sidered overall a first-order reaction. k value, calculated between the 5th and 30th days, is 0.117 days^{-1}.

Data reported here seem to confirm the possible mechanism of regulation of acid phosphatase by phosphate level, according to that demonstrated by other authors in plants[5,6] and for alkaline phosphatase in bacteria[12,13], fungi[3] and mammalians[13,14]. In fact, results obtained from some authors on acid phosphatase of rat kidney, clearly demonstrate that such enzymes in mammalians is not regulated by phosphate level[15–17]. Then, although the regulatory effect of phosphate level on phosphatase activity has been ascertained in all organisms, from simpler to more complex, one could discriminate a mechanism which could function through alkaline phosphatase in mammalians and through acid phosphatase in plants.

Riassunto. Durante la germinazione di *Pinus pinea* i livelli di fosfato libero sono risultati essere correlati a quelli della fosfatasi acida, anche in differenti parti della plantula. I valori di fosfato totale sono costanti in tale periodo.

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[13] A. TORRIANI, Biochim. biophys. Acta *38*, 460 (1960).
[14] A. GAREN and C. LEVINTHAL, Biochim. biophys. Acta *38*, 470 (1960).
[15] F. MELANI, G. RAMPONI, A. GUERRITORE and V. BACCARI, Nature *201*, 710 (1964).
[16] F. MELANI, G. RAMPONI, M. FARNARARO, E. COCUCCI and A. GUERRITORE, Biochim. biophys. Acta *138*, 411 (1967).
[17] F. MELANI, M. FARNARARO and G. SGARAGLI, Boll. Soc. it. Biol. Sper. *43*, 1406 (1968).

6-Hydroxydopamine-Induced Inhibition of Brain Catecholamine Synthesis without Ultrastructural Damage

6-Hydroxydopamine induces a specific degeneration of peripheral sympathetic nerve terminals with a marked depletion of norepinephrine[1–4]. Its intracerebral[5] or intraventricular[6–8] injection also causes a decrease of norepinephrine and to a lesser extent of dopamine in the brain. Degenerative changes of catecholaminergic nerve terminals are also observed[7]. Recently, it has been shown, however, that a small single dose of 6-hydroxydopamine (200 µg) injected into the cerebral ventricles lowers the cerebral catecholamines without inducing ultrastructural damage[9]. Therefore, mechanisms other than destruction of the catecholaminergic nerve terminals might be involved in the catecholamine-depleting action of 6-hydroxydopamine.

In the present paper, evidence is presented that doses of 6-hydroxydopamine which apparently do not cause ultrastructural brain damage inhibit the formation of cerebral catecholamines from tyrosine administered into the cerebral ventricles.

Experimental. In male albino rats of Wistar origin (Füllinsdorf), weighing 250–280 g, 200 µg 6-hydroxydopamine (base, applied as hydrobromide dissolved in 10 µl saline) were injected through a cannula permanently implanted in the right lateral ventricle of the brain[10]. Animals given saline injections served as controls. 2 or 5 days later and 1 h before decapitation, the animals received either 15 µg of L-2-^{14}C-tyrosine (specific activity 175 µC/mg; dissolved in 10 µl 0.1M Na-K-phosphate buffer, pH 7.4) intraventricularly or 3 mg/kg L-2-^{14}C-3,4-dihydroxyphenylalanine (dopa) (specific activity 100 µC/mg) i.p. The brains were rapidly dissected on ice and 10–14 (^{14}C-tyrosine) or 3–5 (^{14}C-dopa) brain stems (including the basal ganglia) were pooled.

In the ^{14}C-tyrosine experiments, the brain tissue was homogenized in 0.4M $HClO_4$, the supernatant adjusted to pH 2 and brought on a column of Dowex 50-X 4 in Na^+ form. As previously described[11], 3 different fractions were obtained: amino acids, catecholamines and metabolic end-products. In order to separate the products of tyrosine transamination from the catechol derivatives, the fraction of metabolic end-products from ^{14}C-tyrosine-treated animals was passed through alumina essentially as described for the separation of dopa and 3-O-methyldopa[11]. The absorbed cakchol metabolites were eluted with 0.2N HCl. This eluate was adjusted to pH 5 and passed through a column of Dowex AG 3 X 4 (0.5 g) from which the dihyroxylated deaminated metabolites (DHDM) were re-eluted with 5N acetic acid. After measurement of the radioactivity of an aliquot of the eluate, the rest was evaporated. The residue was dissolved in 90% (v/v) aqueous methanol and chromatographed on Whatman No. 1 in butanol saturated with 0.5M K-acetate buffer, pH 4.5. The paper chromatogram was cut into strips and measurements of their radioactivity were carried out in a liquid scintillation counter.

In the experiments with ^{14}C-dopa, the brain stems were homogenized in 0.5N HCl and the supernatant, adjusted to pH 5, was passed on Dowex AG 3 X 4 for the absorption of the acidic metabolites which were re-eluted and submitted to paper chromatography as described above. The effluent, adjusted to pH 2, was brought on Dowex 50-X 4 for the separation of amino acids and catecholamines[11].

