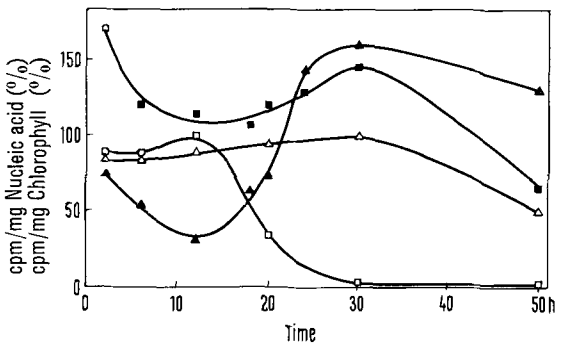
according to KING[16] and the nucleic acids spectrophotometrically by measuring extinction at 260 nm, using calf thymus DNA as a reference. All results were expressed as percent of the control.

Results and discussion. In the Figure the mean of 2 independent experiments is given, showing uptake of ^{32}P into the nucleic acid, as well as the CO_2 fixation, of spinach leaves in the presence of Ioxynil and Prometryne. As demonstrated in the Figure, Ioxynil and Prometryne affect the ^{32}P incorporation into the nucleic acid before CO_2 fixation is influenced. This is particularly pronounced in the case of Ioxynil. The ^{32}P incorporation, taken to be a measure of nucleic acid metabolism, shows a similar pattern for both herbicides: the uptake of ^{32}P shows a minimum after 12–18 h incubation and increases to a maximum level after 30 h.



Time course study on the incorporation of P^{32} into nucleic acid and CO_2 fixation. Results are expressed in percent of values obtained for the herbicide-free controls. □, $C^{14}O_2$ fixation in the presence of Prometryne; ■, P^{32} incorporation in the presence of Prometryne; △, $C^{14}O_2$ fixation in the presence of Ioxynil; ▲, P^{32} incorporation in the presence of Ioxynil. The average rates recorded for the control plants were as follows: CO_2 fixation, 150 μmole CO_2/mg chlorophyll/h; P^{32} incorporation, 1.75 μatom P/mg nucleic acid/h.

With regard to the rate of photosynthetic CO_2 fixation of leaf discs from herbicide incubated plants, it was found that Prometryne inhibited photosynthesis after 12–30 h and Ioxynil after 50 h. These results, compared with those on ^{32}P incorporation into nucleic acid, where rate of incorporation was almost immediately affected, suggest that the herbicides do not interfere primarily with the photosynthetic reaction cycle.

This finding was further substantiated by following $^{14}CO_2$ uptake in the herbicide treated plants. The study revealed that in the range where for both herbicides ^{32}P incorporation into nucleic acid was already affected, the $^{14}CO_2$ fixation rate, as well as the distribution pattern of the assimilates (detected by radio-chromatography), remained unchanged when compared with the untreated control.

Although a proper evaluation of our results is indeed difficult, it might reasonably be speculated that both herbicides interfere primarily with the replica system before the more autonomous functions of the photosynthetic apparatus are affected.

Zusammenfassung. Spinatpflanzen (*Spinacia oleracea* L.) wurden in einer Nährlösung, welche die Herbizide Ioxynil respektive Prometryn enthielten, kultiviert. Es zeigte sich, dass in den Blättern dieser Pflanzen der ^{32}P-Einbau in die Nukleinsäuren beeinflusst wurde, bevor eine Hemmung der photosynthetischen CO_2-Fixierung eintrat.

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Pyridine Nucleotide Oxidized to Reduced Ratio as a Regulator of Muscular Performance

Pyridine nucleotides (DPN, DPNH, TPN, and/or TPNH) have been identified as important cofactors in almost all major metabolic pathways. It seems possible that the absolute levels and the state of the oxidized to reduced moieties could influence the activities of the several metabolic pathways. The pyridine nucleotide oxidized to reduced ratio has been shown to be altered in several metabolic states, including tissue ischemia (BURCH and VON DIPPE[1]), nerve stimulation (GIACOBINA and GRASSO[2]), and starvation (LARDY[3] and GLOCK and MCLEAN[4]). We hypothesized that this ratio could be acting as a factor within the muscle to regulate performance. That is, if a muscle possessed the ability to maintain a higher oxidized-to-reduced ratio it would increase its performance capability.

The well-known ability of physical training to increase one's capacity for muscular performance was utilized in order to broaden the range of responses to an acute exercise stimulation. 2 groups of 75-day-old Sprague-Dawley rats were chosen. The first group had been swum for 8 weeks, twice a day for 2 h each session, with up to 3% of their body weight attached to the tail. The second group was non-trained. At the end of the training period, the animals were lightly anaesthetized with ether, the achilles tendon clipped, and the distal end of the gastrocnemius-plantaris muscle group attached to a linear variable differential transformer. The muscle group was loaded with a 20-g weight. Direct muscle stimulation (0.2 mA) was applied to the in situ muscle preparation at the rate of 2 twitches/sec. At the end of 10 min the contracting muscle group was freeze-clamped with aluminium tongs, precooled in liquid nitrogen. Work performance was determined by summing the distance the 20-g weight was moved during each of the 1200 individual twitches. The pyridine nucleotides (DPN, DPNH, TPN, and TPNH) were assayed by appropriate enzymatic reactions (alcohol dehydrogenase, lactate dehydrogenase, glutamate dehydrogenase, and glucose-6-phosphate dehydrogenase, respectively). The change in absorbance at 340 nm was measured on Beckman Model DU spectrophometer.

