not measured, it is possible that any gastric or oral UR[4-6] may have some influence on RR outside the central nervous system (CNS).

The rationale behind the present study was oriented towards CNS arousal factors in RR control and not necessarily towards changes in metabolic rate per se. But if there is a resting UR of daytime metabolic rate, which may be reflected in the changes in oxygen consumption reported earlier[10], then these small changes in oxygen requirements may be made at the expense of tidal volume and not necessarily in RR[11]. Thus a further study might assess changes in respiration flow rate[12].

[11] F. J. CLARK and C. VON EULER, J. Physiol., Lond. *222*, 267 (1972).

[12] Acknowledgment. We would like to thank Dr RAY MEDDIS of this department for his helpful advice and assistance in the data analysis.

## A Paradoxical Effect of 2,4-Dinitrophenol in Stimulating the Rooting of Hypocotyl Cuttings of *Phaseolus mungo*[1]

K. K. NANDA and A. K. DHAWAN

*Plant Physiology Research Unit, Department of Botany, Panjab University, Chandigarh-160014 (India), 9 December 1975.*

*Summary.* 2,4-dinitrophenol enhanced root formation on dark-grown hypocotyl cuttings of *Phaseolus mungo*. This effect is probably related to uncoupling of oxidative phosphorylation and not through IAA-metabolism as is evident from studies with respiratory inhibitors ($Cd^{2+}$) and non-phenolic uncouplers of oxidative phosphorylation (arsenate).

While examining the effect of some phenolic compounds on hypocotyl cuttings of *Phaseolus mungo*, increased root formation was observed with 2,4-dinitrophenol (DNP). Work was therefore undertaken to investigate this unusual phenomenon of increased rooting with an uncoupler of oxidative phosphorylation[2,3].

Healthy, uniform seeds of *Phaseolus mungo* were germinated in sterilized Petri-dishes (15 cm diam.) lined with cotton pads, maintained at 28 ± 2 °C in the dark. Uniform seedlings were made into cuttings with 3.5 cm hypocotyl and 6.5 cm epicotyl by excising the cotyledons, the upper epicotylar and lower hypocotylar portions. These were planted in holes on tin-foils stretched over specimen tubes (3 × 7.5 cm), each containing 20 ml of the requisite test solution. Cultures were maintained in the dark and the test solutions were changed after every 24 h. Observations on the number of cuttings rooted and the number of roots were recorded after 7 days. The experiment was repeated 3 times with similar trends of results.

The results, together with the treatments presented in the Table, show that all cuttings rooted in water, as well as in 1 µg/ml IAA. 1% sucrose alone or with IAA, enhanced rooting, showing thereby that the optimal production of adventitious roots was limited by the level of endogenous nutrition and that a proper balance between auxin and nutrition was necessary for the process[4-7].

DNP alone, with IAA (1µg/ml), sucrose (1%) or IAA + sucrose enhanced rooting (Table). Auxin activity of the substituted phenols is lost when the position *para* to hydroxyl group is substituted by strong electron attracting groups[8,9]. However, the present investigation reveals that in the case of DNP, a *para* substitution results in an active molecule. It was decided to investigate why a *para* substituted phenol which acts as an uncoupler of oxidative phosphorylation also should enhance rooting.

Phenolic compounds are known to enhance certain auxin-caused responses[10-12]. DNP might, therefore, exert its influence via interaction with some hormonal mecha-

[1] The research has been financed by a grant from United States Department of Agriculture.

[2] H. A. LARDY and H. WELLMAN, J. biol. Chem. *201*, 357 (1953).

[3] M. E. PULLMAN and G. SCHATZ, A. Rev. Biochem. *36*, 539 (1967).

[4] K. GURUMURTI and K. K. NANDA, Phytochemistry *13*, 1089 (1974).

[5] K. K. NANDA, M. K. JAIN and S. MALHOTRA, Physiologia Pl. *24*, 387 (1971).

[6] K. K. NANDA and M. K. JAIN, New Phytol. *70*, 949 (1971).

