Conclusions. DPPD protects when given by mouth against oxygen toxicity. The degree of protection varies directly with the number of applications, indicating that DPPD is accumulated in tissue.

Since the same central nervous system response to hyperbaric oxygen was seen in all mice it does not seem to be involved in the mechanism of DPPD protection. The mechanism is not known, but contrary to the results of JAMIESON and VAN DEN BRENK, the lungs were less damaged in treated animals.

These data, save for the exception noted, substantially confirm those of JAMIESON and VAN DEN BRENK. There is, however, a great strain difference in the tolerance of mice to hyperbaric oxygen. Untreated C_3H mice survived the same pressure much longer than the strain they used.

Some preliminary experiments in which untreated and DPPD fed mice were returned to air at atmospheric pressure after varying intervals in hyperbaric oxygen show that DPPD greatly extends the time they can spend in hyperbaric oxygen without obvious damage[5].

Résumé. Des souris adultes traités au préalable par du *N,N*-diphenyl-*p*-phénylène diamine sont protégés contre la toxicité du oxygène hyperbarié. La protection est dépendente du dosage. Le méchanisme est inconnu.

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Cytokinin and Gibberellin-Like Activity in the Spring Sap of Trees

The hormonal control of the physiological processes by which deciduous trees begin active growth in spring has been the subject of much speculation and investigation[1-3]. Of direct interest to the present work is the demonstration by NANDA and PUROHIT[4] that gibberellic acid will initiate the release of sugars from starch stored in twigs. As measured by the increase in bud burst (dormancy release), applied gibberellic acid will also overcome the effect of a substance causing dormancy in birch[5]. EAGLES and WAREING[5] also observed in *Acer pseudoplatanus* 'significant changes in the levels of endogenous gibberellins during December–April'. Recently SKENE[6] has demonstrated gibberellin activity in the bleeding sap collected from *Vitis vinifera* prior to bud burst.

Kinin-like compounds, together with gibberellins, may also be implicated in bud burst since benzyladenine will break dormancy in *V. vinifera*[7] and in unchilled seeds of a number of species of deciduous trees[8]. Application of kinetin to lateral buds of peas enables them to overcome apical dominance[9], although auxin must be added to the bud if its growth rate is to be of the same magnitude as that of an uninhibited lateral bud[10]. The bleeding sap of *Acer saccharum* was shown by NITSCH and NITSCH[11] to contain substances with cytokinin-like activity shortly before bud burst. Thus in early spring supplies of gibberellins and cytokins may become available to buds either by release or by synthesis within the buds themselves, or as imports, together with nutrients, in the ascending sap. We have already shown that the xylem sap of a number of herbaceous plants contains both kinin-like[12] and gibberellin-like[13] substances (see also[14]).

This communication deals with an examination of the kinin and gibberellin activities of the spring sap of 2 tree species.

Xylem sap was collected from sycamore (*A. pseudoplatanus*) and birch (*Betula pubescens*) in March 1966, immediately prior to bud burst. Sap used for gibberellin determination was obtained by sawing down a 6 m high sycamore tree and collecting the sap bleeding from the remaining stump (30 cm high) and root system. Sap for cytokinin determination was collected by boring a hole (30 cm above ground level) through the bark into the xylem of birch and sycamore trees, and fitting a piece of polythene tube into the hole. In both cases the sap was collected for 12 h, immediately frozen and kept at − 20 °C until required.

Gibberellin determination. After thawing, 100 ml of sap was processed as described elsewhere[15]. The final eluants, from the thin layer chromatograms of the 2 fractions (acidic, and basic + neutral) were assayed for gibberellin content using the barley endosperm assay[16,17], and the Meteor pea bioassay[15]. The results obtained from the fraction extracted at pH 7.5 are shown in Figure 1. Only this fraction showed any significant ($P < 0.05$) amounts of gibberellin-like substances. While the barley endosperm assay showed one zone of activity, Rf 0.3–0.6, 2 peaks (Rf 0.2–0.3 and 0.5–0.6) were obtained using the Meteor

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pea assay. It is known that these assays are differentially sensitive to various characterized gibberellins[18,19].

