tained the following components: tris-HCl buffer, *p*H 7.8, $4.8 \times 10^{-2}M$; $MgCl_2$, $10^{-3}M$; inorganic phosphate (P_i), $10^{-3}M$ (containing P_i^{32}); adenosine diphosphate, $10^{-3}M$; and spinach chloroplast fragments. Chloroplast preparation, chlorophyll determination, and the adenosine triphosphate assay have been described (*5*). Anaerobic conditions were obtained by evacuation of the standard reaction mixture, flushing it with argon, and adding catalase and ethanol. All illuminations were at 2.6×10^4 erg cm^{-2} sec^{-1} and at 22°C. Spectra were determined with a Bausch and Lomb Spectronic 505.

Under anaerobic conditions the oxidized trimethylene dipyridyl, curve 1, Fig. 1, was photoreduced (Fig. 1, curves 2, 3, and 4). Admission of air after the reduction of the dye resulted in complete recovery of the spectrum shown in curve 1. This recovery was quicker than the recording time of the instrument employed. Thus, aerobically the reduced dye does not accumulate. In fact, one observes an oxygen uptake in a Mehler-type reaction in which hydrogen peroxide is formed, an indication that the dye is being reduced by way of the photochemical system and then reoxidized. Concurrent with these reactions, adenosine triphosphate is formed at the rate of 120 μmole per milligram of chlorophyll per hour.

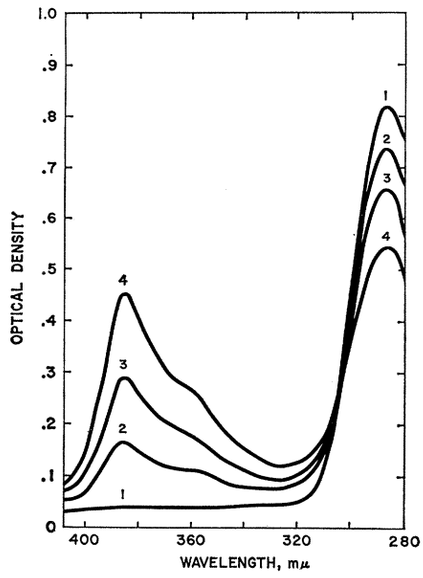

Fig. 1. Anaerobic photoreduction of 1,1′-trimethylene-2,2′-dipyridylium dibromide with spinach chloroplast fragments. Curve 1: zero illumination; curves 2, 3, and 4: after 5, 10, and 20 minutes of illumination, respectively. The standard reaction mixture contained 47 μg of chlorophyll per milliliter. The reduced peak is at 386 mμ and oxidized peak at 287 mμ.

Homer *et al.* (*6*) reported that the E'_0 of trimethylene dipyridyl is -549 ± 3 mv. The exact E'_0 was not determined in these experiments, but the following experiments indicate that the redox potential is lower than that of the hydrogen electrode near neutrality. Attempts to reduce the dye at *p*H 7 or 8 with hydrogen gas and palladium asbestos were not successful. At *p*H values above 10, reduced trimethylene dipyridyl accumulated with hydrogen gassing. Trimethylene dipyridyl was not reduced with hydrogen gas and *Clostridium pasteurianum* hydrogenase, although the hydrogenase readily reduced methyl viologen. After the reduction of trimethylene dipyridyl with the chloroplast photochemical system and addition of the following individual substances in the dark, one could observe their reduced forms spectrophotometrically: spinach ferredoxin; *Clostridium pasteurianum* ferredoxin; NADP; methyl viologen; benzyl viologen; and 1,1′-ethylene-2,2′-dipyridylium. The E'_0 of these compounds at *p*H 7.55 are -432, -417, -320, -446, -359, and -350 mv, respectively. If air was then admitted one observed the oxidized spectrum of each of the above-mentioned substances, with the exception of NADPH.

Homer *et al.* (*6*) have reported that the molar extinction coefficient (E_m) of oxidized trimethylene dipyridyl is 15,600 at 287 mμ. From the data in Fig. 1 the calculated E_m of the reduced trimethylene dipyridyl at 386 mμ is 25,000.

In other experiments under similar conditions no reduction of acridine dyes, E'_0 from -700 to -900 mv, was obtained; nor was oxygen evolved or taken up; nor was adenosine triphosphate formed. Indeed these dyes completely inhibited photophosphorylation catalyzed by methyl viologen in the range of 10^{-5} to $10^{-6}M$.

