The methods employed furnish an estimate of the kinetics of synthesis of the structural protein of ribosomes.

We are indebted to R. M. Bock, F. H. Yin, and A. S. L. Hu for their advice and assistance in certain experiments.

* Published with the approval of the Director of the Wisconsin Agricultural Experimental Station. This investigation was supported in part by research grants from the U.S. Air Force Office of Scientific Research of the Air Research and Development Command (AF 49(638)-314), National Institutes of Health (E-1459), and the National Science Foundation (B-7150).

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*BIOCHEMICAL CONTROL OF STOMATAL OPENING IN LEAVES**

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Communicated by H. B. Vickery, July 25, 1961

In the course of previous studies on the role of glycolic acid in leaf metabolism, it was found that α-hydroxysulfonates are effective inhibitors of the enzyme glycolic oxidase.[1] Treatment of leaves with these substances resulted in rapid and extensive changes in the products formed during photosynthesis.[2, 3] More recently, visible physiological effects have been observed when tobacco leaves are treated under certain environmental conditions. When slightly wilted leaves were supplied with these glycolic oxidase inhibitors during high water stress, in bright sunlight, the water vapor lost by transpiration decreased with the result that such leaves regained their turgidity more rapidly than control leaves.

Most of the water transpired by leaves apparently escapes through the stomata.[4] The width of the stomatal apertures is controlled by osmotic forces in the kidney-shaped guard cells which surround these openings. When the guard cells are

filled with water and are turgid, the stomata are open; when the guard cells lose water and become flaccid, the stomatal apertures are greatly reduced in size.[5] The readily observable effect of glycolic oxidase inhibitors on the water relations of leaves suggested that these compounds rapidly affect the osmotic pressure within the guard cells and thus control the width of the stomata.

An investigation was therefore made of some biochemical aspects of the control of stomatal opening and the effect of stomatal closure on the transpiration of leaves in light. A standardized system for assaying the extent of stomatal opening in tobacco leaf disks was developed, and with this assay a number of metabolic regulators were tested for their ability to close the stomata. This study was facilitated by a new technique for measuring stomatal apertures quickly and accurately. In addition, the effect of artificially induced closure of stomata on reducing water loss by transpiration without affecting CO_2 assimilation during photosynthesis was studied.

Methods.—Measurement of stomatal apertures: The still unpublished technique described here was devised and shown to me by Joan Tucker, Department of Botany, University of Oxford, to whom I am most grateful. A viscous fluid silicone rubber ("RTV-11," Silicone Products Dept., General Electric Company, Waterford, N. Y.) which has been well stirred and then mixed with a sufficient amount of catalyst ("Silicure T-773") is gently spread with a spatula over the area of the leaf or leaf disk to be examined. Enough catalyst is added so that the fluid polymer is converted into a firm piece of rubber within 2 to 3 min. The silicone rubber, which is then peeled from the leaf surface, provides an accurate impression of the epidermis. The impression is dried briefly in a desiccator and is then painted with a thin film of cellulose acetate solution (commercial colorless nail polish diluted with an equal volume of acetone). When the film has dried, it is stripped off and examined under a microscope fitted with an ocular micrometer at a magnification of 1,000 ×. The epidermal cells, guard cells, and stomatal apertures are clearly visible on the film (Fig. 1).

Preliminary experiments with this method indicated that if a tobacco leaf were kept with its base in water in darkness for 1 hour, although narrow slits could almost always be observed, the apertures never exceeded 1 to 1.5 μ in width. If the tobacco leaf were then placed in sunlight in a humid atmosphere, within about 1 hr more than 90% of the stomatal apertures were 2μ in width or greater, and some apertures as wide as 10–12μ (Fig. 1) have frequently been measured. Based upon this experience, although all of the stomatal apertures measured were recorded, those openings 2μ in width or greater are considered to be "open," and those less than 2μ as "closed." The measurements are made, one in each of 100 microscope fields and, by counting the number of apertures 2μ or larger, the results can be conveniently expressed as "per cent stomata opened." With experience, this procedure requires only a few minutes.

The classification of percentage of stomatal apertures as "open" or "closed" correlates well with the mean stomatal width (Table 1); a mean stomatal width of about 1μ corresponds to 0% "open," and an average width of 4μ is approximately equivalent to 100% of the apertures "open."

The silicone impressions can be stored for many months and fresh films prepared for examination as required. As many as four successive impressions have been made in one day on the same area of a leaf still attached to the plant with no apparent damage.

