Modeling Rubisco Reaction with a New Two-Substrate Ordered Model with a Rate-limiting Step.

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Introduction: Oxygenic life begins with photosynthesis. This process controls CO_2 fixation and is responsible for the terrestrial and marine life on the planet. An increase in the global CO_2 concentration would have feedbacks on atmospheric temperature, water holding capacity of the atmosphere, and worst of all a decrease in the CO_2 holding capacity of oceans, all of which lead to accelerated climate change.

Rubisco is the gatekeeper of the natural process of CO_2 sequestration from the atmosphere. The fundamental model of Rubisco kinetics must be sound in order to support all subsequent theories and models that depend on Rubisco kinetics.

Three Kinetic Theories for Rubisco reaction have been discussed over the past three decades:

- Co-limitation Theory (Michaelis-Menten)
- Two-Process Theory (Farquhar, von Caemmerer & Berry, 1980)
- Two-Step Theory (Farazdaghi, 2004)

Of which, the Two-Process theory has been the most widely used.

Co-limitation Theory (Michaelis-Menten): Limitation of substrate at low substrate levels, limitation of enzyme at substrate saturation and co-limitation of enzyme and substrate for intermediate substrate levels.

Critique: This type of model has been experimentally shown to be effective for transitional reactions but inadequate in describing steady state two-substrate ordered reactions. Farazdaghi (2004, www.farazdaghi.com) developed a model for enforcing the effect of the limiting step on the kinetic parameters of two-step reactions.

Two-Process Theory (Farquhar, von Caemmerer & Berry, 1980): This model assumes that carboxylation is limited by two independent processes: by RuBP saturated Rubisco at low CO_2 and by RuBP regeneration rate at high CO_2. A convexity factor represents a small co-limitation. A limitation of TPU is also added to the plateau of the model.

Process 1: Low CO_2

Analysis: According to Blackman, when a factor becomes limiting the rate of reaction stops responding to any other factor, until the limitation is lifted. Thus if photosynthesis is limited by Rubisco at CO_2 compensation point (Γ), then photosynthesis remains ZERO at all levels of CO_2 beyond Γ.

Conclusion: The mixed kinetics of this theory are contradictory and logically do not allow for net photosynthesis to increase above zero, leading to a discontinuation of all life.

Process 2: High CO_2

Analysis: RuBP regeneration is assumed to be a function of total electron transport with a maximum of Jmax. But contrary to this assumption, we observe:

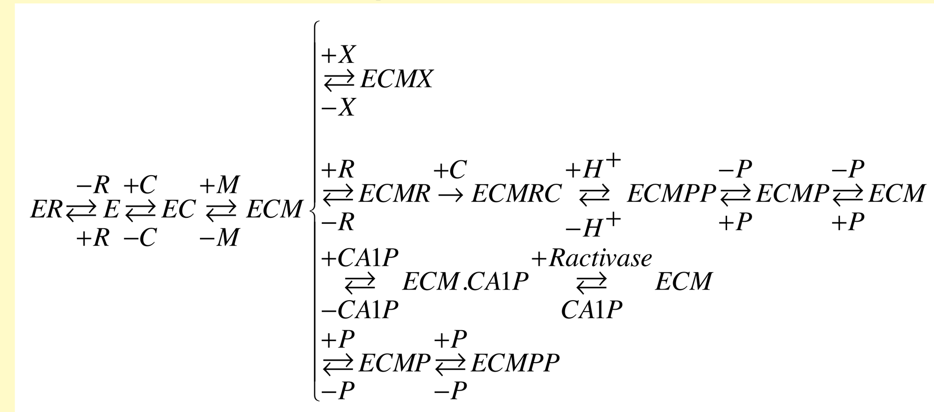
1) RuBP regeneration uses only a small fraction of the electron transport system energy, while the Jmax equation assumes all the energy is used for RuBP regeneration.
2) It is clear from the Calvin Cycle that RuBP is regenerated to replace the RuBP that has been used in Enediol biosynthesis. Therefore energy consumed in RuBP regeneration is equal to the initial amount of energy stored in RuBP (i.e. the RuBP which was released from Rubisco by Rubisco activase). Thus the energy used for RuBP regeneration is cancelled with the energy input of the initial RuBP and the net effect of RuBP regeneration in Calvin cycle will be zero.

