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Absence of Parallelism between Polyamine and Nucleic Acid Contents during Induced Growth of Cucumber Cotyledons

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The cytokinins (benzyladenine or benzyladenosine) decreased spermidine and spermine contents despite increasing putrescine content, when administered to isolated cotyledons of *Cucumis sativus* L. var. Guntur in organ culture. KCl decreased putrescine contents, although marginally increasing polyamine contents. The cytokinins and/or KCl augmented nucleic acid biosynthesis and accumulation, resulting in enhanced growth and differentiation of the isolated cotyledons. These observations show that polyamine accumulation and growth are not always coupled.

It has become common to relate the increase in polyamine concentrations with enhanced growth, cell proliferation and augmented macromolecular biosynthesis. Studies with microbial, animal and plant systems show that (*a*) di- and poly-amines stimulate growth *per se* (Tabor & Tabor, 1964; Clo *et al.*, 1976; Bagni & Fracassini, 1974) and (*b*) increases occur in the activities of both ornithine decarboxylase and *S*-adenosyl-L-methionine decarboxylase accompanied by increased amine concentrations after hormone and drug administration (Raina & Jänne, 1975), and in rapidly growing tissues, including natural or induced neoplasias (Snyder & Russell, 1970; Bachrach, 1976). A close parallelism between polyamine and macromolecular biosynthesis, particularly the relationship of spermidine to RNA synthesis, was observed in a number of those systems (Raina & Jänne, 1975). These observations led to the generalization that 'no growth processes occur without prior stimulation of polyamine biosynthesis' (Russell, 1973*a*) and that 'biosynthesis and accumulation of polyamines appear to be a universal prerequisite for growth' (Russell, 1973*b*). In the present paper we report the lack of correlation between growth, RNA and DNA synthesis on the one hand and the concentrations of putrescine, spermidine and spermine on the other in a higher-plant system induced to grow in organ culture under the influence of growth promoters.

Materials and Methods

The sources of the seeds (*Cucumis sativus* L. var. Guntur), biochemicals and details of culture conditions have been described elsewhere (Suresh *et al.*, 1978). Briefly, about 50 pairs of cotyledons from 60h-etiolated seedlings were washed and transferred to a sterile Petri dish containing a Whatman no. 1 filter-paper disc moistened with 5ml of the freshly prepared hormone and/or KCl solutions or 5ml of water as control. The Petri dishes were incubated under continuous fluorescent light of approx. 1300lx for various times at 23±2°C. No detectable bacterial contamination was observed during the period of the culture. The cotyledons were subsequently washed, homogenized at 0°C with cold $HClO_4$ (final concn. 0.2M) and cooled overnight. The acid-soluble supernatant was processed for di- and poly-amine analysis (Ramakrishna & Adiga, 1973). From the acid-insoluble pellet, lipids were extracted and the macromolecules fractionated and estimated (Ramakrishna & Adiga, 1975*a*).

Results and Discussion

The administration of cytokinins, namely benzyladenine or benzyladenosine, to the isolated cotyledons of *Cucumis sativus* in culture led to pronounced morphological changes and growth and differentiation into leaf-like structures. The changes form the basis of a bioassay for cytokinins (Udayakumar & Krishnasastry, 1973). This effect of cytokinin was also simulated by KCl. The effect of combined treatment with cytokinin and KCl on growth was additive. Addition of putrescine, spermidine or spermine to the culture medium in the range 0.01–100mM did not bring about the growth-promoting effect; in fact concentrations above 1mM were inhibitory. This growth and differentiation induced by cytokinin and/or KCl involve the biogenesis of the chloroplast, increase in cell size and number, vascularization of the tissue and multicellular trichome formation on the adaxial surface resulting in a dorsiventral leaf from the embryonic cotyledon (Suresh, 1978). It may be emphasized that this growth and differentiation also occur during the normal course of germination of the seed and that the cotyledonary leaf photosynthesis has a profound influence on the subsequent growth

