface of the leaf. The amount was in some cases nearly as great as that formed in darkness, and also exhibited about the same ratio of production for the two sides. It is not at present quite clear to what cause this should be attributed, but probably to some experimental error.

Among the conclusions to the paper, we find it stated that the expired quantities of carbon dioxide appear to be in a much closer relation to the distribution of stomata than do those of exhaled water. This is exhibited in most of the cases given above, and is, indeed, the utmost that can be said, for there is but little agreement enough between these stomatic and respiratory ratios. Yet there is, without doubt, a tendency shown for the exhalation of $CO_2$ to follow the distribution of stomata. No attempt to obtain similar numbers has been published during the time that has elapsed between the paper under consideration and the present paper.

In 1867, BOUSSINGAULT contributed a long experimental paper (9). He directed his attention entirely to the gaseous exchange taking place during active assimilation. As this exchange far exceeds in intensity that of respiration, the former subject appears much the more likely to give a decisive experimental answer to the problem of the nature of the path of exchange.

The results, however, led him to take up a view exactly the contrary of the one supported by GARREAU, and to minimise the function of stomata in gaseous exchange. He appeared to prove by trustworthy experiments that the vigorous intake of carbon dioxide during assimilation in bright light proceeded chiefly through the astomatic cuticle. These experiments demand detailed criticism, which must be reserved for a subsequent special section (V.). It may be here pointed out that his critical experimental proof consisted in showing of two otherwise similar leaves, that the one which had its stomata mechanically blocked did not decompose any less carbon dioxide than the leaf with its stomata in open functionable condition.

Between 1829 and 1866, GRAHAM (10) published a series of papers on the various diffusion phenomena presented by gases, to our knowledge of many of which but little has since been added. For the clear exposition of our subject a short consideration of his views on those forms of diffusion which are of biological importance seems quite unavoidable, as many of the views and researches which follow are based directly upon them.

The simplest form of diffusion consists in the intermixing of two different gases or mixtures of gases which are in contact with one another unseparated by any septum. GRAHAM ascertained that gases move into one another at rates inversely proportional to the square roots of their respective densities. This form of diffusion, without an intervening septum, may be called "open diffusion."

Of diffusion through membranes two forms are recognized, one to be known as "porous diffusion," in which the diffusing gas passes through minute hypothetical pores, invisible with the highest magnification, by virtue of the motion of its individual molecules.

The relative rates at which different gases pass in this way through the same thin graphite plate under similar conditions have been again found to be inversely proportional to the square roots of the densities of the gases. For oxygen or carbon dioxide, which are alone of biological importance, this ratio is 1 : 1·17; the times required for equal volumes of the two gases to pass through will be of course the reverse of these. If the pores of the material are larger than this ideal smallness, as are those of unglazed porcelain, dry bladder, &c., the difference of velocities between the two gases is less marked and diminishes with increasing porosity till, with visible holes such as those through which gases can pass nearly instantaneously by difference of pressure, it ceases to be appreciable.

Certain other membranes through which gas can also pass have nothing of this finely porous structure but are theoretically continuous, and are as a class known as colloid membranes; such are caoutchouc, water films, and all membranes saturated with water. The phenomenon of the gas passage is here known as "osmosis," and it is supposed that the gas enters into union, either chemically or by a sort of solid solution, with the substance of the membrane on one side, and passing on through it in this condition is given off on the opposite side in a gaseous form. The relative velocities of different gases are for osmosis quite the reverse of those for porous diffusion, $CO_2$ travels 5·3 times as fast as oxygen. GRAHAM worked out this phenomenon for thin films of caoutchouc, but since his time much has been done on the osmosis of gases through water films, such as soap bubbles, to which, in this relation, many organic membranes saturated with water seem to approximate. For a long series of vapours and gases EXNER (12) finds that the velocity of transmission is proportional to $C/\sqrt{d}$, where C is the absorbing or solvent power of the film for the gas and $d$ the gas density.

For a given permeable membrane the only experimental way of deciding by which process gas passes through it, is by the determination of the relative velocities for two or more different gases. Through a dry stucco plate, as we have said above, oxygen passes slightly faster than carbon dioxide. If, however, the stucco be wetted, the gases both pass very much slower and carbon dioxide then five times as fast as oxygen. The former is due to porous diffusion, the latter to osmosis.

Through capillary tubes, fine openings in thin plates and other narrow spaces of a measurable size, gases pass much quicker than even by porous diffusion, but the passage is not even here a simple mass movement, for increased pressure quickly ceases to cause a proportional increase in the rate of passage, and further, different gases move at their own specific rates. Two sorts of passages have been worked at quantitatively, but only one concerns us at present, this form is known as "effusion," and has been investigated by drawing various gases into a vacuum through a fine hole in a very thin metal plate. The holes used by GRAHAM were a few hundredths of an inch in diameter, and the plate must be so thin that the hole is in no way a capillary tube lest a mixed effect be produced. Such openings as are here in question occur naturally as stomata. Through such a hole different gases pass at the same relative rates as in porous diffusion, though the two are essentially different phenomena, and it can be deduced simply from the laws of falling bodies what relative rates will hold for effusion. The absolute velocities in the two cases are in no way comparable. Gas passes into a vacuum by effusion many thousand times faster than by porous diffusion.

We continue the biological papers with one by BARTHÉLEMY (13) in 1868. After premising on the strength of BOUSSINGAULT's researches that stomata only play a secondary rôle in gaseous exchange, and that there exists no fixed relation between number or size of stomata and assimilatory activity, he brings forward for the first time the view that the cuticle plays a definite specific part in gaseous interchange. This view was suggested to him by GRAHAM'S (10) work on the osmosis of gases through thin films of caoutchouc combined with FREMY'S (14) then accepted determination of the chemical constitution of cuticle, as being very similar to that of caoutchouc. Carbon dioxide is transmitted through caoutchouc with a rapidity far exceeding that of other ordinary gases, and, of course, it is this gas that must make its way with great rapidity to the internal cells of the leaf during assimilation. Cuticle, then, according to BARTHÉLEMY, is to be regarded as a membrane specially fitted by its physical colloid properties to transmit carbon dioxide into the interior of the leaf. Nothing is suggested as to the path by which the equally large quantities of oxygen formed are passed out of the leaf.

If this view be correct, it should be experimentally possible to show that carbon dioxide passes faster through cuticle than do other gases. By the use of GRAHAM'S form of apparatus, BARTHÉLEMY obtained results which, if trustworthy, afford ample and striking evidence that this is so.

He closed the top of a wide tube full of mercury with a leaf 20 sq. centims. in area, supported on a thin porous plug or layer of chamois leather, and carefully luted to the tube at its edges. When the stopcock at the lower end of the tube of mercury was opened, the weight of the column sucked air through the leaf. Employing different gases, the rate of sinking of the mercury was found to vary considerably, that is, the gases passed, according to him by *osmosis*, through the leaf at different rates. The relative times for equal volumes of gas were found to be, $CO_2 = 1$, $O_2 = 9$, $N_2 = 15$. These are quite in harmony with the corresponding numbers 1, 5·4, 13·6, obtained by GRAHAM for caoutchouc, but the absolute rate of passage exceeded any record for the latter substance, as much as 40 cub. centims. passing through 20 sq. centims. in 6–9 minutes. *A priori*, one could not but be sceptical of the trustworthiness of these numbers, so difficult is it to obtain a perfectly unperforated stretch of most leaves 20 sq. centims. in area, and so large are the quantities of gas stated to pass through in a given time.

In 1874, BARTHÉLEMY followed the subject up with a second paper (15), in which further evidence of a similar nature is brought forward. The absence or sparsity of stomata on Cacti, green fruits, spathes, and other active parts, is pointed out, and also the difficulty with which, on account of its high density, carbonic acid would diffuse through stomata. Since the previous paper the author has performed some more exact experiments on the relative rate of osmosis of gases through cuticle. The material he now prefers to employ is very thin withered *Begonia* leaves, which have dried up till they form a pellicle consisting of little more than the cuticularized layers; also strips of epidermis that separate from the subjacent tissue in certain *Begonia* leaves to form the well-known white spots. With this dubious material, further numbers, closely in accordance with GRAHAM'S for caoutchouc, are obtained.

He now concludes, finally, that the entrance at all events, of carbon dioxide into the leaf, takes place almost entirely through the cuticle. As to the stomata, he enunciates the view that they act as valves, allowing gases to pass out when internal pressure is in excess, but never allowing movement of gas in the reverse direction. This valvular function is deduced from anatomical considerations, from the visible opening of the large stomata on the leaf of *Ranunculus ficaria* when air is forced into the petiole and the converse visible closing of the guard cells when suction is applied to the petiole, and also from an experiment first given by HALES, in which a tube dipping into mercury is securely luted to the cut end of a leafy branch, and the branch carefully varnished all over its surface. Under these circumstances a column of mercury may be held up in the tube for over a day, because the stomata admit practically no air through them into the intercellular spaces, and so down into the tube supporting the column of mercury. The accuracy of his observation on *Ranunculus* has been denied, and the experiment by HALES is now interpreted as only a demonstration of the narrowness of the intercellular spaces in wood.

In 1877 and 1878 MERGET contributed three short notes to the French Academy opposing BARTHÉLEMY, and experimentally supporting the stomatic rôle by ingenious though rather inconclusive diffusion experiments.

The first (16) attempts to follow the path of diffusion into leaves of those vapours whose passage can be traced by their discoloration effects. Of these, mercury-vapour is considered to yield the most decisive results, as it is said that it cannot be carried in a water current nor osmose through a colloid membrane. BOUSSINGAULT (17) and others have shown the strong toxic action of this vapour, and that it particularly attacks protoplasm, producing dark discoloration and death. If a leaf be exposed to mercury vapour, even the deeply situated cells turn dark in a short time. From this ready penetration by a substance that cannot osmose, MERGET argues that the vapour must pass through the stomata and penetrate by the intercellular spaces. Also, if a leaf be injected with mercury and pressed between paper sensitized with $AgNO_3$, the blackening of the paper by the vapour only occurs in those parts which bear stomata. The interpretation given is no doubt correct, but it is not logically conclusive, for the very fact that mercury attacks protoplasm shows that it must osmose through a colloid membrane—the cell wall—and so might enter the leaf partly through the cuticle.* One fact the author does establish, namely, that diffusion takes place through stomata with equal facility in either direction.

Several other vapours, such as $H_2S$ and $SO_2$, gave similar results, and $NH_3$ was especially rapid in its action. A leaf, injected with ammonia solution, shows white fumes with HCl and blackening of mercuric nitrate paper in contact with its stomatic surface alone.

To this paper, BARTHÉLEMY replied (18) criticising the abnormal and toxic character of the gases that MERGET employed, and reinforcing his arguments on the difficulty

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[*] From the toxic effect of mercury vapour described above, it would almost seem that we are justified in assuming that mercury, though of course only to a very slight degree, actually is soluble in water, if indeed some poisonous soluble salt of mercury is not formed on contact of the vapour with the moist cell walls. *Cf.*, further, NAEGELI, on so-called oligo-dynamic phenomena.

of assuming that all the immense quantity of carbon dioxide taken in during assimilation, so diluted as it is in our atmosphere, and so slow in its rate of diffusion, should be introduced through stomata, always minute in their dimensions and often retired in their situation. The assumption of the cuticular rôle seems necessary, further, for young organs where stomata are yet unopened and assimilation is said to be active.

In a second note, MERGET (19) replies that he is in the course of obtaining quite similar results with normal uninjurious gases, and states that he has repeated BARTHÉLEMY's osmosis experiments as carefully as possible, consistent with the vague description given of them, and has obtained opposite results.

His third note (20) contains the promised experiments with uninjurious gases, which simply show that the intercellular system of a leafy branch may act as a medium of diffusive exchange between a chamber of hydrogen, in which either the cut end or the leaves are placed, and the external atmosphere: in a longer or shorter time all the hydrogen is replaced by air. Artificial blocking of the stomata prevents this exchange, but coating the astomatic surfaces has no such effect.

Meanwhile, N. J. C. MÜLLER (21), in 1870 and 1873, had described experiments made by him on similar lines to those of BARTHÉLEMY, but carried out with infinitely more care and exactitude. Unfortunately, for the value of his conclusions, it appears, according to WIESNER (23), that his material, the upper epidermis of the leaf of *Hæmanthus puniceus*, which he considered to possess a perfectly continuous cuticle, must be rejected as untrustworthy. The apparatus he uses is rather complicated and based on GRAHAM's diffusiometer. He at first hoped to be able to ignore the occasional stomata that occur on the upper epidermis of many leaves reputed to be free of stomata, by employing the expedient of keeping the material saturated with water so that the stomata should be hermetically closed by the turgidity of the epidermis cells. In this hope he made preliminary experiments on the suction required to force the stomata open and draw bubbles of air through such material, but found that it was not as great as the suction he must employ in his diffusiometer, so that a pure diffusion result could not be thus obtained. His results on this point are, nevertheless, worth recording. The upper epidermis of *Scilla peruviana* required when in air, a column of 120 millims. Hg. to draw air through its stomata; when put in contact with water on its internal cellulose side, the required pressure rose gradually to 222 millims. If the whole experiment was performed in direct sunlight, which dilates the stomata, lower numbers were obtained, 30 millims. in air, 170 millims. in water. When the cuticular surface is kept wet, the effect is less marked.

MÜLLER therefore had to seek for an epidermis entirely free from stomata: this he thought he had found in *Hæmanthus puniceus*, and he regarded the gas that came through under pressure as the result of a purely molecular motion. He alone of all writers on the subject dwells on the compound nature of the isolated epidermis employed in such experiments, how it consists of two layers of cellulose wall and of cell contents, as well as the cuticular membrane to which many experimenters attribute all the effect. He experimented on the epidermis, both moist and artificially dried, but to eliminate the part played by the cell contents he rejects his results on the former, where $CO_2$ passes faster than oxygen in the ratio $\frac{6}{5}$. The rate of passage of gas through artificially dried membranes is absolutely quicker, but in this case oxygen passes slightly quicker than carbon dioxide. These results he accepts as exhibiting the proper physical properties of the cuticle and epidermis wall, and so evidently regards the process as allied rather to porous diffusion than to osmosis.

His results are thus entirely opposed to BARTHÉLEMY's, and certainly would recommend themselves much more strongly by the care with which they are performed were his material unobjectionable.

In 1879 WIESNER (23) published the results of his determination from the purely experimental point of view of the parts played by some of the different diffusion processes in relation to the varying gas-pressure within the plant. He first defines the terms employed, not altogether in an unexceptionable manner, but these need not be further dealt with. The short section devoted to epidermis alone concerns us here. Although aware that the accepted view was that stomata in general are so small that the normal difference of internal and external pressure can only be slowly equalized through them, WIESNER anticipated that such passage of gas would take place according to the law for gaseous effusion.

To confirm this he determined the rates at which equal volumes of gases of various densities pass through stomatiferous epidermis under the suction of a column of mercury. The epidermis of the under surface of the leaf of *Agave americana*, which is very easily stripped off, is most suitable for this experiment. In this case the stomata are of quite exceptional size, the area of the opening being estimated at 0·00019 sq. millim., and through 12 sq. millims. of this tissue about 2 cub. centims. of air could be drawn in a minute by a column of 75 millims. of mercury. Equal volumes (approximately 0·6 cub. centim.) of air, coal-gas, and carbon dioxide of the relative specific gravities 1, 0·74, and 1·42 required respectively 18·5, 16, and 23 seconds for their passage under similar conditions. If this passage takes place by effusion, the times required for equal volumes should be inversely proportional to the square root of the density of the gas employed. The above numbers are very nearly in this relation, from which WIESNER concludes that effusion through stomata is the process by which the gases pass. To this one can assent, but it should be pointed out that, in porous diffusion through the cuticle, the same law for relation of velocity to density holds, as in effusion; so that, theoretically, this form of diffusion is not excluded, though, on other considerations, it hardly seems probable that it should occur here.

When, however, similar experiments are tried with the isolated epidermis of leaves possessing stomata of the usual small size, nothing like so great a quantity of gas can be drawn through, and the truth of SACHS' qualifying remarks (p. 504) is well brought out. Thus, with *Saxifraga sarmentosa*, where the area of the stomatal opening when extended is only 0·00003 sq. millim., a suction of 70 millims. Hg will draw no visible quantity of gas through in several minutes; a column of 200 millims. Hg is required to obtain quantities sufficient to prove the ratio to be in accordance with the law for effusion.