Conclusions:

1) The energy used for steady state RuBP regeneration is not a factor in the electron transport equation.
2) Jmax in this theory cannot be a valid construct.

The total steady state electron transport is used for net CO_2 fixation and other metabolic demands, not for RuBP regeneration.

Two-Step Theory, Farazdaghi, 2004: The Rubisco reaction has two distinct kinetics. The transitional reaction follows a single step Michaelis-Menten model and the steady state reaction follows an ordered two-substrate, two-step, reaction model.



Where, E=Rubisco, R=RuBP, C=CO_2, M=Mg^{++}, X=XuBP, P=PGA.

Synopsis:

Experimentally, four Phases of Rubisco Reaction are observed:

1) Pre-Activation: In darkness Rubisco acts as Storage/Sink for RuBP. Other sugar phosphates like CA1P also inhibit Rubisco.

2) Activation: In light Rubisco Activase frees Rubisco, facilitates its carbamylation with CO_2 and stabilization with Mg^{++}.

3) Initial Transitional Reaction:

i) Active Rubisco reacts with the RuBP that has been released from Rubisco and after deprotonation produces Enediol.

ii) Enediol reacts with CO_2 and O_2 competitively.

Conclusion: Enediol is the true carboxylase/oxygenase; all the reactions are sequential, therefore they constitute only one step; Michaelis-Menten should describe its kinetics, and maximum velocity (Vcmax) is controlled by Enediol capacity.

4) Steady State Reaction: The initial capture of CO_2 and its reaction with Enediol continues with a number of small but sequential steps such as hydration, carbon-carbon cleavage and product (PGA) release.

Steady State Carboxylation requires energy (NADPH and ATP) for steady removal of PGA for the release of Rubisco and partly for RuBP regeneration and Rubisco activase that participate in the biosynthesis of Enediol. Thus, Activation State of Rubisco is controlled by the activities of Calvin cycle and Rubisco Activase.

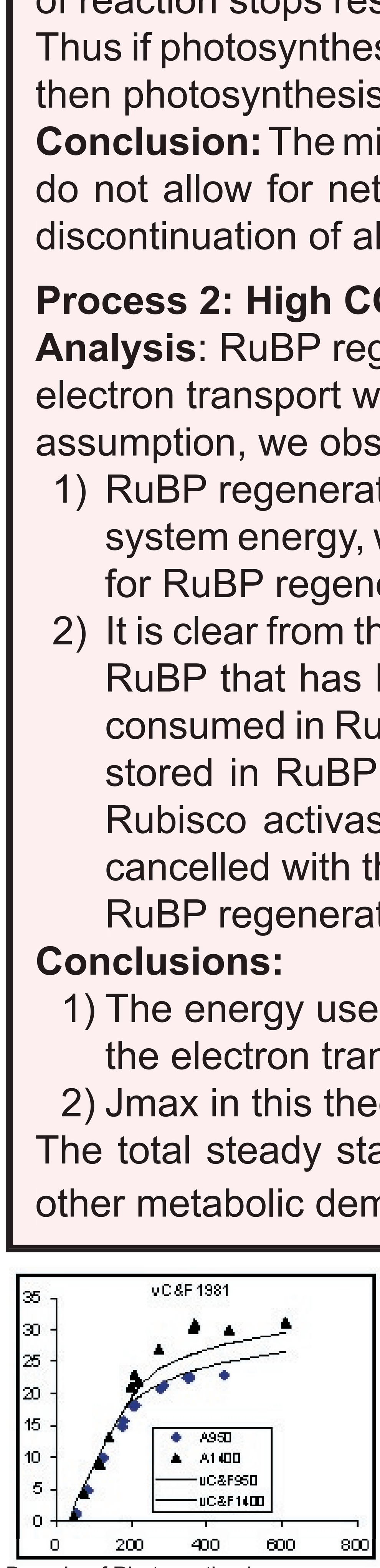
Steady State Carboxylation also requires continuous supply of Enediol, which in turn needs Rubisco. But Rubisco is concurrently distributed in both enediol synthesis and carboxylation steps. Therefore, the reaction has two main steps but its rate is determined by the velocity of the limiting step and the capacity of readily available enzyme that is controlled by a combination of Calvin cycle and Rubisco activase and limited by the capacity of Rubisco.