[7] K. K. NANDA and M. K. JAIN, New Phytol. *71*, 825 (1972).

[8] D. B. HARPER and R. L. WAIN, Ann. appl. Biol. *64*, 395 (1969).

[9] R. L. WAIN and D. B. HARPER, Nature, Lond. *23*, 1155 (1967).

[10] J. P. NITSCH and C. NITSCH, Ann. Physiol. Veg. *4*, 211 (1962).

[11] R. L. WAIN and H. F. TAYLOR, Nature, Lond. *207*, 167 (1965).

[12] C. E. HESS, Plant Physiol. *40*, Suppl. 45 (1965).

Effect of DNP, DNP + $Cd^{++}$ and arsenate alone and in combination with IAA, sucrose and IAA + sucrose, on the number of dark-grown *Phaseolus mungo* hypocotyl cuttings with excized apex and cotyledons that rooted out of 10 and the number of roots produced per rooted cutting (Figures within parentheses)

| Treatment | Combination with | | | |
|---|---|---|---|---|
| | Water | IAA (1 µg/ml) | Sucrose (1%) | IAA (1 µg/ml) + sucrose (1%) |
| Water | 10 (4.0 ± 0.3) | 10 (4.3 ± 0.1) | 10 (6.0 ± 0.4) | 9 (7.0 ± 0.3) |
| DNP (5.0 µg/ml) | 9 (9.3 ± 0.9) | 10 (11.7 ± 0.9) | 10 (10.3 ± 0.9) | 10 (19 ± 1.0) |
| DNP (5.0 µg/ml) + $Cd^{++}$ ($2 \times 10^{-6}$ *M*) | 10 (4.8 ± 0.4) | 5 (4.1 ± 0.4) | × | 9 (3.1 ± 0.2) |
| Arsenate ($10^{-6}$ *M*) | 9 (4.4 ± 0.2) | 10 (6.0 ± 0.2) | 10 (8.2 ± 0.7) | 10 (11.5 ± 1.0) |

±, Standard error. ×, Cuttings decayed.

nism. Such a possibility, however, appears to be remote in this case as: a) A combination of DNP and IAA is not synergistic to rooting (Table). b) DNP is not believed to exercize its effect through IAA metabolism, as DNP exerted no influence on in vitro IAA-oxidase activity (Figure 1); nor did the treatment with DNP have any considerable effect on the in vivo IAA-oxidase activity of the cuttings, except for a 'delaying effect' on the enzyme activity peak (Figure 2). Protection of IAA from oxidation[13] or enhanced IAA synthesis[14] by DNP has not been observed.

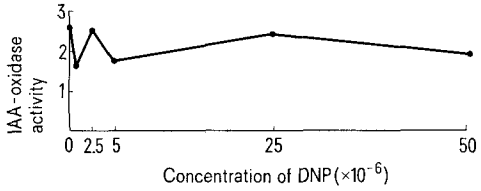


Fig. 1. In vitro effect of different concentrations of DNP on the activity of IAA-oxidase of the dark-grown *Phaseolus mungo* hypocotyl cuttings with excized apex and cotyledons. IAA-oxidase activity was assayed as described earlier and expressed in terms of µg IAA oxidized under specified conditions.

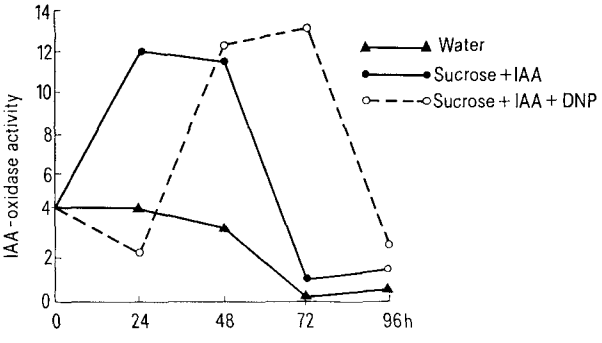


Fig. 2. Effect of DNP in combination with IAA+sucrose on the in vivo IAA-oxidase activity of the dark-grown *Phaseolus mungo* hypocotyl cuttings with excized apex and cotyledons. IAA-oxidase activity was assayed as described earlier and expressed in terms of µg IAA oxidized under specified conditions.