Cytokinin determination. After thawing, 45 ml of sap from each species were partitioned between butanol and water, chromatographed as previously described[12] and assayed using MILLER's soybean callus bioassay[20]. The results (Figure 2) demonstrate 3 peaks of cytokinin-like activity; Rf 0.2–0.4 in the aqueous fraction, and Rf 0.1–0.5, 0.8–1.0 in the butanol fraction. Thus these compounds have properties similar (butanol solubility, Rf, kinin activity) to those of substances isolated from maize

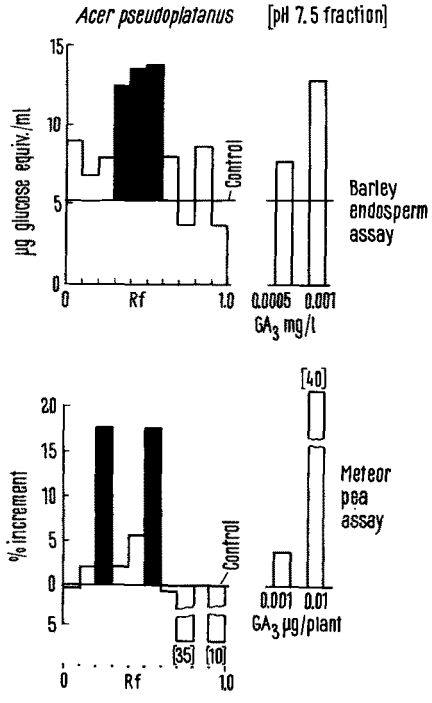

Fig. 1. Barley endosperm and meteor pea assay for presence of gibberellin-like substances in a fraction extracted at pH 7.5 from the bleeding sap of *A. pseudoplatanus*. Black portion of the histograms indicates significant difference from controls at, at least P of 0.05.

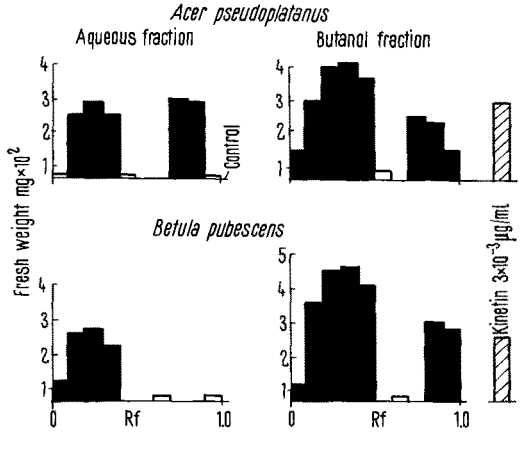

Fig. 2. Soybean callus bioassay for presence of kinin-like substances in aqueous and butanol fractions of bleeding sap of *A. pseudoplatanus* and *B. pubescens*. Black portion of the histograms indicates significant difference from controls at, at least P of 0.05.

by MILLER[21], suggestive of the presence of a zeatin-like compound with its nucleoside and nucleotide. The Rf of known zeatin (kindly supplied by Prof. D. S. LETHAM) is 0.2–0.4 in the borate system, i.e. roughly corresponding with the compounds of low Rf in the butanol-soluble fraction. Work is in progress on the chemical identity of the unknown factor.

These results clearly show that before bud burst in spring, the xylem sap of some tree species contains cytokinins and gibberellin-like substances. The source of these substances in the tree is not readily established. They may originate in either the roots or the xylem parenchyma of the trunk and may be synthesized de novo or released from storage[22]. If they originate from the roots, the roots must be either growing, or at least in a non-dormant state just before bud burst: a suggestion which is supported by ENGLER[23], COCKERHAM[24] and JONES[25] who all report active root growth in *A. pseudoplatanus* throughout most of the winter. Release of hormones from the xylem parenchyma would be consistent with the observation that dormant buds, on detached rootless twigs of beech can be induced to break dormancy by continuous illumination[26], an observation that has been confirmed by the present authors.

The activities of cytokinins and gibberellins in bud burst are likely to be diverse since they are involved in the regulation of phenomena ranging from the mobilization of assimilates and inorganic substances[4,27] to cell division[28] and protein synthesis[29]. However it seems likely that if the roots synthesize and export cytokinins[12] and gibberellins[15] the roots must act in concert with the buds themselves. The buds are known to be sites of auxin synthesis, the basipetal movement of which may in turn partially control root growth[30–33].

Zusammenfassung. Die Xylemsäfte von *Acer pseudoplatanus* und *Betula pubescens* enthalten vor dem Austreiben der Knospen im Frühjahr Cytokinine und Gibberellin-ähnliche Stoffe. Diese stammen entweder aus den Wurzeln oder aus dem Xylemparenchym.

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