Since steady state Rubisco activation state is primarily dependent on the Calvin cycle for the removal of PGA and Rubisco release, any limitation on the Calvin cycle by any factor (e.g. electron transport or RuBP regeneration) will have a negative feedback on the Rubisco reaction.

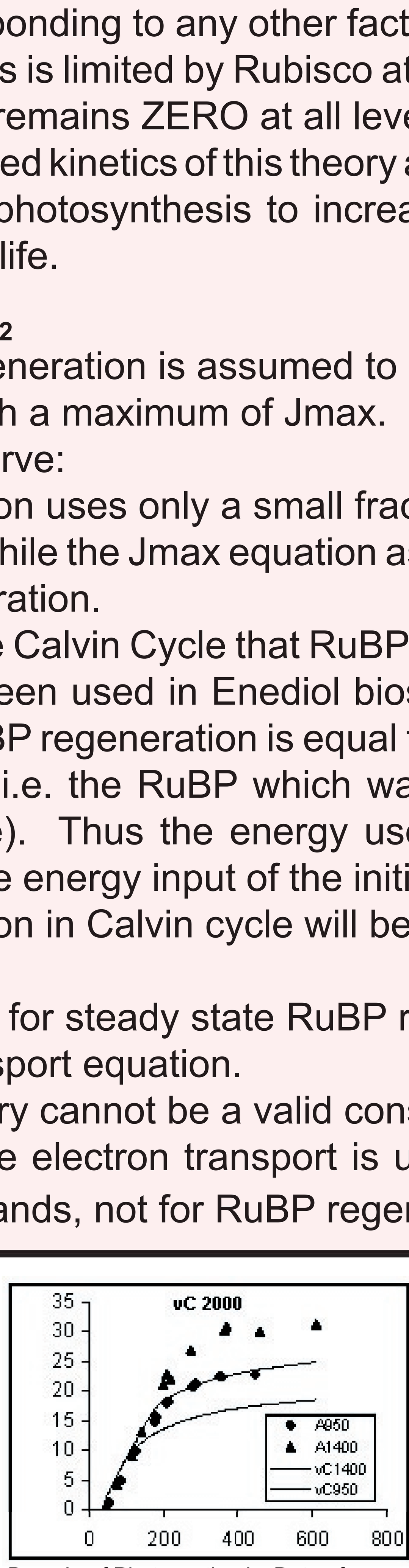
Following the presentation of the two-step model (Farazdaghi, 2004, 2005), in a sharp departure from the Rubisco-limited theory, Tcherkez, Farquhar and Andrews (2006) gave a theory that "all ribulose bisphosphate carboxylases may be nearly perfectly optimized."

This led to a new "kinetic model" by McNevin, von Caemmerer & Farquhar (2006). The new model is expected to calculate estimates of values of kinetic constants based on co-limiting model of Michaelis-Menten. This model is subject to the same limitations enumerated by Collatz, Berry, Farquhar & Pierce (1990), who in response to the co-limiting model of Farazdaghi & Edwards (1988) pointed out that: "This problem (co-limitation) and the absence of a strong mechanistic basis caused FvC&B (Farquhar, von Caemmerer & Berry 1980) and others (...) to abandon approaches based on kinetics of a single enzyme."