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The Table shows the total (free and bound) pyridine nucleotide concentrations found in the 2 groups of rats at the end of the 10-min contraction period. The value for muscle DPNH showed a statistically significant difference between the 2 groups. The alteration in the DPNH value was sufficient to cause a significant difference in the pyridine nucleotide oxidized-to-reduced ratio. As a result of intra-group variation in work performance and oxidized-to-reduced ratio, the sum of the oxidized pyridine nucleotides divided by the sum of the reduced nucleotides did not (and mathematically should not) equal the mean of the individually calculated oxidized-to-reduced ratios.

The statistical relationships between the muscle oxidized-to-reduced ratio (y) and work performance (x) were as follows: non-trained animals, $y = 7.7811 - 0.0041x$; trained animals, $y = 9.9797 - 0.0006x$. Work performance equaled 2645 ± 142 meter-grams for the non-trained animals and 2715 ± 145 for the trained animals. These relationships indicate that, within each group, the greater the work performance the more capable the cell was of tolerating a more reduced state. Furthermore, for any given work performance level, the muscle of the trained animal had a higher oxidized-to-reduced ratio than the muscle of the non-trained animal.

It seems likely that the higher oxidized-to-reduced ratio in the muscles of the trained rats indicates an increased capacity of the mitochondria to keep the cell in a more oxidized state. Currently, we are investigating alterations in the oxidation-reduction state of the various subcellular compartments within the muscle cell[5].

Pyridine nucleotide concentrations

	Non-trained	Trained
DPN	970.8 ± 75.4	1027.3 ± 90.0
DPNH	262.8 ± 51.5[a]	124.9 ± 19.4
TPN	8.1 ± 1.5	10.2 ± 3.1
TPNH	106.4 ± 30.9	66.9 ± 15.9
Oxidized/reduced	4.4 ± 0.7[a]	7.3 ± 1.2
Total pyridine nucleotides	1348.1 ± 109.4	1229.3 ± 94.2

Values are in μmoles/kg of tissue ± S.E.M. $N = 18$. The pyridine nucleotide oxidized to reduced ratios represent the mean of the ratios (DPN+TPN)/(DPNH+TPNH) for each experiment. [a] $p < 0.05$.

Zusammenfassung. Die Pyridinnukleotid-Gehalte von trainierten und untrainierten Muskeln werden nach zehnminütiger Arbeit miteinander verglichen. Das Verhältnis der oxidierten zu reduzierten Pyridinnukleotiden ist beim trainierten Muskel stärker oxidiert als beim nicht trainierten Muskel.

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Inactivation of Cystathionase and of Cysteine Sulfinic Acid Decarboxylase by Proteolytic Enzymes: Effect of Pyridoxal Phosphate

It has been reported[1,2] that the activities of crude and of partially prufied preparations of rat liver cystathionase (L homoserine hydro-lyase EC 4.2.1.15) and cysteine sulfinic acid (CSA) decarboxylase (L cysteine sulfinate carboxy-lyase EC 4.1.1.29) are, at least in part, protected against heat denaturation and urea inactivation by their coenzyme, pyridoxal phosphate (PLP), whereas pyridoxine and other derivatives (pyridoxamine, pyridoxal, pyridoxamine phosphate) afford no protection. It appeared of interest to undertake studies which would attempt to determine whether a similar protection can be observed in other situations, more closely related to the conditions of physiological degradation of tissue proteins. In this paper, studies of the action of proteolytic enzymes, trypsin (Worthington), α-chymotrypsin (Seravac Lab.) and pronase (Calbiochem) on partially purified cystathionase and CSA decarboxylase are described. Furthermore, to gain additional insight as to the role that PLP plays, the effect of PLP on these reactions of proteolysis was investigated and is also described. A preliminary report of some of these findings has already appeared[3].

Cystathionase and CSA decarboxylase were extracted from Wistar male rat liver and partially purified according to the procedures already described[2] with slight modifications: in the preparation of CSA decarboxylase, the supernatant was heated to 55 °C for 5 min and centrifuged before addition of ammonium sulphate[4]; in the preparation of cystathionase, dithiothreitol (DTT) was omitted in the solutions used and the preparation was in each case lyophilized.

It is noteworthy that cystathionase was obtained essentially in the form of apoenzyme, for, before or after lyophilization, it exhibited a very small activity unless PLP was added to the incubation mixture, whereas the activity of purified CSA decarboxylase was the same when measured with or without addition of PLP.

The measurements of enzymic activities were carried out according to the procedures previously described[5,6], and the modifications already reported[1,2].

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