fact is an expression of the large amount of work which is done by the egg. The question is how ATP is utilized. Contractile processes on the egg cortex, connected with the actomyosin-like ATPase activity, bring about considerable changes in the shape of the egg and movements of the cortex toward the vegetal pole[1,2,6]; the ooplasmic components are mixed up and segregated into 5 different regions[3], while mitochondria collect at the vegetal pole[4,5]. All those modifications require energy expense which is supplied by the ATP present in the unfertilized egg.

Moreover the role of ATP in amino acid activation for the protein synthesis must be taken into account; a noticeable increase of activated amino acids has been found in the egg of *C. intestinalis* after fertilization[15]. The rise in permeability of the egg membrane could also be responsible for ATP consumption, since ATPase present in cell membrane plays a role in the active transport of sodium and potassium[16].

ATP is closely linked to all the phosphorilating processes in the cell; it has been reported that in sea urchin eggs after fertilization there is a marked increase in some of the phosphorilated substrates of glycolisis[17]; moreover a noticeable increase of NADP has been detected at the expense of NAD[18]. An investigation of the activity of phosphorilating processes in the ascidian egg is now in progress in our laboratory.

If we compare the oxygen consumption with the ATP level in unfertilized and fertilized eggs, it is clear that in the former a low level of oxygen consumption corresponds to a high ATP content, while in the latter high oxygen consumption is accompanied by a low ATP level. It seems, therefore, that the release of respiratory control following fertilization is related to ATP consumption. In the sea urchin egg such a situation has been found: a noticeable ATP decrease occurs at fertilization[19,20].

An analysis of ADP level revealed a low concentration in the unfertilized egg and a high level in the fertilized egg[20]. Since it is known that the concentration of high energy phosphate acceptors is a regulatory factor in respiration[21,22], the low level of ADP could, according to the authors, be responsible for the low level of oxygen consumption of the unfertilized egg. Measurements of ADP content in unfertilized and fertilized eggs of ascidians which are in progress in our laboratory could throw light on this problem.

Riassunto. Il dosaggio dell'ATP presente nelle uova è stato fatto col sistema luciferin-luciferasi misurando la luminescenza mediante un contatore a scintillazione liquida. È stato rilevato che 10 min dopo la fecondazione il livello di ATP nelle uova si abbassa di ben sette volte. Il risultato è discusso sulla base delle modificazioni morfologiche e biochimiche che intervengono nell'uovo alla fecondazione.

T. D'Anna

Istituto di Zoologia dell'Università,
Palermo (Italy), 2 December 1968.

[15] M. Molinaro, Rend. Accad. naz. Lincei *34*, 8, 563 (1963).

[16] H. J. Schatzmann, Experientia *20*, 551 (1964).

[17] K. Aketa, R. Bianchetti, E. Marré and A. Monroy, Biochim. biophys. Acta *86*, 211 (1964).

[18] N. Isono and I. Yasumasu, Expl Cell Res. *50*, 616 (1968).

[19] M. Rossi, F. Aiello, E. Scarano, in *Fertilization* (Ed. Ch. B. Metz and A. Monroy; Acad. Press N.Y. 1967), vol. I, p. 387.

[20] A. I. Zotin, V. S. Faustov, L. I. Radzinskaja and N. D. Ozernyuk, J. Embryol. exp. Morph. *18*, 1 (1967).

[21] H. A. Lardy and H. Wellman, J. biol. Chem. *195*, 215 (1952).

[22] J. Immers and J. Runnström, Devl. Biol. *2*, 90 (1960).

Differentiation of Haustoria in the Germinating Embryos of Mistletoe Without Host Stimulus

In recent years morphogenesis of embryo of parasitic angiosperms has attracted much attention[1–4]. The seedlings of parasites raised in cultures, in the absence of the host, usually fail to differentiate haustoria. This communication describes in vitro differentiation of haustoria from the excised embryo and endosperm of *Scurrula pulverulenta*, in the absence of host tissue or host extract.

Mature fruits of the parasite were surface-sterilised with absolute ethyl alcohol for 10 min. The 'seeds' (lacking a seed coat; Figure A) were excised and planted on modified White's semi-solid medium (WM) without IAA[5] containing 2% sucrose. In some treatments WM was supplemented with casein hydrolysate (CH), watermelon juice (WMJ), IAA, and cytokinins, individually and in various combinations.

On White's semi-solid medium the seeds germinated in 45% of the cultures. Although the addition of 400 ppm CH did not improve the percentage of germination, the seedlings appeared robust (Figure B). On WM as well as WM + CH, 46% seedlings formed haustoria. The addition of $10^{-5} M$ benzyladenine (BA) to WM increased the percentage of germination to 90; and 60% of the seedlings developed haustoria. The percentage of germination on WM + 10% WMJ was comparable to that on WM + BA, but the seedlings on the former medium failed to form haustoria. The differentiation of haustoria was also inhibited by coconut milk.

In 45% of the cultures raised on WM + 400 ppm CH + 1 ppm IAA, after 5 weeks the embryo proliferated into a callus upon coming in contact with the nutrient medium. The resulting callus was compact, green and slow-growing. Several tracheid-like cells were observed in a 14-week-old callus. After 20 weeks the callus bore shoots, or haustoria, or both (Figure C).

Proliferation of the embryo also occurred if 1 ppm kinetin was added to WM + CH + IAA. However, the callus was friable, non-chlorophyllous, and either a shoot or a haustorium failed to differentiate.

