Integrating phytohormone metabolism and action with primary biochemical pathways. II. Interrelationships between disturbed nitrogen and carbon metabolism and changes in hormonal concentrations and sensitivities in neoplastic tissue cultures

GASPAR, Thomas, et al.

Abstract
The paper begins with a review of the concept of neoplastic progressions in plant tissue cultures, with the progressive acquisition of (a relative) independence to the hormones auxins and cytokinins. It takes advantage of the deviations of carbon and nitrogen metabolisms shown in these particular cases, to illustrate the interdependence with the metabolisms of the hormones (and increased sensitivities to) polyamines and ethylene. These results provide additional examples of the changing concepts in hormonology.

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Integrating phytohormone metabolism and action with primary biochemical pathways. II. Interrelationships between disturbed nitrogen and carbon metabolism and changes in hormonal concentrations and sensitivities in neoplastic tissue cultures.

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Summary

The paper begins with a review of the concept of neoplastic progressions in plant tissue cultures, with the progressive acquisition of (a relative) independence to the hormones auxins and cytokinins. It takes advantage of the deviations of carbon and nitrogen metabolisms shown in these particular cases, to illustrate the interdependence with the metabolisms of the hormones (and increased sensitivities to) polyamines and ethylene. These results provide additional examples of the changing concepts in hormonology.

Abbreviations

ACC: l-aminocyclopropane-1-carboxylic acid; ADC: arginine decarboxylase; ALA: aminolevulinic acid; CHA: cyclohexylamine; DFMA: α-difluoromethyl-arginine; DFMO: α-difluoromethylornithine; GABA: γ-aminobutyric acid; HNO: habituated non-organogenic; α-KG: α-cetoglutarate; MDH: malate dehydrogenase; MGBG: methylglyoxal-bis-(guanylhydrazone); N: normal; OAT: ornithine amino-transferase; ODC: ornithine decarboxylase; PA(s): polyamine(s); PEP: phospho-enolpyruvate; PPP: pentose phosphate pathway; Put: putrescine; SAM: S-adenosylmethionine; Spd: spermidine; Spm: spermine; TCA: tricarboxylic acid.
Introduction

While studying the physiology of different tissue cultures and organogenic programs *in vitro*, we made a thorough comparative study of the processes of habituation and vitrification (see below) which affect cultured cells and calli, and micropropagated shoots respectively. We first established a reciprocal relationship between both phenomena [69]: habituated tissues are hyperhydric and vitrified organs may become habituated. We also came to conclude that the two processes were steps of neoplastic progressions (see below) leading to true cancerous states (see below) in the absence of introduced and detected pathogens [59,60,62,63,65,73]. The auxin- and cytokinin-autonomy of habituated tissues could not be explained simply by an overproduction of these hormones and alternative possibilities of hormonal control are now being discussed and tested (see below + [99] for reviews). We very early were confronted with three main characteristics of both habituated calli and vitrified tissues as compared with normal tissues: overproduction of polyamines [20,77,80,97,106], lower empirism emission of ethylene [71,79,80] and deficiency of tetrapyrrole-containing compounds, including chlorophylls [17,44,84], cytochromes [84] and peroxidases [10,23,71,101,102] (Table 1). It is interesting to notice that exogenous polyamines induce cancer in animal cells and that animal cancer cells accumulate PAs [134,140,143].

The present paper aims at showing, how, by investigating the mechanisms of polyamine accumulation, relationships with the other peculiarities, nitrogen and carbon metabolism, and the endogenous hormones were established.