Thus plant chloroplasts can reduce substances with E'_0 at least 120 mv lower than that of spinach ferredoxin. The lower limit of the reducing capacity of illuminated chloroplast is unknown but appears to be above -900 to -700 mv since the acridine dyes were not reduced. It is relevant to note that in these studies we have only been successful in reducing trimethylene dipyridyl about 50 percent, whereas methyl viologen is completely reduced; this reduction indicates that -550 mv may be near the lower limit of chloroplast-reducing capacity at equilibrium. After the trimethylene dipyridyl is reduced with illuminated chloroplasts, oxidized ferredoxin and NADP may be added in the dark and the terminal reaction sequence (scheme 1) of photosynthetic electron transfer can be observed. There is also the possibility that some substance(s) in plant chloroplasts have an E'_0 lower than that of the hydrogen electrode.

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Psychrometric Measurement of Leaf Water Potential: Lack of Error Attributable to Leaf Permeability

Abstract. *A report that low permeability could cause gross errors in psychrometric determinations of water potential in leaves has not been confirmed. No measurable error from this source could be detected for either of two types of thermocouple psychrometer tested on four species, each at four levels of water potential. No source of error other than tissue respiration could be demonstrated.*

A fundamental requirement of psychrometric methods for determining leaf water potential (*1*, *2*) is that the leaf be brought to vapor pressure equilibrium with the small space in an equilibration chamber. A thermocouple psychrometer is used to measure the

Table 1. Comparison of water potentials (in bars) at four levels (A, B, C, D) determined with a Spanner psychrometer; either a 10- or 60-second cooling period was used (Ψ_{10} and Ψ_{60}, respectively). All results have been corrected for respiration.

	A		B		C		D	
	Ψ_{60}	Ψ_{10}	Ψ_{60}	Ψ_{10}	Ψ_{60}	Ψ_{10}	Ψ_{60}	Ψ_{10}
Pepper	−3.5	−3.4	−5.5	−5.5	− 9.2	− 9.3	−16.1	−16.1
Sunflower	−3.7	−3.9	−7.6	−7.4	−11.5	−11.6	−20.5	−21.0
Tobacco	−3.7	−3.9	−6.3	−6.4	−11.4	−11.6	−15.7	−15.6
Geranium	−2.1	−2.1	−5.5	−5.6	−10.9	−10.9	−15.3	−15.4

Table 2. Comparison of water potentials (in bars) at four levels (A, B, C, D) determined with either Spanner or Richards and Ogata psychrometers (Ψ_S and Ψ_R, respectively). All values include a correction for respiration. The corrections applied are given for the Richards and Ogata psychrometer only, and are enclosed in parentheses.*

	A		B		C		D	
	Ψ_S	Ψ_R	Ψ_S	Ψ_R	Ψ_S	Ψ_R	Ψ_S	Ψ_R
		(−0.5)		(−0.5)		(− 0.8)		(− 1.6)
Pepper	−4.3	−4.5	−6.0	−5.8	−12.2	−12.6	−16.0	−15.9
		(−0.6)		(−1.2)		(− 1.6)		(− 2.0)
Sunflower	−4.5	−4.8	−7.0	−7.2	−14.1	−13.8	−16.7	−17.2
		(−0.2)		(−1.3)		(− 1.8)		(− 1.4)
Tobacco	−3.6	−3.6	−6.1	−6.5	−10.5	−10.9	−13.1	−13.6
		(−0.4)		(−0.5)		(− 1.8)		(− 2.2)
Geranium	−3.3	−3.3	−5.1	−5.4	−11.6	−11.6	−14.4	−14.7

* The uncorrected Ψ_R value is the value in the table minus the corresponding value in parentheses, for example, for pepper $-4.0 = -4.5 - (-0.5)$.

water potential of this space, and it is assumed that the psychrometer does not alter the water potential within the equilibration chamber. Rawlins (*3*) has suggested that this may not be true, and he reported an error (his Table 2) of at least 60 percent in the determination of the water potential of pepper leaves when he used the Richards and Ogata psychrometer (*4*). This psychrometer has a permanently wet junction and, as Rawlins points out, water will distill continuously from the wet junction to the leaf, provided it is not fully turgid. If the leaf were to offer a significant resistance to water-vapor diffusion, the vapor pressure at the leaf surface would become significantly greater than that within the leaf. This would reduce the psychrometer reading and result in an underestimation of the water potential of the leaf (that is, give a value closer to zero than the leaf).

