Table II. Effect of heat inactivation and L-methionine inhibition on the activity of LAP and CAP

Samples	Remaining activity (%)			
	LAP		CAP	
	Heating[a]	Methionine[b]	Heating[a]	Methionine[b]
Normal serum	96.2	21.5	—	—
Pregnancy serum	32.0	65.8	8.6	77.6
Lysosomal extract	18.3	64.5	8.2	72.5
Microsomal extract	91.4	21.2	88.7	12.8

[a] 60 °C for 30 min. [b] 0.02 *M*.

reported. Our preliminary experiments revealed different rates of heat inactivation and L-methionine inhibition among serum and placental enzymes (Table II). Successively, we examined and compared effects of heating at 60 °C for 30 min as well as 0.02 *M* L-methionine on serum and placental AP isozymes with the use of disc electrophoresis. As can be seen in Figure (B) and (C), it is evident that normal serum LAP band and microsomal band were heat-stable and sensitive to L-methionine inhibition, while 2 CAP bands of pregnancy sera and lysosomal extracts were heat-labile and insensitive to inhibition by this amino acid.

RYDÉN[13] described the subcellular localization of CAP in human placenta. However, no experiments were performed on the characterization of properties of each enzyme. From the results obtained in our study, the 2 bands (CAP_1 and CAP_2) of pregnancy sera and the 2 lysosomal bands shared the same enzymatic characteristics, such as heat stability, L-methionine inhibition and electrophoretic pattern. This finding suggests that the increased AP in pregnancy sera may be derived from the lysosmes of placenta. These 2 lysosomal bands might represent 2 different conformational forms of a single enzyme, whose rate of migration is in a pH dependent equilibrium with each other, as discussed on retroplacental CAP by SJÖHOLM and YMAN[14]. The difference in the enzymatic properties between the lysosomal and the microsomal enzymes indicates the presence of multiple molecular forms of AP within a single tissue, i.e. the placenta. From the fact that LAP band in all human sera has practically no CAP activity, the normal serum LAP seems to be distinct from the placental enzymes.

The supernatant showed exactly the same electrophoretic behavior as the pregnancy serum. This observation is supposed to be due to the contamination of retroplacental blood, which contains a large amount of AP and in addition the soluble enzyme derived from ruptured lysosomes during fractionation procedures.

Conclusions. Evidences for the existence of 2 AP isozymes in human placenta were presented: the lysosomal and the microsomal isozymes, which are distinct from the normal serum LAP. The increased AP in pregnancy sera possessed the same enzymatic properties as the lysosomal enzyme in human placenta, with regard to heat resistence, sensitivity to L-methionine inhibition and electrophoretic pattern. These similarities suggest that the pregnancy serum AP may originate from the placental lysosomes.

Zusammenfassung. Charakterisierung zweier Arten von Aminopeptidase (AP)-Isozymen in den Lysosomen und Mikrosomen der menschlichen Plazenta. Die im Serum schwangerer Frauen auftretende AP hatte ähnliche Eigenschaften wie die Lysosomen-AP. Die Erhöhung der Aminopeptidase beruhte folglich auf dem Austritt lysosomaler Enzyme aus der Plazenta.

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Stimulation of Phosphoenolpyruvate Carboxylase Activity in Rust-Infected Wheat Leaves

Rust infected wheat leaves retain chlorophyll at the periphery of uredosori in regions termed 'Green Islands'[1]. This area of the infected leaf is active metabolically and can be considered a 'sink' for all metabolites[2–4]. *Erysiphe graminis* infected wheat leaves showed no decline in photosynthetic activity per unit of chlorophyll[5]. Since the chlorophyll content of leaves is reduced by infection, it is only the overall photosynthetic activity that decreases[3, 5, 6]. Therefore it is entirely possible that the chlorophyll retained[7] or reformed[5], in the green islands may be more efficient in photosynthesis. Stimulation of the photosynthetic CO_2 uptake was reported in infected organs of bean and safflower prior to the sporulation of the fungus[8]. LIVINE speculated that the flow of carbon during the photosynthesis of the green island, follows a