Standard assay of stomatal closure in tobacco leaf disks: Leaves were taken from greenhouse grown tobacco plants (var. Havana Seed or Connecticut Shade). For stomata to open fully, I have found it to be essential that the plants constantly receive an abundant supply of water. A leaf, 7–12-gm fresh weight, is cut and its base placed in water in darkness for at least 1 hr to insure complete closure (in terms of the assay) of the stomata. A sufficient number of disks, 1.6 cm in diameter, are then cut with a sharp punch from the central region of the leaf, avoiding large veins. Disks chosen at random are placed in pairs right side up in 50-ml beakers. Exactly 3 min after the cutting of the disks commences, 5 ml of water or of the solution to be tested are added to the beaker. The disks are immersed in the fluid, and the beakers are placed in a water bath maintained at 24°. The disks float on the surface and are illuminated from above with

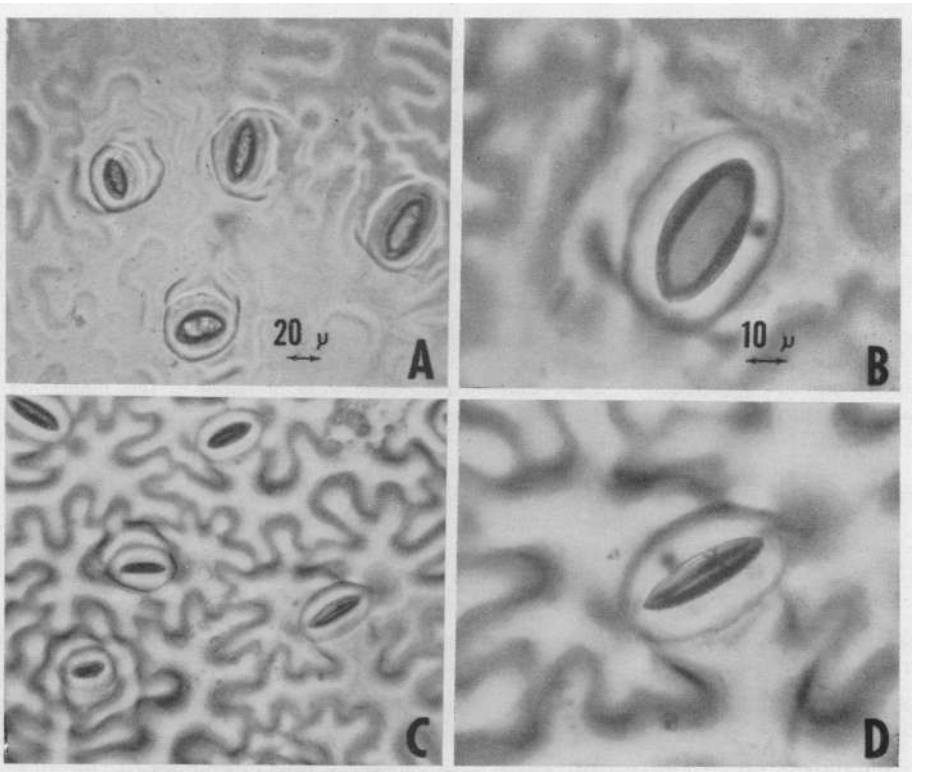

FIG. 1.—Photomicrographs of lower epidermis of tobacco leaf disks prepared from silicone rubber impressions. *A* and *B* are taken from cellulose acetate films prepared from impressions of a leaf disk floated on water under the conditions of the standard assay described under *Methods*. *A* represents a microscopic field viewed at 430 ×, and *B* is the stoma on the extreme right viewed at 1000 ×. *C* and *D* are analogous photomicrographs taken from a tobacco leaf disk floated on a solution of α-hydroxy-2-pyridinemethanesulfonic acid, and illustrate that "closed" stomata, according to the definition given under *Methods*, still have an aperture of 0.5 to 1 μ in width.

