than the percentage increase in the rate of urine excretion. The rate of potassium excretion was only slightly increased. These renal responses to 4-leucine-oxytocin were consistently demonstrated in four experiments with a dose range from 0.5 μg per 100 g to 0.7 μg per 100 g of body weight. Figure 1 shows the results from one typical experiment.

In three other experiments, rats with marked water diuresis were given a continuous infusion of arginine-vasopressin (synthetic) to suppress water diuresis. The infusion rate was adjusted to produce and maintain a 50- to 60-percent inhibition of water diuresis. Under our experimental conditions, the priming dose of arginine-vasopressin was between 0.025 and 0.04 milliunit. The infusion rate was between 0.0035 and 0.006 milliunit/min, with a flow rate always less than 0.05 ml/min delivered by a Harvard infusion pump.

Arginine-vasopressin infusion reduced the output of urine, increased the urinary osmolality to hyperosmotic levels, and reversed clearance of free water to reabsorption of free water. It had either no effect or caused a slight increase in osmolar clearance (C_{osm}) and excretion rates of sodium, potassium, and chloride.

When, under the influence of vasopressin infusion, a steady state was achieved, simultaneous infusion of 4-leucine-oxytocin (priming dose 0.5 μg per 100 g of body weight; an infusion rate of 0.05 to 0.20 μg per 100 g per minute) caused a marked diuresis. Urine output was increased to or above the initial level of water diuresis before vasopressin infusion. Osmolality of the urine decreased, and the urine became hypoosmotic. Free-water reabsorption was decreased in all three experiments, and in two of these experiments, it was reversed to free-water clearance; in effect the antidiuretic action of vasopressin was inhibited. Since the unavoidable effect of alcohol on the determination of osmolality tends to minimize the calculated reduction in free-water reabsorption, the absolute effect of 4-leucine-oxytocin on this is even greater. Osmolar clearance and excretion rates for sodium, potassium, and chloride were all increased. Natriuresis and chloruresis were the most prominent features of the response to 4-leucine-oxytocin infusion. When 4-leucine-oxytocin infusion was stopped, the urinary excretion pattern returned to that seen under vasopressin infusion. The duration of 4-leucine-oxytocin infusion varied from 15 to 60 minutes, and after its cessation, the anti-ADH effect subsided accordingly in 10 to 30 minutes. Figure 2 shows the results of one experiment.

Thus 4-leucine-oxytocin is a potent natriuretic-diuretic polypeptide and also has an antivasopressin activity. It has only a slight kaliuretic activity. The natriuretic-diuretic property of 4-leucine-oxytocin is not unique. Oxytocin at low doses can produce natriuresis and diuresis in rats (*2, 4, 5*); but at high doses, it is definitely antidiuretic. The uniqueness of 4-leucine-oxytocin is its lack of antidiuretic activity and its antivasopressin activity.

Brunner *et al.* (*5*) and Sawyer and Valtin (*6*) have shown that at certain doses oxytocin inhibits the antidiuretic response of rats to vasopressin. In these experiments, the inhibition was partial, and the free-water reabsorption was not measured. Therefore, Sawyer and Valtin (*6*) could not ascertain whether the inhibition is a specific antagonism or is simply the impact of oxytocin natriuresis-diuresis superimposing on the vasopressin antidiuresis.

Although the experiments reported herein are not extensive, we do have sufficient data to provide a meaningful discussion of the mechanism of the renal actions of 4-leucine-oxytocin.

First, it can be concluded that the natriuretic-diuretic activity of 4-leucine-oxytocin is not vasopressin-dependent, since this effect was demonstrable during water diuresis in water-loaded rats anesthetized with ethanol. It can be assumed with reasonable certainty that the release of endogenous vasopressin in rats during water diuresis was effectively blocked by the water load and the added ethanol.

Second, 4-leucine-oxytocin has an antivasopressin activity. This is shown by the ability of 4-leucine-oxytocin to reduce the vasopressin-induced free-water reabsorption and to reverse it to free-water clearance. The present experiments, however, do not provide data to indicate whether the 4-leucine-oxytocin inhibition of vasopressin is at a receptor level or is due to the inhibitory activity of 4-leucine-oxytocin on renal sodium reabsorption at a certain segment or segments of the nephron which, in turn, affects the concentrating capacity of the kidney.