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pathway other than the usual Calvin cycle. In other words, a more efficient pathway of CO_2 fixation, such as the HATCH and SLACK cycle[9], may begin to operate to meet the increased demand for carbohydrate building blocks. According to HATCH and SLACK, CO_2 fixation is mediated by PEP-carboxylase (E.C.4.1.1.31) as follows: Phosphoenolpyruvate + CO_2 + H_2O → Oxalacetate + Pi. If the HATCH and SLACK cycle occurs in rust infected leaves, then it should also be possible to demonstrate a PEP-synthetase (E.C.2.7.1.40) which mediates the following reaction: Pyruvate + ATP + Pi → PEP + AMP + PP.

Two species of wheat, *Triticum compactum* Host var. Little Club and *Tricticum aestivum* L. var. Marquis were grown in the green house at ca. 21 °C. Primary leaves of the plants were infected with *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and Henn. Race 10, 38 and 48-1 respectively, 8 to 10 days after sowing.

Variety Little Club with a type 4 infection is susceptible to all stem-rust races employed whereas Marquis is moderately resistant to Race 10 and 38 and very resistant to Race 48-1.

Table I. Activity of phosphoenolpyruvate synthetase in healthy and infected wheat leaves

Material	System	Δ O.D./min	Protein content (mg/0.1 ml)
Healthy	Complete	0.015	3.2
Healthy	— ATP	0.020	3.2
Healthy	— Pyruvate	0.016	3.2
Infected	Complete	0.024	3.16
Infected	— ATP	0.018	3.16
Infected	— Pyruvate	0.010	3.16

Red Bobs wheat leaves inoculated with *Puccinia graminis* Race W-56 were used in this experiment. The complete assay system contained in 3.0 ml: *Tris*-HCl buffer, pH 7.6, 50 m*M*; Na-pyruvate, 5 m*M*; ATP, 5 m*M*; $MgCl_2$, 16 m*M*; $NaHCO_3$, 10 m*M*; NADH, 48 μ*M*; P-enolpyruvate carboxylase, 0.1 ml (1.9 mg protein); enzyme preparation, 0.1 ml (ca. 3 mg protein). The reaction was started by the addition of pyruvate. P-enolpyruvate carboxylase was a partially purified preparation from leaves of *Zea mays* var. Early King.

Table II. Activity of phosphoenolpyruvate carboxylase in healthy and infected wheat leaves

Material	Δ O.D./min	Protein (mg/ml)	Specific activity
Little club			
Healthy	0.08	4.80	166
Infected with			
Race 10	0.204	4.84	421
Race 38	0.196	5.24	374
Marquis			
Healthy	0.042	4.00	105
Infected with			
Race 10	0.285	7.20	396
Race 38	0.240	6.60	363

The assay system contained in each 3.0 ml: *Tris*-HCl buffer pH 7.6, 50 m*M*; P-enolpyruvate, 4 m*M*; $MgCl_2$, 16 m*M*; $NaHCO_3$, 10 m*M*; NADH, 260 μ*M*; Malic Dehydrogenase, 1 international unit; and enzyme preparation 0.1 ml (400–700 μg protein). The reaction was started by the addition of P-enolpyruvate. Protein was determined by the Folin-phenol method of LOWRY et al.[14].

Inoculated plants were covered with plastic bags for 24 h to maintain 100% humidity. Symptoms of infection appeared after 7 to 10 days; another 3 to 5 days later uredial pustules and surrounding green islands, if any, appeared. In summer when the light intensity was higher, the leaves were inoculated about 7 days after sowing and sporulation occurred earlier.