On WM supplemented with BA, kinetin, 6-(γ,γ-dimethylallylamino)-purine, or zeatin, each at $10^{-5} M$, shoot buds differentiated from the peripheral cells of the un-

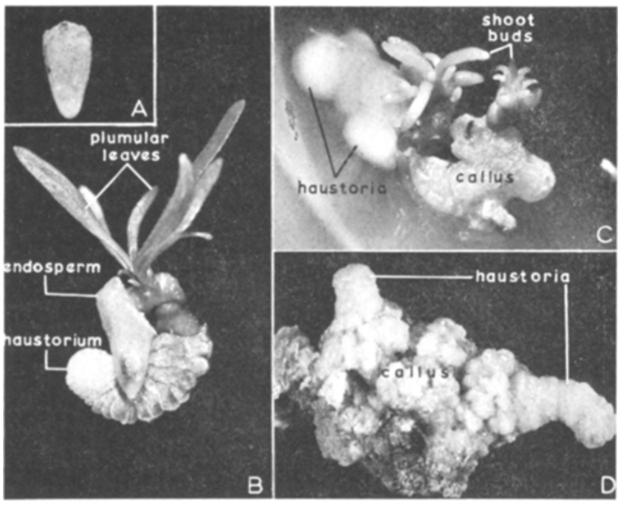
[1] E. H. Truscott, Am. J. Bot. *53*, 739 (1966).

[2] Y. P. S. Bajaj, Can. J. Bot. *44*, 1127 (1966).

[3] J. M. Bonga and C. Chakraborty, Can. J. Bot. *46*, 161 (1968).

[4] T. S. Rangan and N. S. Rangaswamy, Can. J. Bot. *46*, 263 (1968).

proliferated endosperm. If these were excised and planted on WM + 400 ppm CH + 1 ppm IAA + 1 ppm kinetin, they proliferated into a callus and subsequently differentiated shoots and haustoria (Figure D).



(A) 'Seed' (endosperm with enclosed embryo) at culture. × 2.5. (B) 9-week-old seedling on WM + 400 ppm CH; note the worm-like haustorium and several plumular leaves. Endosperm is on left. × 2.5. (C) 20-week-old seed culture on WM + 400 ppm CH + 1 ppm IAA, showing embryonal callus which has differentiated shoot buds and haustoria. × 2. (D) 15-week-old culture of shoot buds of endospermic origin, on WM + CH + IAA + kinetin. The callused bud has differentiated haustoria. × 2.5.

The haustoria, either from the embryo or the endosperm, were worm-like. Anatomically, they were comparable to the haustoria formed inside the host tissue in vivo, and showed a glandular epidermis, characteristic collapsed layers, and centrally arranged vascular bundles.

Thus, seeds of the parasite *Scurrula pulverulenta* can germinate on a simple nutrient medium and the differentiation of haustoria is not at all host-dependent. It is concluded that in this plant the cells of the endosperm and embryo have the inherent potentiality for the development of haustoria but this must be evoked by suitable chemical milieu[6].

Zusammenfassung. Die Samen von *Scurrula pulverulenta,* einem Baumparasiten, keimen auf einfachen Nährböden. Aus den embryonalen und endospermalen Geweben entwickeln sich in den In-vitro-Kulturen, auch bei Abwesenheit von Wirtsgewebe, Haustorien.

S. S. Bhojwani

Department of Botany, University, Delhi 7 (India), 23 December 1968.

---

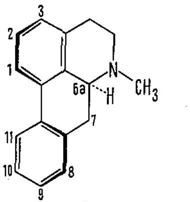
[5] N. S. Rangaswamy, Phytomorphology *11*, 109 (1961).

[6] Acknowledgments. I am grateful to Professor B. M. Johri and Professor H. Y. Mohan Ram for valuable suggestions, and to Dr. M. A. Rau for supplying fresh fruits of *Scurrula pulverulenta*.

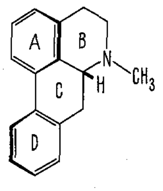
STUDIORUM PROGRESSUS

The Relationship Between the Ring D-Substituents and the Absolute Configuration for the Aporphine Alkaloids

All aporphines possess a permanently twisted biphenyl system, and the sign of the specific rotation at 589 nm is usually a good criterion for the absolute configuration[1,2]. If an aporphine is dextrorotatory its absolute configuration is as in I, while if it is levorotatory the absolute configuration is represented by expression II. Positions 1 and 2 are always substituted, while substituents may also be present at C-3, 7, 8, 9, 10, or 11.



I



II

In 1962, one of us made some tentative generalizations relating the position of the substituents in ring D of the naturally occurring aporphines to the absolute configurations of the molecules[3]. Since this time a large number of new aporphines have been isolated, so that a reconsideration of this relationship is warranted.

1, 2, 9, 10-Substituted aporphines. C-1, 2, 9, 10-substituted aporphines are generally dextrorotatory, and hence of absolute configuration I. Approximately a dozen such alkaloids were known in 1962, and this number has now more than doubled. Table I lists the names, substituents, and specific rotations for the aporphines belonging to this group.

The only exception appears to be the rarely occurring base phanostenine which exhibits $[\alpha]_D$ −36.7° ($CHCl_3$). (±)-Isoboldine has been found together with (+)-isoboldine in *Glaucium* spp.[4]. Aporphines are not usually found in a racemic form, and it is probable that the racemic isoboldine was formed in the plant by oxidation of (+)-isoboldine to the corresponding immonium salt or eneamine, which was then reduced non-stereospecifically back to isoboldine.

---

[1] M. Shamma, Experientia *16*, 484 (1960).

[2] C. Djerassi, K. Mislow and M. Shamma, Experientia *18*, 53 (1961).

[3] M. Shamma, Experientia *18*, 64 (1962).

[4] T. Slavik, Colln Czech. chem. Commun *33*, 323 (1968).