From these results it seems that BARTHÉLEMY was perhaps partly correct in his view of the valve-like action; for slight differences of pressure cause no passage of gas, but in all cases, on increasing the pressure, gas passes. WIESNER does not seem to have tried the effect of reversing the epidermis, so that gas might be drawn in the opposite direction; any difference in the two results should throw a clear light on this uncertainty.

WIESNER gives his reader the impression that the normal exchange of gases through stomata takes place by *effusion* (*cf.* p. 7 of 'Sep. Ab.'); and further, he has defined effusion, p. 3, as including "den Ausgleich zweier verschiedener Gase" through a fine opening, as well as the passage of one gas into a vacuum through such an opening. In reality, however, the interdiffusion of two different gases through an opening is a slow process of pure diffusion without a septum, which we have called "open diffusion," and quite different from the hundred times faster process of effusion, although in each case different gases move at the same relative velocities. Effusion in nature only comes into play when the manometric pressure within a leaf is greater or less than that of the external atmosphere.

We have no evidence that during life such differences of pressure do occur to any extent within the leaf, which seems, in fact, peculiarly adapted by its structure to equalize such differences. Such assimilatory or respiratory exchange, as we shall decide does take place through stomata, takes place, then, by "open diffusion."

No further papers bearing directly on this subject appeared till 1887, when MANGIN published two summary notes (26), (27), and then followed them up in 1888 by a detailed paper (28) of careful experiments on diffusion through cuticle.

The fundamental object of this paper is the determination, firstly, of the quantity of gas that might diffuse under given conditions across the isolated cuticle; and secondly, of the quantity of this gas absorbed or exhaled by the organ to which the cuticle belongs, in full activity. If the former is less than the latter, stomata must come into use, but if the former is the greater, then osmosis may be sufficient to account for all the exchange.

In the introduction MANGIN handles BOUSSINGAULT'S quite genuine experiments rather roughly, but as he fails to perceive that their error lies in a fallacious interpretation only, he cannot suggest the correct way of bringing them into harmony with his own results, and so attributes their divergence to experimental imperfections of the means used to mechanically block the stomata (starch paste and lard), and suggests as an improvement vaseline, which I shall show later on gives identically the same results.

MANGIN also objects to SACHS' well-known experiment of sucking gases in through the stomata and intercellular spaces of a leaf-lamina, and out through the cut end of the petiole, as no proof of a natural ventilatory function, and states that since GARREAU has shown that cuticle free from stomata is pervious to $CO_2$, "il est certain que les expériences de M. SACHS donneraient les mêmes résultats si on les répétait avec des membranes ou des feuilles dépourvues de stomates." This seems a startling statement, and must be incorrect.

The diffusion experiments are carried on in an ingenious apparatus invented by the author, in which the cuticle under investigation serves as a diaphragm between two chambers, one of which is kept filled with $CO_2$ while the other is closed, contains some other gas, and is connected with an adjustable manometer, the movements of which record the amount of gas diffusing into or out of the chamber through the diaphragm of cuticle.

From the ratio of the manometric change observed when the one chamber contains some solid caustic potash, to absorb the carbon dioxide diffusing through the cuticle, to that observed when it contains none, the rate of diffusion of the gases can be calculated.

The method appears excellent, but the materials used seem to be open to objections. The cuticle of various leaves is isolated, by allowing the leaf to remain macerating for some weeks in a culture of Bacillus Amylobacter, which gradually destroys the rest of the tissues. Pieces of cuticle,* 2 to 4 centims. square, are thus obtained and employed. In order to render them more resistant, they are painted over with a ten per cent. warm solution of glycerine jelly. Of this it is alleged that it produces no appreciable increase of the resistance of the cuticle to the passage of gases, and that it will serve to block up the stomata and cracks which have hitherto been the great stumbling-block in such diffusion experiments. Surely it must still be objected, that, since it is so permeable, gas will pass much quicker through those spots where there is nothing but a film of glycerine jelly covering a stoma or crack than through the unperforated cuticle. The osmosis numbers obtained one would expect to be much too high. Thus, prepared cuticle gives numbers for the rates of diffusion of equal volumes of different gases agreeing closely with those obtained by GRAHAM for caoutchouc.

| | | | | |
|---|---|---|---|---|
| Cuticle . . . | $CO_2 = 1$ | $H = 2{\cdot}75$ | $O = 5{\cdot}5$ | $N = 11{\cdot}5$ |
| Caoutchouc . . | $CO_2 = 1$ | $H = 2{\cdot}47$ | $O = 5{\cdot}3$ | $N = 13{\cdot}5$ |

Fallacious as may be the absolute numbers, the relations between different cuticles are of interest. Thus the cuticle of the lower surface of the leaf is always found to be more permeable than that of the upper.

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* WIESNER reasonably suggests that this so-called cuticle is really, in most cases, epidermis.

Subjoined are some of the quantities of $CO_2$ that diffuse through the cuticles of the respective surfaces, expressed in cubic millimetres per hour and square centimetres.

$$\text{Holly } \frac{\cdot 005}{\cdot 009}, \qquad \text{Pear } \frac{\cdot 052}{\cdot 269}, \qquad \text{Privet } \frac{\cdot 082}{\cdot 107}.$$

The lower "cuticle" thus never exceeds the upper in permeability by more than five times, in spite of the fact that the lower is usually riddled with stomata covered only with a film of glycerine jelly, while there are but few of these weak spots on the upper cuticle. This shows that the actual permeability of the cuticle of the lower surface, apart from the stomata, is never as great as five times that of the upper cuticle. I hope to make use of this later.

In no case does the author give any diffusion results with a cuticle perforated with stomata taken before as well as after the coating with glycerine jelly, so that a real quantitative estimate of the effect of this latter might be made.

In the second part of the paper, from experiments on the respiration and assimilation of a piece of leaf, the author comes to the conclusion that the amount of osmosis through the cuticle, calculated from the above data, is quantitatively insufficient to account for the whole of the necessary gas exchange.

Moreover, as we have seen, the osmosis numbers accepted are probably too high for isolated cuticle, not to mention that in natural circumstances the cellulose walls and protoplasmic and aqueous contents of the epidermis cells, each offering its own resistance, have all to be traversed before the gases reach the active palisade cells.

The experimental results on respiration and assimilation are given with complete absence of absolute numbers and conditions, so that it is quite impossible to judge of their value, therefore, without quoting any of them; it may be stated that from the reduction in the respiratory exchanges in living leaves when the stomata are blocked with glycerine jelly, the author concludes that the part played by the stomata is one-half to one-third that of the cuticular exchange. During assimilation, however, he holds that the stomata must carry on practically the whole of the exchange, as the tension of $CO_2$ in the air is too small to produce much osmosis through the cuticle.

In a note to a paper on Starch Formation from Sugar and other substances, BOEHM, in 1889 (29), mentions that he has performed experiments, which go to prove the preponderating rôle of stomata in the gaseous exchange of assimilation. He exposed thick hypostomatic leaves, laid with their lower faces on the surface of water, to bright light in an atmosphere rich in carbon dioxide, and found that starch was only formed in small areas. He concludes that gases diffuse only extremely slowly through the epidermis cells, and the stomatic path of gaseous exchange must be much the more important.

According to the work of NAGAMATZ (46) this experiment should only succeed with those leaves whose surfaces can be wetted and the stomata so blocked. Other leaves which retain a sensible film of air on them from bloom or other causes, should be able presumably to assimilate in the given conditions. According to GERNEZ (49) most substances, including leaves, unless especially cleaned, retain an imperceptible film of air on their surface when under water, but this does not seem to come into play in assimilation by submerged plants.

In 1889 WIESNER, in conjunction with MOLISCH, published a second paper (30) on the movements of gases in plants. It is practically all devoted to the solution of two problems: (1) Can gases be filtered by pressure through cell walls? (2) To what extent do gases diffuse through cell walls, dry and wet?

As he points out, the most diverse views on these points and as to whether gases pass through cell walls more easily wet or dry, find supporters. Only tissues that are devoid of intercellular spaces can give really trustworthy experimental results, and all previous experiments are rejected as either made with imperfectly continuous tissue or fallacious in interpretation.

Such are the experiments of LEITZMANN (31). Those of MANGIN, WIESNER regards as completely vitiated by the fallacious use of glycerine jelly and of no physiological import.

(1.) On the first point all sorts of material were investigated, often with pressure differences of several atmospheres applied perhaps for days, and all gave negative results:—bottle cork, potato periderm, grape and apple-skins, thallus of ulva, endosperm and pith with occluded interspaces.

Dealing with the epidermis of leaves, WIESNER says that even in the absence of stomata it nearly always contains fine openings, which would invalidate the experiments. A previous paper has shown that gases pass through such openings at rates inversely proportional to the square root of the density of the gas; consequently, on obtaining experimentally such rates of passage with different gases, a leaf may be rejected as being perforated with fine openings. Leaves which withstand this test allow no air to pass through them, even under considerably increased pressures. The delicate leaves of *Mnium*, only one cell in thickness, fulfil this condition, if supported on some coarse porous material. A pressure of 250 millims. Hg continued for 30 days drew no air through them. Leaves of *Potamogeton crispus* gave the same result. Pieces of Ivy leaf were similarly tested for 14 days with a pressure of 600 millims. Hg, or 3 atmospheres for 24 hours. The leaf of *Stratiotes aloides* alone allowed air to pass through under a pressure of 3 atmospheres. The gases in this case do not follow the law of velocities for effusion, but for osmosis, to which process, made possible by the exceptionally large amount of water in the cell-walls, the passage of the gas is attributed.

Thus, of the astomatic epidermis, as of all other plant membranes, it must be said that it does not allow an appreciable mechanical passage of gas under pressure. This means that it is in no way porous or comparable to graphite as PFEFFER had held.

(2.) On the question of the permeability by osmosis of cell membranes to gases, an equally general but positive answer is returned. Firstly as to cork: BOEHM (32) has shown that cork absorbs $CO_2$ as other gases, and of course will give it off again when in an atmosphere free from $CO_2$. From this one would expect that, contrary to common belief, cork is in very thin layers permeable to gases by osmosis, and this WIESNER proved to be the case. He also proves that moistened cork allows gas to pass faster than air-dry cork. Other investigated tissues allow practically no gas to pass when dry, but when moistened become very permeable. The same materials as before were experimented on; no satisfactory lignified material could however be found on which to perform trustworthy experiments, but it appears, like corky tissue, to be somewhat permeable when dry.

We thus see that corked membranes, among which isolated cuticle should be included, differ in their behaviour from cellulose walls.

WIESNER points out that, rather than graphite, cell-walls resemble caoutchouc, in that they absorb and transmit $CO_2$ more readily than other gases, and are practically impervious to gas under pressure; caoutchouc, however, cannot imbibe water. A colloid membrane, such as gelatine, fulfils this and the other conditions as well, but does not fall into analogy with corked cell-walls in being pervious when dry.

Cuticle and corked cell-walls, I would suggest, are largely imbibed with some substance of a waxy nature, in which $CO_2$ may be dissolved (*cf.* Appendix). The wax, then, would play here the same part as does the water in cellulose walls; but, inasmuch as the wax cannot be "dried" out of the walls in the same way as water can, so air-dry cuticle and corky tissue are pervious to gases by osmosis. For the passage of gases through cell-walls imbibed with water the conditions approximate closely to those in the passage through films of water, such as soap-bubbles, which have been worked out by EXNER and others.

The most recent work on our subject is by STAHL, 1894 (33), who has anticipated myself in publication of one class of experiments that go to prove the almost absolute *rôle* of stomata in assimilatory exchange.

These consist in showing the local absence of starch formation when a limited area of a leaf is coated on its stomatiferous side with some substance that mechanically blocks the stomata. This forms an easy demonstration experiment; STAHL tried it on numerous leaves, with the uniform result that, while starch was plentifully formed in normal parts of the leaf, no starch appeared in the coated parts after long exposure to light (*cf.* Section V., p. 550).

We may now shortly sum up the bearing of these various researches. Our history commences with two pieces of direct experimental work on the living leaf. That of GARREAU showed that the exhalation of respiratory $CO_2$ was locally related to stomatic distribution; the other, by BOUSSINGAULT, showed, in opposition, that the inhalation of assimilatory $CO_2$ is locally independent of stomata. Unless the view were accepted that respiration took place by one path and assimilation by another, one of these opposed pieces of work had to be discarded. That by GARREAU, as the earlier, as performed by an obviously experimentally inexact method, and on a function which quantitatively and qualitatively was much inferior to that worked on by Boussingault, fell into the background, and Boussingault's results were generally accepted. Of the various researches on diffusion which followed, the first, by Barthélemy, strongly supported Boussingault's view. Then Merget took up the view that stomata are the important paths of exchange, and performed a few ingenious, but not very conclusive, experiments on the effect of mercury vapour and diffusion through whole branches. In the controversy with Barthélemy that ensued, the latter's influence gradually faded away, but that of Boussingault remained. Other workers then investigated the physical properties of isolated cuticle. Of these, Müller and Mangin, fifteen years apart, determined the relative rates of passage of different gases through this material. Müller did but little more than this, and, of his different results, accepted the incorrect one that oxygen passes through quicker than $CO_2$. Mangin reversed this relation, with results similar to, but infinitely more trustworthy than, those of Barthélemy. But Mangin did more than this, for, from his numerical estimates of the rate at which gases do diffuse through cuticle, fallaciously high as they are, he concluded that this diffusion would be insufficient for the constant needs of the plant, and that stomata must play some part as paths of gaseous exchange; this he estimates at a half to a third of the total for respiration, and nearly the whole for assimilation. Wiesner worked only indirectly on the questions before us; he showed that when gases pass through stomata under pressure they obey the laws for effusion through fine holes, but, further, incorrectly extended this to signify that normal gaseous exchange through stomata takes place by effusion. Wiesner and Molisch again, in direct work, proved that continuous cell-walls are pervious to gases by osmosis, and more readily when wet. Cuticularized and suberized walls alone allow gases to pass when dry.

The two other more recent papers, those by Boehm and Stahl, are limited to confirming experimentally Mangin's view that stomata play a great part in assimilatory exchange. Boehm supposed that he had blocked stomata by water, and found that under these conditions very limited starch formation took place. Stahl covered the stomatic surface of leaves with a coat of wax and found that no starch formation took place on the coated areas. In spite of these various experiments, possibly explicable in some other way than that suggested, Boussingault's work still remained firmly established.

A year and a half previous to this point—in 1893—I commenced the researches to be described in the present paper. In the two succeeding sections I hope to prove that nearly the whole gaseous exchange takes place through the stomata both in respiration and in assimilation. In a further one I shall completely explain away Boussingault's results, the only experimental support of the cuticular exchange theory, and afterwards provide a few data as to what possibility there is of any exchange at all taking place through unperforated epidermis and cuticle.

### Section III.—*Experiments on the Paths of Exit of Carbonic Acid from Leaves.*

Experimental comparison of the amounts of $CO_2$ passing into or out of the two surfaces of living leaves, on which the distribution of stomata is known, must be held capable of giving a more direct answer to the problem of what part is played by the stomata during life in gaseous exchanges and what part by other paths, than can possibly be drawn by induction from diffusion experiments on isolated epidermis or from similar indirect considerations.

Such actual determinations it is my part to supply. The only previous attempts, those of GARREAU (*cf.* p. 506), were made by such an untrustworthy method, and give results so irregular in their relation to stomatic distribution, that they afford no real basis for generalization. In these determinations the chief difficulty lies in the smallness of the quantities that must be estimated. The total amount of carbon dioxide given off by a leaf rarely exceeds one-tenth of a cubic centimetre for 10 square centimetres in an hour, and may be only half this quantity. Nor can the area that is employed be much increased beyond this without seriously limiting, by reason of the magnitude of the leaves required, the range of plants that can be utilized.