The enhancement of respiration by DNP by removing the obligatory link to phosphorylation is well known[2,3,15,16]. Promotion of rooting by DNP might, therefore, involve the uncoupling of oxidative phosphorylation and increased respiration; $2\times10^{-6}$ *M* Cadmium ($Cd^{++}$), a known inhibitor of respiration[17], checked the DNP-caused promotion of rooting (Table), thus lending support to such a possibility. That the uncoupling of oxidative phosphorylation and not the interaction with auxin metabolism is likely to be involved in the DNP-caused promotion of rooting, is further supported by the fact that arsenate ($10^{-6}M$), a non-phenolic uncoupler of phosphorylation[18], also enhanced rooting (Table), contrary to the earlier observations[19].

Thus, increased root production by DNP, a *para* substituted phenol, appears to be related to uncoupling of oxidative phosphorylation and is considerably different from that by other phenolic compounds which act through IAA metabolism[10-12].

[13] P. L. GOLDACER, A. W. GALSTON and R. L. WEINTAUB, Arch. Biochem. Biophys. *43*, 358 (1953).
[14] S. A. GORDON and L. G. PALEG, Plant Physiol. *36*, 838 (1961).
[15] H. A. LARDY, P. WITOUSKY and D. JOHNSON, Biochemistry *4*, 552 (1965).
[16] H. A. LARDY, J. L. CONNELLY and D. JOHNSON, Biochemistry *3*, 1961 (1964).
[17] H. R. MAHLER and E. H. CORDES, *Biological Chemistry* (Harper and Row Publishers, New York 1971), p. 686.
[18] H. F. TERWELLE and E. C. SLATER, Biochim. biophys. Acta *89*, 385 (1964).
[19] W. R. KRUL, Plant Physiol. *43*, 439 (1968).

## Relation of the Character of Capacitance Vessel Responses in Spleen and Small Intestine Elicited by Electrical Stimulation of the Sympathetic Fibres to the Level of Oxygen Exchange in the Organs

B. I. TKACHENKO, P. K. POZDNJAKOV and N. JA. MEDVEDEVA

*Institute of Experimental Medicine, Laboratory for Circulation, Leningrad P–22 (USSR), 10 October 1975.*

*Summary.* There is a correlation between character and magnitude of capacitance vessel responses and venous blood oxygen saturation under electrical stimulation of sympathetic fibres in the spleen and small intestine.

It was shown[1] that the electrical stimulation of sympathetic fibres caused both constrictory and dilatory responses of the spleen and small intestine capacitance vessels, resistance vessel response being always constrictory. The lack of uniformity of the capacitance vessel responses was not due to the resistance vessel responses, capillary filtration and changes in arteriovenous anastomotic flow. In spite of the fact that the dilatory responses of the capacitance vessels were observed in small number of experiments (21% for spleen and 16% for intestine), it was necessary to study mechanisms of these reactions. This study was intended to compare the dynamics of changes of venous blood oxygen saturation and the character of the capacitance vessel responses.

*Method.* The experiments were performed on cats (30) anaesthetized with urethane (1 g/kg) under artificial respiration. A spleen and a section of small intestine were humorally isolated and autoperfused with a constant blood volume pumps. The resistance and capacitance vessel reactions were studied by the method described previously[2-4]. The arterial pressure, the perfusion pressure and the venous outflow were recorded with an electromanometer on the optical oscillograph. Simultaneously with the resistance and capacitance vessel responses, the dynamics of the venous blood oxygen saturation was recorded with an oxymeter (model 057) using a probe attached to the special glass cuvette. The stimulation of the splanchnic or spleen sympathetic fibres was accom-