Habituation

The phenomenon of plant tissue habituation, discovered in carrot and called "anergie" or "accoutumance aux hormones" by Gautheret [74] consists of the acquired and hereditary capacity for autonomous growth, in the absence of exogenously supplied auxins or cytokinins in the tissue culture [41,91,128,129,148]. Habituation has been observed, in some cases, as a gradual process while in others, it occurs spontaneously. The phenomenon of habituation bears a striking similarity to tumour transformation in crown gall disease where tumour tissue grows independent of exogenous hormones. This common loss in the requirement of exogenous growth factors has led to consider habituated tissues as hormone autotrophic, i.e. capable of autonomous production of auxins and cytokinins [127]. Major production sources of auxins and cytokinins in habituated cells have not been confirmed however [99]. An altered sensitivity to endogenous hormones has been suggested [131,150], as well as the accumulation of metabolites (dehydroidiconiferyl alcohol glucosides) that could replace cytokinins in the control of cell divisions [12,151]. Another explanation may come from altered metabolisms of ethylene [18,34,79,80,112] and of polyamines [4,13,77,80,97,117,118,120-122,144]. Alterations in gene express-ion might render cell division independent of hormonal regulation [34]. An increased content of diacylglycerol as well as increased levels and turnover of inositol phosphates in habituated tissues as in animal cancerous cells might account for the latter hypothesis [52]. In most cases, the process of habituation appears to be reversible,
Table 1. Compared levels of PAs, ethylene emission, proline, soluble POD activity, catalase activity and chlorophylls in normal and HNO sugarbeet calli (at the end of the culture cycle) and its normal and vitrified shoots (at the end of the vitrifying cycle) of wild cherry. Cytochromes= b5 + P420 + P450.

<table>
<thead>
<tr>
<th></th>
<th>Calli</th>
<th>Shoots</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>HNO</td>
<td>N</td>
</tr>
<tr>
<td>Total free PAs (nmol.g⁻¹ DW)</td>
<td>3562 ±363</td>
<td>10866 ± 935</td>
<td>4144 ± 305</td>
</tr>
<tr>
<td>Ethylene emission (nmol g⁻¹FWh⁻¹)</td>
<td>82.7 ± 1.3</td>
<td>4.2 ± 1.5</td>
<td>105.3 ± 9.5</td>
</tr>
<tr>
<td>Proline (µmol.g⁻¹ DW)</td>
<td>2.7 ± 0.2</td>
<td>13.1 ± 0.9</td>
<td>21.4 ± 2.5</td>
</tr>
<tr>
<td>Soluble POD activity (mg.mg⁻¹ prot)</td>
<td>2.24 ± 0.09</td>
<td>0.16 ± 0.02</td>
<td>0.027 ± 0.002</td>
</tr>
<tr>
<td>Catalase activity (nkat.mg⁻¹prot) (%H₂O₂ destroyed. min⁻¹; mg⁻¹prot)</td>
<td>58.37 ± 3.44</td>
<td>4.16 ± 0.21</td>
<td>187.2 ± 40.0</td>
</tr>
<tr>
<td>Cytochromes (µmol.mg⁻¹prot)</td>
<td>3.71 ± 0.41</td>
<td>0.58 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Chlorophylls a+b (µg.g⁻¹FW)</td>
<td>12.79 ± 1.02</td>
<td>3.89 ± 0.24</td>
<td>480 ± 28</td>
</tr>
</tbody>
</table>
and habituated cells keep their totipotency, as do genetic tumours [8,61], and can regenerate roots, buds or somatic embryos. Habituation thus is generally regarded to be an epigenetic phenomenon [126,128]. It may also be considered as a neoplastic step towards cancer (see below + Table 2). Neoplastic transformation to the fully habituated state and to cancer indeed is not accomplished in a single step, but occurs gradually [61].

Table 2. A. A few steps of an in vitro cellular neoplastic progression issuing in fully habituated nonorganogenic callus cells. B. A few steps of an in vitro organismic neoplastic progression issuing in cancerized shoots and fully habituated nonorganogenic callus (according to [73].