Most of the experimental difficulties have been overcome by the use of the apparatus described in the previous paper,* which, indeed, was designed chiefly with the view of directly attacking this problem. The detailed description there given will not be reproduced here; it need only be said that employing this apparatus, two constant currents of air, generated by two aspirators and either freed from carbon dioxide or charged with a given percentage of it, can be drawn over the respective surfaces of the same or different leaves, and the amount of carbon dioxide that is present in the current after this passage be continuously determined through any period of time without stopping or altering the current. For the respiration experiments now to be considered, it suffices to free from carbon dioxide the currents of air, and this is accomplished, without producing a negative pressure that might draw gases out of the leaf, by the potash tower (B. I., fig. 4). The current from this passes over the leaves attached to the two capsules to be immediately described, and so through either the "absorption chambers" or the "resistance bottles" to the aspirators. The capsules, of which there are a pair, one for each surface of the leaf, were made by the Cambridge Scientific Instrument Company to my design. Each (fig. 1, B), consists of a stout circular brass rim, to one face of which a thin plate of glass is hermetically cemented. Through the rim are drilled two diametrically opposite small holes. Into each of these a fine copper tube 1 millim. in bore is soldered. One tube is curved half-way round the rim till it reaches its fellow, and they then both proceed away from the capsule for a length of 10 centims., to where they pass through

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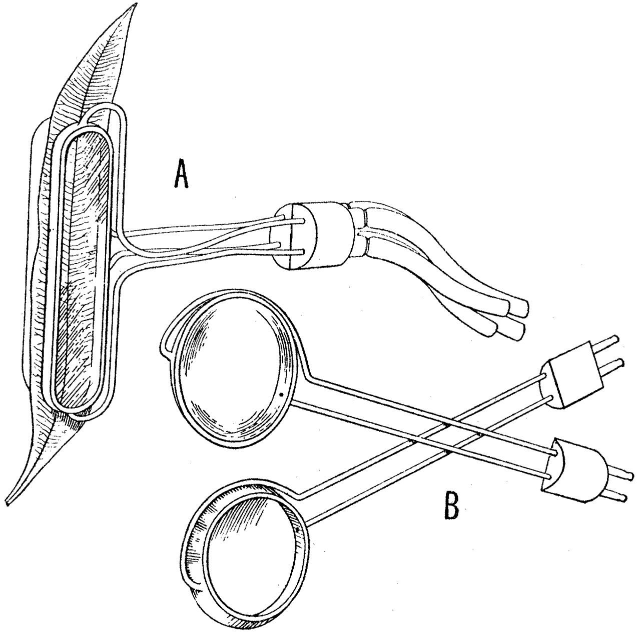
* "Experimental Researches. No. I. On a New Method for Investigating the Carbonic Acid Exchanges of Plants." This will be afterwards referred to as B.

a short hemi-cylindrical block of brass, and terminate in rounded ends to which india-rubber tubing can be attached. When the two capsules are placed face to face, these brass blocks are just in contact, and so form together a complete cylinder which can be firmly gripped in an ordinary retort-stand clamp; thus the pair can be secured in any desired position. The depth of each chamber is 5 millims. and the diameter 36 millims. If any leaf sufficiently large be slipped between them and be secured hermetically by a ring of wax to the capsules on either side, the arrangement is complete. One tube from each chamber is attached by india-rubber tubing to the glass tube L of the apparatus (B. I., fig. 1), which supplies air freed from carbon dioxide; the other two tubes are connected to the two glass tubes projecting from the switchboard (G, F) which are in connection with the absorption chambers. When the current of air is circulating it then enters each capsule by one hole in the rim and leaves it by the other opposite hole, taking up carbonic acid in its passage. In addition to these circular capsules, I had made also a pair of oblong ones 75 millims. long by 15 millims. wide, with rounded corners, and with the holes for the circulation of the air-current at the opposite ends. These are shown with a leaf *in situ* in fig. 1, A. It will be noticed that both pairs cover approximately the same area, 10 sq. centims., so that the results obtained with them are directly comparable. Armed with these two shapes, either long narrow leaves or roundish ones can be experimented on easily; and very jagged and deeply-cut leaves are the only ones that cannot be employed. With these convenient capsules the manipulation is comparatively simple, but making an air-tight wax union between the leaf and the

Fig. 1.



rim of the capsule requires a little care. The wax which I have found most appropriate is a mixture of beeswax 50 parts, vaseline 35 parts, and resin 15 parts (36). This melts at 52° C., is soft enough to be extremely easily moulded, and is very tenacious, never cracking when cold.

The necessary amount of wax is attached to the rim of the capsules first, and then the leaf is gently pressed between the two and so a preliminary adhesion obtained. The brass blocks are now screwed up in their clamp and the whole can thus be easily handled; by holding each side uppermost in its turn and going round the wax with a hot wire an air-tight union is secured with surprisingly little damage to the leaf employed.

The leaves investigated are usually taken from branches removed with al precautions against withering, and cut down under water till only the desired leaf remains. The choice of leaf is determined by careful examination with a lens to see that no perforations occur through the substance of the leaf. In a few cases the leaves have been left *in situ* on a plant in a pot, and in some others the lamina has been cut closely round the edge of the capsules after the leaf has been waxed in, and the projecting rim of leaf carefully waxed over. All the methods seem to give the same results for the first hours of investigation. Except where otherwise stated, it will be understood that the first of the above methods has been employed.

When the leaf is securely waxed on, the capsules are tested to see if all the unions are air-tight, by clamping the tubes leading to them and noting whether this completely stops the air current through the pressure bottles. A similar test is performed at the conclusion of the experiment. Everything being ready, the current is allowed to flow for an hour or more at some constant fixed rate between 50 and 100 cub. centims. per hour, through the capsules and the pressure bottles, in order that the amount of carbon dioxide the current is taking up from the leaf may become constant. It is, of course, understood that in all experiments on respiration the leaf is darkened by a black cloth.

At the end of some time the two currents are simultaneously switched through the two absorption chambers for some period varying from two to sixteen hours, at the end of which an estimation of the carbon dioxide absorbed is made. In many cases the experiment is started in the evening and allowed to go on all through the night till the next morning, this gives a convenient and relatively large amount of carbon dioxide for estimation.

We may start by first considering some cases where the contrast between cuticle and stomata is most marked, that is, where the cuticle is very thick. Evergreen leaves furnish the best examples of this; I have experimented on the Ivy. Oleander, and Cherry-laurel.

EXPERIMENT I.—*Nerium oleander*. Mature leaf of previous year.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| April 24–25, 9.14 P.M. to 10.44 A.M., 13 hrs. 30 mins. | Upper | 0·025 | 0·002 | $\frac{3}{100}$ |
| | Under | 0·875 | 0·065 | |
| „ 25, 11.43 A.M. to 3.43 P.M., 4 hrs. . . . . . . | Upper | 0·025 | 0·006 | $\frac{6}{100}$ |
| | Under | 0·365 | 0·091 | |

EXPERIMENT II.—*Prunus laurocerasus*. Fully developed leaf.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| July 1–2, 8.20 P.M. to 10.35 A.M., 14 hrs. 20 mins. . | Upper | 0·03 | 0·002 | $\frac{4}{100}$ |
| | Under | 0·76 | 0·053 | |
| „ 2, 12.5 P.M. to 3.20 P.M., 3 hrs. 30 mins. . . . | Upper | 0·00 | 0·000 | $\frac{0}{100}$ |
| | Under | 0·30 | 0·090 | |

EXPERIMENT III.—*Hedera helix*. Mature leaf. These determinations were taken on only one side at a time, and on two different leaves.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| 11.10 A.M. to 3.10 P.M., 4 hrs. . . . . . . . . . . | Upper | 0·00 | 0·000 | $\frac{4}{100}$ |
| 3.30 P.M. to 10.30 A.M., 19 hrs. . . . . . . . . . | Upper (same leaf) | 0·04 | 0·002 | |
| 4.30 P.M. to noon, 19 hrs. 30 mins. . . . . . . . | Lower (different leaf) | 1·08 | 0·054 | |

In all these cases the amount of carbon dioxide given off from the upper surface never exceeds a trace. The allowed experimental error in estimating the carbon dioxide amounts to one-hundredth of a cubic centimetre, so that for short periods an accurate determination for the upper surface cannot be expected. Neglecting these short experiments and considering only those that outlast twelve hours, we find for the upper surfaces a uniform amount of 0·002 cub. centim. per hour for the 10 square centims. of area, which is only 3 to 4 per cent. of the amount for the lower surface. None of these leaves have stomata on their upper faces, and we see, then, clearly, that here the stomata are practically the only paths of exit of carbonic acid from the leaf.

The resistance, then, which a thick cuticle offers to the passage of water vapour is more than equalled by its resistance to the passage of carbon dioxide, and GARREAU (12) was quite right when he held that stomata distribution is more closely quantitatively linked to exhalation of this gas than to that of water vapour. STAHL'S recent researches on transpiration (33), made by the aid of cobalt chloride paper, point strongly to a much greater divergence between the water exhalation on the two surfaces of hypostomatiferous leaves than GARREAU found. The latter rarely found the lower surfaces transpire more than four times as much as the upper, whereas the times required for the reddening of cobalt chloride paper, given by STAHL, indicate an almost negligible transpiration from the upper surface of thick leaves. We may anticipate that numbers with a ratio approaching those for respiration more closely than the numbers of either GARREAU or VON HOHNEL (36) will be obtained by future investigations.

Recalling that VON HOHNEL (36) has shown that in young hypostomatiferous leaves, before cuticularization is complete, the transpiration is greater from the upper surface than it is in mature leaves, I experimented on the respiration of young leaves of the above three plants, as soon as they had grown large enough to be used. In all cases the leaves were of a bright light-green colour, and had but just expanded.

EXPERIMENT IV.—*Nerium oleander.* Very young leaf, just the size of the oblong capsules.

| Duration of Experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| April 25–26, 5.56 P.M. to 10.11 A.M., 16 hrs. 15 mins. | Upper. | 0·02 | 0·001 | $\frac{1}{100}$ |
| | Lower. | 2·26 | 0·140 | |
| ,, 26, 11.5 A.M. to 12.50 P.M., 1 hr. 45 mins. . . . | Upper. | 0·00 | 0·000 | $\frac{0}{100}$ |
| | Lower. | 0·27 | 0·155 | |

EXPERIMENT V.—*Prunus laurocerasus.* Youngest leaf but one of branch ; of just sufficient length to take the oblong capsules.

| Duration of Experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| May 22, 1.20 P.M. to 6 P.M., 4 hrs. 40 min. . . . . . | Upper. | 0·005 | 0·001 | $\frac{1}{100}$ |
| | Lower. | 0·55 | 0·115 | |
| ,, 22–23, 6.46 P.M. to 12.46 P.M., 18 hrs. . . . . . | Upper. | 0·025 | 0·001 | $\frac{2}{100}$ |
| | Lower. | 1·01 | 0·055 | |

EXPERIMENT VI.—*Hedera helix*. Young terminal leaf just large enough to take the circular capsules.

| Duration of Experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| May 20–21, 3.19 P.M. to 8.19 A.M., 17 hrs. . . . . . | Upper.<br>Lower. | 0·02<br>1·28 | 0·001<br>0·075 | $\frac{2}{100}$ |

From these experiments it is clear that, instead of a greater percentage of carbon dioxide being exhaled from the upper surface in the young leaves, the ratio is, if anything, less, as indeed is also the absolute amount, while the total amount exhaled altogether by the two sides is, as would be expected in still growing leaves, larger than in mature leaves.

It thus seems that the young cuticle of these leaves presents no greater permeability to carbon dioxide than the fully developed. This is opposed to the results for water obtained by VON HOHNEL (36). The leaves which I employed were not, however, very young, and when a more suitable material is found I hope to do further experiments on still younger leaves, and correlate the results with the development of the stomata. STAHL, in his recent paper, referred to above, finds, though his work is only qualitative, no approach to equality of transpiration on the two surfaces of hypostomatiferous leaves, even in their youngest stages within the bud, such as VON HOHNEL and MERGET (37) affirmed to exist.

Contrasting the mean results of both groups of experiments, we get for the carbon dioxide evolved, in cub. centims. per hour :—

TABLE II.

| Plant. | Developing leaves. | | | Mature leaves. | | |
|---|---|---|---|---|---|---|
| | Upper surface. | Lower surface. | Ratio. | Upper surface. | Lower surface. | Ratio. |
| *Nerium oleander* . . . . | 0·001 | 0·147 | $< \frac{1}{100}$ | 0·002 | 0·078 | $\frac{3}{100}$ |
| *Prunus laurocerasus* . . . | 0·001 | 0·085 | $< \frac{2}{100}$ | 0·002 | 0·076 | $\frac{3}{100}$ |
| *Hedera helix* . . . . . | 0·001 | 0·075 | $< \frac{2}{100}$ | 0·002 | 0·054 | $\frac{4}{100}$ |

We may now turn to some herbaceous and thin leaves which have all their stomata on the lower surface. I have investigated the thin leaves of *Platanus* and *Ampelopsis hederacea* and the extremely delicate leaves of *Polygonum sacchalinense*.

EXPERIMENT VII.—*Platanus occidentalis.* Fully developed leaf cut closely round the margin of the capsule.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| Aug. 3–4, 8.9 P.M. to 11.9 A.M., 15 hrs. . . . . . . | Upper<br>Lower | 0·02<br>0·80 | 0·001<br>0·058 | $\frac{2}{100}$ |
| In morning, testing disclosed a small rupture of the wax round the midrib on lower surface; carefully re-waxed. | | | | |
| Aug. 4, 1.35 P.M. to 6.35 P.M., 5 hrs. . . . . . . . | Upper<br>Lower | 0·015<br>0·225 | 0·003<br>0·045 | $\frac{6}{100}$ |

EXPERIMENT VIII.—*Platanus occidentalis.* A different mature leaf.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. |
|---|---|---|---|
| | | cub. centim. | cub. centim. |
| Aug. 10–11, 7.35 P.M. to 10.35 A.M., 15 hrs. . . . . . . . . . . | Upper | 0·02 | 0·001 |

EXPERIMENT IX.—*Ampelopsis hederacea.* A single large leaflet experimented on.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| 1 P.M. to 4.30 P.M., 3 hrs. 30 mins. . . . . . . . . | Upper | 0·01 | 0·003 | $\frac{3}{100}$ |
| 4.45 P.M. to 8.15 P.M., 3 hrs. 30 mins. . . . . . . | Lower (different leaflet) | 0·31 | 0·10 | |

EXPERIMENT X.—*Ampelopsis hederacea.* Another leaflet.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| Aug. 4–5, 8.19 P.M. to 11.49 A.M., 15 hrs. 30 mins. | Upper<br>Under | 0·04<br>1·44 | 0·003<br>0·096 | $\frac{3}{100}$ |

EXPERIMENT XI.—*Polygonum sacchalinense.* A large fully developed leaf.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| Aug. 11–12, 8.11 P.M. to 9.41 A.M., 13 hrs. 30 mins. | Upper | 0·03 | 0·002 | $\frac{8}{100}$ |
| | Lower | 0·37 | 0·027 | |
| Aug. 12, 10 A.M. to 6.45 P.M., 8 hrs. 45 mins. | Upper | 0·01 | 0·001 | $\frac{3}{100}$ |
| | Lower | 0·305 | 0·035 | |

This last leaf is the most delicate one known to me that carries all its stomata on its lower surface.

If we tabulate the results for these thinner leaves, taking the mean of all the determinations for the lower surface and neglecting those for the upper surface, which are shorter than 12 hours, we get in cubic centims. per hour :—

## TABLE III.

| Plant. | Upper surface. | Lower surface. | Ratio. |
|---|---|---|---|
| | cub. centim. | cub. centim. | |
| *Platanus occidentalis* | 0·001 | 0·05 | $\frac{2}{100}$ |
| *Ampelopsis hederacea* | 0·003 | 0·10 | $\frac{3}{100}$ |
| *Polygonum sacchalinense* | 0·002 | 0·03 | $\frac{6}{100}$ |

From these we cannot but conclude that, practically speaking, no more $CO_2$ passes through the cuticle normally in these thinner leaves than in the thick ones. That in *Polygonum* the ratio reaches 6 per cent. is due to the very small total activity of this very thin leaf; the absolute quantity from the upper surface is no larger than in other cases, and the internal tension may be the same.

It must be recalled too, that the amounts estimated on the upper surface, even in a twelve-hour experiment, are only two or three times the allowed error of observation in titration, and further, that if a current of air freed as far as practicable from $CO_2$ has been drawn through the baryta solution in the apparatus for a corresponding period, a glass plate having been substituted for the leaf, a certain, though minute amount of carbonic acid will have found it way into the baryta solution by the end of the experiment. The amount of this error of working may be more than half as much (0·02 cub. centim. for twelve hours) as a leaf appears to give out by the upper surface. So it seems that delicate as is the method of estimating the $CO_2$, the amount given out by 10 square centims. of upper leaf surface during many hours is so minute as to rank almost with the errors of working and observation. Further there is a possible source of error in the occurrence of occasional isolated stomata on the upper surface of these leaves.