TABLE 1

EFFECT OF α-HYDROXYSULFONATES SUPPLIED THROUGH THE BASE OF THE LEAF ON STOMATAL OPENING AND TRANSPIRATION

	Experiment 1: 30 min			Experiment 2: 60 min		
	Leaf in water	Leaf in 0.01 *M* A*	Leaf in 0.01 *M* B*	Leaf in water	Leaf in 0.01 *M* A*	Leaf in 0.01 *M* B*
Initial leaf wt, gm	12.35	13.75	13.75	9.45	10.85	12.50
Wt change of leaf, gm	−0.10	+0.25	+0.80	+0.40	+0.75	+1.05
Fluid change, gm	−1.85	−1.90	−1.75	−3.50	−2.55	−2.70
Transpiration rate, gm H_2O per gm leaf per hr	−0.32	−0.24	−0.14	−0.33	−0.17	−0.13
Per cent stomata "open" zero time	4	0	2	4	12	10
Mean stomatal width zero time, μ	0.5	0.5	1.0	1.1	1.3	1.3
Per cent stomata "open" at end	58	6	14	96	18	12
Mean stomatal width at end, μ	2.0	1.2	1.4	4.3	1.5	1.4

* Compound A is α-hydroxy-2-pyridinemethanesulfonic acid, and B is sodium hydroxymethanesulfonate.

Tobacco plants were permitted to wilt slightly in bright sunlight before the start of the experiment. In each experiment, successive leaves from the same plant were chosen, and stomatal opening was determined from silicone rubber impressions prepared from a small area of the upper surface of each leaf before the leaves were excised at zero time. The leaves were weighed and placed in previously weighed beakers containing water or the solution to be tested. At the end of the experimental period, silicone rubber impressions of the upper surface of the leaves were again prepared, and the leaves and beakers were weighed separately to obtain the transpiration rates.

In Experiment 1, the leaves were maintained in an upright position in 4,700 ft-c of sunlight at 24° and 75% relative humidity for 30 min. In Experiment 2, the leaves were kept at about 3,700 ft-c of sunlight at 32° in high relative humidity for 60 min.

300-watt photoflood lamps that provide 4,000 ft-c at the upper surface. Mirrors placed below the beakers provide about 2,000 ft-c to the lower surface. A fan is fitted to blow air across the beakers so that the temperature of the fluid in contact with the leaf disks remains at about 25°.

At the end of the assay period, 90 min, the disks are blotted and impressions are made of the lower epidermis. Since it was found that stomata of tobacco leaf disks floated on water open at the same rate in the upper and lower epidermis, and there are 2 to 3 times as many stomata in the lower epidermis, it is more convenient to utilize the lower surface for the assay. The "per cent of stomata opened" is estimated by measuring 50 stomatal apertures from impressions made of each of the 2 disks. Control disks placed in water furnish a standard of comparison for test solutions, the stomata of the control disks being generally 80–100% "open." All assay results are expressed as "per cent of stomata closed" in comparison with these controls. This assay provides a measure of the effectiveness of chemical compounds in reducing stomatal opening.

Materials.—Of the α-hydroxysulfonates used, those containing 10 carbon atoms or less, and the bisulfite addition compound of 2,4-dichlorobenzaldehyde were prepared in this laboratory by reaction of the corresponding aldehyde with an excess of sodium bisulfite.[2] The compounds were recrystallized from aqueous alcohol and their purity was established by analysis for sodium. The acetylated hydroxymethanesulfonate, sodium acetoxymethanesulfonate, was prepared as described by Lauer and Langkammerer[6] and gave the correct analysis for sodium and saponification equivalent. The α-hydroxysulfonates with more than 10 carbon atoms, and the pyridine aldehyde derivatives used were purchased from the Aldrich Chemical Company. The plant growth regulators tested were commercial samples; each was recrystallized from an appropriate solvent before use and was found to have the correct melting point.

Results.—Physiological effects of α-hydroxysulfonates on whole leaves: The first indication that inhibitors of glycolic oxidase influence water relations was the observation that wilted leaves recover more quickly when a solution of an α-hydroxysulfonate was supplied through the base of sunlit tobacco leaves. These visible effects on turgidity were easily confirmed by measurements of the increase in weight of treated leaves. When the method for determining stomatal openings became available, it was possible to investigate the effect of α-hydroxysulfonates on stomatal behavior directly (Table 1).

Although most of the water vapor which diffuses from the plant moves through the stomatal apertures because they offer the path of least resistance, there is still a difference of opinion concerning the absolute relationship between stomatal opening and rate of transpiration.[4] From the water budget calculated from the experiments in Table 1, however, under conditions favorable for the opening of closed stomata in tobacco leaves, α-hydroxysulfonates supplied through the base of the leaf inhibited stomatal opening in turgid leaves with a concomitant reduction in transpiration of 25 to 60%. Treated leaves gained in fresh weight and became turgid more rapidly than control leaves. In experiments of 30 min duration, the amount of fluid taken up by an excised leaf was approximately the same whether it was placed in water or α-hydroxysulfonate solution. The stomata in treated leaves did not open as much, and such leaves transpired significantly less water. The action of α-hydroxysulfonates in closing stomata in whole leaves was exerted within 30 min, an observation which is reminiscent of the time scale of glycolic acid accumulation in intact leaves under similar conditions.[2, 3] It should be pointed out, however, that in Table 1 sodium hydroxymethanesulfonate appears to be more effective in reducing transpiration than the pyridine analogue. This is the reverse of the order of the relative reactivity of these two compounds in bringing about the accumulation of glycolic acid in sunlight in whole leaves,[2, 3] an observation which suggests that the guard cells are more responsive to chemical control