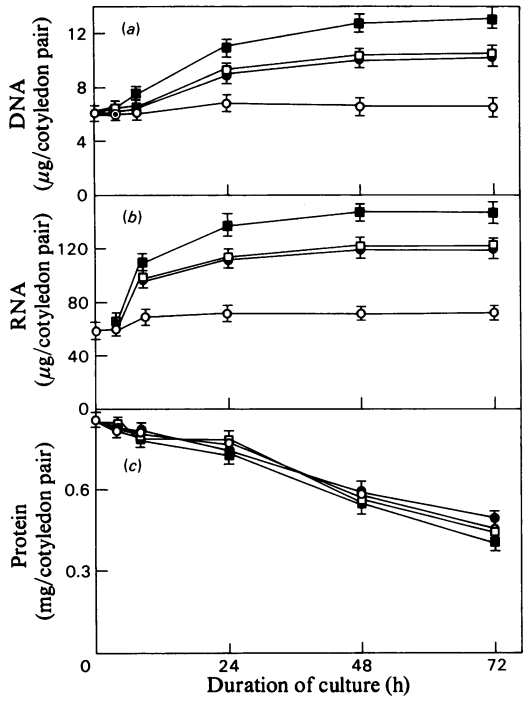

Fig. 1. *Changes in DNA (a), RNA (b) and protein (c) contents with time during growth and differentiation of cotyledons of Cucumis sativus L. var. Guntur induced by benzyladenosine and/or KCl in organ culture*
○, Control; ●, 13.4μM-benzyladenosine; □, 50mM-KCl; ■, benzyladenosine+KCl. The experimental points represent means±S.D. ($n = 4$). For other details see the text.

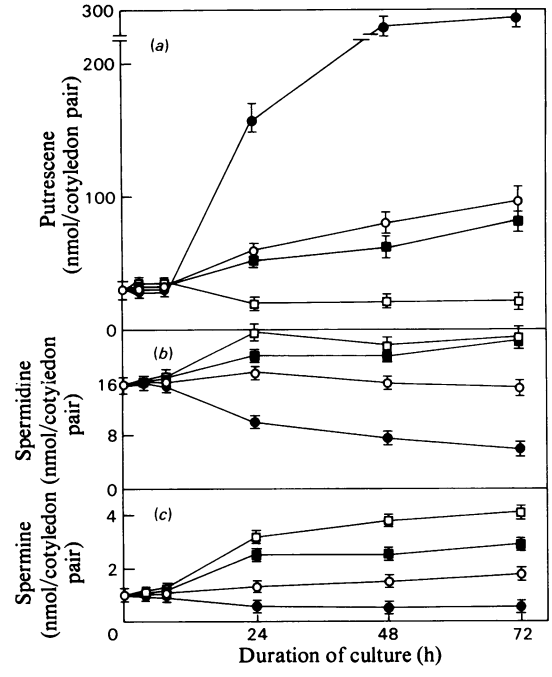

Fig. 2. *Changes in putrescine (a), spermidine (b) and spermine (c) contents with time during growth and differentiation of cotyledons of Cucumis sativus L. var Guntur induced by benzyladenosine and/or KCl in organ culture*
○, Control; ●, 13.4μM-benzyladenosine; □, 50mM-KCl; ■, benzyladenosine+KCl. The experimental points represent means±S.D. ($n = 4$).

of the cucumber seedling (Penny *et al.*, 1976). It is pertinent that in *Cucumis sativus* L. var. Delicatess increased RNA and protein synthesis on administration of benzyladenine and KCl respectively have been reported (Knypl, 1971).

The changes in total DNA and RNA contents induced by benzyladenosine and/or KCl are shown in Figs. 1(*a*) and 1(*b*). Significant increases ($P < 0.001$) in RNA by 8h and DNA by 24h were observed after treatment with cytokinin or KCl. The nucleic acid contents were consistently higher on combined treatment with cytokinin and KCl, a feature that was in agreement with the morphological observations. A similar response in the concentrations of nucleic acids was observed when either KCl, benzyladenine or benzyladenosine was added. A 50% increase in ^{32}P incorporation into RNA was evident by 3h after administration of the growth promoters even after correction for the enhanced intracellular pool size of the precursor (results not given). The total protein content (Fig. 1*c*) did not alter with the different treatments, although a uniform decrease (approx. 50%) was observed by 72h. Despite this mobilization of the reserve protein, a significantly increased incorporation (25%) of ^{14}C-labelled amino acids was observed after 6h in the presence of the growth stimulators (results not given).