We may, therefore, conclude generally that those leaves which have all their stomata on the lower surface, exhale nearly the whole of their carbonic acid on the same surface, and that only really the merest traces pass through the epidermis and cuticle of the upper surface.

Before dealing with those leaves which have stomata on both surfaces, I must present the results obtained with very young leaves of *Platanus*. This leaf, when quite young is densely pubescent; indeed at the size at which they were experimented on—not more than 5 centims. in diameter and so only about one-tenth of their mature size—the green surface can hardly be seen through the hairs. On this material I determined the $CO_2$ given off from both surfaces in two different cases.

EXPERIMENT XII.—*Platanus occidentalis.* Very young leaves, densely crowded with hairs.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| June 11–12, 4.20 P.M. to 11 A.M., 18 hrs. 40 mins. . . | Upper | 0·115 | 0·006 | $\frac{10}{100}$ |
| | Lower | 1·135 | 0·060 | |
| May 19–20, 9.45 P.M. to 12.15 P.M., 14 hrs. 30 mins. . | Upper | 0·155 | 0·01 | $\frac{16}{100}$ |
| | Lower | 0·955 | 0·06 | |

This is in such decided contrast to the results for the young leaves of the plants of the first group, that I think the large amount of $CO_2$ given off above must be connected with the plentiful hairs.

Curious to see what would be the result of removing them, I shaved them all off the upper surface with the greatest care, employing a blunt silver knife. The result was an interesting increase in the amount for the first few hours, possibly due to the opening of the bases of the hair cells and their contents.

EXPERIMENT XIII.—*Platanus occidentalis.* Another similar leaf; down removed from the upper surface.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| June 19, 3.56 P.M. to 8.16 P.M., 4 hrs. 20 mins. . . . . | Upper | 0·125 | 0·029 | $\frac{29}{100}$ |
| | Lower | 0·435 | 0·100 | |
| June 19–20, 9.7 P.M. to 11.27 A.M., 14 hrs. 20 mins. . . | Upper | 0·195 | 0·01 | $\frac{16}{100}$ |
| | Lower | 0·845 | 0·06 | |

After the first few hours the amount of $CO_2$ given off averaged about the same as in an uninjured leaf (Experiment XII).

These leaves of *Platanus* were, I think, rather younger than the leaves of *Nerium*, *Hedera,* and *Prunus*, which gave an entirely different result, so it may possibly be that these latter exhale $CO_2$ from both surfaces when examined in a younger stage than I could employ them. I am inclined, though, to think that with glabrous leaves it would not be so. I propose, however, to make further experiments on this point in due season.

Having seen how closely the surface exhalation of carbon dioxide is limited to the areas bearing stomata in leaves with these openings only on one surface, we may feel sure that leaves with stomata on both surfaces will liberate carbon dioxide through both surfaces. It only becomes then of interest to see how far a proportional relation holds between stomatic distribution and the exhalation of carbon dioxide.

The majority of these leaves have more stomata on the lower than the upper surface; I have found, however, one suitable leaf with more stomata above, and one with an equal number on either surface.

EXPERIMENT XIV.—*Alisma plantago.* The stomatic ratio is given as $\frac{135}{100}$ (38). The leaves were cut off under water and experimented on in the vertical position with the stalk in water. Two different leaves were used.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| June 1–2, 8.25 P.M. to 10.31 A.M., 14 hrs. . . . . . . | Under | 0·38 | 0·027 | $\frac{135}{100}$ |
| ,, 2–3, 8.58 P.M. to 10.10 A.M., 13 hrs. 20 mins. . | Upper (different leaf) | 0·27 | 0·020 | |

EXPERIMENT XV.—*Alisma plantago.* Similar conditions: a series of double experiments, all taken on the same region of one leaf.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| July 12–13, 8.40 P.M. to 9.40 A.M., 13 hrs. . . . . | Upper | 0·395 | 0·030 | $\frac{120}{100}$ |
| | Lower | 0·320 | 0·025 | |
| ,, 13, 4 P.M. to 6.30 P.M., 2 hrs. 30 mins. . . . | Upper | 0·075 | 0·030 | $\frac{115}{100}$ |
| | Lower | 0·065 | 0·026 | |
| ,, 13–14, 8.12 P.M. to 9.42 A.M., 13 hrs. 30 mins. . | Upper | 0·295 | 0·022 | $\frac{113}{100}$ |
| | Lower | 0·265 | 0·019 | |

These two experiments show a ratio of $CO_2$ exhalation between the two surfaces which may be said to be almost identical with that of the distribution of the stomata.

The falling-off in the absolute amount of respiration during the second night in Experiment XV. is, naturally, under the conditions considerable, yet the ratio between the two surfaces is nearly unaltered. This was the only one of the various leaves known to have more stomata on the upper surface, that was at once obtainable and large enough for my capsules. But the results are so clear as to hardly need further confirmation.

For a leaf with practically the same number of stomata on the two surfaces I made use of the isobilateral leaves of *Iris germanica.*

EXPERIMENT XVI.—*Iris germanica.* The leaf was cut off low down and kept vertical and dipping into water. The round capsules were applied at opposite spots, half way up the leaf.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| May 21, 10.13 to 5.53 P.M., 7 hours 40 mins. | . . . | 0·225 | 0·029 | $\frac{110}{100}$ |
| | . . . | 0·205 | 0·026 | |
| May 21–22, 6.30 P.M. to noon, 14 hours 30 mins. | . . . | 0·345 | 0·021 | $\frac{105}{100}$ |
| | . . . | 0·340 | 0·020 | |

Here again the $CO_2$ exhalation follows with undoubted proportionality the distribution of the stomata.

We may now consider a few cases of the more common occurrence; where the stomata on the lower surface are in varying excess over those on the upper.

In establishing a relation between the distribution of stomata and evolution of $CO_2$ there is considerable difficulty in deciding what numbers to take as representing the ratios of stomatic distribution on the two surfaces of the leaf. Those determined by different observers or by different methods rarely agree. The numbers given by WEISS seem the most reliable and they are the means of widely different numbers (39). Seeing that it is then impossible to get accurate determinations for a given leaf, we cannot look for any very close numerical agreement between stomata and $CO_2$. I have therefore been contented with comparing the numbers for the evolution of $CO_2$ obtained by myself with those given for the stomata by trustworthy authorities.

The most detailed example that I have to offer is that of *Ricinus communis,* foliage leaves and cotyledons.

Experiment XVII.—*Ricinus communis.* The first foliage leaf above the cotyledons of a young plant in which the cotyledons appeared to be still in full activity.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| June 10, 1.14 P.M. to 6.44 P.M., 5 hours 30 mins. | Upper | 0·080 | 0·015 | $\frac{100}{260}$ |
| | Lower | 0·205 | 0·037 | |
| June 10–11, 7.29 P.M. to 7.49 A.M. 12 hours 20 mins. | Upper | 0·185 | 0·015 | $\frac{100}{240}$ |
| | Lower | 0·435 | 0·035 | |

Experiment XVIII.—*Ricinus communis.* Fully expanded cotyledon of the same plant as above. The cotyledon still attached to the small plant in a pot.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| June 5–6, 8.49 P.M. to 11.19 A.M., 14 hrs. 30 mins. | Upper | 0·180 | 0·012 | $\frac{100}{190}$ |
| | Lower | 0·340 | 0·023 | |
| June 6, 12.36 P.M. to 5.46 P.M., 5 hrs. 10 mins. | Upper | 0·065 | 0·012 | $\frac{100}{180}$ |
| | Lower | 0·15 | 0·022 | |

Experiment XIX.—*Ricinus communis.* The opposite cotyledon of the same plant was cut off and experimented on with the stalk in water.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| June 7, 12.26 P.M. to 7.56 P.M., 7 hrs. 30 mins. | Upper | 0·095 | 0·012 | $\frac{100}{160}$ |
| | Lower | 0·155 | 0·020 | |

The uniformity of the results obtained when there is a reasonable amount of $CO_2$ evolved from the surfaces under investigation seems to clearly exhibit the value of the method of estimation.

The mean respiratory ratio for the foliage leaves is thus about 10/25 and for the cotyledons 10/18.

Now Haberlandt has determined the number of stomata for both the cotyledon and foliage leaf in connection with some experiments on transpiration (40). He finds on a square millimetre for the foliage leaf 70/177 and for the cotyledon 106/146. With the numerator 10 these become 10/25 and 10/14. Thus in this case the agreement between stomata and $CO_2$ exhalation is as close as could be expected. In discounting stomatic ratios it must be borne in mind that the stomata are often of more or less different sizes on the two surfaces, which must modify the ratio regarded simply as an expression of the penetrability of the surface to gases by diffusion.

It is interesting to note that the sum of the number of stomata on the two surfaces is the same in foliage leaf and cotyledon; it is only the distribution which varies. While HABERLANDT found that the cotyledon transpired more than the foliage leaf in proportion of 18 to 11, I find that the foliage leaf respires more than the cotyledon in proportion of 17 to 11, so that these two functions seem quantitatively independent of one another.

In other leaves that I have experimented on the ratios between exhalation of carbon dioxide and distribution of stomata on the two surfaces are also in fairly close agreement.

EXPERIMENT XX.—*Populus nigra*. Stomata 100/575 (25). This is one of the few trees that have stomata on both sides of the leaf.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| 9.15 P.M. to 10.35 A.M., 13 hrs. 20 mins. . . . . . | Upper | 0·135 | 0·010 | $\frac{100}{375}$ |
| | Lower | 0·500 | 0·037 | |

Further, a leaf, of the distribution of the stomata on which I can find no record. My own rough determination gives 5/12, which is quite close to the exhalation ratio.

EXPERIMENT XXI.—*Helianthus tuberosus*. Leaf attached to a large shoot in water. Stomata, 100/240.

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| July 3–4, 8.5 P.M. to 9.45 A.M., 13 hrs. 40 mins. . . . | Upper | 0·315 | 0·023 | $\frac{100}{273}$ |
| | Under | 0·86 | 0·063 | |

Another example :-

EXPERIMENT XXII.—*Tropæolum majus.* Capsules applied to the peripheral part of the lamina petiole in water. Stomata about 100/200 (38).

| Duration of experiment. | Surface. | Total $CO_2$. | $CO_2$ per hour. | Ratio. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| July 28–29, 9.30 P.M. to noon, 14 hrs. 30 mins. | Upper | 0·20 | 0·014 | $\frac{100}{260}$ |
| | Lower | 0·52 | 0·036 | |
| Aug. 3–4, 8.46 P.M. to 10.46 A.M., 14 hrs. | Upper | 0·22 | 0·015 | $\frac{100}{270}$ |
| | Lower | 0·55 | 0·040 | |

A factor which ought to come into play and affect these ratios is the varying power possessed by the stomata of closing in the dark (34). In *Alisma* it is known that the stomata remain wide open in the dark. If the stomata become actually hermetically closed in any cases, it is hardly to be supposed that the ratios for exhalation of carbon dioxide would remain unaffected. I hope to make some experiments on this point and on the effect of withering, which rapidly closes the stomata in some cases.

A well-marked broad relation between stomatic distribution and the exhalation of carbon dioxide is, however, clearly proved by the examples given in this section, and we may now turn our attention to the relation between stomatic distribution and assimilatory intake of $CO_2$.

### Section IV.—*On the Path by which Carbon Dioxide passes into Leaves during Assimilation.*

The experiments on this point are conducted on essentially the same plan as those on respiration, with the difference that the air coming into the capsules is not freed from $CO_2$, but is loaded with a definite percentage of this gas by the "constant $CO_2$ generator" described in the previous paper (B. I., fig. 5).

After the leaf has been fitted up carefully in the capsules described in Section III., the glasses of which have been previously well cleaned, the inlet tubes are attached by a T-tube to the exit tube B of the "$CO_2$ generator," and the aspirator currents allowed to run for a couple of hours, in order that the percentage of $CO_2$ may attain constancy. During this time the leaf with the capsules is kept completely darkened, but it must be so arranged that when the covering is removed a bright light—either direct sunshine, or, much preferably, the reflected light from a heliostat —falls on the capsules at right angles to their surface, so that the leaf within is fully illuminated.

At the end of the two hours it is expected that the streams of air passing through the two capsules are moving at constant and equal rates, and contain equal and constant proportions of $CO_2$. These expectations can be verified by taking two pairs of preliminary estimations. For each pair the two currents are switched through the two absorption chambers for a given time, and at the end switched back through the pressure bottles while the titration is performed. The duration of the passage need be but quite short, generally fifteen minutes, but its length depends on the amount of $CO_2$ with which the air current has been charged; this, however, never much exceeds 1 per cent. If the amounts of $CO_2$ absorbed in the two chambers be found to be equal, within the error of observation, and the second pair, taken a little later, show no increase in $CO_2$ content, everything is ready, and the cover is removed from the leaf capsules. After a certain length of illumination, a new estimation of the $CO_2$ is made for the same length of time as before. If any $CO_2$ has been taken up by either surface of the leaf, the current from that chamber will show a falling-off in its $CO_2$ content; and, if it does not show this, then no $CO_2$ has been taken up. Subsequently, the illumination may be altered or stopped, and, after a short time, another estimation made, and so on, ending with a pair of estimations in darkness, when, if all has gone well, the original amounts of $CO_2$ should be again obtained.

To get a complete set of readings of this description requires much watchfulness, and a favourable concurrence of circumstances such as is rarely accorded.

A reasonable expectation of unclouded sunshine is necessary to justify the preliminary setting up of the experiment, and the fulfilment of this expectation is equally essential. No considerable variations of temperature must occur during the time, as these, at least under my former conditions of work, affect the rate of the aspirator flow and the working of the $CO_2$ generator. No experimental hitch of any kind must occur; a wrong tap turned may produce a lasting error; and the experimenter must always admit exactly the right amount of baryta solution to give a resistance equal to that in the resistance bottles, or the rate of flow will not be quite steady.

I can only present a few experiments in which everything has gone absolutely correctly.

Of these we may first take one with a fairly thin leaf which possesses stomata only on its lower surface.

EXPERIMENT XXIV.—*Ampelopsis hederaceæ.* Petiole cut down several times under water and four of the leaflets removed. The round capsules attached to the two surfaces of the other leaflet.

The capsules were attached at 2.15 P.M., the $CO_2$ generator being supplied with N/50 HCl, and the aspirators running at 88·5 cub. centims. per hour each. The leaf was darkened at 2.30 and the current drawn through till 5.4 to get everything constant. Each estimation lasts 15 minutes.

| Number. | Conditions of illumination. | Baryta solution neutralized. | | $CO_2$ absorbed. | |
|---|---|---|---|---|---|
| | | Upper surface. | Lower surface. | Upper surface. | Lower surface. |
| | | cub. centim. | cub. centim. | cub. centim. | cub. centim. |
| 1 | Dark from 2.30 to 5.19<br>Estimation, 5.4 to 5.19 | 0·74 | 0·76 | 0·00 | 0·00 |
| 2 | Diffuse light, 5.19 to 6.5<br>Estimation, 5.50 to 6.5 | 0·74 | 0·68 | 0·00 | 0·04 |
| 3 | Sunlight (feeble), 6.5 to 6.32<br>Estimation, 6.17 to 6.32 | 0·74 | 0·48 | 0·00 | 0·14 |
| 4 | Dark, 6.32 onwards<br>Estimation, 6.57 to 7.12 | 0·74 | 0·68 | 0·00 | 0·04 |
| 5 | Dark. Estimation, 7.34 to 7.49 | 0·76 | 0·78 | 0·00 | 0·00 |

The third and fourth columns contain the numbers of cubic centimetres of the standard baryta solution neutralized by the carbon dioxide in the current for each estimation. This is, of course, given by the difference between the actual amount of the hydrochloric acid required to neutralize the solution in the absorption chamber, and the standard amount calculated from preliminary comparisons of the strengths of the two solutions.

The baryta solution employed is of such a strength as to unite with exactly half its volumn of carbon dioxide gas. On this relation, the figures given in the last two columns are calculated from those in the two before. The slight variations in the first and last pairs of estimations are no larger than the error of estimation and may be neglected.

From this table the result comes out most clearly that no $CO_2$ is taken up by the upper astomatic surface of the leaf, even in direct sunshine; the whole amount that is absorbed enters by the under surface.

It will be observed that it takes some little time for the $CO_2$ content to reach its maximum again on darkening after light, and that the ·04 cub. centim. "absorbed" in reading 4 is only an expression of this.