than is the mechanism which results in glycolic acid synthesis in the tissues of the whole leaf.

These results clearly demonstrated the feasibility of effectively reducing transpiration by artificially induced stomatal closure in bright sunlight, and stimulated a further investigation of this phenomenon.

Control of Stomatal Opening in Leaf Disks.—Effect of light: The disk technique provided a convenient method of obtaining uniform samples of leaf tissue for studying stomatal opening. When disks from leaves previously kept in the dark for 1 hr in order to close the stomata were floated on water and exposed to 4,000 ft-c of light, the stomata opened fully in about 90 min as shown in Figure 2. For this reason a 90-min period was used routinely in the standard stomatal assay When leaf disks with opened stomata were returned to darkness, the stomata closed within 30 min, and when brought into the light for a second time they opened again at the initial rate.

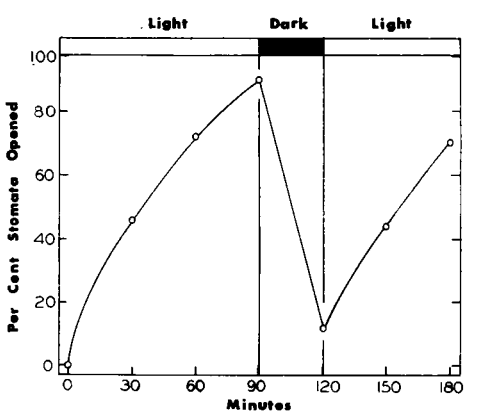

FIG. 2.—Effect of time and light on stomatal opening of tobacco leaf disks. Disks were floated on water under the conditions of the standard assay. During the dark period, the beakers containing the disks were covered with aluminum foil.

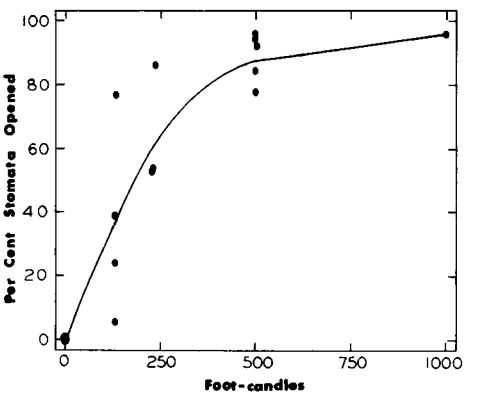

FIG. 3.—Effect of light intensity on stomatal opening of tobacco leaf disks. The various light intensities were obtained by covering the beakers used in the standard assay with various thicknesses of glass cloth. Each point at the same light intensity represents a different experiment with disks cut from another leaf. The results are expressed as percentages of similar observations on control disks kept at 4,000 ft-c.

Under the conditions of the standard assay, the stomata of leaf disks on water remained opened for many hours when provided with continuous light. In one such experiment, after 1.5 hr, 93% of the stomata were found opened; after 3 hr, 85%; after 5 hr, 81%; and after 10 hr, 85%.

The necessity for light in order for the stomata of tobacco leaf disks floating on water to open is striking, and attempts were therefore made to estimate the light intensity required to bring about stomatal opening under the conditions of the standard assay (Fig. 3). At light intensities of about 140 ft-c, leaf disks from different leaves varied in their response. In all experiments, the stomata were opened more than 50% at a light intensity of 250 ft-c, and at 500 ft-c almost maximal opening was achieved. The light intensities required to open stomata in

tobacco leaf disks are accordingly considerably lower than those necessary to obtain maximal rates of CO_2 assimilation in intact leaves.[7]

Disks half in light and half in darkness: I have also studied stomatal opening in tobacco leaf disks which were floated on water under the standard assay conditions except that one-half of each disk was completely shaded from the light from above and below with a light-shield constructed of two thin stainless steel plates. In one such experiment, disks completely in the dark had 0% stomata open at the end of 90 min; disks all in the light had 74% open; disks half in light and half in dark had 85 and 0% of the stomata open in the respective parts. Moreover, it was determined that within a distance of 1 mm from the line separating light from dark on the same disk receiving both treatments simultaneously, the stomata were either completely opened or closed.

