Effect of different concentrations of sodium metabisulfite, IAA and sucrose alone and in combinations, on the number of mung bean hypocotyl cuttings that rooted out of 10 and the number of roots produced per cutting (figures in parentheses)

Treatment	Concentration of sodium metabisulfite (mg/l)					
	0	10	20	40	80	100
IAA (0, 1, 3 or 5 mg/l)	0	0	0	0	0	0
Sucrose (1%)	10 (5.4 ± 0.2)	10 (6.1 ± 0.4)	10 (8.0 ± 0.8)	10 (9.2 ± 0.9)	10 (13.7 ± 1.5)	10 (11.3 ± 1.0)
IAA (1 mg/l + sucrose 1%)	10 (15.9 ± 1.7)	10 (26.5 ± 2.1)	10 (38.3 ± 2.1)	10 (36.8 ± 2.1)	10 (26.4 ± 1.5)	10 (21.9 ± 1.8)
IAA (3 mg/l + sucrose 1%)	10 (29.8 ± 0.2)	10 (38.1 ± 2.3)	10 (40.2 ± 1.2)	10 (37.5 ± 4.8)	10 (31.7 ± 1.8)	10 (25.4 ± 2.2)
IAA (5 mg/l + sucrose 1%)	10 (33.4 ± 1.2)	10 (42.0 ± 1.7)	10 (48.0 ± 2.5)	10 (33.9 ± 2.4)	10 (18.7 ± 1.2)	10 (18.3 ± 1.8)

± Standard error

the epicotylar and hypocotylar portions, leaving behind only 6 cm in each case with 3 cm length of epicotyl and of hypocotyl. The cuttings were divided into 48 groups of 10 each, to be cultured in grade tubes, each containing 30 ml of test solution and only hypocotylar portion was dipped in the medium. The test solutions consisted of varying concentrations of IAA, sucrose and metabisulfite, singly and in different combinations, and were prepared in 30 μM chloramphenicol to prevent microbial infection, an equivalent being added to water to serve as control. Observations of the number of rooted cuttings and roots produced were recorded after 7 days. The experiment was repeated three times with similar results.

The results, together with the treatments, are presented in the Table. Cuttings did not root either in water or in IAA alone but rooted in sucrose and more profusely in IAA + sucrose, showing that the production of adventitious roots was limited by the level of endogenous nutrition and that a proper balance between auxin and nutrition was necessary for optimal effect [7–10].

Sodium metabisulfite acted synergistically with IAA. Thus, the number of roots produced on cuttings cultured in 1 mg/l IAA + 1% sucrose + 20 mg/l sodium metabisulfite was higher than on those cultured in 5 mg/l IAA + 1% sucrose, the highest being on cuttings grown in 5 mg/l IAA + 1% sucrose + 10 or 20 mg/l sodium metabisulfite. These results clearly show that enhancement in the enzymatically produced oxidative products of IAA stimulated by sodium metabisulfite[3,6] considerably increased the production of adventitious roots. The inhibitory effect of higher concentrations of sodium metabisulfite in the medium may be ascribed to the supra-optimal concentrations of oxidation products. It is thus apparent that these oxidation products exhibit, like all growth regulators, a diphasic concentration response. A high synergism of monophenols that enhance the activity of IAA oxidase with applied auxins has also been demonstrated earlier[11]. The reduction in Avena coleoptile curvature with chlorogenic acid[12], a polyphenol that inhibits, but its enhancement by monophenol parahydroxybenzoic acid[13] that promotes the activity of IAA oxidase, lends support to the postulate that enzymatic oxidation of IAA is essential for a positive growth response and refute the detoxification role ascribed to IAA-oxidase.

The complete suppression of rooting on mung bean hypocotyl cuttings by even as low as 100 µg of a specific proteinaceous inhibitor of IAA-oxidase, that has been isolated in this laboratory from stem cuttings of *Ipomoea fistulosa*[14] when it is added to the medium containing IAA + sucrose, lends further support to this postulate.

Zusammenfassung. Nachweis, dass die Adventivwurzelbildung an Bohnenhypocotylen (*Phaseolus mungo*) in Anwesenheit von Indolylessigsäure gefördert wird bei gleichzeitigem Na-metabisulfit Angebot. IES und Na-bisulfit haben einen synergistischen Effekt, welcher Oxidationsprodukten der ersteren zugeschrieben wird.

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Adenosine, a Sex-Linked Excretory Product of a Lepidopteran, *Pieris brassicae*

The main excretory purine compounds of insects are known as uric acid (and its metabolites) and pteridines. Some minor compounds, intermediates in uric acid production have also been detected, e.g. hypoxanthine and xanthine[1,2]. All these compounds may be present simultaneously, but their relative importance may vary among species. In the case of the pupal/adult stages of the Lepidopteran *Pieris brassicae*, the most abundant products are uric acid in the body[3,4], leucopterin and isoxanthopterin in the wings[4–7]. HARMSEN[4] described an important accumulation of xanthine in the body (1,200 µg/animal) prior to adult emergence. We tried various metabolic

experiments to understand the reason for such an accumulation, but, surprisingly, we found only traces (less than 50 µg) of this compound in our strain of *P. brassicae*. However, we found important sex-linked amounts of another compound, not earlier reported in Lepidoptera, that we identified as adenosine.