The actual percentage of $CO_2$ present in the capsules at various times may be calculated from the amount of baryta solution neutralized, and the rate of flow of the current. Thus, the current flows $\frac{1}{4}(88{\cdot}5) = 22{\cdot}12$ cub. centims. for every fifteen minutes. The amount of $CO_2$ passing in this time is for the top surface of the leaf, $\frac{1}{2}(0{\cdot}74) = {\cdot}37$ cub. centim. of $CO_2$, so that the strength is 1·67 per cent. of $CO_2$. During the most active absorption (Reading 3) the amount is reduced on the lower surface to $\frac{1}{2}(0{\cdot}48)$ cub. centim. of $CO_2$ in 22·12 cub. centims. of air, which gives 1·09 per cent. So the leaf has assimilated 0·58 per cent. of $CO_2$ from the air supplied to it.

Another experiment with a leaf of *Platanus* gave similar results, though the circumstances were not steady, and the amount of $CO_2$ liberated by the $CO_2$ generator rose considerably towards the end of the experiment.

EXPERIMENT XXV.—*Platanus occidentalis.* Stomata all below. Very much weaker acid, about N/250, was used in the $CO_2$ generator. The aspirators ran 90 cub. centims. per hour each. The preliminary current in the dark lasted for 4 hours. Each estimation lasted 20 minutes.

| Number. | Conditions of illumination. | Baryta solution neutralized. | | $CO_2$ absorbed. | |
|---|---|---|---|---|---|
| | | Upper surface. | Lower surface. | Upper surface. | Lower surface. |
| | | cub. centim. | cub. centim. | cub. centim. | cub. centim. |
| 1 | Dark, 1.35 to 5.35<br>Estimation, 5.15 to 5.35 . . . . | 0·17 | 0·19 | 0·00 | 0·00 |
| 2 | Sunshine (feeble), 5.35 to 6.58<br>Estimation, 6.5 to 6.25 . . . . . | 0·20 | 0·06 | 0·00 | 0·07 |
| 3 | Sunshine (the same)<br>Estimation, 6.38 to 6.58 . . . . . | (Spoilt) | 0·08 | .. | 0·06 |
| 4 | Dark, 6.58 onwards<br>Estimation, 8.11 to 8.31 . . . . | 0·21 | 0·26 | 0·00 | 0·00 |

The continuous rise in the $CO_2$ content, apparently on both surfaces, due to external circumstances, leaves the conclusion unshaken that all the $CO_2$ absorbed is taken up by the lower stomatic surface.

A further fragmentary experiment with a delicate leaf quite confirms this. The experiment was cut short by an accident.

EXPERIMENT XXVI.—*Polygonum sacchalinense.* Stomata all on lower surface. N/100 acid in generator.

| Number. | Conditions of illumination. | Baryta solution neutralized. | | $CO_2$ absorbed. | |
|---|---|---|---|---|---|
| | | Upper surface. | Lower surface. | Upper surface. | Lower surface. |
| | | cub. centim. | cub. centim. | cub. centim. | cub. centim. |
| 1 | Dark, 10 to 12.31<br>Estimation, 12.16 to 12·31 . . . . | 0·36 | 0·44 | 0·00 | 0·00 |
| 2 | Sunlight, 12.31 to 1.31<br>Estimation, 1.16 to 1.31 . . . . | 0·37 | 0·11 | 0·00 | 0·16 |

I have so far only obtained very few sets of estimations on leaves with stomata on both surfaces. The summer of 1894 has hardly afforded a single day suitable for these prolonged experiments.

With *Alisma plantago* I have obtained one excellent complete series of readings. In this experiment all the estimations lasted fifteen minutes, and the intervals between them, during which two titrations had to be performed and the chambers refilled, were also all exactly fifteen minutes. This gives the series great regularity, and the conditions of illumination were changed every thirty minutes, just at the end of each absorption period.

EXPERIMENT XXVIII.—*Alisma plantago.* Leaf in oblique position with stalk in water. Stomata 135/100. N/100 HCl in the $CO_2$ generator. Current rate = 100 cub. centims. per hour.

| Number. | Conditions of illumination. | Baryta solution neutralized. | | $CO_2$ absorbed. | |
|---|---|---|---|---|---|
| | | Upper surface. | Lower surface. | Upper surface. | Lower surface. |
| | | cub. centim. | cub. centim. | cub. centim. | cub. centim. |
| 1 | Dark, 11.45 to 2.5<br>Estimation, 1.50 to 2.5 . . . . . | 0·30 | 0·28 | 0·00 | 0·00 |
| 2 | Sun through ground glass, 2.5 to 3.5<br>Estimation 2.20 to 2.35 . . . . . | 0·09 | 0·17 | 0·10 | 0·06 |
| 3 | Sun, as above. Estimation, 2.50 to 3.5 . | 0·00 | 0·07 | 0·15 | 0·11 |
| 4 | Sun through thin paper, 3.5 to 3.35<br>Estimation, 3.20 to 3.35 . . . . | 0·10 | 0·14 | 0·10 | 0·07 |
| 5 | Shaded on the upper surface; sun on the lower surface, 3.35 to 4.5<br>Estimation, 3.50 to 4.5 . . . . . | 0·15 | 0·14 | 0·07 | 0·07 |
| 6 | Dark, 4.5, onwards<br>Estimation, 4.30 to 4.45 . . . . | 0·24 | 0·29 | 0·03 | 0·00 |
| 7 | Dark. Estimation 5.5 to 5.20 . . . . | 0·28 | 0·28 | 0·00 | 0·00 |

The assimilation is, in this experiment, very vigorous; in Reading 3 the whole of the carbon dioxide supplied to the upper surface was absorbed. It can be reckoned out as before that the stream of air entering the capsule contained 0·60 per cent. of carbon dioxide, and that the capacity of the capsule being 5 cub. centims., any particle of the gas may be considered to remain inside the capsule for three minutes only, during which short time all the molecules of carbon dioxide diffuse through the stomata into the leaf.

It will also be noticed that there is a slight but constant tendency for the absorption of $CO_2$ to be greater on the upper surface where there are more stomata. Thus, in 2, 3, and 4 this is clearly exhibited, while in 5, when the leaf is lighted from below, the absorption by the lower surface only becomes equal to but not greater than that by the upper surface.

An incomplete experiment with a leaf of *Tropæolum* showed absorption of carbon dioxide by both faces of the leaf when the leaf was illuminated on the upper surface from a mirror. The distribution of the stomata is varyingly given by different observers from $\frac{1}{2}$ to $\frac{1}{8}$. It is sufficient at present that there are more below.

EXPERIMENT XXIX.—*Tropæolum majus.* A large leaf with the lamina horizontal, and the capsules on peripheral part of the leaf. Stalk in water. N/60 HCl in $CO_2$ generator. Aspirators both running 86 cub. centims. per hour. Preliminary current started at 11.15. Estimations each last 30 minutes.

| Number. | Conditions of illumination. | Baryta solution neutralized. | | $CO_2$ absorbed. | |
|---|---|---|---|---|---|
| | | Upper surface. | Lower surface. | Upper surface. | Lower surface. |
| | | cub. centim. | cub. centim. | cub. centim. | cub. centim. |
| 1 | Dark, 11.15 A.M. to 3.4. P.M.<br>Estimation, 2.34 P.M. to 3.4 P.M. . . | 0·81 | 0·82 | 0·00 | 0·00 |
| 2 | Sunlight, 3.4 P.M. to 4.2 P.M.<br>Estimation, 3.32 P.M. to 4.2 P.M. . . | 0·68 | 0·62 | 0·07 | 0·10 |

Although lighted on the upper surface, distinctly more carbon dioxide is taken in by the lower surface, and on this surface the stomata are more abundant.

With these experiments may be compared some made on assimilation in which no carbon dioxide is artificially supplied, but instead, the assimilation of the carbon dioxide formed by respiration is noted.

EXPERIMENT XXXI.—*Alisma plantago.* Stomata, 135/100.

| Number. | Conditions of illumination. | Total $CO_2$ escaping. | | $CO_2$ per hour. | |
|---|---|---|---|---|---|
| | | Upper surface. | Lower surface. | Upper surface. | Lower surface. |
| | | cub. centim. | cub. centim. | cub. centim. | cub. centim. |
| 1 | Dark all night to 9.40 A.M.<br>Estimation, 13 hrs., 8.40 P.M. to 9.40 A.M. | 0·395 | 0·32 | 0.030 | 0·025 |
| 2 | Diffuse light, 9.40 A.M. to 3.16 P.M.<br>Estimation, $3\frac{3}{4}$ hrs., 11.31 A.M. to 3.16 P.M. | 0·005 | 0·01 | 0·001 | 0·003 |
| 3 | Dark, 3.16 P.M. to 6.30 P.M.<br>Estimation, $2\frac{1}{2}$ hrs., 4 P.M. to 6.30 P.M. . | 0·075 | 0·065 | 0·030 | 0·026 |

The total amounts of $CO_2$ estimated for the $3\frac{3}{4}$ hours of illumination are not greater than the allowed error of observation, so it may be that the whole of the respiratory carbon dioxide is assimilated in this diffuse light.

A similar experiment with the stomatic under surface of another leaf shows apparently a complete assimilation in sunlight, but a not quite complete assimilation in diffuse light.

Experiment XXXII.—*Acer platanoides.* Stomata, 0/S. Illuminated on its upper surface.

| Number. | Conditions of illumination. | Total $CO_2$ escaping. | $CO_2$ per hour. |
|---|---|---|---|
| | | cub. centim. | cub. centim. |
| 1 | Direct sunshine, 11.6 to 3.6<br>Estimation, 12.6 to 3.6 . . . . . | 0·00 | 0·00 |
| 2 | Dark, 3.6 P.M. to 5.35 P.M.<br>Estimation, 3.55 to 5.35 . . . . | 0·135 | 0·08 |
| 3 | Diffuse light, 5.35 to 8.1<br>Estimation, 5.46 to 8.1 . . . . . | 0·04 | 0·018 |

The third estimation was however commenced rather too soon after the period of darkness for this to be quite conclusive evidence of escape of $CO_2$ in diffuse light.

The first reading of Experiment XXXII. and the third of Experiment XXVIII. show conclusively that the classical experiment by Garreau (41), demonstrating the supposed liberation of carbon dioxide in bright light is misleading. That a little $CO_2$ continually escapes from an entire branch put in a closed chamber with baryta water in the sun is not to be wondered at. The shaded parts of assimilating tissues, together with the non-assimilating tissues of petioles, stems, buds, &c., might quite account for this, but it is difficult to see how any carbonic acid should escape from a completely illuminated leaf surface of normal assimilatory activity. The result of Garreau's experiment is then only an expression of the uncritical conditions in which it was made. Corenwinder (42) showed in 1878 that with young but expanded leaves the chlorophyll of which was not yet fully developed, an evolution of carbon dioxide takes place in the sun, and that this is clearly due merely to the imperfect development of the assimilatory function, for the amount of the $CO_2$ liberated increases in the dark. With mature leaves he found that no liberation of $CO_2$ takes place in sunshine.

From the combined consideration of the concordant results obtained on respiration and assimilation, we see definitely that, at least, as far as carbon dioxide is concerned, gaseous exchange closely follows stomatic distribution on the two surfaces of leaves. From this it results that this exchange takes place practically entirely through the openings of the stomata into the intercellular spaces. The only alternative hypothetical possibility, that in every case the exchange takes place across the epidermis cells and cuticle of those *areas which bear stomata*, and yet not through the stomatal openings, can hardly be seriously entertained. This supposition would involve, among others, the assumption that the cuticle on the lower surface of hypostomatiferous leaves is often fifty to a hundred times more permeable than that of the upper surface.

Mangin (27) has, however, experimentally shown that the cuticle of the lower surface of leaves, well known to be thinner and more delicate than that of the upper, is never more than four or five times as permeable as the latter. Though his absolute results on artificially isolated cuticle may be of little application, yet we may trust them to give reliable comparisons between different cuticles, such as those of the upper and under surface when treated both in the same way. The difference shown by his investigations is in no way capable of explaining the normal differences of $CO_2$ liberation, which are almost of a higher order of magnitude.

The experiments in the subsequent sections bear on the gaseous exchange under abnormal conditions, but we may now definitely conclude that, under normal conditions, where stomata are present, their openings form almost the sole path of gaseous exchange.

## Section V.—*On the Gaseous Exchange in Leaves when the Stomata are Occluded.*

As stated in the historical treatment of our subject (p. 508), Boussingault has been held to have proved that the carbon dioxide taken up by assimilating leaves passes, not through the stomata, but through the cuticle and epidermis of the stomata-free upper surface of the leaf.

This section will be devoted to the full exposition of the fallacy that underlies the accepted interpretation of his results. His work merits this special consideration, as it has been hitherto the chief experimental prop of the "cuticular exchange" theory, and, as the principal experiment, is perfectly trustworthy in itself, in spite of several suggestions to the contrary.

The experiments occur in his "Étude sur les Fonctions des Feuilles," 1867 (9), in a long section of it entitled "Action comparée de la lumière sur les faces opposées d'une feuille . . ."

Although not so divided by the author, the experiments really fall into two classes: one deals with the localization of gaseous exchange on the surfaces of the leaf, and the other with the action of light on the two surfaces.

The first class consists of a single important experiment, No. VIII.* (p. 374), which is the foundation for the whole conclusion. In this experiment one of two similar leaves of *Nerium oleander* was painted over with lard on its upper astomatic surface, and the other similarly treated on the lower surface, thus blocking the stomata. These were then compared as to their assimilating power by being exposed side by side to bright diffuse light in glass tubes containing a known mixture of air and carbon dioxide. After eight hours it was found that the leaf with its stomata open had decomposed 10 cub. centims. of carbon dioxide, while the other, with the stomata blocked by the lard, had decomposed 17·5 cub. centims. of this gas. This

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* The experiments are not numbered in his text, but the numbers are quoted from the *précis* of the experiments given at the end of Boussingault's article.

result was then clearly to be taken as proof that the carbon dioxide passed into the leaf by the upper astomatic surface, and not through the stomata, or blocking them would have diminished the amount of carbon dioxide assimilated.

For some time after I had carried out the experiments of the previous section which prove conclusively that, during assimilation, carbon dioxide enters the leaf not through the cuticle but through the stomata, this result of BOUSSINGAULT's was a complete enigma to me.

It was impossible to explain it, as MANGIN had attempted, by the discontinuity of the coating of lard, for if this existed and so only some fraction of the stomata were blocked up, the effect became still more remarkable, as it must be assumed that blocking the whole of the stomata would cause a still profounder difference between the two leaves.

That the cuticle of leaves was slightly permeable to $CO_2$ was well-known, but how was one to explain the greater intake of $CO_2$ in this condition than in the leaf with its stomata open? It occurred to me that the explanation might lie in the large $CO_2$ content of the gas in which the leaves were exposed. The $CO_2$ formed more than 30 per cent. of the mixture, and the less decomposition of $CO_2$ in the leaf with stomata open might be due to its receiving not *less* $CO_2$ than the other leaf, but *more*, indeed so much more above the optimum for assimilation that it decomposed much less than the leaf inside which the $CO_2$ was not so abundant. Within the latter, $CO_2$ would never become very plentiful, diffusing slowly as it must through the upper surface of the leaf, as the stomata were blocked with lard. I then turned to GODLEWSKI's well-known paper (43) on the optimum strength of $CO_2$ for assimilation, and found that he had worked on this same leaf, *Nerium*, and that although his results are not very decisive, there was evidence that the Boussingault result could be interpreted in this way. Thus GODLEWSKI found the optimum strength of $CO_2$ for assimilation in *Nerium* to be decidedly lower than the 8 per cent. which is the number for other leaves. On looking through his experiments with *Nerium* there will be found one, No. XXXIV., which just seems to fit the case in question. In this experiment he exposed two leaves to the light in 28 per cent. $CO_2$ and 3·6 per cent. $CO_2$ respectively, and the amounts of $CO_2$ decomposed were as 10 : 17·8. Assume that from an environment of 30 per cent. $CO_2$, the $CO_2$ diffuses through the cuticle of the leaf with blocked stomata at such a slow rate that there is only as much $CO_2$ inside the leaf as would result from about 3·6 per cent. outside were the stomata open, and the whole contradiction is gone. I shall later adduce experiments to prove that some such relation as this does hold.