<table>
<thead>
<tr>
<th>A Plant material</th>
<th>Independence to auxins and cytokinins</th>
<th>Presence or capacity to form primary meristems</th>
<th>Organogenic totipotency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organs and tissues of a plant</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Normal callus</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Habituated organogenic callus</td>
<td>+</td>
<td>+</td>
<td>partial</td>
</tr>
<tr>
<td>Habituated nonorganogenic callus cells as cancerous cells</td>
<td>+</td>
<td>-</td>
<td>(irreversible)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B Plant material</th>
<th>Hormonal autonomy (auxins + cytokinins)</th>
<th>Presence or capacity to form primary meristems (M)</th>
<th>Organs formed</th>
<th>State of the meristems (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explants from a whole normal plant or from an in vitro proliferating cluster</td>
<td>-</td>
<td>Well functioning M</td>
<td>Normal stems and leaves Roots</td>
<td>Normal</td>
</tr>
<tr>
<td>Vitrified cluster</td>
<td>-</td>
<td>Well functioning M</td>
<td>Abnormal stems and leaves Less roots</td>
<td>All of the M still capable of normal functioning</td>
</tr>
<tr>
<td>Vitrified cluster subcultured in vitrifying conditions</td>
<td>+</td>
<td>Bad functioning of the M</td>
<td>Fasciated stem, abnormal leaves Rare roots</td>
<td>Only some of the M capable of normal functioning</td>
</tr>
<tr>
<td>Continued subculture in vitrifying conditions</td>
<td>+</td>
<td>Abnormal functioning</td>
<td>Aberrant organs Callus at the place of roots</td>
<td>Irreversible loss of M through necrosis</td>
</tr>
<tr>
<td>Callus formed at stem basis</td>
<td>+</td>
<td>-</td>
<td>Aberrant organs Callus at the place of roots</td>
<td>Irreversible loss of M through necrosis</td>
</tr>
</tbody>
</table>
Neoplastic progression in callus cell cultures and habituation as a step

Table 3. Characteristics which indicate that cells from a particular fully habituated nonorganogenic sugar beet callus are made of true cancerous cells, in the absence of pathogens. See Gaspar et al. [66]

<table>
<thead>
<tr>
<th>Biological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>- monoclonal origin</td>
</tr>
<tr>
<td>- full hormonal independence <em>in vitro</em></td>
</tr>
<tr>
<td>- high rate of cell division</td>
</tr>
<tr>
<td>- polyploid and aneuploidy</td>
</tr>
<tr>
<td>- reduced cell-to-cell adhesion (fiability)</td>
</tr>
<tr>
<td>- susceptibility to necrosis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Morphological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>- deficient cell wall differentiation</td>
</tr>
<tr>
<td>- deficient chloroplast and mitochondria differentiation</td>
</tr>
<tr>
<td>- large nuclei with irregular shape, with many nucleoli +</td>
</tr>
<tr>
<td>micronuclei</td>
</tr>
<tr>
<td>- apoptic bodies (programmed cell death?)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>- hyperhydricity</td>
</tr>
<tr>
<td>- deficiency of tetrapyrrole-containing compounds</td>
</tr>
<tr>
<td>- permanent oxidative stress</td>
</tr>
<tr>
<td>- low ethylene production</td>
</tr>
<tr>
<td>- accumulation of polyamines</td>
</tr>
<tr>
<td>- enhance pentose phosphate pathway</td>
</tr>
<tr>
<td>- enhanced alternative respiratory pathway</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Typical plant cancer trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>- irreversible loss organogenic totipotency, i.e. the capacity for</td>
</tr>
<tr>
<td>such cells to reorganise primary organogenic meristems at the</td>
</tr>
<tr>
<td>end of a neoplastic progression</td>
</tr>
</tbody>
</table>

A so-called normal callus still is defined by its apparent "anarchic proliferation of undifferentiated cells". This classical definition should be revised taking into account that its growth proceeds through meristematic centres [51,53] and thus is not as anarchic as considered at first sight, and also considering that its cells differentiate, into xylem cells for instance [44]. Nevertheless, absence of visible tissue and organ organisation makes some resemblance with a tumour and therefore a normal callus should be considered as a primitive neoplastic growth. Hormonal manipulation of this normal callus allows the recovery of organogenic potential through bud and root formation notably. A green fully habituated organogenic callus as described by De Greef and Jacobs [45] and Kevers et al. [102] represents additional neoplastic steps with the acquired independence to auxins and to cytokinins, and the loss of rooting.
capacities: such a callus is only capable of shoot formation. Additionally, the shoots formed by the callus, are abnormal [45,66], most often dichotomic. A white fully habituated callus (called HNO, for Habituated Non-Organogenic) was isolated from white cell clumps appearing at the surface of the preceding one [101]: it lacks cell differentiation and is incapable of organizing primary meristems to grow and to form adventitious organs. It can be considered the terminal phase of the neoplastic progression, as confirmed with the series of morphological [43,78] and biochemical traits similar to those of animal cancerous cells (Table 3). The concept of plant cancer cells thus involves the complete loss of organized and organogenic meristematic structures, that is an irreversible loss of totipotency, which makes cancer cells different from those of tumours, where cell differentiation still takes place and where organogenesis is still possible [31,61] (see below).

