

The mode of action of 6-hydroxydopamine in inhibiting the formation of ^{14}C -catecholamines from tyrosine remains to be elucidated. The compound possibly interferes directly with the enzyme tyrosine hydroxylase. This might be the first manifestation of a damage induced by 6-hydroxydopamine which occurs before changes become apparent on electron microscopy. On the other hand, an indirect action of the compound, e.g. by a negative feed-back on tyrosine hydroxylation, cannot be excluded^{15,16}.

In conclusion, doses of 6-hydroxydopamine which do not cause ultrastructural damage to the brain neurons seem to impair the biosynthesis of catecholamines. This effect is probably involved in the decrease of endogenous dopamine and norepinephrine in the brain due to intraventricular injection of 6-hydroxydopamine⁹.

Zusammenfassung. Intraventrikulär verabreichtes 6-Hydroxydopamin in Dosen, die keine ultrastrukturellen Hirnveränderungen erzeugen, vermindert bei Ratten die durch ^{14}C -Tyrosin bedingte Zunahme von ^{14}C -Catecholaminen und von desaminierten ^{14}C -Catecholaminmetabo-

liten im Gehirn. Die ^{14}C -Dopa bedingte Anhäufung von cerebralen ^{14}C -Catecholaminen wird durch Vorbehandlung mit 6-Hydroxydopamin leicht vermindert, während der Anstieg von ^{14}C -Catecholamin-Metaboliten keine signifikante Veränderung erfährt. Es wird geschlossen, dass 6-Hydroxydopamin wahrscheinlich neben einer Verdrängung von Catecholaminen auch eine Hemmung der Hydroxylierung von Tyrosin bewirkt.

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¹⁵ A. ALOUSI and N. WEINER, *Proc. natn. Acad. Sci., USA* **56**, 1491 (1966).

¹⁶ R. C. LIN, N. H. NEFF, S. H. NGAI and E. COSTA, *Life Sci.* **8**, 1077 (1969).

Influence of Prometryne and Ioxynil on Photosynthesis and Nucleic Acid Metabolism in Plants

The herbicides Prometryne¹ and Ioxynil² are both extensively used for weed control in crops. It is well established that Prometryne, as well as other derivatives of s-triazines, interferes with the photosynthetic reactions in plants³. Somewhat contradictory reports on the incorporation of s-triazines into nucleic acid have already been presented. TEMPERLI et al.⁴ demonstrated the incorporation of ^{14}C -labelled Prometryne into bacterial nucleic acid. In a recent paper MÜCKE et al.⁵ challenged the validity of these results. However, a recent report by GRÄSER⁶ even demonstrated incorporation of ^{14}C -labelled Simazine [2-chloro-4,6-bis(diethylamino)-s-triazine] into the nucleic acid of *Zea Mays*, thus further supporting the findings of TEMPERLI et al.⁴.

Regarding Ioxynil, it has been shown that this herbicide uncouples the oxidative phosphorylation in pea-shoot⁷ and rat liver mitochondria⁸. According to PATON and SMITH⁹, two active sites of inhibition in the photosynthetic reaction cycle are to be found, while FRIEND and OLSSON¹⁰ report only one active site. The former findings could be confirmed by BERÜTER¹¹.

In contrast to similar investigations carried out on isolated plant organelles, the present study deals with the effect of these 2 herbicides on photosynthesis and nucleic acid metabolism in whole plants. In order to clarify which metabolic pathway is primarily affected, photosynthesis and nucleic acid metabolism were determined simultaneously by following CO_2 fixation and ^{32}P incorporation, respectively.

Methods. Spinach plants (*Spinacia oleracea* L.) were cultivated in ARNON's¹² culture solution in the green-house: night temperature 15 °C, day temperature 20 °C and 2500 Lux light intensity for 8 h a day. At the end of 8 weeks, 6 samples were taken, each consisting of 7 plants. Each sample was immersed in 650 ml of fresh culture solution to which the following components were added: Incubation was carried out at 20 °C and at a light intensity of 2500 Lux. Leaves from each sample were harvested at definite time intervals. Applying the WARBURG-technique as described by KALBERER et al.¹³, CO_2 fixation of leaf discs from samples 1–3 was measured. The values obtained were expressed as percentages of the C^{14}O_2 fixation

rate obtained for the control sample 3. In samples 4–6 the ^{32}P -labelled nucleic acids were isolated according to a modified SCHMIDT-TANNHAUSER¹⁴ method.

The radioactivity of the extracted ^{32}P -labelled nucleic acid was measured according to CLAUSEN¹⁵ in a Tricarb liquid scintillation counter. Phosphorus was estimated

Sample	Component
1	$4.9 \times 10^{-6} \text{ M}$ Ioxynil
2	$8.3 \times 10^{-6} \text{ M}$ Prometryne
3	None (control)
4	$4.9 \times 10^{-6} \text{ M}$ Ioxynil + 1 mCi $\text{NaH}^{32}\text{PO}_4$
5	$8.3 \times 10^{-6} \text{ M}$ Prometryne + 1 mCi $\text{Na}_2\text{H}^{32}\text{PO}_4$
6	1 mCi $\text{Na}_2\text{H}^{32}\text{PO}_4$ (control)

¹ Trade name for 2,4-bis-(isopropylamino)-6-methylmercapto-s-triazine manufactured by J. R. Geigy AG, Basel (Switzerland).