The truth of the above explanation of BOUSSINGAULT's result will be confirmed if it be shown that in an atmosphere which contains an amount of $CO_2$, that is but little, if at all, above the optimum for assimilation, the reverse result is obtained and the leaf with open stomata decomposes more $CO_2$ than the one with the stomata blocked. I have, therefore, performed a series of experiments similar to BOUSSINGAULT's, but with leaves of *Nerium* in varying percentages of $CO_2$, and have found that in a high percentage the leaf with its stomata blocked always decomposes more than the normal leaf, while in low percentages it decomposes less.

These experiments were all performed, with slight modification, by the method brought to perfection by PFEFFER, and described in detail with all its possible sources of error in his well-known paper, "Die Wirkung farbigen Lichtes auf die Zersetzung der Kohlensäure in Pflanzen" (44). It will, therefore, be only necessary to mention the departures from the procedure there described.

An ordinary cylindrical 100 cub. centim. gas tube, 20 millim. wide, graduated and closed at one end, was employed instead of the special tube, with an ungraduated dilatation at its upper end, which PFEFFER introduced. PFEFFER'S tube was indeed unnecessary for the narrow leaves of *Nerium* on which all the experiments were made, but it is also impracticable for experiments in high percentages of carbon dioxide, as only the lower third of it can be graduated.

Then again, in either tube, the suction of the lungs is not sufficient to raise the mercury up to the height required for the subsequent introduction of more than 20 per cent. of $CO_2$. The simple method that I used to effect this is really more convenient for all cases than the suction through a washing bottle described by PFEFFER, provided that a suction pump of any sort is at hand. A length of india-rubber tubing of small bore, and thick-walled to prevent its occlusion by collapse, is connected with the pump at one end and the other end is pushed up into the assimilation tube till it is above the height to which it is desired to raise the mercury. The pump is gently started, and when the mercury is drawn up to the right level the tubing is tightly gripped in the fingers and drawn down out of the assimilation tube. This method does away with the necessity for a special opening at the upper end of the assimilation tube, and also with the trouble of making this air-tight before each experiment.

In other respects the standard procedure was followed, and the same corrections and reduction formula made use of, the gas-volumes being in all cases reduced to 0° C., 760 millims. Hg., and dryness.

I have not attempted to measure my results beyond tenths of a cub. centim., as I feel sure that the method does not possess any further potentiality of accuracy, chiefly on account of the condensed water which forms on the sides of the tube.

I performed altogether twelve double experiments, in the sunny spring of 1894, all of which gave concordant results. Leaves of *Nerium*, of the previous year, in perfect condition were alone employed. They were in all cases opposite leaves of similar colour and size. The experiments may be given in the ascending order of the relative amounts of carbon dioxide in the air within the assimilation tube.

A preliminary experiment made with two similar leaves both in normal condition and in the same strength of carbon dioxide confirmed the trustworthiness of the method by giving practically the same decomposition of $CO_2$ per unit area for the two leaves.

In a second preliminary experiment (XXXIII.) one of a pair of opposite leaves was vaselined on the upper surface, the other leaf not at all. They both decomposed sensibly the same amount of $CO_2$, which shows that vaseline on this surface has no direct or indirect effect, provided that the stomata are left open, and so may be omitted in the control leaves in the rest of the experiments.

EXPERIMENT XXXIII.—Two opposite leaves of *Nerium oleander*. One vaselined on the upper surface only. The other leaf normal. 3½ hours' exposure. Sky variable.

| *Nerium oleander.* | Before exposure. Air + $CO_2$. | Volume after KHO. | $CO_2$ decomposed. | | Mean percent. of $CO_2$. |
|---|---|---|---|---|---|
| | | | Total. | Per hour and sq. centim. | |
| | cub. centims. | cub. centims. | cub. centims. | cub. centim. | |
| Normal leaf . . . . .<br>Vol., 0·7 cub. centim.<br>Area, 13·2 sq. centims. | 61·3 + 26·0 | 68·8 | 7·5 | 0·020 | 26 |
| Leaf vaselined above . .<br>Vol., 0·8 cub. centim.<br>Area, 12·4 sq. centims. | 62·1 + 27·0 | 69·0 | 6·9 | 0·019 | 26 |

The first column gives the condition of the leaves together with their respective volumes and areas; the second, the absolute corrected amounts of air and carbon dioxide introduced into the assimilation tube at the beginning of the experiment. After the stated exposure to bright light the leaf is withdrawn and the $CO_2$ still undecomposed is absorbed by introducing caustic potash. The volume of gas left on cooling after the action of the potash is given in the third column. The excess of this over the primary volume of air in the tube gives the total amount of oxygen formed by the assimilating leaf; this figures in the fourth column and in the fifth column it is reduced to a common unit of one hour and a square centimetre of leaf area. The sixth column contains the mean percentage of carbon dioxide present during the assimilation, and so gives us a sufficient method, though not so accurate a one as might be desired, for the comparison of the different experiments.

EXPERIMENT XXXIV.—Two opposite leaves of *Nerium oleander*, as equal as possible in size and colour, were selected; the lower surface of one was well greased with vaseline, rubbed gently into it with the finger. The other leaf was untreated. They were similarly exposed in gas tubes, side by side, in a south window from 11.15 A.M. to 4 P.M., the sun shining most of the time.

| *Nerium oleander.* | Before exposure. Air + $CO_2$. | After KHO. | $CO_2$ decomposed. | | Mean percent. of $CO_2$. |
|---|---|---|---|---|---|
| | | | Total. | Per hour and sq. centim. | |
| | cub. centims. | cub. centims. | cub. centims. | cub. centim. | |
| Normal leaf . . . . . .<br>Area, 20 sq. centims.<br>Vol., 0·9 cub. centim. | 67·8 + 5·9 | 74·2 | 6·4 | 0·07 | 4·0 |
| Vaselined leaf . . . . .<br>Area, 20 sq. centims.<br>Vol., 1·0 cub. centim. | 66·4 + 5·9 | 67·3 | 0·9 | 0·01 | 7·5 |

EXPERIMENT XXXV.—Leaves as in Experiment XXXIV. Exposure, 11.25 to 3.45. Day dull, with little sun.

| *Nerium oleander.* | Before exposure. Air + $CO_2$. | After KHO. | $CO_2$ decomposed. | | Mean percent. of $CO_2$. |
|---|---|---|---|---|---|
| | | | Total. | Per hour and sq. centim. | |
| | cub. centims. | cub. centims. | cub. centims. | cub. centim. | |
| Normal leaf . . . . . .<br>Area, 19·3 sq. centims.<br>Vol., 0·9 cub. centim. | 64·2 + 4·1 | 68·8 | 4·6 | 0·055 | 3·0 |
| Vaselined leaf . . . . .<br>Area, 19·3 sq. centims.<br>Vol., 1·1 cub. centim. | 64·2 + 7·3 | 65·1 | 0·9 | 0·01 | 9·6 |

EXPERIMENT XXXVI.—Leaves as in Experiment XXXIV. Exposure, 12.30 to 3.30. Sky variable.

| *Nerium oleander.* | Before exposure. Air + $CO_2$. | After KHO. | $CO_2$ decomposed. | | Mean percent. of $CO_2$. |
|---|---|---|---|---|---|
| | | | Total. | Per hour and sq. centim. | |
| | cub. centims. | cub. centims. | cub. centims. | cub. centim. | |
| Normal leaf . . . . . .<br>Area, 16·80 sq. centims.<br>Vol., 1·4 cub. centim. | 63·1 + 6·2 | 65·4 | 2·3 | 0·046 | 7·3 |
| Vaselined leaf . . . . . .<br>Area, 15·95 sq. centims.<br>Vol., 1·2 cub. centim. | 63·3 + 5·9 | 64·1 | 0·8 | 0·017 | 8·0 |

EXPERIMENT XXXVII.—Leaves as in Experiment XXXIV. Exposure, 11.30 to 3.30; behind a slightly ground glass plate. Continuous sunshine.

| *Nerium oleander.* | Before exposure. Air + $CO_2$. | After KHO. | $CO_2$ decomposed. | | Mean percent. of $CO_2$. |
|---|---|---|---|---|---|
| | | | Total. | Per hour and sq. centim. | |
| | cub. centims. | cub. centims. | cub. centims. | cub. centim. | |
| Normal leaf . . . . . .<br>Area, 20·99 sq. centims.<br>Vol., 1·0 cub. centim. | 64·1 + 14·7 | 79·2 | 15·1 | 0·18 | 9·3 |
| Vaselined leaf . . . . .<br>Area, 20·62 sq. centims.<br>Vol., 1·2 cub. centim. | 63·8 + 14·7 | 67·0 | 3·2 | 0·04 | 17·9 |

In all these the $CO_2$ decomposed by the normal leaf exceeds that decomposed by the vaselined leaf. In Experiments XXXVIII.–XL., made in stronger mixtures of carbon dioxide, this relation is reversed.

EXPERIMENT XXXVIII.—Leaves as in Experiment XXXIV. Exposure, 12 to 3.30. Much sunshine.

| *Nerium oleander.* | Before exposure. Air + $CO_2$. | After KHO. | $CO_2$ decomposed. | | Mean percent. of $CO_2$. |
|---|---|---|---|---|---|
| | | | Total. | Per hour and sq. centim. | |
| | cub. centims. | cub. centims. | cub. centims. | cub. centim. | |
| Normal leaf . . . . . .<br>Area, 22·43 sq. centims.<br>Vol., 1·08 cub. centim. | 36·6 + 40·4 | 40·1 | 3·5 | 0·043 | 50·2 |
| Vaselined leaf . . . . .<br>Area, 22·73 sq. centims.<br>Vol., 1·25 cub. centim. | 35·1 + 40·1 | 40·6 | 5·5 | 0·069 | 49·7 |

EXPERIMENT XXXIX.—Leaves as in Experiment XXXIV. Exposed behind ground glass; 11 A.M. to 3.15 P.M. Continuous sunshine.

| *Nerium oleander.* | Before exposure. Air + $CO_2$. | After KHO. | $CO_2$ decomposed. | | Mean percent. of $CO_2$. |
|---|---|---|---|---|---|
| | | | Total. | Per hour and sq. centim. | |
| | cub. centims. | cub. centims. | cub. centims. | cub. centim. | |
| Normal leaf. . . . . .<br>Area, 19·11 sq. centims.<br>Vol., 0·90 cub. centim. | 33·9 + 48·3 | 37·9 | 4·0 | 0·049 | 56·3 |
| Vaselined leaf . . . . .<br>Area, 18·45 sq. centims.<br>Vol., 1·00 cub. centim. | 34·1 + 47·0 | 39·4 | 5·3 | 0·067 | 54·7 |

EXPERIMENT XL.—Leaves treated as in Experiment XXXIV. Exposed in practically pure $CO_2$.

| *Nerium oleander.* | Before exposure. | After KHO. | $CO_2$ decomposed. | | Mean percent. of $CO_2$. |
|---|---|---|---|---|---|
| | | | Total. | Per hour and sq. centim. | |
| | | cub. centims. | cub. centims. | cub. centim. | |
| Normal leaf . . . . .<br>Area, 20·87 sq. centims. | Pure $CO_2$ | 4·1 | 4·1 | 0·033 | 98 |
| Vaselined leaf . . . . .<br>Area, 17·89 sq. centims. | Pure $CO_2$ | 6·4 | 6·4 | 0·060 | 96 |

In these three experiments, as in BOUSSINGAULT'S, the vaselined leaf decomposed more $CO_2$ than the normal leaf.

EXPERIMENT XLI.—Two leaves were both vaselined on the lower face and exposed at the same time, one in a low percentage of $CO_2$, the other in a high percentage.

| *Nerium oleander.* | Before exposure. Air + $CO_2$. | After KHO. | $CO_2$ decomposed. | | Mean percent. of $CO_2$. |
|---|---|---|---|---|---|
| | | | Total. | Per hour and sq. centim. | |
| | cub. centims. | cub. centims. | cub. centims. | cub. centim. | |
| Leaf vaselined below . .<br>Vol., 1·2 cub. centim.<br>Area, 20 sq. centims. | 50·4 + 32·2 | 53·3 | 2·9 | 0·036 | 37·2 |
| Leaf vaselined below . .<br>Vol., 1·3 cub. centim.<br>Area, 20 sq. centims. | 78·0 + 8·8 | 78·9 | 0·9 | 0·01 | 9·6 |

The result shows that with leaves that have their stomata blocked, the ordinary relation between the assimilation and the presence of superoptimal amounts of $CO_2$ around the leaves is reversed.

Tabulated below is a comparison of the relation given by these experiments of the assimilation of normal and vaselined leaves to the composition of the atmosphere in which they are placed.

TABLE IV.

| No. of experiment. | Absolute amounts of $CO_2$ decomposed per unit area. | | Ratio of the amounts of $CO_2$ decomposed per unit area. | | Mean percentage of $CO_2$ present in each experiment. |
|---|---|---|---|---|---|
| | Normal leaf. | Vaselined leaf. | Normal leaf. | Stomata blocked. | |
| | cub. centim. | cub. centim. | | | |
| XXXIV. | 0·07 | 0·01 | 1 | 0·14 | 6 |
| XXXV. | 0·055 | 0·01 | 1 | 0·20 | 6·3 |
| XXXVI. | 0·046 | 0·017 | 1 | 0·21 | 7·5 |
| XXXVII. | 0·18 | 0·04 | 1 | 0·37 | 14 |
| XXXVIII. | 0·049 | 0·067 | 1 | 1·3 | 55 |
| XXXIX. | 0·043 | 0·069 | 1 | 1·5 | 50 |
| XL. | 0·033 | 0·060 | 1 | 1·8 | 97 |

The behaviour of the ratios between the two amounts in each experiment is very obvious. As the fifth column shows, it continually increases till it becomes ten times as great. It might possibly be suggested that this was all due to the falling off of the assimilation in the normal leaf, as the $CO_2$ is increased above the optimum, while perhaps the assimilation of the leaf with its stomata blocked remained constant, or even also fell off, though very slightly. Experiment XLI. was specially designed to show that this is not so. Here, under the same conditions, a leaf in 37 per cent. $CO_2$ assimilated over three times as much as a leaf in 10 per cent. $CO_2$ when both had their stomata blocked.

After this it cannot but be clear that the whole phenomenon bears the interpretation that I have put upon it. A consideration of the absolute numbers given in the two other columns of the table quite supports this view. Of course, as the conditions of illumination, &c., varied for each experiment, only a general comparison can be made, but evidently the vaselined leaves XXXIV., XXXV., XXXVI. in low percentages of $CO_2$ decomposed only minute quantities of this gas, while those in high percentages—XXXVIII., XXXIX., XL.—averaged five times as much. It seems possible from the numbers, that within these latter leaves the amount of $CO_2$ was approaching the physiological optimum, which, with open leaves, occurs right at the other end of the scale of percentages.

A due consideration of this difference seems to bring out the relativity of that which we speak of as the *optimum* percentage of $CO_2$ for assimilation. The numbers experimentally obtained for this function really express the *external optimum* of carbon dioxide content—a number very convenient to know, but not of direct physiological significance. The *internal optimum* for the atmosphere surrounding the assimilating cells themselves must be a smaller number, which might well be constant for all cases. The external optimal number would then be a combined expression of this constant and a variable, which would depend on the structure of the leaf in question, and would be large for those leaves which, by reason either of the smallness or scarcity of their stomata, the narrowness of their intercellular spaces or the thickness of the leaf offered considerable resistance to the diffusion of carbon dioxide.

How variable the size and numbers of stomata may be, the more recent paper of Weiss (39) plentifully exhibits, and Unger (45) has shown that the volume of the intercellular spaces in leaves may vary from 3 per cent. to 71 per cent. of the total volume of the leaf. Whether all this variation really does produce a marked effect on the penetrability of leaves we cannot yet say.

In the vaselined leaves under consideration with their stomata blocked, carbon dioxide might reach the intercellular spaces round the assimilating cells by two possible paths, by diffusion through the cuticle and epidermis cells, or through the film of vaseline blocking the mouths of the stomata.

Experiment XXXIV. shows that only one-seventh as much $CO_2$ reaches the assimilating cells by the conjoint action of these paths as passes by the open stomata, and in a future section (VI.) I hope to give some facts tending to show what share each path takes.

Having thus fully cleared up the experiments of Boussingault's first class, which deal with the localization of gaseous exchange, we may turn to those of the second class, which really deal chiefly with the localization of the action of light on the leaf. Two expedients were adopted by Boussingault to effect, for the purposes of comparison, an isolation of the action of light on either surface of the leaf.