Vitrification in a neoplastic progression to cancer and death at the organismic level

Vitrification was the term generally used to characterize the hyperhydric malformations frequently affecting plants, during their in vitro vegetative propagation (under the effect of cytokinins at high concentrations, on flabby soft culture media, in confined atmosphere with high relative humidity). The so-called vitreous or vitrified shoots appear turgid or hyperhydric, watery at their surface, and hypolignified. They indeed contain much water but in their intercellular spaces [105] and less lignin [104] than normal shoots. The vitrified organs are somehow translucent, in some cases less green than normal, and brittle [55,58,100,160]. In the most common cases of vitrification, organs are not really malformed but cell differentiation is limited which, for woody plants, has led to the consideration of vitrification as a way of rejuvenation [42,92]. In other cases, leaves are malformed: frequently very elongated, wrinkled and/or curled, and brittle. In general, stems of vitrified shoots are broad, thick in diameter, with shorter internodes. Necrosis of leaf margins and of some stem apices may occur [94]. In most cases, vitrification is still reversible at that stage, which means that some apices of vitrified shoots placed in non-vitrifying conditions can function normally and give rise to normal plants.

In some cases, hyperhydric shoot clusters have been described as teratomas with morphological deviations similar to those induced by bacteria [123]. Abnormal leaves with deviating phyllotaxis and occasional fasciated stems are produced. These symptoms denote malfunctioning of the primary meristems. Different situations may result from the continued subculture of such vitrified clusters. It may happen that the clusters suddenly degenerate through simultaneous necrosis of all meristematic apices. Individualized shoots of such clusters do not survive at subculture and they become brown and die. These (humid) apex necroses of vitrified shoots are to be distinguished from the other must known (dry) apex necroses which result from hormone deficiency [94]. In some other cases, the subculture of vitrified clusters is still possible by use of clusters and not by individual shoots. Such explants provide other teratoma-like clusters with more or less distinguishable shoots. They can generally be subcultured and continue to proliferate in the absence of growth regulators which means that they became habituated (personal observations on long term cultures of Prunus and
Rhododendron). Zoglauer et al. [161] and Pierik [139] mention other examples of habituated shoot clusters. Shoots from these clusters, insensitive to auxin application, do not root. This may mean that such habituated shoots have lost part of their totipotency: the rooting capacity. In a few cases, habituated shoots form at their bases, cauliflower or broccoli-like structures, in which true stems and leaves are no longer recognizable. Meristems at that stage have lost their normal way of forming structured stems and leaves, and vitrification at that stage is irreversible. Whole-plant structures have disappeared but the "broccoli-like" mass still greatly increases in volume because of extremely rejuvenated cells. Such teratomas, with a progressive loss of organogenc capacity to form stem apices, are thus also the result of a sort of neoplastic progression (Table 2) [63,65]. This illustrates plant cancer at the organismic level, where progressive rejuvenation of some cell populations causes the death of the whole organism by rendering meristems incapable of continuing to maintain the normal structures and to play their organogenic and physiological roles. This situation apparently results from a progressive loss of cell-to-cell adhesion which renders dissociable tissues breakable, as in the above cancerous friable calli.

This finally means that plants are not more resistant to neoplastic transformation than animals. The features are simply different.

Oxidative stress, the initiation and maintenance of tumorous and cancerous states

There is convincing evidence that oxidants and agents, which induce a cellular prooxidant state, can act as carcinogens, in particular as promoters and progressors [39]. Reactive oxygen species (ROS) play roles as cell signals and are implicated in the redox regulation of cell proliferation but also in apoptosis occurring frequently in cancer cells [32]. Oxidative stress is classically known as one of the causes of cancer initiation in animals [1,64,157]. Oxidative signals trigger cell death in plants as they may in animals [75]. Sustained oxidative damage of nucleic acids, proteins and lipids caused by ROS, is considered to be a major factor in the general functional decline of tissues associated with age-associated degenerative diseases and cancer [85]. ROS may also be indirectly responsible of the defects in the cell cycle which lead to the genomic instability of cancer cells [85'].