Chemical control of stomatal opening in leaf disks: In light, under conditions otherwise favorable for the opening of stomata, a number of compounds were found to prevent closed stomata in tobacco leaf disks from opening (Table 2). In general, the effective compounds found thus far can be divided into several groups: certain α-hydroxysulfonates, which are known to be inhibitors of glycolic oxidase; some plant growth regulators; and several metabolically active metal-chelating compounds.

The results in Table 2 show that there is a great difference in the effect of stomatal closure in leaf disks by α-hydroxysulfonates depending on the length of the carbon

TABLE 2

EFFECTIVENESS OF VARIOUS COMPOUNDS ON STOMATAL CLOSURE OF TOBACCO LEAF DISKS

Compound	Per Cent Stomata Closed* 0.01 *M*	0.0033 *M*	0.001 *M*	0.0001 *M*
Sodium bisulfite	A	A	C	..
Na hydroxymethanesulfonate	C	..	D	..
Na acetoxymethanesulfonate	C	..	D	..
Na α-hydroxyethanesulfonate	C	..	..	..
Disodium sulfoglycolate	D	..	..	..
Na α-hydroxyhexanesulfonate	..	D	D	..
Na α-hydroxyoctanesulfonate	..	A	D	..
Na α-hydroxydecanesulfonate	..	A	A	D
Na α-hydroxyhexadecanesulfonate	..	..	D	..
Na α-hydroxyoctadecanesulfonate	..	..	D	..
Na α-hydroxy-2,4-dichlorobenzene-methanesulfonate	..	..	A	D
α-hydroxy-2-pyridinemethanesulfonic acid	..	A	B	D
α-hydroxy-3-pyridinemethanesulfonic acid	..	..	C	D
α-hydroxy-4-pyridinemethanesulfonic acid	..	..	D	D
Glycolic acid	C	..	..	..
Na 2,4-dichlorophenoxyacetate	..	A	B	D
Indole-3-acetic acid	D	..	..	..
Na 1-naphthaleneacetate	..	..	A	D
β-naphthoxyacetic acid	..	..	A	D
Phenoxyacetic acid	..	..	C	..
Methoxyacetic acid	C	..	..	..
2,4-Dinitrophenol			..	D
Sodium cyanide			C	D
8-Hydroxyquinoline sulfate			A	D
Na dimethyldithiocarbamate			D	D
Disodium ethylenediamine tetraacetate			D	D
Sodium azide			A	D

* A represents 100 to 76% stomatal closure, B is 75 to 51% closure, C is 50 to 26% closure, and D is 25 to 0% closure.

The compounds tested were supplied to tobacco leaf disks as described under *Methods* for the standard assay of stomatal closure.

chain. Of the various straight chain homologues of α-hydroxysulfonates tested, the most effective appeared to be the decanesulfonate.

Among the three substituted pyridine homologues examined, the relative order of decreasing effectiveness is substitution in the 2-, the 3-, and the 4-positions. This latter relationship is similar to unpublished results obtained earlier in this laboratory in experiments carried out on the accumulation of glycolic acid by detached leaves in sunlight.[2, 3] When 5×10^{-3} M solutions of each of the α-hydroxysulfonic acid derivatives indicated were supplied through the base of tobacco leaves, the glycolic acid found per gm of leaf at the end of 1 hr was 6.6 μmoles with the 2-, 1.4 μmoles with the 3-, and 1.1 μmoles with the 4-substituted derivative, respectively. Thus in this series, the effectiveness in stomatal closure is in the same order as the ability to block the glycolic oxidase reaction *in vivo*.

There are several reports in the literature on the effect of plant growth regulators on the closing of stomata. Ferri and Levy[8] have indicated that spraying an aqueous solution of β-naphthoxyacetic acid at a concentration of 1.5×10^{-3} M on nasturtium leaves, or using this solution to water the soil of potted plants, brought about stomatal closure and reduced transpiration as compared with control plants. Bradbury and Ennis[9] reported that dipping kidney bean leaves in solutions of 2,4-dichlorophenoxyacetate at concentrations ranging from 4×10^{-5} M to 4×10^{-3} M gave progressively increasing stomatal closure. As shown in Table 2, both of these growth regulators brought about large reductions in stomatal apertures in the standard tobacco leaf disk assay at approximately the same concentrations described by these workers. Naphthaleneacetate was also effective, but indole-3-acetic acid and phenoxyacetic acid were completely ineffective in this assay.