For one, a strip of opaque black paper was fixed by a very thin layer of starch paste to that surface of the leaf which he wished to remove from the action of light.

For the second, the two similar surfaces of a pair of leaves are fixed together, also by starch paste. If the two upper faces are joined together, it is assumed that the two lower only are exposed to the action of light, and *vice versâ*.

In all cases the leaves to be compared were exposed at the same time to light in separate vessels, containing known mixtures of air and $CO_2$, and the amount of $CO_2$ decomposed in a given time determined.

A control leaf, with neither of its surfaces covered with black paper, is employed at the same time, and is said to have both its surfaces exposed to light. The only satisfactory conclusion that these experiments do really bring out is that, *cæteris paribus*, a leaf assimilates more when light falls on its upper surface than when the light falls on the lower surface. With thin leaves this difference is only slightly marked, but with thick ones it is considerable; yet, of course, nothing approaching to a real separation is obtained, and the very conclusion drawn by the author that the sum of the amounts of carbon dioxide decomposed by the two faces acting "separately" exceeds that of the two faces acting together in one uncovered leaf, might have shown that the method was untrustworthy.

In all the assimilation experiments that he performed, Boussingault used high proportions of $CO_2$, and this is undoubtedly one of the several sources of error in those experiments of the second class in which leaves were pasted together in couples and compared. These untrustworthy experiments with their dubious conditions of illumination and diffusion, would have been passed over here had they not been recently particularly referred to as supporting the view that carbon dioxide is normally absorbed through the cuticle of the leaf. In one of these experiments (VII.) a pair of Oleander leaves, fastened together with starch paste by their upper surfaces, decomposed 12·7 cub. centims. $CO_2$, while a similar pair fastened together by their lower surfaces decomposed 18·3 cub. centims. This greater decomposition in the latter might be entirely due to the excess of $CO_2$ being kept off by the blocked stomata, as detailed above, or might be aided by the different faces illuminated in the two cases. How far, again, the result is militated against by the imperfect blocking of the stomata which must result when two leaves with such prominent mid-ribs are fastened together by their lower surfaces, can hardly be determined.

BOUSSINGAULT regarded his experiments, as a whole, as proving that the upper surface of a leaf is more active in *gaseous exchange* than the lower stomatic surface, but only Experiment VIII., the significance of which we have entirely reversed, ought to have been held to yield clear evidence on this point. Most of the experiments of the second class do not furnish any evidence on this point, but only as to the greater activity of the upper surface *in relation to light*. These two functions are widely different, but are mixed up together in his conclusions; while the localization of the latter of them still holds good, that of the former has been destroyed. Although in the introduction to this very paper BOUSSINGAULT states that DE SAUSSURE has determined that one-eighth or one-twelfth of $CO_2$ in an atmosphere forms the optimum for $CO_2$ decomposition, yet the state of this knowledge was hardly ripe enough for him to be expected to grasp the fatal bearing of it on most of his own results. One may be permitted to wonder, however, that the fallacy has remained for so long unexposed.

As a corollary to the experiments given in this section, mention must be made of the effect of exposing leaves that have their stomata blocked with vaseline to so small a percentage of carbon dioxide as that occurring in the atmosphere. Here the difference between the vaselined leaf and the normal leaf is very marked, and according to the usual phraseology, the vaselined leaf does not assimilate at all, that is to say, it forms no starch on exposure to bright light.

If a leaf with its stomata all on the lower surface be freed from starch by darkness and then, with half the under surface coated with vaseline, be exposed for a whole day in a good light, no starch will be found in this half, while the other has formed starch freely. Coating the upper surface has no effect on starch formation. I performed such experiments in the summer of 1893 and showed them to some botanists. Nearly at the close of my research, in July, 1894, STAHL (33) published similar experiments. He found a mixture of cocoa-butter and bees-wax preferable to vaseline. His observations are much more extended than mine, and he gives some interesting figures of the starch formation that takes place round wounds or openings in the upper epidermis under these circumstances. I obtained the best results with leaves of *Vitis*, *Polygonum sacchalinense*, and especially with *Sparmannia*. In its suitability for demonstration purposes this experiment should be of great service in dispelling the established English view as to the paths of gaseous exchange.

## Section VI.—*On the Artificial Diffusion of Carbonic Acid through the Living Leaf.*

With a view of obtaining more detailed information on the possible paths of gaseous exchange in leaves, I have performed some quantitative diffusion experiments on leaves of *Nerium*, which are specially suitable for such work since they are very strong, rarely damaged or perforated by insects, and quite devoid of stomata on the upper surface.

In previous sections, III. and IV., we have proved that normally, gaseous exchange takes place through the stomata. In Section V. we have shown that $CO_2$ diffuses into a leaf with its stomata blocked with vaseline when it is present in large quantities outside, but we have not yet decided by what path this takes place. So far we cannot say whether the cuticle is absolutely impervious to $CO_2$ or whether it is pervious, though but slightly.

The following experiment gives a clear answer on this point. A leaf of *Nerium* is carefully fitted up between the pair of oblong capsules. To the chamber on the upper surface, air freed from $CO_2$ by the potash tower is supplied and the current passing from it is led through one of the resistance bottles in the usual way. The tubes of the capsule attached to the lower surface of the leaf are, however, disconnected from the apparatus and a current of air containing a very high percentage of $CO_2$ (25 to 30 per cent.) is passed continuously through them over the lower surface of the leaf. This is accomplished in a way to be presently described. After both currents have been passing over the two surfaces for some hours in order that everything may become constant, the current from the upper surface which will contain any $CO_2$ that may diffuse through the leaf is switched into one of the absorption chambers. Everything is allowed to go on in this way for over twelve hours and then the amount of $CO_2$ that has diffused through the leaf in this time is estimated, and the exact strength of the current, rich in $CO_2$, going over the lower surface also determined. This latter cannot be determined by passing it through the absorption chamber of the apparatus, because it contains so much $CO_2$ that it would neutralize all the baryta in it in a few minutes and much $CO_2$ would escape unabsorbed.

The production and analysis of this current are conducted as follows. A large bottle holding a dozen litres is filled with air mixed with 30 per cent. of $CO_2$. It is closed above by a cork, with two glass tubes through it. Through one of these water slowly enters the bottle and drives out the gas through the other, which is connected with the capsule on the lower surface of the leaf. The water enters in a constant succession of drops, the rate being regulated by a capillary tube and a constant head of water. This may be kept up for a whole day without attention, but as the water entering will take up some of the carbonic acid, it is necessary to test the actual strength of the current passing the leaf. This is done by collecting the outflowing current from the capsule in a graduated tube over mercury, and determining the $CO_2$ by potash absorption. Such a determination is taken at the beginning and the end of the diffusion experiment, and the mean is taken to represent the strength of the gas on the lower surface of the leaf.

In one case this was 25 per cent. $CO_2$, and the current which was drawn over the upper surface was passed through the absorption chamber from 8.48 P.M. to 11.28 A.M. On titrating, 0·45 cub. centim. of baryta had been neutralized by $CO_2$, and the aspirator had run out 1275 cub. centims. of water. The amount of $CO_2$ in this current was therefore 0·02 per cent., or 0·016 cub. centim. of $CO_2$ had diffused through the 10 sq. centims. of leaf surface in each hour.

A similar experiment in which 31 per cent. $CO_2$ was supplied to the lower surface of the leaf gave a strength of 0·035 per cent. $CO_2$ on the upper surface, or 0·034 cub. centim. per hour traversing the leaf.

EXPERIMENT XLII.—Diffusion through the leaf of *Nerium oleander*.

| Percent. $CO_2$ on lower surface. | Duration of estimation. | Total $CO_2$ diffused. | Percent. $CO_2$ on upper surface. |
|---|---|---|---|
| | hours. | cub. centim. | |
| 25 | $14\frac{1}{3}$ | 0·225 | 0·02 |
| 31 | 19 | 0·655 | 0·035 |

The amount of $CO_2$ that is found on the upper surface has traversed the whole thickness of the leaf, but we may regard the stomata and intercellular spaces as offering very little resistance to diffusion compared to the actual passage through the substance of the upper epidermis and cuticle.

From these experiments on the living leaf we find that the thick cuticle and epidermis of the leaf of *Nerium* are not quite impervious to $CO_2$. Two deductions may be drawn from these experiments: one that, imagining the direction of diffusion reversed, the placing of a leaf with its stomata really blocked in an atmosphere of 30 per cent. $CO_2$ and in the light is hardly more generous from the point of view of assimilation than placing it in the ordinary atmosphere with its stomata open. The other, that when the upper surface of a leaf exhales $CO_2$ to this amount (0·035 per cent. of current) in the dark with its stomata blocked, the internal atmosphere of the leaf must contain nearly 30 per cent. $CO_2$.

To fully analyse the conditions of diffusion in leaves with stomata blocked by vaseline, we must also determine how much diffusion can take place through a film of vaseline.

For this purpose an experiment quite similar to the above was performed, except that in place of the leaf between the two capsules, there was a piece of filter paper most carefully saturated with vaseline and coated with a film of it about as thick as that applied to the leaves in Section V. The result was as follows :—

EXPERIMENT XLIII.—Diffusion through vaseline.

| Percent. on one surface. | Duration of estimation. | Total $CO_2$ diffused. | Percent. $CO_2$ on other surface. |
|---|---|---|---|
| | hours. | cub. centim. | |
| 40 | 13 | 0·825 | 0·065 |
| 35 | 7½ | 0·455 | 0·061 |

There is no doubt that the amount of $CO_2$ that had passed, really diffused (by osmosis) through the substance of the vaseline and not through some unvaselined holes in the filter paper, because the 40 per cent. $CO_2$ current was under a slight excess pressure (5 millims. of water) and if there had been the slightest porosity of the film a very much larger amount of $CO_2$ must have passed through. Vaseline then in thin films is much more pervious to $CO_2$ (*cf.* Appendix) than the cuticle of the leaf of *Nerium*. We see therefrom what caution must be employed in all inductions drawn from experiments on gaseous exchange when the stomata are blocked by vaseline. What is true for vaseline is more or less true also for other waxes and unguents (*cf.* Appendix). We now need to know something of the relative permeability of the *stomatic* surface of a leaf coated with vaseline and the normal uninterrupted cuticle. Information on this point can be obtained by estimating the $CO_2$ evolution from the two surfaces when the leaf is vaselined below and kept dark. This is carried out in exactly the same way as the respiration experiments detailed in Section III., only the lower surface of the leaf is carefully coated with vaseline some hours before the experiment is commenced.

EXPERIMENT XLIV.—*Nerium oleander.* Leaf coated on lower surface with vaseline on afternoon of May 31. Estimation made for the upper surface only.

| Date. | Total $CO_2$ from upper surface. | $CO_2$ evolved per hour. | Per cent. $CO_2$ in current. |
|---|---|---|---|
| | cub. centim. | cub. centim. | |
| May 31 to June 1, 8.30 P.M. to 10.30 A.M., duration 14 hours | 0·10 | 0·0035 | 0·006 |
| June 1–2, 11.40 A.M., to 12.40 P.M., duration 25 hours | 0·41 | 0·008 | 0·011 |
| June 2–3, 1.18 P.M. to 11.48 A.M., duration 22½ hours . | 0·31 | 0·007 | 0·009 |

The first estimation may be ignored as being too low, the $CO_2$ not having yet arrived at its full strength in the internal atmosphere of the leaf. Again, in a later experiment estimations of the $CO_2$ given off were taken on both surfaces of the leaf at once.

Experiment XLV.—*Nerium oleander.* The leaf coated with vaseline on its lower surface on August 16; estimations taken on both surfaces at once.

| Number. | Time. | Total $CO_2$. | $CO_2$ per hour. | Per cent. $CO_2$ in chamber. |
|---|---|---|---|---|
| | | cub. centim. | cub. centim. | |
| 1 | Aug. 18, $6\frac{2}{3}$ hours . . . . . . | $\frac{0\cdot055}{0\cdot160}$ | $\left.\frac{0\cdot008}{0\cdot024}\right\} = 0\cdot032$ | $\frac{0\cdot016}{0\cdot045}$ |
| 2 | Aug. 18–19, $17\frac{1}{2}$ hours . . . . | $\frac{0\cdot145}{0\cdot280}$ | $\left.\frac{0\cdot008}{0\cdot016}\right\} = 0\cdot024$ | $\frac{0\cdot015}{0\cdot055}$ |

In all the fractions the numerator denotes the upper surface of the leaf and the denominator the lower surface. The figures for the upper surface of the leaf agree very well and show definitely that in a leaf which has its normal outlet, the stomata, blocked up, part of the carbon dioxide of respiration passes out through the upper surface. In the normal leaf 0·002 cub. centim. per hour was the maximum amount of $CO_2$ that appeared to pass out from the upper surface; if, indeed, it be not all due to working errors. With the vaselined leaf the amount is too large to be attributed to error, and four times that from the normal leaf. This must be attributed of course to the greater strength which the $CO_2$ reaches in the internal atmosphere under these conditions. The smaller total respiration of the leaf (0·3 cub. centim. instead of 0·8 cub. centim.) is due presumably to the scarcity of oxygen within the leaf. The amount of $CO_2$ diffusing out above does not however rise to the ·02 per cent. which 25 per cent. $CO_2$ supplied to the lower surface of the leaf causes.

Experiment XLV. serves to convince us of that which the proving of the diffusibility of $CO_2$ through vaseline suggested, namely, that the lower surface is by no means rendered impervious by coating it with vaseline. About one-fourth (·020 cub. centim.) as much passes through the vaselined surface as passes through this surface with its stomata open (·078 cub. centim.) (*cf.* Table II., p. 525), and, which is most striking, the lower surface is even in this condition more permeable than the normal upper surface by some two or three times.

Injecting a leaf produces a somewhat similar effect. Thus a leaf of *Nerium* was injected with water by means of a vacuum pump, and when it was found by inspection that the air had been completely removed, it was wiped carefully dry and put up between the oblong capsules, its petiole being in water and the whole kept quite dark.

EXPERIMENT XLVI.—*Nerium oleander*. Leaf injected with water at 3.30 P.M.

| Duration of experiment. | Total $CO_2$. | $CO_2$ per hour. |
|---|---|---|
| 7.23 P.M. to 10.53 A.M., 15 hrs. 30 mins. . . . . . | cub. centim<br>$\frac{0 \cdot 065}{0 \cdot 61}$ | cub. centim.<br>$\left.\frac{0 \cdot 004}{0 \cdot 04}\right\} = 0 \cdot 044$ |

In the morning the leaf was still completely injected. In this leaf the normal diffusion through intercellular spaces can no longer take place. All the gases travel in solution, and the stomatal surface has no longer an immense advantage in the matter of gaseous exchange. So, though the total gas exchange is small, yet a tenth part takes place through the upper epidermis. Comparing the numbers obtained for relative output of $CO_2$ on the two surfaces with normal injected and vaselined leaves of *Nerium*, we have :—

$$\text{Normal } \frac{\cdot 002}{\cdot 078} \text{ cub. centim.} = \frac{1}{39}. \quad \text{Injected } \frac{\cdot 004}{\cdot 040} \text{ cub. centim.} = \frac{1}{10}.$$

$$\text{Vaselined below } \frac{\cdot 008}{\cdot 023} \text{ cub. centim.} = \frac{1}{3}.$$

We are led to conclude that the path of gaseous exchange is not, so to speak, a fixed specialized route, but depends on the balance of permeability. The permeability of the epidermis with its cuticle is small but fixed. If intercellular spaces filled with air and unblocked stomata, be the alternative path, then all the exchange goes that way, and at most 3 per cent. takes place through the upper epidermis. When the resistant water-logged stomata and intercellular spaces are the alternative route, then 10 per cent. of the $CO_2$ may pass through the upper epidermis. If vaseline take the place of water the resistance is increased again, and 33 per cent. may pass through the upper surface. That is to say, if we take the permeability of the continuous epidermis as unity, that of stomatic epidermis will be 39, falling to 3 when coated with vaseline.

This seems to give a clear conception of the balance of the possible paths of gaseous exchange. Were a leaf to consist of two surfaces of continuous epidermis without any stomata, its potentiality of gaseous exchange would be extremely limited and its respiration proceed with difficulty. I hope to carry out some experiments on such organs with continuous epidermis.