A psychrometer devised by Spanner (*1*) does not have a permanently wet junction. Instead, a water drop is formed anew by condensation after the passage of a Peltier cooling current through the psychrometer immediately before each reading. The quantity of water is very much smaller than that in the Richards and Ogata instrument, and it evaporates completely between readings. There is therefore no net transfer of water from the Spanner psychrometer to the leaf, and leaf water potentials should not be underestimated. However, leaf permeability could still affect the results if water vapor passes from the leaf to the chamber space when the wet junction is formed by cooling and condensation of water from the chamber space. Flow would be in the direction opposite to that considered by Rawlins and, with a sufficiently low leaf permeability, vapor pressure in the chamber space would become lower than that in the leaf, so that the leaf water potential would be overestimated. The possibility that this will occur is increased by the necessity to observe the maximum temperature depression due to the wet junction; this depression is fleeting and occurs shortly after the cooling current is passed. Waister (*5*) has reported satisfactory results from the Spanner psychrometer, and Barrs (*6, 7*) found it preferable to the Richards and Ogata psychrometer because the readings could more readily be corrected for an error due to liberation of heat by the respiring tissue into the equilibration chamber. Since these workers found the Spanner psychrometer useful, and Rawlins did not consider it in his report, it seemed worthwhile to establish whether leaf water potentials obtained by this psychrometer were measurably influenced by leaf permeability. This is the principal object of the present report.

If leaf permeability did influence readings of the Spanner psychrometer in the way suggested, then the error should increase with increase in the amount of water condensed out of the system by the cooling current. When the cooling current was passed for 60 seconds, approximately 5 to 7 times as much water was condensed out (depending on the actual water potential) as when a 10-second period was used. This was estimated from the ratio of the areas under recorder traces of the total outputs of the psychrometer from the moment when the cooling current was stopped until the wetted junction had dried completely. Observed wet-bulb depression was slightly lower when the cooling period was shorter so the psychrometer was calibrated over a range of water potentials for both cooling periods, and the appropriate calibration curve was applied subsequently to readings obtained with leaves.

Data were collected for four species: pepper (*Capsicum frutescens* L. 'Californian Wonder'), tobacco (*Nicotiana tabacum* L. 'Hicks'), geranium (*Pelargonium zonale* L. 'Paul Crampel'), and sunflower (*Helianthus annuus* L. 'Lange Grey'); the 10- and 60-second cooling periods were used. These plants were chosen because Rawlins (*3*) had considered the first two, Barrs (*7*) had previously reported an absence of effect of leaf permeability on psychrometric determinations of water potential in geranium, and sunflower water potentials have been measured psychrometrically by Ehlig and Gardner (*8*). Data were obtained for each species at four different water potentials since Rawlins had reported an influence of water potential on leaf permeability, permeability declining as water potential dropped. The range of water potentials was obtained by allowing detached leaves to dry in the laboratory for varying periods before they were put in the equilibration chambers. The results (Table 1), corrected for respiration (*7*), show excellent agreement between water potentials measured with either a 10- or 60-second cooling period for all species at all levels of water potential, from almost full turgor to severe wilt. This suggests that either a negligible quantity of water is transferred from the leaf during condensation onto the wet junction or that there is negligible resistance to the movement of the water.

Rawlins's conclusion (*3*) that low leaf permeability could lead to underestimates of leaf water potential (with the Richards and Ogata psychrometer) was partly based on data uncorrected

for tissue respiration. Since this correction (*7*) would have tended to reduce errors attributable to low leaf permeability, it was considered worthwhile to make corrected measurements. Comparison with similar measurements for the Spanner psychrometer would indicate the residual error attributable to low leaf permeability. Tests were carried out with paired samples from opposite halves of the same leaf, the samples being placed in separate chambers with one of the two types of psychrometer. The same four species were tested at approximately the same water potentials (Table 2). Each correction for respiration was made by combining the output of the dry psychrometer (with no water on the silver ring) with the normal output (with water on the silver ring), the required temperature difference of chamber dry-bulb minus chamber wet-bulb being given. The dry-bulb reading was obtained by removing the psychrometer from the chamber after a constant wet-bulb reading had been obtained, drying the silver ring, and replacing the dry psychrometer in the chamber. The pairs of corrected water potentials agree well, although not as closely as the values shown in Table 1. This is probably largely because observations were made on pairs of samples with different psychrometers, rather than on one sample with the same psychrometer. However, differences are similar in size to the experimental errors found by Waister (*9*) for the Spanner psychrometer (standard error for mean of duplicate samples less than ± 0.25 bars) and by Ehlig (*2*) for the Richards and Ogata psychrometer (less than ± 0.3 bars), and are randomly distributed. The results offer no evidence that the Richards and Ogata psychrometer underestimates water potential, provided a correction is applied for respiration, and Rawlins's conclusion that this instrument can be grossly affected by leaf permeability is not substantiated for the four species investigated.