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References and Notes

1. G. Flouret, V. J. Hruby, V. du Vigneaud, in preparation.
2. W. Y. Chan, *Endocrinology* **77**, 1097 (1965).
3. W. H. Sawyer, *Meth. Med. Res.* **9**, 210 (1961).
4. W. H. Sawyer, *Amer. J. Physiol.* **169**, 583 (1952); H. Croxatto, R. Rosas, L. Barnafi, *Acta Physiol. Latinoamer.* **6**, 147 (1956); B. Berde and A. Cerletti, *Helv. Physiol. Pharmacol. Acta* **19**, 135 (1961); R. Rosas, L. Barnafi, T. Pereda, H. Croxatto, *Amer. J. Physiol.* **202**, 901 (1962); J. Kramer, E. H. Grinnel, W. M. Duff, *Amer. J. Med. Sci.* **252**, 331 (1966).
5. H. Brunner, G. Kuschinsky, G. Peters, *Arch. Exp. Path. Pharmakol.* **228**, 457 (1956).
6. W. H. Sawyer and H. Valtin, *Endocrinology* **76**, 999 (1965).
7. Supported in part by NIH grants HE-09795, HE-01675, and HE-11680. We thank Mrs. Jessie Lawrence and Miss Margitta Wahrenburg for technical assistance.

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Carbon Dioxide Exchange in Cotton: Some Anomalous Fluctuations

Abstract. *Anomalous depressions in carbon dioxide exchange were observed in cotton leaves that were exhibiting oscillations in transpiration under controlled conditions of environment. The depressions occurred only when leaf temperature exceeded 37.5°C and when the leaf diffusive resistance was minimum. Stomatal control of the supply of carbon dioxide to the leaf does not seem to be implicated in the effect.*

Several observers have noted oscillations, with periods of 20 to 30 minutes, in the rate of transpiration and carbon dioxide exchange in various species of plants (*1*). It has been confirmed that these oscillations are associated with fluctuations in the diffusive resistance of the stomata. This suggests that the net CO_2 exchange during the oscillations is normally controlled by the supply of CO_2 to the chloroplasts. We report fluctuations in the CO_2 exchange which are not directly related to the oscillations in diffusive resistance.

Measurements were made of CO_2 exchange (by differential infrared gas

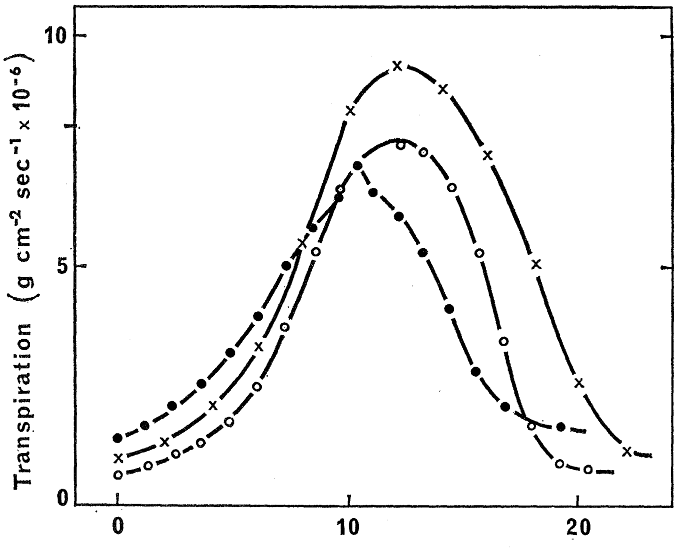


Fig. 1 (above). Oscillations in the rate of transportation as influenced by leaf temperature; X, 37.5°C; ○, 40°C; and ●, 42.5°C.

