that erythropoietin binds to the chromatin, probably by a protein-protein bound and then renders a portion of the DNA available as a template for the synthesis of RNA. In vivo stimulation of the RNA polymerase may also be explained in this manner. PINTO[11] described the formation of a complex between erythropoietin and DNA from tissues like bone-marrow, liver, spleen and thymus. The binding occurs in a particular region of the DNA and probably involves a potein-protein interaction in which the hormone could play the role of an allosteric ligand in respect to the other protein.

In a recent paper GROSS and GOLDWASSER[1] have reported that shortly after erythropoietin is made available to marrow cells in vitro, a very large RNA (150 S) is synthesized. This RNA cannot be detected in control samples without hormone. The 150 S RNA has a short half-life of approximately 6 min. All the data presented suggest the following sequential molecular events induced by the erythropoietin.

The first molecular action of the hormone would be related to a specific binding to a protein receptor at the chromatin level. As a result of this action, a specific region of the DNA is susceptible to be used as a template. The second molecular event is related to the action of the RNA polymerase and the synthesis of a 150 S RNA. The following step may be related with the degradation process of the 150 S RNA and the preparation of the biochemical units for the protein synthesis.

The sequential nuclear reactions discussed above are consistent with the idea that the information for the synthesis of haemoglobin must be prepared during the early stages of the erythropoietic process[12,13]. The evidence presented firmly supported the hypothesis that the erythropoietin acts at molecular level in the transcription stage. Further studies of this idea are in progress and will be reported later on[14].

Resumen. La eritropoyetina estimula in vivo la actividad de la RNA polimerasa de médula ósea de rata. In vitro, la hormona no parece aumentar la actividad de la enzima. Las características de la acción enzimática demuestran que la actividad medida corresponde a la de la RNA polimerasa descrita en otros tejidos. Se sugiere que el sitio primario de acción de la hormona está en la etapa de transcripción y se discuten los eventos moleculares que produce la eritropoyetina a este nivel.

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Role of Phosphate and Acid Phosphatase During Germination of *Pinus pinea* Seed[1]

A striking feature of seed germination can be considered to be the mobilization of storage energetic material accumulated in reserve tissues, and the following translocation and utilization of the same for the development of embryo axis. Phosphate plays an extremely important role in a variety of reactions in germinating seed, where it appears primarily in a linked organic form and very little seems to be present as free inorganic orthophosphate[2], so that it represents a limiting factor in many reactions.

Correlated with this fact, the increase in activity of typical hydrolitic enzymes as phosphatases appears particularly significant and the change in free and total phosphate level observes in the same period. Researches on this biological mechanism have been made extensively during the life cycle of algae[3], fungi[4] and in germinating seed of some angiosperms[5,6]. Recently an extensive plan of research on the metabolism of Gymnosperms led us to investigate the alternance of metabolic pathways during seed germination of different species of conifers[7–10] but our data are lacking in the role of phosphate in this systematic group.

In this paper we have followed the time sequences of the changes in free and total phosphate compared with the levels of acid phosphatase in seedling and in the endosperm, root and shoot of germinating seed of *Pinus pinea*.

Experimental. Seeds of *Pinus pinea* were cultivated on moistened sand at 20 °C for a 9 h photoperiod. Preparation of extracts and determination of acid phosphatase, free and total phosphate and protein content was performed as previously indicated[6].

Results and discussion. Results are reported in Figures 1 (a, b, c, d) and 2, where the values of acid phosphatase, free and total phosphate are reported during the first 20 days of germination in different parts of germinating seed. In general total phosphate remains constant in the experi-

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mental period, whereas a decrease in free phosphate is correlated with an increase in acid phosphatase or vice versa. In Figure 2 the exponential and logarithmic plot of formation of free phosphate in the whole seedling is reported.

The linear plot has an exponential behaviour. Assuming this curve can be represented by the equation

$$y = a \cdot e^{nx} \qquad \text{(a)}$$

where y is concentration of phosphate and x stands for day of experiment, a and n two constants which can be easily calculated; by analytical procedure the following expression can be obtained:

$$y = 2.70 \cdot e^{0.105x} \qquad \text{(b)}$$

where $a = 2.70$ and $n = 0.105$. In this way the curve can be easily calculated theoretically (dotted curve).

From data reported, it becomes evident that the systematic group of origin plays a fundamental role on the development of germinating seed. Literature data[6,11,12] show clearly that in Angiosperms phosphatase activity reaches the maximum, in the major part of cases, at the 4th day of germination and never after the 10th day. In the conifer group, the time of germination is longer. Sometimes on the 18th day of germination the endosperm is still present and active. Correlated to this fact is the activity of acid phosphatase which reaches the maximum on the 20th day. This behaviour, valid also for other enzymes[8–10], seems to represent a constitutive characteristic of this systematic group.