Results. (1) One hour after intraventricular injection of ^{14}C-tyrosine, the accumulation of radioactive amino acids

[1] C. C. PORTER, J. A. TOTARO and C. H. STONE, J. Pharmac. exp. Ther. *140*, 308 (1963).
[2] R. LAVERTY, D. F. SHARMAN and M. VOGT, Brit. J. Pharmac. *24*, 549 (1965).
[3] J. P. TRANZER and H. THOENEN, Experientia *24*, 155 (1968).
[4] T. MALMFORS and CH. SACHS, Europ. J. Pharmac. *3*, 89 (1968).
[5] M. UNGERSTEDT, Europ. J. Pharmac. *5*, 107 (1968).
[6] N. J. URETSKY and L. L. IVERSEN, Nature *221*, 557 (1969).
[7] F. E. BLOOM, S. ALGERIE, A. GROPPETTI, A. REVUELTA and E. COSTA, Science *166*, 1284 (1969).
[8] W. P. BURKARD, M. JALFRE and J. BLUM, Experientia, *25*, 1295 (1969).
[9] G. BARTHOLINI, J. G. RICHARDS and A. PLETSCHER, Experientia *26*, 142 (1970).
[10] J. F. HYDEN, L. R. JOHNSON and R. P. MAICKEL, Life Sci. *5*, 1509 (1966).
[11] G. BARTHOLINI and A. PLETSCHER, J. Pharmac. exp. Ther. *161*, 14 (1968).

was not substantially changed in the brain stem of 6-hydroxydopamine-treated animals as compared to controls. On the other hand, the fractions of ^{14}C-catecholamines and ^{14}C-DHDM were significantly diminished ($p < 0.01$) (Table I). As established by paper chromatography, the fraction of ^{14}C-DHDM consisted of 3 components, i.e. ^{14}C-3,4-dihydroxyphenylacetic acid, ^{14}C-3,4-dihydroxymandelic acid and an unidentified compound. After pretreatment with 6-hydroxydopamine, ^{14}C-3,4-dihydroxyphenylacetic acid and ^{14}C-3,4-dihydroxymandelic acid were reduced, whereas the unknown component was not significantly changed ($p > 0.05$) (Table I).

(2) After i.p. injection of ^{14}C-dopa, the concentration of labelled amino acids and metabolic end-products in the brain stem of rats pretreated with 6-hydroxydopamine was slightly but not significantly ($p > 0.05$) increased, whereas the ^{14}C-catecholamine fraction was significantly ($p < 0.01$) decreased (Table II). According to paper chromatography, the fraction of metabolic end-products consisted of ^{14}C-3,4-dihydroxyphenylacetic acid, ^{14}C-homovanillic acid and traces of ^{14}C-vanillylmandelic acid. No difference in the composition of the ^{14}C-phenolcarboxylic fraction was found in controls and in 6-hydroxydopamine-treated animals (Table II).

(3) Electronmicroscopical examination showed no apparent degenerative changes of brain neurons, as previously described.

The same results were obtained 2 and 5 days after administration of 6-hydroxydopamine.

Discussion. According to the present experiments, 6-hydroxydopamine injected into the cerebral ventricle in doses previously shown not to cause ultrastructural changes[9] markedly inhibits the ^{14}C-tyrosine-induced accumulation of ^{14}C-catecholamines in the brain stem. Furthermore, 6-hydroxydopamine causes a decrease in ^{14}C-DHDM which results from a diminution of the ^{14}C-catecholamine metabolites, ^{14}C-3,4-dihydroxyphenylacetic acid and ^{14}C-3,4-dihydroxymandelic acid. This finding indicates that the decreased accumulation of ^{14}C-catecholamines is at least in part due to inhibition of their biosynthesis. According to preliminary results, 6-hydroxydopamine increases the endogenous cerebral tyrosine only to a small extent (by about 10%). Therefore, the markedly diminished formation of ^{14}C-catechols by 6-hydroxydopamine cannot be due to a mere 'dilution' of ^{14}C-tyrosine.

In the fraction of acidic ^{14}C-tyrosine metabolites, only the dihydroxylated deaminated compounds (DHDM) have been determined since these can be separated (on alumina) from the products of tyrosine transamination. No method has been available for a reliable separation of the methoxylated products, e.g. homovanillic acid. However, there is a correlation between dihydroxylated and methoxylated derivatives. In fact, part of the dihydroxyphenylacetic acids is converted to homovanillic acid[12]. Moreover, chlorpromazine, which induces a marked increase of endogenous homovanillic acid, also enhances the tyrosine-induced rise of dihydroxyphenylacetic acid[13]. On the other hand, after ^{14}C-dopa administration, homovanillic acid is formed to the same extent in controls and in 6-hydroxydopamine-treated animals (Table II). It is therefore likely that the 3,4-dihydroxyphenylacetic acid and probably also the 3,4-dihydroxymandelic acid may be representative of the acidic metabolites of catecholamines.