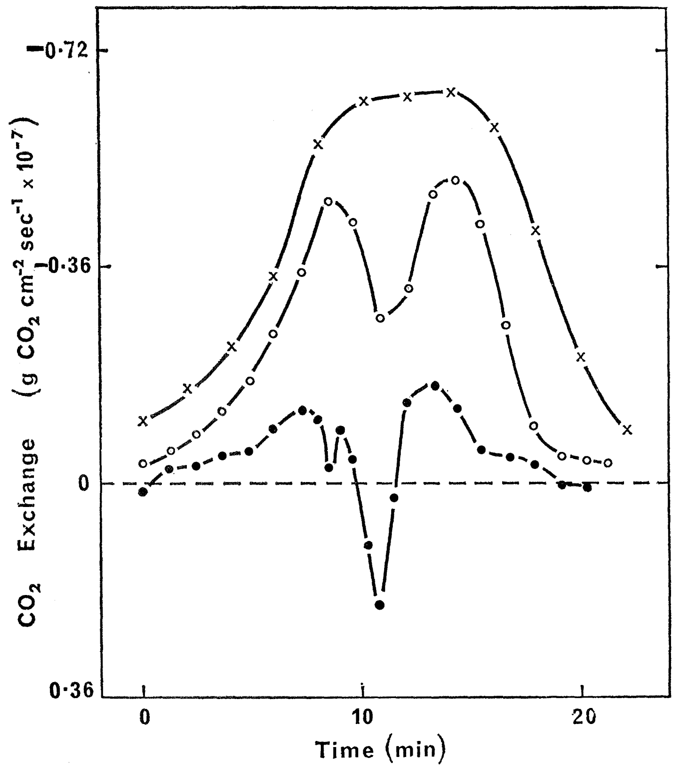


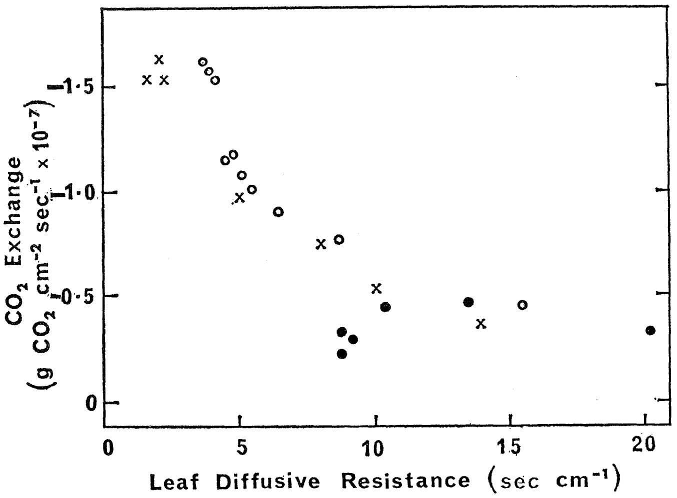
Fig. 2 (right). Oscillations in the rate of CO_2 exchange as influenced by leaf temperature; X, 37.5°C; ○, 40°C; and ●, 42.5°C.

analysis), transpiration, leaf temperature, and β-ray transmission (to indicate variation in water content of the leaf) of the third of six leaves of a cotton plant previously grown for 2 months in a controlled environment. This leaf was enclosed in a chamber in which the light level (wavelength 0.4 to 0.7 μ) was 100 watt m^{-2} and CO_2 concentration was 300 ± 10 volumes per million. The occurrence of oscillations was irregular, but in this experiment oscillations were initiated by a 5-minute dark period during normal light hours.

The leaf resistance was calculated by the vapor-exchange method (*2*) from data on leaf temperature, specific humidity of the air, and the rate of transpiration. This resistance, which includes stomatal and cuticular components, is expressed as the resistance to water vapor exchange in units of seconds per centimeter.

During the experiment, leaf temperature was increased from 32.5° to 42.5°C in steps of 2.5° and then decreased in the opposite sequence, the temperature at each level being maintained constant throughout three or four successive oscillations in the rate of transpiration. Temperature of the leaf was controlled by continuous adjustment of the temperature of the ambient air; temperature of the stem and remaining leaves of the plant was approximately 23°C and that of roots 30°C throughout the experiment. The roots were in an aerated, modified Hoagland nutrient solution.