Some commonly used metabolic inhibitors, such as 2,4-dinitrophenol and cyanide, did not influence stomatal closure in the leaf disk assay, while the fungicide and metal-chelating compound 8-hydroxyquinoline sulfate, and the respiratory inhibitor sodium azide were highly effective, preventing stomatal opening at concentrations of 1×10^{-3} M.

The adequate reproducibility of the standard leaf disk assay, and its ability to discriminate between inhibitors of stomatal opening is illustrated in greater detail in Figure 4. From such dosage-response curves, it is clear that sodium azide will close 50% of the stomata in disks at a concentration of about 2×10^{-4} M, while α-hydroxy-2-pyridinemethanesulfonic acid brings about 50% closure at a concentration of approximately 1×10^{-3} M. The slopes of the dosage-response curves for these two compounds are evidently different, thus suggesting that they influence stomatal closure by interfering with different biochemical mechanisms, as might be expected. By comparison, as shown in Figure 4, about 0.68 M sucrose is required to prevent the opening of half of the stomata. These results indicate that the osmotic pressure in the guard cells, which is responsible for the increase in water in these cells during stomatal opening, must be approximately 15.2 atmospheres. The molar concentration of sucrose required is thus 680 times as high as the equally effective concentration of α-hydroxy-2-pyridinemethanesulfonic acid and 3,400 times greater than that of sodium azide under the same conditions.

Chemical control of closure of opened stomata: Since a number of the compounds tested could effectively prevent the opening of stomata in leaf disks under standard conditions, it became of interest to determine whether an active compound could

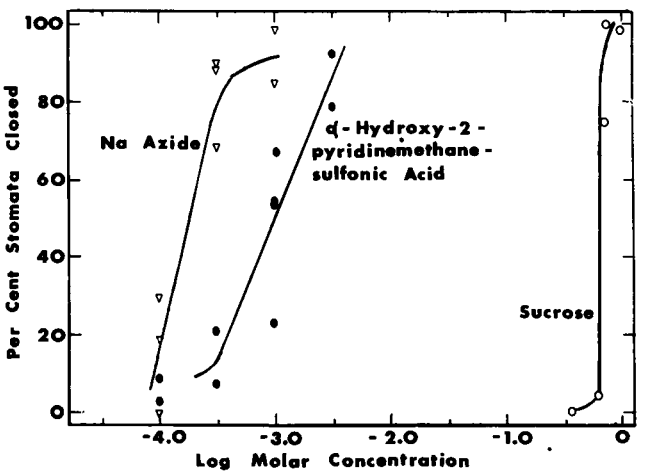

FIG. 4.—Effect of different concentrations of sodium azide, α-hydroxy-2-pyridinemethanesulfonic acid, and sucrose on stomatal closure of tobacco leaf disks. Each replicate point shown at a given concentration represents a different experiment with disks from separate leaves. The data were obtained under the conditions of the standard assay.

also close already opened stomata under environmental conditions otherwise favorable for them to remain open. The experiments shown in Table 3 clearly demonstrate that α-hydroxy-2-pyridinemethanesulfonic acid at a concentration which largely prevents stomatal opening (Fig. 4) also rapidly caused opened stomata to close.

Effect of stomatal closure on transpiration and photosynthesis in whole leaves: In previous experiments with $C^{14}O_2$ in which α-hydroxysulfonates were supplied through the base of the leaf in sunlight, CO_2 assimilation was inhibited about 33%.[3] Similar reductions in photosynthesis have now been observed in experiments in which a CO_2 gas analyzer was used. Such inhibitions probably arise from metabolic effects of these compounds on mesophyll cells and are unrelated to the effect of α-hydroxysulfonates on stomatal closure.

In order to test this view, I have attempted to close stomata by spraying α-hydroxysulfonates on both surfaces of leaves. Under these conditions the guard cells in the epidermis might be more readily influenced than the mesophyll tissue. Results are shown in Table 4. It is clear that completely reproducible stomatal

TABLE 3

CLOSURE OF OPENED STOMATA IN LEAF DISKS

Treatment	Per cent stomata open	Stomata closed as percentage of water control
Experiment 1		
90 min water	99	..
90 min water; 90 min water	82	..
90 min water; 60 min A*	12	86
90 min water; 90 min A*	4	95
Experiment 2		
90 min water; 90 min water	96	..
90 min water; 90 min A*	44	54

* A is 0.001 *M* α-hydroxy-2-pyridinemethanesulfonic acid.
The experiments were carried out under the conditions described in the text for the standard assay with tobacco leaf disks. In some treatments, after the usual 90 min, the disks were blotted and returned for another period as indicated in either water or α-hydroxysulfonate solution.