Phosphoenolpyruvate synthetase was isolated according to the procedure of HATCH and SLACK[10]. 20 g of leaves were homogenized at 5 °C in a Waring blender with 100 ml of 0.1 *M Tris*-HCl buffer, pH 8.3 containing 5 m*M* 2-mercaptoethanol, 5 m*M* $MgCl_2$, 1 m*M* EDTA and with or without 2.5 m*M* pyruvate. Since the enzyme is inactivated by cold, all subsequent operations were conducted at room temperature (ca. 25 °C). The homogenate was filtered through 4 layers of cheesecloth and the filtrate was brought to 30% $(NH_4)_2SO_4$ saturation. The filtrate was centrifuged at 27,000 × *g* for 20 min and the percipitate discarded.

The supernatant was brought to 60% $(NH_4)_2SO_4$ saturation, and the precipitate obtained after centrifugation as above was dissolved in 5.0 ml of the grinding medium (diluted 2-fold with dist. water). The enzyme was assayed by coupling with P-enolpyruvate carboxylase and malic dehydrogenase and measuring the oxidation of NADH at 340 nm in a Unicam spectrophotometer Model SP800. An increase of ca. 60% (Table I) activity of the PEP-synthetase was detected in the extracts of infected leaves as compared to extracts of healthy leaves.

Phosphoenolpyruvate carboxylase was isolated essentially according to the procedure of SANDHU and WAYGOOD (unpublished). Wheat leaves, 10 to 15 g, were harvested after 19 days, chilled and homogenized in a Waring blender with 80 ml of 50 m*M* KH_2PO_4, pH 7.5, for 2 min. The homogenate was filtered through 4 layers of cheese cloth and the filtrate was brought to 20% $(NH_4)_2SO_4$ saturation. The filtrate was centrifuged at 27,000 × *g* for 20 min and the precipitate was discarded. The supernatant was brought to 55% $(NH_4)_2SO_4$ saturation centrifuged as above and then the precipitate was dissolved in 40 ml of grinding medium. The enzyme was re-precipitated at 55% $(NH_4)_2SO_4$ saturation and finally dissolved in 4 to 5 ml *Tris*-HCl buffer, pH 7.5. All operations were conducted in the cold (5 °C). The enzyme was assayed by coupling with malic dehydrogenase and measuring the decrease in optical density of NADH at 340 nm. Almost a 4-fold increase in the specific activity of the enzyme, PEP-carboxylase, was detectable in the extracts of leaves infected with rust (Table II). However, there was a significant increase in the protein content as well, especially in the case of Marquis wheat, which is moderately resistant to the pathogens.

There is considerable evidence accumulating that rust-infection tends to increase the key components NAD (P),[11] ATP[12] of photosynthesis in rust-infected leaves. In fact, recalculation of the data of SCOTT and SMILLIE[13] show that photosynthesis per mg chlorophyll is 50% higher in *Erysiphe graminis*-infected barley leaves as compared to healthy leaves. While it has been believed that overall photosynthesis is decreased in rust-infected leaves as compared to healthy leaves the opposite may be

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true on a chlorophyll basis[13]. One can speculate that there may be a switch from the Calvin cycle in which ribulose diphosphate, is the acceptor of CO_2 to the HATCH and SLACK cycle in which P-enolpyruvate serves as the initial CO_2 acceptor. The latter cycle is found in more efficient photosynthetic plants such as *Zea mays, Saccharum officinarum* and other species in the tropical tribes of the Graminae. Accordingly, it was of interest to find that P-enolpyruvate carboxylase activity increased 3-4-fold in rust-infected leaves as compared to their healthy controls. Associated with this enzyme of the HATCH and SLACK cycle pathway is PEP-synthetase (not found in Calvin cycle photosynthetic plants) and while preliminary experiments indicated only a weak activity of this enzyme in rust-infected plants it was greater than that demonstrable in healthy plants. It will be necessary to devise a technique enabling one to separate the metabolism of green island tissue from that of the adjacent tissue and correct for the contribution of the parasite, before the question can be answered as to whether the HATCH and SLACK pathway is predominent in 'green island'[15,16].