At all temperatures, oscillations in the rate of transpiration were closely approximated by a simple harmonic wave with a period varying between 22 and 26 minutes (Fig. 1). Oscillations



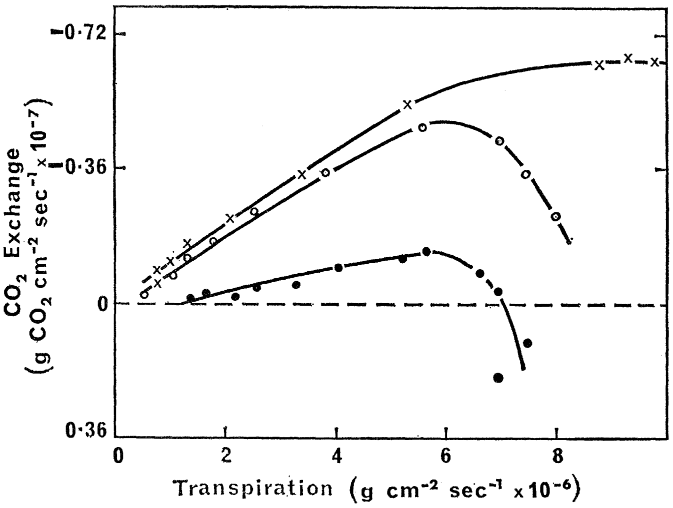


Fig. 3 (left). Influence of leaf diffusive resistance on the rate of CO_2 exchange under steady-state and oscillating conditions and at different leaf temperatures; X, 30°C steady-state; ○, 40° and 42.5°C steady-state; and ●, 40°C oscillating conditions. Fig. 4 (right). Relationship between the rate of CO_2 exchange and transpiration at three leaf temperatures; X, 37.5°C; ○, 40°C; and ●, 42.5°C.

of CO_2 exchange and transpiration were similar at leaf temperatures up to 37.5°C. At 37.5°C the peak of the curve representing CO_2 exchange became flattened, and at 40°C a sharply decreased rate of exchange occurred over a period of 4 minutes when the rate of transpiration was near its maximum (Fig. 2). The decrease was still more marked at 42.5°C, the exchange being positive during an interval of about 2 minutes. The behavior of the plant at any given temperature did not exhibit hysteresis, the phenomena observed being independent of the direction of the preceding change of temperature. The depressions were noted in ten oscillations.

These surprising fluctuations in CO_2 exchange by the leaf when the level of light, concentration of CO_2, and leaf temperature were constant, and the leaf diffusive resistance minimum, could be due to partial inhibition of photosynthesis, a stimulation of respiration, or both. Respiration is known to increase with an increase in temperature (*3*) but leaf temperature was constant during any one oscillation. The anomalous fluctuations occurred only at the higher temperatures. While the influence of temperature on the rate of respiration could account for a general reduction in the rate of CO_2 exchange, it cannot be invoked to explain the sudden efflux of CO_2, unless this efflux was under stomatal control.

Stomatal control of CO_2 diffusion offered the main control of CO_2 exchange at leaf temperatures up to 37.5°C and during part of each oscillation at higher temperatures. It is clear, however, that another mechanism limited CO_2 exchange at higher temperatures, as this exchange was depressed when the leaf diffusive resistance was minimum (Fig. 3). Other resistances to CO_2 transfer are involved in CO_2 exchange (*4*), and it may be that they are temperature-dependent. If the rate of photosynthesis was limited by another resistance, then a leveling rather than a periodic depression would be expected.

The influence of leaf temperature on the rate of CO_2 exchange was also observed under conditions when the leaf diffusive resistance was constant. In these experiments the stomatal diffusive resistance was the major component of the leaf diffusive resistance. Under steady-state conditions CO_2 exchange at both 30° and 42.5°C was virtually the same at the same leaf diffusive resistance (Fig. 3). Furthermore, in one experiment leaf temperature was maintained at 42.5°C for 6 hours with a low leaf diffusive resistance without a significant change in CO_2 exchange. In contrast, there was a significant reduction in the rate of CO_2 exchange at the lowest leaf resistance during oscillations in stomata at 42.5°C.