TABLE 4

EFFECT OF α-HYDROXYSULFONATE SPRAY ON STOMATAL APERTURES, TRANSPIRATION RATE, AND PHOTOSYNTHETIC RATE OF DETACHED TOBACCO LEAVES

Expt. no.	Initial wt of leaf, gm	Fluid sprayed	Mean width of stomata in upper surface at end, μ	Transpiration rate, μmoles H_2O per gm of leaf per hr	Photosynthetic rate, μmoles CO_2 per gm of leaf per hr	Ratio transpiration: photosynthesis
1	11.94	water	4.7	8,670	172	50
	11.87	0.0033 *M* A*	3.9	6,890	168	41
2	10.60	water	3.6	8,610	164	53
	9.33	0.0033 *M* A*	5.0	9,330	186	50
3	12.04	water	3.0	6,940	148	47
	11.50	0.001 *M* B*	2.3	5,510	172	32
4	12.22	water	2.9	5,610	189	30
	10.97	0.0033 *M* B*	1.1	1,110	181	6
5	11.23	water	1.0	8,390	173	49
	14.80	0.001 *M* A*	1.2	5,830	173	34

* Compound A is α-hydroxy-2-pyridinemethanesulfonic acid and compound B is sodium α-hydroxydecanesulfonate.

Adjacent tobacco leaves from the same plant were kept with their bases in water in the dark for 1 hr to insure closure of the stomata before the start of each experiment. Each leaf was then weighed, and a fine mist of the solution indicated was sprayed on both surfaces. The excess liquid was shaken off, and the base of the leaf was then placed in a small previously weighed beaker containing water. The experiments were conducted in a glass chamber, volume 15.5 liters, maintained at about 28° with high relative humidity, and illuminated with approximately 7,000 ft-c provided by 300-watt photoflood lamps immersed in a running water bath.[10] Normal air was always available to the leaf, and for one 5-min period out of every 15 min, an airtight glass cover was placed on the chamber. The air was continuously sampled with the aid of a circulating pump and passed through a Beckman infrared CO_2 analyzer fitted with a recorder, from which the rate of carbon assimilation was determined. At the end of the experiment, 60 min, except for Experiment 5 which lasted 90 min, the leaf and beaker were again weighed to determine the rate of transpiration, and silicone rubber impressions were simultaneously taken of the upper and lower surfaces of the central portion of the lamina in order to determine the stomatal opening.

closure cannot yet be attained by the procedure used with the compounds tested. However, in 3 of the 4 experiments of 60 min duration, significantly more of the stomata of the upper surface of the leaf were closed at the end in treated leaves than in control leaves (Experiments 1, 3, and 4). In these same experiments, stomatal apertures in the lower surfaces of all leaves were unaffected, and the water lost by transpiration in treated leaves was diminished by 20, 20, and 80%, respectively. In spite of the reduction in water loss, there was no significant reduction in CO_2 assimilation, so that the ratio of transpiration to photosynthesis was also lowered. In Experiment 2, there was an 8% increase in transpiration in the treated leaf, but the photosynthetic rate was also higher so that the ratio of transpiration to photosynthesis remained unchanged.

Experiment 5, Table 4, proceeded for 90 min, and although at the end of this period the stomata were "closed" in both the treated and control leaves, the photosynthetic rates were high, being about 20 times as great as the anticipated rate of oxygen uptake in darkness by the same tissue.[3] The treated leaf transpired 30% less water. The rates of CO_2 assimilation in both leaves were approximately equal during the entire 90-min period, and were constant after the first 15 min. Even at high light intensities, the rate of diffusion of CO_2 through the stomatal aperture clearly does not limit the rate of CO_2 assimilation.

Although the ideal α-hydroxysulfonate, or the optimal conditions for supplying it to leaves in order to obtain efficient and specific stomatal closure are still uncertain, the data in Table 4 demonstrate that at high light intensities it should be possible to reduce water loss of leaves without interfering seriously with their CO_2 uptake.