The experiments with ^{14}C-dopa indicate that 6-hydroxydopamine does not markedly interfere with dopa decarboxylation, since the cerebral accumulation of ^{14}C-phenolcarboxylic acid is not reduced. The considerable diminution of the ^{14}C-tyrosine-induced increase of the ^{14}C-3,4-dihydroxyphenylcarboxylic acids and in part also of the ^{14}C-catecholamines by 6-hydroxydopamine might therefore be the consequence of an inhibition of tyrosine hydroxylation. An additional effect of 6-hydroxydopamine, i.e. an interference with the amine storage mechanism, may, however, be involved. This is indicated by the (relatively slight) diminution of the ^{14}C-dopa-induced accumulation of ^{14}C-catecholamines. Furthermore, preliminary experiments indicate that ^{14}C-norepinephrine administered intraventricularly disappears more rapidly from the brain in animals pretreated with 200 µg 6-hydroxydopamine than in controls. A displacement mechanism has also been postulated in extracerebral adrenergic nerves[14].

Table I. Effect of 6-hydroxydopamine (6-OHDA) on radioactive metabolites in brain stem of rats injected with ^{14}C-tyrosine

Fraction	Controls Radioactivity	6-OHDA Radioactivity	% of controls[a]
Amino acids	2.56 ± 0.09	2.71 ± 0.06	106.1 ± 2.5
Catecholamines	0.34 ± 0.03	0.075 ± 0.005	22.9 ± 3.5[b]
DHDM	0.0150 ± 0.0015	0.0095 ± 0.001	64.0 ± 3.3[b]
DOPAC	0.0085 ± 0.0005	0.0029 ± 0.0004	28.3 ± 2.3[b]
DOMA	0.0042 ± 0.0017	0.0028 ± 0.0011	58.0 ± 8.5[b]
Unknown	0.0037 ± 0.001	0.0040 ± 0.0008	96.3 ± 6.0

[a] Averages of the % values obtained in each individual experiment.
[b] $p < 0.01$.
^{14}C-Tyrosine (15 µg) was injected into the lateral ventricle of the brain 48 h after intraventricular administration of 200 µg 6-OHDA and 1 h before sacrifice. The radioactivity (per g brain tissue) is expressed in percent of the total injected radioactivity. The values represent averages and S.E. of 3 experiments. DHDM, dihydroxylated deaminated metabolites; DOPAC, 3,4-dihydroxyphenylacetic acid; DOMA, 3,4-dihydroxymandelic acid.

Table II. Effect of 6-hydroxydopamine (6-OHDA) on radioactive metabolites in brain stem of rats injected with ^{14}C-dopa

Fraction	Controls Radioactivity	6-OHDA Radioactivity	% of controls[a]
Amino acids	5.98 ± 0.41	7.16 ± 0.90	119.1 ± 12.4
Catecholamines	1.15 ± 0.14	0.72 ± 0.04	65.2 ± 6.4[b]
PCA	2.01 ± 0.23	2.14 ± 0.08	110.0 ± 9.2
DOPAC	0.60 ± 0.08	0.69 ± 0.07	102.8 ± 10.9
HVA	1.35 ± 0.15	1.38 ± 0.04	91.7 ± 11.6

[a] Averages of the % values obtained in each individual experiment.
[b] $p < 0.01$.
^{14}C-L-Dopa (3 mg/kg) was administered i.p. 48 h after injection of 200 µg 6-OHDA into the lateral ventricle of the brain and 1 h before sacrifice. The radioactivity (per g brain tissue) is expressed in percent of the injected radioactivity per g body weight. The values represent averages and S.E. of 4 experiments. PCA, phenolcarboxylic acids; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid).

[12] H. Alton and McC. Goodall, Biochem. Pharmac. *18*, 1373 (1969).
[13] G. Bartholini and A. Pletscher, Experientia *25*, 919 (1969).
[14] H. Thoenen and J. P. Tranzer, Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. *261*, 271 (1968).

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-hydroxydopamine[9].

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-Catecholaminmetaboliten 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 Prometryne[1] and Ioxynil[2] 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 plants[3]. 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 GRÄSER[6] 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-shoot[7] and rat liver mitochondria[8]. According to PATON and SMITH[9], two active sites of inhibition in the photosynthetic reaction cycle are to be found, while FRIEND and OLSSON[10] report only one active site. The former findings could be confirmed by BERÜTER[11].

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's[12] 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-TANNHAUSER[14] method.

The radioactivity of the extracted ^{32}P-labelled nucleic acid was measured according to CLAUSEN[15] in a Tricarb liquid scintillation counter. Phosphorus was estimated

Sample	Component
1	$4.9 \times 10^{-6} M$ Ioxynil
2	$8.3 \times 10^{-6} M$ Prometryne
3	None (control)
4	$4.9 \times 10^{-6} M$ Ioxynil + 1 mCi $NaH^{32}PO_4$
5	$8.3 \times 10^{-6} M$ Prometryne + 1 mCi $Na_2H^{32}PO_4$
6	1 mCi $Na_2H^{32}PO_4$ (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. TÜRLER 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).

[12] D. I. ARNON, Pl. Physiol. *24*, 1 (1949).

[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).