The only parameters, beside leaf temperature, we could link to this depression were a lowered level of plant water and high rate of transpiration. Carbon dioxide exchange was closely related to transpiration up to a rate of 6×10^{-6} g cm^{-2} sec^{-1}, but at higher rates of transpiration the rate of exchange declined (Fig. 4). The rate of transpiration may have influenced photosynthesis through lowered water content of the leaf, but the depressions of CO_2 exchange often occurred without any consistent reduction in leaf water. If the depressions are directly related to the water relations of the plant, it would appear that the rate of flow of water rather than the content of water in the leaf has influenced the photosynthetic process. This suggestion cannot be valid unless we suppose that the effect is dependent on temperature, as high rates of transpiration occurred without influencing the rate of CO_2 exchange at lower temperatures. Under steady-state conditions, the leaf responds to increasing water stress mainly by increasing stomatal diffusive resistance, so that the special condition, noted during the oscillations, of a high transpiration rate with a low content of plant water is unlikely to occur under steady states.

The effect is difficult to explain but may be due to variation in water content between organelles within the leaf, encouraged by temperature-dependent permeability changes, high transpiration rates, and low content of plant water. This could alter the volume, structure, or the capacity for ion exchange of the chloroplasts, and thus influence the photosynthetic capacity of the leaves. A particular explanation could be the effect of water content of chloroplasts on the reversible uncoupling of photophosphorylation from electron transport of the photosynthetic process (see *5*).

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References and Notes

1. V. G. Karmanov and V. N. Savin, *Dokl. Akad. Nauk SSSR* **154**, No. 4 (1964); V. G. Karmanov, S. N. Meleschenko, V. N. Savin, *Biofizika* **10**, 155 (1965); W. L. Ehrler, F. S. Nakayama, C. H. M. van Bavel, *Physiol. Plant.* **18**, 766 (1965); F. E. Cox, *J. Exp. Bot.*, in press; G. F. Howe, *Ohio J. Sci.* **64**, 378 (1964); N. A. Maximov and T. A. Krassnoselsky-Maximov, *Ber. Deut. Bot. Ges.* **46**, 383 (1928).
2. R. O. Slatyer and J. F. Bierhuizen, *Australian J. Biol. Sci.* **17**, 115 (1964).
3. E. E. I. Rabinowitch, *Photosynthesis and Related Processes* (Interscience, New York, 1956), vol. 2, part 2.
4. R. O. Slatyer, *Plant-Water Relationships* (Academic Press, New York, 1967), pp. 292–295.
5. K. A. Santarius and R. Ernst, *Planta* **73**, 91 (1967).
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Limb Regeneration: Induction in the Newborn Opossum

Abstract. *The marsupial* Didelphys virginiana *(the North American opossum) is uniquely suited for studies of mammalian limb replacement. By transplanting nervous tissue to the limb, regeneration has been successfully induced in this mammal.*

In lower vertebrates at least two factors, nervous (*1*) and hormonal (*2*), are decisive in determining whether or not regeneration will occur after limb amputation. Investigators have used this knowledge to induce regeneration in typically nonregenerating appendages of adult anurans (frogs) (*3*) and reptiles (lizards) (*4*). However, previous attempts to induce mammalian limb regeneration have been disappointing (*5*).

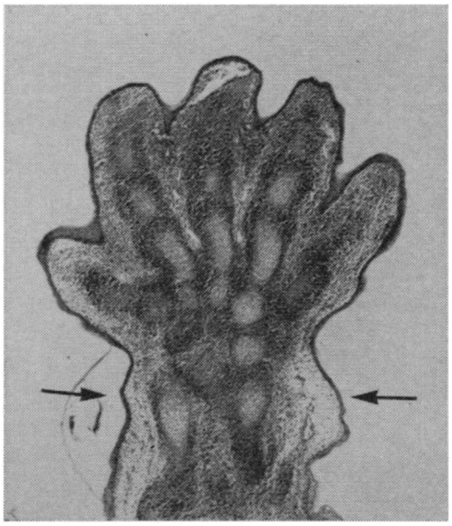

Fig. 1. Histological detail of newborn opossum hindlimb. Arrows indicate level of amputation (× 36).