² Trade name for 3,5-diiodo-4-hydroxy-benzonitrile manufactured by May & Baker Ltd. (England) and Amchem Products Inc. (USA).

³ H. GYSIN and E. KNÜSLI, *Advances of Pest Control Research* (Academic Press, New York 1960), vol. 3, p. 289.

⁴ A. TEMPERLI, H. TÜRRLER and C. D. ERCEGOVICH, *Z. Naturforsch.* **21b**, 903 (1966).

⁵ W. MÜCKE, P. W. MÜLLER and H. O. ESSER, *Experientia* **25**, 353 (1969).

⁶ H. GRÄSER, Abstr. No. 900, 6th Meeting Fedn. Europ. Biochem. Soc., Madrid 1969.

⁷ M. W. KERR and R. L. WAIN, *Ann. appl. Biol.* **54**, 441 (1964).

⁸ V. H. PARKER, *Biochem. J.* **97**, 658 (1965).

⁹ D. PATON and J. E. SMITH, *Nature* **207**, 1211 (1965).

¹⁰ J. FRIEND and R. OLSSON, *Nature* **214**, 942 (1967).

¹¹ J. BERÜTER, in preparation (1970).

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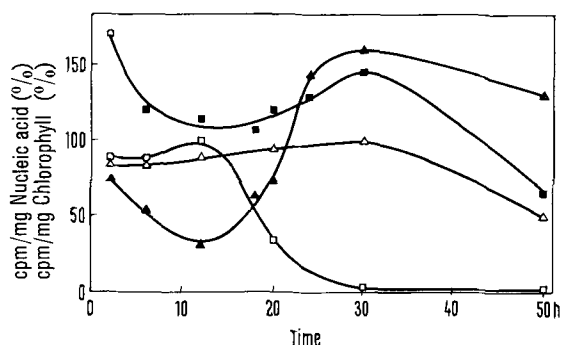
¹³ P. KALBERER, B. B. BUCHANAN and D. I. ARNON, *Proc. natn. Acad. Sci.* **57**, 1542 (1967).

¹⁴ E. P. TYNER, C. HEIDELBERGER and G. A. LE PAGE, *Cancer Res.* **13**, 186 (1953).

¹⁵ T. CLAUSEN, *Analyt. Biochem.* **22**, 70 (1968).

according to KING¹⁶ 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 ³²P into the nucleic acid, as well as the CO₂ fixation, of spinach leaves in the presence of Ioxynil and Prometryne. As demonstrated in the Figure, Ioxynil and Prometryne affect the ³²P incorporation into the nucleic acid before CO₂ fixation is influenced. This is particularly pronounced in the case of Ioxynil. The ³²P incorporation, taken to be a measure of nucleic acid metabolism, shows a similar pattern for both herbicides: the uptake of ³²P shows a



Time course study on the incorporation of P³² into nucleic acid and CO₂ fixation. Results are expressed in percent of values obtained for the herbicide-free controls. □, C¹⁴O₂ fixation in the presence of Prometryne; ■, P³² incorporation in the presence of Prometryne; △, C¹⁴O₂ fixation in the presence of Ioxynil; ▲, P³² incorporation in the presence of Ioxynil. The average rates recorded for the control plants were as follows: CO₂ fixation, 150 μmole CO₂/mg chlorophyll/h; P³² incorporation, 1.75 μatom P/mg nucleic acid/h.

minimum after 12–18 h incubation and increases to a maximum level after 30 h.

With regard to the rate of photosynthetic CO₂ 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 ³²P 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 ¹⁴CO₂ uptake in the herbicide treated plants. The study revealed that in the range where for both herbicides ³²P incorporation into nucleic acid was already affected, the ¹⁴CO₂ 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 ³²P-Einbau in die Nukleinsäuren beeinflusst wurde, bevor eine Hemmung der photosynthetischen CO₂-Fixierung eintrat.

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¹⁶ E. J. KING, *Biochem. J.* 26, 292 (1932).

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 DIPPE¹), nerve stimulation (GIACOBINI and GRASSO²), and starvation (LARDY³ and GLOCK and McLEAN⁴). 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 spectrophotometer.

¹ H. BURCH and P. VON DIPPE, *J. biol. Chem.* 239, 1898 (1964).

² E. GIACOBINI and A. GRASSO, *Acta physiol. scand.* 66, 49 (1966).

³ H. LARDY, *Control of Energy Metabolism* (Ed. B. CHANCE, R. ESTABROOK, J. WILLIAMSON; Academic Press, New York 1965), p. 246.

⁴ G. GLOCK and P. McLEAN, *Biochem. J.* 61, 388 (1955).