Rawlins reported an error of at least 60 percent in the determination of the water potential of pepper leaves with the Richards and Ogata psychrometer, which he attributed to low leaf permeability. The results reported here do not support this explanation, and suggest an error of only 10 to 20 percent, which has been attributed to respiration effects. Since this leaves a possible further error of 40 percent unaccounted for, and our conclusions have been reached as a result of different types of experiment, it seemed worthwhile to try to establish whether the differing approaches adopted were responsible. Accordingly, Rawlins's pepper-plant experiment (*3*) was repeated. A pepper plant was grown for 3 months in nutrient solution, transferred to nutrient solution containing carbowax 4000 late one afternoon, and kept in the dark overnight prior to sampling. The low transpiration conditions would be expected to bring about approximate equality between water potentials in the leaves and in the culture solution. With the Spanner psychrometer, and with corrections being made for respiration to the leaf water potentials, values found were 10.2 bars for the nutrient solution and 9.6, 10.2, and 10.9 bars (average 10.2 bars) for three leaves. There was reasonable agreement between the two sets of data and no evidence of any large error in leaf water-potential measurement in addition to that of the respiration effect. Although there is no obvious explanation for the agreement reported here between leaf and solution water potentials, in contrast to Rawlins's result, it should perhaps be pointed out that the correction for leaf respiration can be much higher than the 10 to 20 percent (Table 2) when more than one layer of leaf tissue lines the equilibration chamber (*6*).

Thus for the four species examined, leaf permeability is not sufficiently low to affect determinations of leaf water potential when either the Spanner or the Richards and Ogata psychrometer is used. Good agreement exists between the two instruments provided corrections for respiration effects are applied. The results, from the culture-solution experiment, were not significantly affected by any additional residual errors, such as extraneous moisture sinks (*3*).

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Insulin: Inducer of Pyruvate Kinase

Abstract. *The hepatic pyruvate kinase activity markedly decreased when rats were made diabetic by alloxan injection. Insulin treatment induced new synthesis of pyruvate kinase which was prevented by injection of ethionine and actinomycin D. The evidence indicated that the increased pyruvate kinase activity induced by insulin entails at a certain stage a stimulation of the synthesis of certain RNA species relevant to the production of this enzyme.*

From the point of view of their strategic metabolic role and similar responsiveness to hormonal regulatory action, the four key hepatic gluconeogenic enzymes appear to be produced on the same functional genome unit (*1*). The evidence for their strategic role was presented by Krebs (*2*) and the regulatory responsiveness in terms of the action of adrenocortical hormone as inducer and insulin as suppressor of the biosynthesis of the key gluconeogenic enzymes was summarized (*1, 3*).

Another group of key enzymes, glucokinase, phosphofructokinase and pyruvate kinase, is thought to be rate-limiting for glycolysis (*1, 3*). These enzymes have low activities, govern one-way reactions and operate in the opposite direction to the function of the key gluconeogenic enzymes. The work of Weinhouse (*4*) and, subsequently, other investigators (*5*) indicated that liver glucokinase decreased in diabetic rats and was returned to normal by insulin injections. Since the rise in glucokinase activity was blocked by inhibitors of protein synthesis, it was concluded that the insulin-induced elevation of glucokinase activity was *de novo* enzyme biosynthesis (that is, synthesis of new enzyme) (*6*). Glucokinase was not affected by adrenocortical hormone treatment (*4, 6*). We suggested that the key glycolytic enzymes—glucokinase, phosphofructokinase, and pyruvate kinase—might occupy the same functional genome unit (*1, 3*), and in consequence a similar hormonal response was expected from all three enzymes. In order to subject this concept to experimental studies the behavior of pyruvate kinase was examined first under conditions similar to that reported for glucokinase. Our results show that hepatic pyruvate kinase