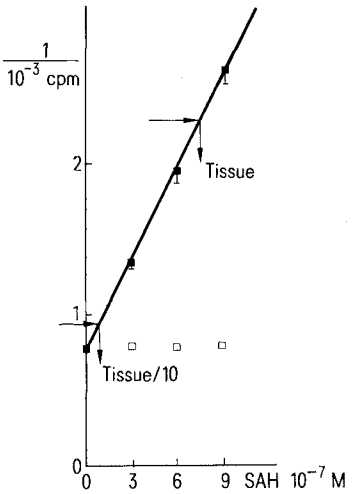
assayed for binding. Another aliquot was treated by SAH hydrolase/adenosine deaminase mixture and then assayed for binding. This 2nd aliquot was used as a tissue blank in order to show whether any endogenous product inhibited SAH binding; in the case of rat brain this blank was always zero, but it could be useful for tissues with a high level of SAM or where unknown interfering nucleosides might be present.

An internal standard curve performed with rat brain extract, where endogenous SAH had been removed, was identical with the external standard curve (results not shown).

The value of SAH level in rat brain was assayed using a concentrated (0.5 g/ml) or a 10-fold diluted (0.05 g/ml) homogenate; we observed respectively 1.52 ± 0.08 nmoles/g tissue or 1.66 ± 0.42 nmoles/g tissue. These values were compatible with the values previously determined by HPLC of 1 to 3 nmoles/g tissue[1,3].

In conclusion, the method described is more sensitive and specific than the previous methods because SAH binding protein (though undefined, and present in a crude extract) is as specific as any methylase; specificity could be further enhanced by using SAH hydrolase, the structural specificity of which differs from that of the binding. Our method is very rapid and 20-50 assays can be run simultaneously (in 1 day). The necessary materials, ^{3}H-SAH, SAH hydrolase and brain membranes (it is important to underline that calf brain can be used instead of rat brain) can be stored at least 3 months at $-20\,^\circ$C; thus, a large stock of the different materials can be prepared at 1 time. Finally, as we are presenting our results as a function of SAH concentration, the lowest measurable amount can be decreased using smaller volumes of reagents; for example, using a 100 µl final incubation volume, we measured 10 pmoles of SAH with satisfactory precision.



^{3}H-SAH binding to brain membranes in the presence of various amounts of cold SAH. 1 ml SAH solution was added to 100 µl ^{3}H-SAH (200,000 cpm per sample, $5 \cdot 10^{-9}$ M) and 900 µl brain homogenate. After 10 min incubation at 0 °C, the samples were filtered of GF/B glass filters and the radioactivity determined by liquid scintillation counting. Each point was the mean of 3 determinations ± SD. ■ Untreated assays; □ treated with SAH hydrolase (50 µl) + adenosine deaminase (4 units), 2 h at 37 °C. For tissue assay, brain tissue was homogenized in Tris buffer pH 7.4, 50 mM (0.5 g/ml) and the proteins were heat denatured (10 min at 100 °C). 1 ml aliquots were assayed: cpm = 438 ± 16 (3 determinations); other 1 ml aliquots were treated by SAH hydrolase, adenosine deaminase mixture and analyzed: cpm = 1240 ± 20 (3 determinations). In another experiment, the homogenate was diluted 10-fold; we obtained 1065 ± 14 cpm. The concentrations of SAH calculated from the radioactivity determination were 1.52 ± 0.08 nmoles/g tissue and 1.66 ± 0.42, respectively.

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A modified leaf chamber assembly to determine the rate of CO_2 exchange for upper and lower sides of leaves

G. Kulandaivelu, A.M. Noorudeen[1], P. Sampath[1] and S. Periyanan

School of Biological Sciences, Madurai Kamaraj University, Madurai-625 021 (India), August 25, 1981

Summary. Using a simple leaf chamber and a differentially calibrated IR gas analyzer the photosynthetic CO_2 uptake rate has been determined separately for upper and lower sides of a leaf.