The question of gaseous exchange in assimilation is rather a different one. In this case the maximum difference of tension that can arise for carbon dioxide, between the inside and outside of the leaf, is fixed under normal circumstances and so minute (·04 per cent. outside and ·00 per cent. inside) that alternative paths do not come into play, and if the stomatal path be not open, practically no carbon dioxide enters the leaf.

## Section VII.—*Conclusion and Summary.*

In conclusion, but little can be added to what has been said in the previous section. It must be noticed that, throughout, only the carbon dioxide of the gaseous exchange, and not the oxygen, has been localized. Chemical analysis hardly permits us to attempt any such work with this latter gas. It can, however, hardly be doubted that its movement is normally limited to the stomata as much as that of carbon dioxide. For, on the one hand, it must theoretically diffuse more readily through small openings such as stomata, since its density is less, and on the other hand, it has been experimentally proved to pass through cuticle less readily than carbon dioxide.

The internal structure of leaves is generally in accordance with the view that all gaseous exchange takes place through stomata, for the intercellular spaces narrow towards the palisade cells, forming a sort of funnel with the wide end at the stomatal region. The active cause of the divorce which results in the palisade cells and stomata, whose united action is necessary for vigorous assimilation, being found on opposite surfaces of the leaf, is, of course, the double necessity for at once limiting transpiration and illuminating the assimilating cells.

Numbers are available which allow us to form a slight quantitative conception of the process of diffusion which must go on during active assimilation. From SACHS' paper "Die Ernährungsthätigkeit der Blätter" (24) we extract, that for a square metre of leaf, and an hour, the gain in weight by assimilation is in *Helianthus annuus* 1·88 grams. Reckoning this material as all starch, it necessitates an absorption of 3 grams. or 1530 cub. centims. of $CO_2$, that is, 0·15 cub. centim. of $CO_2$ traverse the surface of each square centimetre of the leaf per hour. Now, according to the measurements of WEISS (25) this leaf possesses 17,500 stomata in this area of the upper surface, and 32,500 on the lower. As regards diffusion through the leaf, the size of the stomatal slits is the important point; these have not been measured in this particular plant, but taking the mean measurements for other plants with similar stomata, we may assume the area of a single opening to be about 0·000077 square millim. the openings will then constitute 1·25 per cent. of the upper surface, and 2·5 per cent. of the lower. This adds up to a total opening of 3·75 sq. millims. into each sq. centim. of leaf area. Through this congeries of minute holes, 0·15 cub. centim. of $CO_2$—(the total $CO_2$ in 400 cub. centims. of air)—which is present in the atmosphere at a tension not exceeding one-third of a millim. of mercury (0·04 per cent. of 760 millims. Hg)—must diffuse every hour.

The passage of $CO_2$ into the leaf during assimilation is the most difficult and crucial of the four movements of gases, two of respiration and two of assimilation, that take place. In any of the other three the differences of tension between the inside and outside of the leaf may increase to almost any extent, while for this case the difference of tension never exceeds 0·3 millim. Hg. The cuticle of land plants is practically impenetrable to this small tension. Wetting the cuticle does not assimilate it in function at all to that of water plants, for it still continues impervious, as NAGAMATZ' (46) experiments show. He found that with submerged leaves of land plants which can be wetted, no starch formation is effected. I have found, however, that the cellulose-walled cells readily conduct their gaseous exchange under water. If a leaf (*e.g.*, *Ailanthus glandulosa*) be injected with water containing carbon dioxide, and be then placed in the sun, still under water, the water is rapidly driven out of the leaf, owing to the formation by assimilation of more oxygen than the water in the intercellular spaces can dissolve. If the leaf is kept in the dark it remains injected for days, and so also if it is injected with water carefully freed from $CO_2$ and then exposed to the sun.

Some of the errors into which BOUSSINGAULT and other writers on this subject have fallen, have been due to the use of too concentrated $CO_2$, which can at that tension diffuse through cuticle in sufficient quantity to give apparently normal phenomena. With approximately normal strengths of $CO_2$ no appreciable diffusion takes place.

I hope, shortly, to examine whether on parts devoid of stomata, gaseous exchange, especially in assimilation, is as vigorous as it seems generally held to be.

To sum up briefly my general results:

(1.) Under normal conditions, practically the sole pathway for $CO_2$ into or out of the leaf is by the stomata.

(2.) In young leaves the cuticle seems to be no more permeable to $CO_2$ than in mature leaves.

(3.) If the stomata be mechanically blocked an appreciable osmosis of $CO_2$ may take place through the cuticle provided that the tension of the $CO_2$ be great enough.

(4.) The normal amount of $CO_2$ in the atmosphere is not sufficient to produce any appreciable osmosis into a leaf with its stomata blocked; assimilation therefore cannot continue under these conditions.

(5.) The experimental *optimum* of $CO_2$ for assimilation depends on the structural porosity of the leaf, so that if this be reduced by blocking the stomata, even pure $CO_2$ may not quite effect optimal assimilation.

(6.) To this, and not to the stomata being inoperative in gaseous exchange (which was BOUSSINGAULT'S view), is due the fact that in concentrated $CO_2$ a leaf with its stomata open assimilates less than one with the stomata blocked.

(7.) In bright light a fully green leaf assimilates all the $CO_2$ that it is forming by respiration and none escapes from it. GARREAU'S demonstration to the contrary is only an expression of the imperfection of the conditions under which it was performed.

Finally I should like to express my thanks to Deputy-Professor DARWIN and other colleagues for putting my time as far as possible at my own disposal for the prosecution of this somewhat laborious research and for much other encouragement.

## APPENDIX.

### *On the Solubility of Carbon Dioxide in Oils and Waxes.*

In various modern scientific books, it is stated or implied that carbonic acid is insoluble in oils and waxes and will not diffuse through them.

In WATTS' 'Dictionary of Chemistry' (1883), in the article on "Diffusion of Gases," p. 819, it is stated that neither anise oil nor olive oil absorb carbonic acid. The common practice of covering alkaline solutions with a layer of paraffin oil, to prevent the absorption of carbonic acid from the air, largely implies this; as does also the use of olive oil in the academic experiment of placing water plants in a vessel of water freed from carbonic acid and covered with a layer of oil, in order to exclude atmospheric $CO_2$ and prevent the formation of starch. Further, in 1885, THORNER (47) introduced an apparatus for analysing mixed gases in which water is employed, and is only prevented from absorbing the gases, especially $CO_2$, under analysis, by a thin layer of olive oil. Physiological experiments on the respiration of fishes (48) have also been based on the use of a film of oil to separate the gases liberated in the water from those liberated above it. The employment of films of lard in experiments on gaseous exchange involved tacitly the same assumption.

Having had it forced upon me experimentally that carbon dioxide is soluble in oils, I experimented and found that every oil and wax that I tried absorbed considerable quantities of carbon dioxide, and that in some the gas was more soluble than in water.

I employed vaseline, lard, cocoa-butter, paraffin oil, and olive oil, introducing them into graduated tubes full of carbon dioxide over mercury, and noting the diminution of the volume of the gas.

Of the solids vaseline absorbs most $CO_2$, and cocoa-butter least. Both the oils dissolve over one-and-a-half times their own volume of $CO_2$ at atmospheric pressure and 15° C., but with olive oil the absorption takes place very slowly. A table of results (A) is appended.

As of biological experimental interest, I examined further the effect of a thin film of oil in preventing the passage of carbon dioxide above into water below it. This was also tried with two gas tubes full of $CO_2$ inverted over water; on to the surface of the water, in one of them, a layer of olive oil 4 millims. thick was passed up with a curved pipette. The level in this tube rose much slower than that in the other, but 6 cub. centims. of $CO_2$ had passed through the oil in the first 24 hours, while 74 cub. centims. had been absorbed by the free water surface in the other tube (*cf.* Table B). This result is opposed to that given by THORNER (*op. cit.*), who says that a layer of olive oil 2 millims. in thickness over water prevented any noteworthy absorption of pure $CO_2$ in 9 hours. Through a layer of paraffin oil the passage is still quicker.

This explains why in the practice of volumetric analysis an alkaline solution is found to slowly deteriorate, in spite of a supernatant layer of paraffin oil. In biological experiments it must also be allowed for.

The facts that I present are by no means new, but they seem to have quite dropped out of general knowledge, and, as seen above, have been in some cases denied. After my enquiries had been answered in the laboratory, I came upon an early clue in the literature, which led me to GEHLER's 'Physikalisches Handwörterbuch,' 1825, in which it is stated that PRIESTLEY discovered the solubility of $CO_2$ in olive oil, and another investigator determined it quantitatively for linseed and lavender oils. The former takes up 1·5 vols. of $CO_2$ at 18° C., and the latter 1·6 vols., while water only dissolves 1·0 vol. I also found that twenty years after this DRAPER independently determined that $CO_2$ would pass through oils, and that in his "Treatise on the Forces which Produce the Organization of Plants," published in New York in 1844, he uttered the same protest, which I desire to revive and extend, that "contrary to the apparent opinions of botanists, gases *can* pass through fixed oils, and even lamp oil, apparently in accordance with their solubilities." I append a table of my results, all determined at a temperature between 17° C. and 18° C.

TABLE A.

| Number. | Solvent. | Amount of $CO_2$ absorbed by 1 cub. centim. | Time required to effect saturation. |
|---|---|---|---|
| | | cub. centims. | |
| 1 | Water . . . . | 1·0 | Longer than 2. |
| 2 | Paraffin oil . . | 1·5 | A few hours. |
| 3 | Olive oil . . . | 1·6 | Over 48 hours. |
| 4 | Vaseline . . . | 0·9 | 30 hours, but with a much larger surface. |

TABLE B.—Rate of Absorption of $CO_2$ by Equal Surfaces of Water (3 sq. centims.)

| Duration of experiment. | Free surface. | Through a film of 7 millims. paraffin. | Through a film of 4 millims. olive oil. |
|---|---|---|---|
| | cub. centims | cub. centims. | cub. centims. |
| After 6 hours . . | 21 | 12·5 | 2·5 |
| ,, 24 ,, . . | 73 | 40·0 | 6·0 |
| ,, 48 ,, . . | .. | .. | 12·0 |
| ,, 72 ,, . . | .. | .. | 17·0 |

## BIBLIOGRAPHY.

1. SACHS. Handbuch der Experimentalphysiologie der Pflanzen, 1865, § 70.
2. —— Lehrbuch, 2nd English Ed., pp. 693 and 694.
3. —— Vorlesungen, English Ed., 1882.
4. PFEFFER. Pflanzenphysiologie, 1881, I., § 15.
5. HABERLANDT. Physiologische Pflanzenanatomie, 1884, Sect. 9, § 2.
6. VINES. Physiology of Plants, 1882, pp. 72 and 73.
7. DUTROCHET. Sur les organes aérifères des végétaux. Ann. Sci. Nat., S. I., T. XXV., 1832.
8. GARREAU. Rec. sur l'absorption et l'exhalation des surfaces aériennes des plantes. Ann. Sci. Nat., S. III., T. XIII., 1850.
9. BOUSSINGAULT. Étude sur les fonctions des feuilles, pp. 359–397. Agronomie, T. IV., 1868.
10. GRAHAM. Chemical and Physical Researches, 1829–1866. Collected by ANGUS SMITH. Edinburgh, 1876.
11. RAFFENEAU-DELILE. Evidence du mode respiratoire des feuilles. Ann. Sci. Nat., S. II., T. XVI., 1841.
12. EXNER. Über den Durchgang der Gase durch Flüssigkeitslamellen. Sitz. Wien. Akadem., Bd. 70, Abt. 2, 1874.
13. BARTHÉLEMY. Du rôle que joue la cuticule dans la respiration des plantes. Ann. Sci. Nat., S. V., T. IX., 1868.
14. FRÉMY. Comptes Rendus, T. XLVIII., p. 669, 1859.
15. BARTHÉLEMY. De la respiration et circulation des gaz dans les végétaux. Ann. Sci. Nat., S. V., T. XIX., 1874.
16. MERGET. Sur les fonctions des feuilles dans les phénomènes d'échanges gazeux; rôle des stomates. Comptes Rendus, February, 1877.
17. BOUSSINGAULT. Action délétère de la vapeur du mercure sur les plantes. Agronomie, T. IV., 1868, pp. 343–359.
18. BARTHÉLEMY. Du rôle des stomates et de la respiration cuticulaire. Comptes Rendus, March, 1877.
19. MERGET. Sur les échanges gazeux entre les plantes et l'atmosphère. Comptes Rendus, March, 1877.
20. —— Same title. Comptes Rendus, June, 1878.
21. MÜLLER. Über die Diffusion atmosphärischer Gase in der Pflanze. Pringsheim Jahrbücher, Bd. 17, 1870.
22. VON MOHL. U. über die Ursachen, welche das Offnen und Schliessen der Spaltöffnungen bewirken. Botan. Zeit., Bd. 56.
23. WIESNER. Ausgleich des Gasdruckes in den Geweben der Pflanzen. Sitz. Wien. Akadem., Bd. 79, Abt. 1, 1879.

24. Sachs. Ernährungsthätigkeit der Blätter. Arbeiten des Botanischen Instituts in Würzburg, Bd. III., 1884.

25. Weiss. U. über die Grössen- und Zahlenverhältnisse der Spaltöffnungen. Pringsheim Jahrbücher, Bd. 4, 1866.

26. Mangin. Sur la diffusion des gaz à travers les surfaces cutinisées. Comptes Rendus, November, 1887.

27. —— Sur la perméabilité de l'épidermis des feuilles pour les gaz. Comptes Rendus, November, 1888.

28. —— Re. sur la pénétration ou la sortie des gaz dans les plantes. Ann. de la Science Agronomique Française et Étrangère, 1888.

29. Boehm. Stärkebildung in den Blättern von Sedum spectabile. Botanisches Centralblatt, Bd. 37, 1889.

30. Wiesner and Molisch. U. über Gasbewegung in der Pflanze. Sitz. Wien. Akadem., Bd. 98, 1889.

31. Leitzmann. Permeabilität vegetabilisher Zellmembranen in Bezug auf atmosphärische Luft. Flora, 1887.

32. Boehm. Das Verhalten von vegetabilischen Geweben und von Stärke und Kohle zu Gasen. Botanische Zeitung, 1883.

33. Stahl. Einige Versuche über Transpiration und Assimilation. Botanische Zeitung, 1894.

34. Leitgeb. B. z. Physiologie der Spaltöffnungsapparate. Mitt. Graz, Bd. I., 1886.

35. Acton. Assimilation of Carbon from certain organic compounds. Proc. Roy. Soc., vol. 47, 1889.

36. von Hohnel. Über den Gang der Transpiration bei der Entwickelung des Blattes. Wollny Forschung, Bd. 1, 1878.

37. Merget. Rôle des stomates dans l'exhalation des vapeurs aqueuses. Comptes Rendus, August, 1878.

38. Darwin. On the relation between bloom and the distribution of stomata. Journal Linnean Soc., vol. XXII.

39. Weiss. Weitere U. über Spaltöffnungen mit Einschluss der eigentlichen Spalte derselben. Sitz. Wien. Akadem., Bd. 99, 1890.

40. Haberlandt. Schutzeinrichtungen in der Entwickelung der Keimpflanze. Wien, 1877, Section V.

41. Garreau. Nouvelles recherches sur la respiration des plantes. Ann. Sci. Nat., S. III., T. XV., 1851.

42. Corenwinder. R. sur la composition chimique et les fonctions des feuilles. Ann. Sci. Nat., S. VI., T. VI., 1878.

43. Godlewski. Abhängigkeit der Sauerstoffausscheidung der Blätter von dem Kohlensäuregehalt der Luft. Arbeiten des Botanischen Instituts in Würzburg, Bd. 1.

44. Pfeffer. Wirkung farbigen Lichtes auf die Zersetzung der Kohlensäure in Pflanzen. Arbeiten des Botanischen Instituts in Würzburg, Bd. 1.

45. Unger. Bestimmung der in den Intercellulargängen der Pflanzen enthaltenen Luftmenge. Sitz. Wien. Akadem., Bd. 12, 1854.

46. Nagamatz. Beiträge zur Kenntniss der Chlorophyllfunktion. Arbeiten des Botanischen Instituts in Würzburg, Bd. 3, 1887.

47. Thorner. Apparat zur direkten Analyse im Wasser gelöster Gase. Repertorium für Analytische Chemie, 1885.

48. Gage. Address on Physiology of Respiration. Nature, vol. 46, p. 600 (1892).

49. Gernez. Rs. sur les solutions gazeuses sursaturées. Ann. de l'École Normale Supérieure, 1875.