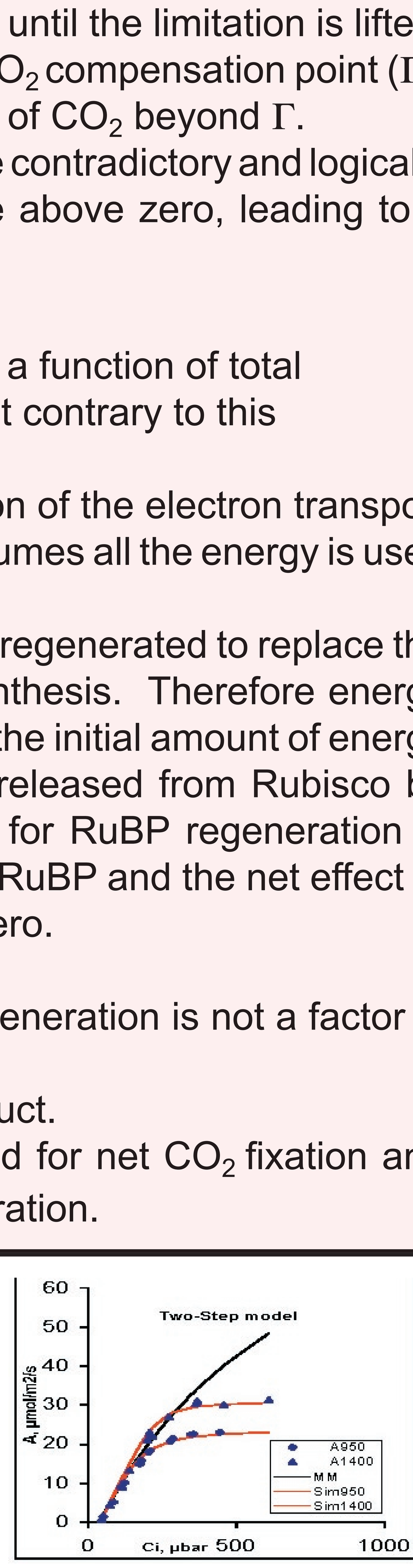
Conclusion: Michaelis-Menten type models are not equipped with a mechanism for enforcement of the effect of the rate-limiting step. The model of Farazdaghi (2004) has provided the required mechanism. Its application to the carboxylation data and its comparison with the Two-Process model is self-explanatory from the graphs. The equations for the two-step model of Farazdaghi (2004) can describe the system efficiently.