Zusammenfassung. Nachweis, dass rostinfizierte Weizenblätter (*Puccinia graminis*) höhere PEP-Carboxylaseaktivität aufweisen als gesunde, und Feststellung einer PEP-Synthetase. Annahme, dass in den grünen Inseln der infizierten Blätter CO_2 nicht mehr nach dem Calvinzyklus, sondern auf dem HATCH und SLACK-Weg fixiert wird.

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Salivary Electrolytes, Protein and pH during Transcendental Meditation

The present investigation concerns the relationship of mineral metabolism with states of consciousness. Transcendental meditation, as taught by MAHARISHI MAHESH YOGI[1], is a world-wide practice of great uniformity and provides an ideal model for experimental study. WALLACE et al.[2,3] have shown that certain physiological and biochemical changes take place during the state of consciousness induced by the practice. Alterations in physiology include decreases in respiration, heart rate, O_2 consumption, CO_2 elimination with increased skin electrical resistance and EEG α-energies[2,3]. Also found were decreases in blood pH, base excess and lactic acid[3], suggesting a state of mild metabolic acidosis. In this study the effects of meditation on salivary minerals and pH was investigated as a preliminary to studies on blood chemistry during the practice.

Materials and methods. The subject, a male university student, had been practicing transcendental meditation for about 6 months and was investigated during 10 sessions over a 2-week-period. Meditation was performed in the late afternoon, after at least 6 h of fasting, in the subject's own home. Unstimulated, mixed saliva was collected by free flow immediately before, and immediately after, a 20-min meditation, and again 10 min after meditating.

The pH of the samples was measured with a Radiometer pH meter and cationic electrolytes were quantitated in 5% trichloroacetic acid (TCA) extracts by atomic absorption spectrophotometry. Inorganic phosphate[4] and protein[5] were measured by classical procedures. TCA-soluble protein was precipitated by shaking with diethyl ether and subsequent aspiration of the ether layer. For statistical evaluation the data was analysed by the paired *t*-test with pre-meditational values acting as control.

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Effects of meditation on salivary electrolytes, protein and pH

	During meditation	*P*	Before meditation (control)	*P*	After meditation
K (µg/ml)	1320 ± 44[a]	< 0.001	1070 ± 57	n.s.[b]	980 ± 35
Pi (µg/ml)	417 ± 16	< 0.001	286 ± 10	n.s.	269 ± 12
Na (µg/ml)	300 ± 13	< 0.001	176 ± 11	n.s.	200 ± 14
Ca (µg/ml)	118 ± 5.1	< 0.001	87 ± 1.6	n.s.	88 ± 4.2
Mg (µg/ml)	14.2 ± 0.9	< 0.001	10.1 ± 0.7	n.s.	9.6 ± 0.3
Zn (µg/ml)	9.8 ± 1.1	n.s.	8.8 ± 0.3	n.s.	10.2 ± 1.5
Acid-soluble protein[c] (mg/ml)	2.05 ± 0.10	< 0.001	1.06 ± 0.02	< 0.025	0.93 ± 0.05
Acid-insoluble protein[d] (mg/ml)	2.04 ± 0.12	< 0.005	1.50 ± 0.09	< 0.02	1.85 ± 0.11
Total protein[e] (mg/ml)	4.09		2.56		2.78
pH	6.54 ± 0.06	< 0.001	6.91 ± 0.04	< 0.02	7.01 ± 0.04

[a] Mean ± S.E.M. $N = 10$. [b] Not significant ($P > 0.05$) by paired *t*-test. [c] Protein not precipitated by 5% trichloroacetic acid. [d] Protein precipitated by 5% trichloroacetic acid. [e] Calculated (sum of values for acid soluble and insoluble protein).