The introduction of a plant tissue or organ in culture, and their subcultures, are accompanied by a series of stresses:
- wounding due to tissue or organ excision
- application of unusual hormonal concentrations and combinations
- probable excess of sucrose and nitrogen supply
- high atmosphere humidity
- accumulation of gases in the confined atmosphere of the vessel
- osmotic shock due to infiltration of the culture medium in the intercellular spaces [27,105].

The changes in the levels of PAs, ethylene emission and proline (Table 1) are indicators of such stresses.

We have shown that tissues introduced in vitro react by adapting their stress defense enzymes and soluble reductants against activated forms of oxygen [56,57,81]. Time-
course changes in these indicators show that tissues or organs, which will grow normally, recover a normal metabolism after the stress reactions, those which do not recover will become abnormal [56,57]. There are indications of cell "destabilization" through such stresses, for instance through the abundance of free fatty acids and of thiobarbituric acid reactive substances (malondialdehyde) in the HNO callus (non-chlorophyllous fully habituated non-organogenic callus), where a decrease of the unsaturation level of fatty acids might be indicative of high lipid peroxidation [3,82]. Thiobarbituric acid reactive substances are considered as mutagenic agents in animal cells [7,33,47,154]. Do such compounds contribute to transform some of the normal hormone dependent plant cells into habituated cells and/or some of the habituated organogenic cells into nonorganogenic ones (visibly they have a monoclonal origin), or do they simply contribute to maintain the habituated and/or the nonorganogenic state? This problem is as yet not quite clear since extracts from the same HNO calli appear with a high anti-lipoperoxidant potential [76] and since the same intact cells manifest a higher reducing power at their surface [36]. Such discrepancies also exist in the results collected from vitrifying shoots [57]. There are on the other hand indications that cells of the HNO callus are under permanent stress [119] and that light might have become a stress agent for these white calli [98].

Factors and metabolisms contributing to PAs accumulation in neoplastic cells

a) Ammonia detoxification

As shown from Table 4, in addition to PAs, HNO tissues also accumulate NO$_3^-$ and NH$_4^+$ ions, proline and glutamate. The idea is that the HNO callus was confronted to ammonia stress from the beginning of the culture. Ammonia detoxification was associated to an increase in glutamate dehydrogenase activity and to an accumulation of glutamate which is the precursor of both proline and polyamine syntheses. Experiments using gabaculine which is an inhibitor of ornithine aminotransferase (OAT) have shown (Table 5) a blockage of polyamine synthesis (85% inhibition) which is completely reverses by an ornithine supply [120]. This result revealed that OAT links proline degradation to polyamine synthesis, via ornithine production as indicated in Figure 1. This important deviation of nitrogen metabolism appears to be an essential feature of the fully habituated callus, and probably of vitrified shoots.

Table 4. Characteristics of nitrogen metabolism in habituated (HNO) and normal (N) nonorganogenic sugarbeet cells (according to Kevers et al. [97]).

<table>
<thead>
<tr>
<th>Biochemical characteristics</th>
<th>N</th>
<th>HNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4^+$ (mM)*</td>
<td>1.45</td>
<td>5.40</td>
</tr>
<tr>
<td>NO$_3^-$ (mM)*</td>
<td>1.9</td>
<td>8.8</td>
</tr>
<tr>
<td>glutamate (µmol [g DW]$^{-1}$)</td>
<td>7</td>
<td>47</td>
</tr>
<tr>
<td>proline (µmol [g DW]$^{-1}$)</td>
<td>2.7</td>
<td>13.1</td>
</tr>
<tr>
<td>glutamate dehydrogenase (nmol</td>
<td>58</td>
<td>135</td>
</tr>
<tr>
<td>NADH ox.min$^{-1}$[gFW]$^{-1}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values expressed on cellular water basis
Table 5. Effect of gabaculine (GAB) and ornithine (ORN) treatments on polyamine (PAs) content in habituated callus culture of Beta vulgaris (L.). Measurements were made after 14 days of culture. Values are means of two independent measurements. According to Le Dily et al.[120].