At various intervals during pharate adult and adult stages, groups of 5 animals were collected (males and females separately), and extracted either with water or methanol-pyridine-water (5:1:4, v/v). The extractions were repeated several times, giving a final volume of 80 ml for every lot. An aliquot of 5 ml was concentrated and evaporated on a thin-layer chromatogram (cellulose with luminescer: SCHLEICHER and SCHÜLL 144LS254). Chromatoplates were run with 0.1 *M* ammonium formate, pH 5.3. The 2 main absorbing compounds (Figure 1) were eluted with 10 ml 0.01 *N* HCl on a glass filter and UV-analyzed. The first one was identified as uric acid and the latter showed a maximal absorbance at 257–258 nm; UV-spectra of this compound at various pH's and cochromatography in 6 solvent systems with some purine derivatives led to its identification as adenosine.

Adenosine is localized in the fat body, and noticeable amounts were only found in males. Its appearance is time-related (Figure 2) and correlated with the recovery of uric acid synthesis by the fat body[7,8], that corresponds to a de novo synthesis of purines by the way of IMP[9]. We may therefore assume that IMP is metabolized in the males by two different pathways (Figure 3), leading either to uric acid (fat body contains high levels of guanine deaminase and xanthine dehydrogenase) or to adenosine, not further metabolized at this stage. In the females, adenosine might be used by developing ovaries for nucleic acids or uric acid syntheses.

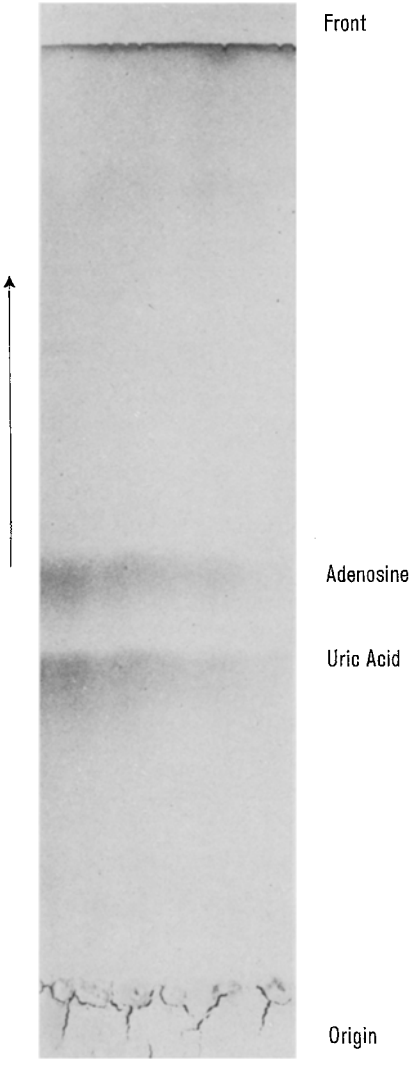

Figure 1. UV-photography of a chromatoplate, showing the 2 major excretory products.

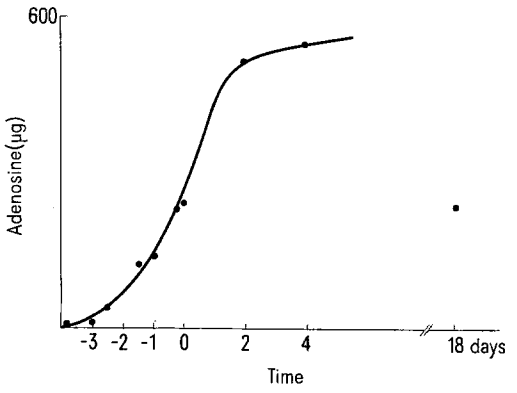

Figure. 2. Appearance of adenosine in the body of male *Pieris*; time-scale starts at adult emergence.

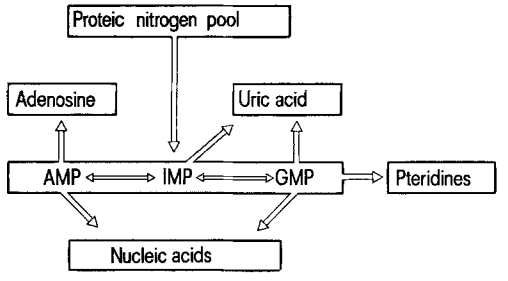

Figure 3. Simplified relationships between purine compounds.

Résumé. Un nouveau composé d'excrétion azotée, l'adénosine a été mis en évidence chez le mâle adulte de *Pieris brassicae*. Il apparaît peu avant l'émergence et s'accumule dans le corps gras, où il est vraisemblablement synthétisé.

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