Discussion.—Heath[5] has recently pointed out that although more than a century has elapsed since the first investigation on the mechanism of the opening and closing

of leaf stomata was conducted, the only certainty is that, at least in many species, this process is controlled by the turgor differences between the guard cells and adjacent cells. Now that a simple standard system for controlling and measuring the opening of stomata in leaf disks is available, it should be easier to define more clearly the biochemical events responsible for these rapid turgor changes in the stomatal apparatus.

In the tissue studied here, the requirement for light and the rapidity of its action in bringing about stomatal opening have been most impressive (Figs. 2 and 3). From the results obtained with disks divided into segments receiving either a light or a dark treatment, it must be concluded that whatever may be the substance produced under the influence of light and which changes the turgidity of the guard cell, this substance must be generated either within or closely adjacent to the guard cell it affects.

There appear to be a number of possible ways in which metabolic regulators can influence stomatal closure in the light and bring about the closure normally observed only in the dark or during wilting. The induction of some alternative pathway of carbon metabolism could inhibit the formation of a solute essential for the increased turgidity of the guard cells. The effect of α-hydroxysulfonates in rapidly altering the products formed in photosynthesis in whole leaves,[3] suggests that these compounds operate in this way. The reactions by which chloroplasts in light produce adenosine triphosphate,[11] a substance essential for carbohydrate synthesis, may also be influenced. The effect of light in stimulating stomatal opening could be brought about directly or indirectly by this photosynthetic phosphorylation, and any method of interfering with the synthesis of adenosine triphosphate near the guard cells or of stimulating its breakdown might then cause stomatal closure. Darkness would of course also accomplish this result. The varied kinds of metabolic regulators (Table 2) which close stomata in leaf disks suggest that there are many biochemical approaches available for investigating the mechanism responsible for control of turgidity in the guard cells. This view is supported by the differing slopes in the dosage-response curves of compounds which would be expected to influence stomatal closure by affecting different biochemical pathways (Fig. 4).

With use of the present leaf disk assay, it should be possible to find even more effective compounds that could be applied to plants to control stomatal opening. In addition to the experiments in Table 4, which support the feasibility of reducing transpiration without affecting photosynthesis, the experiments of Moss *et al.*[12] on photosynthesis in corn plants under field conditions are relevant. These workers found that raising the concentration of CO_2 in the atmosphere around the plants diminished the stomatal apertures of corn leaves. At a CO_2 concentration of 575 ppm, for example, compared with plants at 310 ppm of CO_2, they observed a reduction in transpiration of 23% with a concomitant increase in CO_2 assimilation of 36%.

The CO_2 taken up in photosynthesis, in addition to passing through the stomatal aperture, must also diffuse through an aqueous phase of the mesophyll cell wall before it can react. As pointed out by Hill and Whittingham,[13] stomatal diffusion must therefore represent a much greater proportion of the total diffusion path in the case of transpiration, and stomatal control might be expected to result in a greater decrease of loss of water vapor than CO_2 uptake.

The possibility of conserving water normally lost through plant leaves by biochemically reducing stomatal apertures does not seem to have been considered seriously. The rapidity with which many compounds close stomata in leaf disks suggests that practical methods should be sought under which equally efficient control of stomatal apertures can be achieved under field conditions.

Summary.—The effect of α-hydroxysulfonates, inhibitors of glycolic oxidase, supplied to detached tobacco leaves in sunlight in markedly reducing the water lost by transpiration by stomatal closure is reported. This study was aided by a new technique for measuring stomatal apertures without altering the leaf environment by means of silicone rubber impressions made of the leaf surface. A standard assay for measuring the extent of stomatal closure by compounds supplied to tobacco leaf disks in the light is described. With this leaf disk assay, a number of metabolic regulators, including α-hydroxysulfonates, plant growth regulators, and metal-chelating compounds have been found to prevent stomatal opening at concentrations of about 1×10^{-3} *M*. One of the α-hydroxysulfonates has also been shown to close already opened stomata.

Evidence is presented that by inducing stomatal closure in intact leaves, it is possible to reduce water lost by transpiration at high light intensities without diminishing photosynthetic CO_2 assimilation. These studies offer a new approach to the conservation of water through the biochemical control of leaf stomata.

Grateful acknowledgment is made to Hubert B. Vickery for helpful discussion, to Dale N. Moss for cooperation in use of the CO_2 gas analyzer, and to Maria Borsanyi for skilful technical assistance.

* This investigation was supported in part by a grant from the National Science Foundation and was also aided by a Fellowship from the John Simon Guggenheim Memorial Foundation at the University of Oxford.

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