The changes in the amounts of putrescine, spermidine and spermine during growth and differentiation of cotyledons are depicted in Figs. 2(*a*), 2(*b*) and 2(*c*). The modulation of arginine decarboxylase, the first enzyme in the polyamine biosynthesis in higher plants (Ramakrishna & Adiga, 1975*b*), and changes in the putrescine concentrations by various growth promoters, inhibitors, KCl and H^+ have been studied in detail (Suresh *et al.*, 1978). Unlike the dramatic early changes in ornithine decarboxylase of mammalian systems, the increase in arginine decarboxylase was not as rapid. This may be related to the longer half-life of arginine decarboxylase (3–4h) compared with ornithine decarboxylase (11–20min) (Raina & Jänne, 1975). It is evident from Fig. 2 that the cytokinin decreases

spermidine concentrations despite the large increase in putrescine. However, KCl marginally decreased the amount of putrescine (Fig. 2), whereas significantly increasing polyamines. KCl-induced decrease in putrescine content has been observed earlier in tobacco (Smith, 1975). On the other hand, K^+ deficiency is known to be associated with substantial putrescine accumulation in several plant systems (Smith, 1971, 1975). The decrease in polyamines was observed even when benzyladenosine-treated tissue was extracted in 6M-HCl, supernatants hydrolysed at 110°C for 18h before amine determination (Russell, 1971). This excludes the possibility that the cytokinin elicits the synthesis and storage of any acid-labile polyamine derivative. An essentially similar but magnified pattern was obtained (especially with cytokinin treatment) when the amine content was expressed per unit amount of RNA or DNA (see Figs. 1 and 2). In view of a comparable degree of stimulatory influence on growth and nucleic acid synthesis, the opposite effects of cytokinin and KCl on amine accumulation were unexpected. It is pertinent that in this system during K^+ or cytokinin treatment there exists an inverse relationship between the concentrations of the diamine and of the polyamines despite the probably operation of the putrescine → spermidine → spermine pathway in higher plants (Smith, 1975; Ramakrishna & Adiga, 1975*b*). The fairly constant ratio of RNA/DNA in these experiments indicates the absence of any abnormal growth.

The above findings represent a unique instance of a hormone that promotes growth and differentiation yet decreases polyamine content, showing thereby that amine concentrations and growth are not always obligatorily coupled. Supporting evidence for this premise stems from studies with inhibitors (unlike the present study with growth promoters) of amine or nucleic acid biosynthesis. Methylglyoxal bis(guanylhydrazone) {1,1′[(methylethanediylidine)dinitrilo]diguanidine}, a potent inhibitor of putrescine-dependent *S*-adenosyl-L-methionine decarboxylase, inhibited polyamine accumulation in concanavalin A-stimulated lymphocytes in culture, without impairing the synthesis, processing and accumulation of RNA (Fillingame & Morris, 1973). This drug only partially inhibited DNA synthesis and the rate of entry of cells into mitosis (Fillingame *et al.*, 1975). Similarly, in cultured rat hepatomas and normal rat liver, α-hydrazino-ornithine, a specific inhibitor of ornithine decarboxylase, blocked net putrescine synthesis, but failed to inhibit RNA and DNA synthesis (Harik *et al.*, 1974). On the other hand, in primary mouse kidney cells infected with polyoma virus, polyamine biosynthesis was either unimpaired or stimulated when nucleic acid biosynthesis was blocked by 5-fluorodeoxyuridine or actinomycin D (Goldstein *et al.*, 1976).

These observations, including those now described on the lack of association between polyamine and nucleic acid concentrations during stimulated growth, do not, however, argue against the role played by polyamines in growth and related processes. It is conceivable that, under conditions of limited availability of one of these amines, others could effectively substitute for the physiological function (Fillingame & Morris, 1973; Fillingame *et al.*, 1975). Alternatively a redistribution of the available amines present at the basal concentrations could perform the essential function under such conditions. The amine concentrations fluctuate on treatment with hormones, inhibitors and stress; a true physiological effect due to amine deficiency would precipitate only after decrease below a certain critical threshold value. Thus it would appear that an emphasis on the strict parallelism between polyamines and growth is unwarranted at present, although the two are adventitiously coupled in a number of systems.

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