The rate of CO_2 exchange in plants has long been studied using either entire plants or leaves. At the present date no literature is available about the measurement of the CO_2 exchange rate separately for the upper and lower sides of a leaf[2,3]. Fluorescence induction studies indicate that the photosynthetic efficiencies of the upper and lower sides of a leaf differ significantly[4]. In this communication we describe a modified leaf chamber IR gas analyzer system to determine the CO_2 exchange rate separately for the upper and lower sides. The CO_2 exchange response in this system was relatively faster than those seen for whole leaves or seedlings.

Phaseolus mungo L. and *Ipomoea pentaphylla* Jacq. seedlings were grown in pots under field conditions. All experiments were performed on fully expanded leaves. The leaf chamber was bell shaped (25 mm diameter and 30 mm in height) with an air inlet and outlet (fig.). The broad mouth had a soft rubber ring to ensure complete sealing against air leakage when fixed on the leaf. The chamber was placed on one side of the leaf, the other side of which

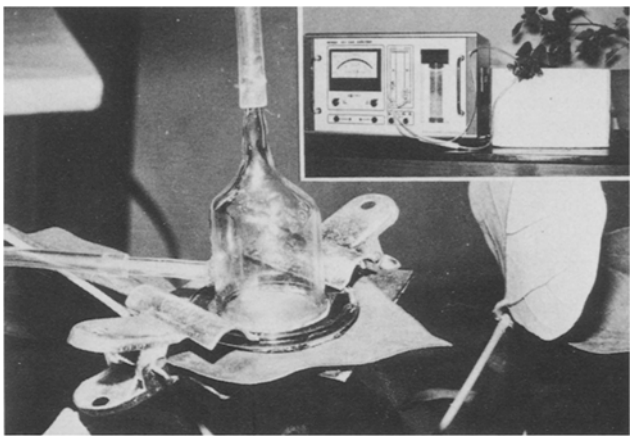
The stomatal frequency and rate of CO_2 uptake for upper and lower leaf sides of 3 plants. Average of 5 measurements

Species	Leaf side	Number of stomata/mm^2	mg $CO_2 \cdot dm^{-2} \cdot h^{-1}$	mg $CO_2 \cdot 10^5$ stomata $\cdot h^{-1}$
Phaseolus	Upper	56	60.4	10.79
	Lower	85	56.1	6.60
Ipomoea	Upper	66	53.9	8.17
	Lower	84	51.8	6.17
Morinda	Upper	7	10.7	15.29
	Lower	103	82.0	7.96

was supported by a black rubber pad, and fixed tightly by means of 2 clips. The air outlets from the leaf chamber were connected to the shorter analyzing cell of a differentially calibrated[5] IR gas analyzer (Series 225, Analytical Development Co. Ltd, U.K.). The rate of CO_2 exchange was monitored continuously on a recorder. Saturating white light was provided by two 100-W reflector lamps. A 5-cm water filter was placed in front of each lamp to eliminate heat. The temperature inside the leaf chamber was kept at 30 ± 2 °C during the measurements.

Typical values for the rate of net photosynthetic CO_2 uptake for the upper and lower leaf sides of several plants are given in the table. The stomatal frequency in these plants varied greatly. Among the plants studied, *Morinda* leaves showed the maximum difference between the upper and lower sides in the number of stomata. This wide variation in stomatal frequency was also reflected in the rate of net CO_2 uptake, calculated on the basis of unit leaf area. Contrary to this, in both *Phaseolus* and *Ipomoea* the rate of CO_2 uptake in the lower leaf side was less than the value for the upper sides. Since CO_2 exchange in such systems is mostly through the stomata, to obtain the true photosynthetic value we calculated the rate of CO_2 uptake on the basis of the number of stomata. This clearly demonstrated lower photosynthetic rates in the lower sides of the leaf in all the plants studied.

Based on the modification of fluorescence induction kinetics Schreiber et al.[4] have suggested that the photosynthetic capacity of the lower side of the leaf is less than that of the upper side. Our direct CO_2 measurements support this suggestion.



Modified leaf chamber to measure the rate of CO_2 exchange in upper and lower leaf sides. Inset shows the complete experimental set-up.

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