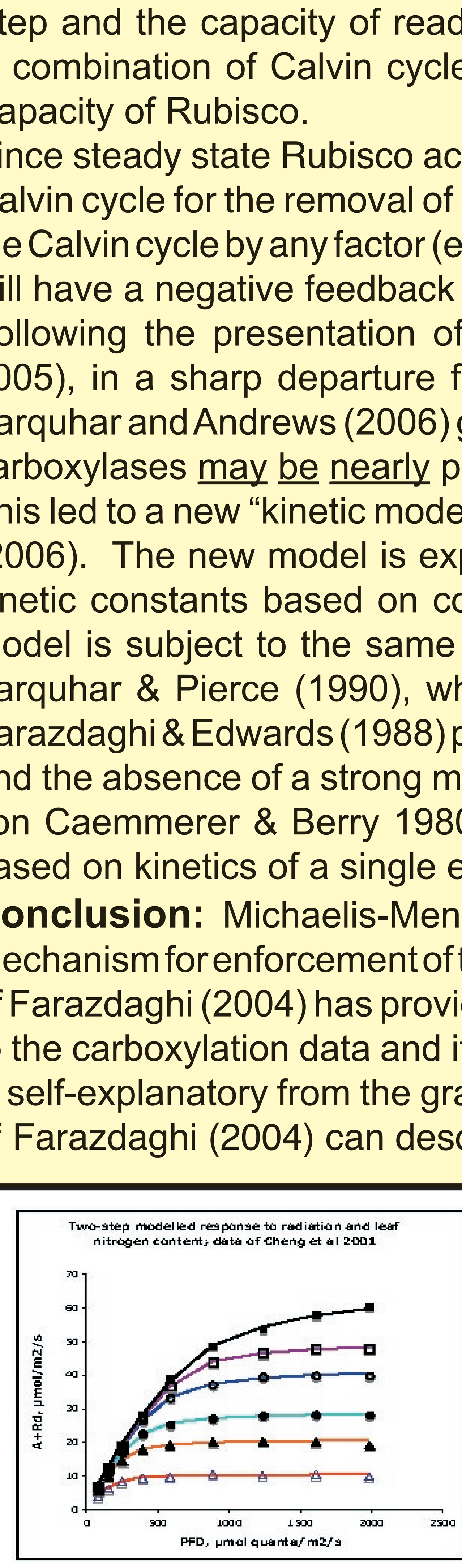
Bean Leaf Photosynthesis Data and Model of von Caemmerer & Farquhar, 1981



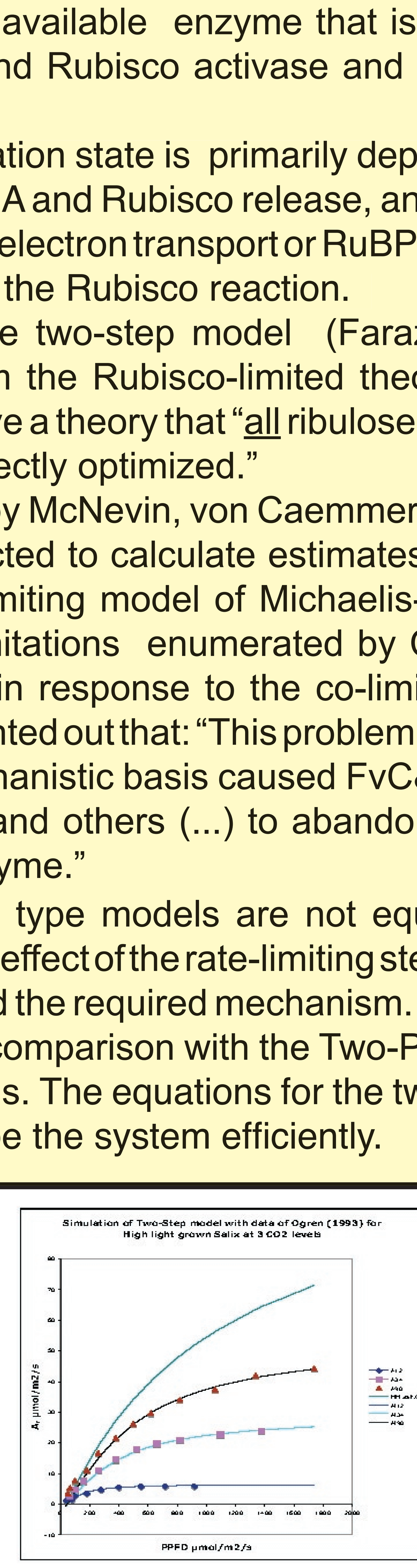
Bean Leaf Photosynthesis, Data of von Caemmerer & Farquhar, 1981, Model of von Caemmerer, 2000



Bean Leaf Photosynthesis, Data of von Caemmerer & Farquhar, 1981, Model of Farazdaghi, 2004



Farazdaghi, 2004, Model.
Note: Lower curves correspond to lower leaf nitrogen levels.



Farazdaghi, 2004, Model.
Note: CO_2 concentration levels of 12, 34 and 90 Pa as shown on graph legend.