From data reported in Figures 1 and 2, one can see that free phosphate is in equilibrium with total phosphate since, when free phosphate is required, it could be released from storage tissues according to the reaction: $XP \rightarrow X + P_i$. A mechanism of this type seems to be responsible for free phosphate increase from the 5th to 30th day of germination. Acid phosphatase could act at this level recovering phosphate from any molecule where it is conserved.

The plot of concentration (C) against time (t), reported in Figure 2, gives a typical exponential profile, whereas the plot *ln* (C) against time gives a straight line which could indicate that free phosphate release is to be con-

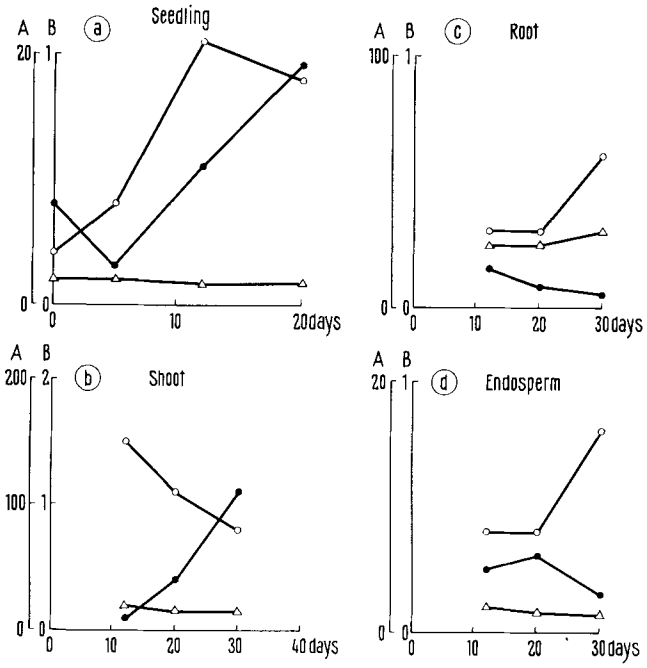

Fig. 1. Levels of acid phosphatase (○), total phosphate (△) and free phosphate (●) in seedling (a), shoot (b), root (c) and endosperm (d) during germination of *Pinus pinea*. Values reported are representative of several experiments, all in agreement with each other.

a) A: scale for free phosphate (μmoles/g of fresh tissue weight). B: scale for acid phosphatase (μmoles of substrate hydrolyzed/mg of total protein) and total phosphate (μmoles/mg of fresh tissue).

b) scale for free phosphate (μmoles/g of fresh tissue weight). B: scale for acid phosphatase (μmoles of substrate hydrolyzed/mg of total protein) and total phosphate (μmoles/mg of fresh tissue).

c) A: scale for total phosphate (μmoles/g of fresh tissue weight) and free phosphate (μmoles/g of fresh tissue weight). B: scale for acid phosphatase (μmoles of hydrolyzed substrate/mg of total protein).

d) A: scale for free phosphate (μmoles/g of fresh tissue weight). B: scale for acid phosphatase (μmoles of hydrolyzed substrate/mg of total protein) and total phosphate (μmoles/mg of fresh tissue weight).

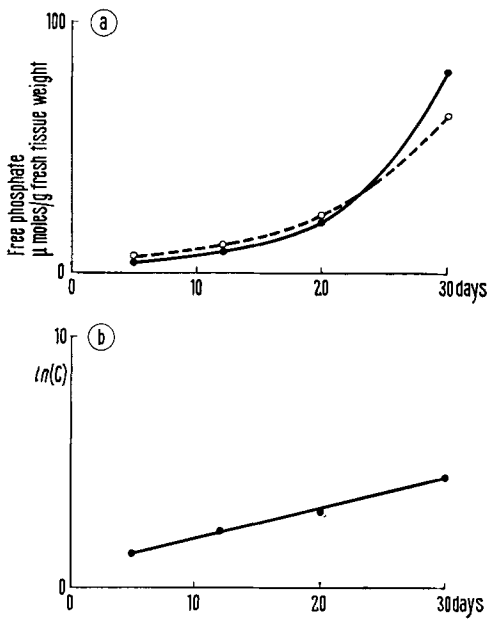

Fig. 2. Exponential (a) and logarithmic plot (b) of formation of free phosphate in seedling of *Pinus pinea*; ———, values found experimentally; – – – – –, values found by calculation.

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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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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

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