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>Total PAs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol (g dry weight)³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.86</td>
<td>1.71</td>
<td>0.20</td>
<td>2.78</td>
</tr>
<tr>
<td>GAB 100µM</td>
<td>0.08</td>
<td>0.22</td>
<td>0.07</td>
<td>0.37</td>
</tr>
<tr>
<td>GAB 100µM + ORN</td>
<td>1.04</td>
<td>1.60</td>
<td>0.24</td>
<td>2.88</td>
</tr>
<tr>
<td>5mM sugars cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Proposed scheme of the disturbed nitrogen metabolism in the fully habituated sugar beet callus. The bold arrows indicate the tight linkage between glutamate, proline and ornithine, which leads to PAs synthesis. ARG: arginine; GDH: glutamate dehydrogenase; GK: glutamyl kinase; GLU: glutamate; GPR: glutamylphosphate reductase; GSA5: glutamate-5-semialdehyde; α-KG: α-ketoglutarate; OAT: ornithine aminotransferase; ORN: ornithine; P5C: pyrroline-5-carboxylate reductase; PO: proline oxidase; PRO: proline.
b) Non photosynthetic CO₂ fixation

The HNO cells of the white habituated callus are totally achlorophyllous and thus are unable to fix CO₂ by photosynthesis [17]. However, they fix CO₂ in a non-photosynthetic manner to phosphoenolpyruvate (PEP) and at a higher rate than N green cells (Table 6) [15]. They had also been shown to have a higher malate dehydrogenase activity (Table 6) [35,36]. The increased PEP-CO₂ fixation thus might account for the measured higher PEP-carboxylase and malate dehydrogenase (MDH) activities but also for PAS accumulation through replenishment of the Krebs' cycle (or TCA cycle) with oxaloacetic acid and malic acids as tentatively illustrated in figure 2. This anaplerotic pathway indeed supports a continuous deviation of α-ketoglutarate (α-KG) from the Krebs' cycle to the glutamate-proline-PAs pathway. A relationship between nonphotosynthetic CO₂ fixation through PEP-carboxylase and a greater NH₄⁺ assimilation has been established in other C₃ plants [142].

| Table 6. Label incorporation in N and HNO calli, fed with ¹⁴CO₂ per jar of equivalent weight, malate dehydrogenase and PEP carboxylase activities (nmol NADH oxidized. mg prot⁻¹. min⁻¹) after 14 days of culture. From Kevers et al. [97]. |
|-----------------|-----------------|
|                  | N               | HNO              |
| Incorporated label in light (dpm jar⁻¹) | 94674           | 17387            |
| Incorporated label in darkness (dpm jar⁻¹) | 8865           | 12561            |
| Malate dehydrogenase | 0.73 ± 0.03   | 2.41 ± 0.21      |
| PEP-carboxylase     | 2.92 ± 0.41    | 3.77 ± 0.09      |

c) Preferential pentose phosphate pathway

The HNO cells display lower glycolytic enzyme activities than the N cells, and these are counteracted by higher activities of the enzymes of the pentose phosphate pathway (PPP) (Table 7) [24] Such a preferential sugar catabolism has already been observed in animal tissues submitted to oxidative stress [90]. The activation of the pentose phosphate pathway would provide the NAD(P)H surplus needed for the reduction of nitrate to nitrite and also facilitate the functioning of MDH as indicated in Figure 2. This is another contribution to PAs accumulation.

In these conditions, the question of the availability of PEP, a product of glycosis and a substrate for PEP-carboxylase, can be posed. Plant mitochondria are characterised by the presence of a second enzyme of malate oxidation, namely the NAD-malic decarboxylating enzyme (ME), in addition to the MDH [135]. The ME allows the production of pyruvate from malate, thus facilitating the turnover of the TCA cycle (even under conditions when ME is activated by CoA, which accumulates in these conditions). Such a non photosynthetic fixation of CO₂ with ME and MDH activities thus appears to play a key role in the synthesis of carbon skeleton for the biosynthesis of amino acids and of respiration substrates. Figures 2 and 3 illustrate this preferential pathway.
Integrating phytohormone metabolism and action with biochemical pathways. II.

Figure 2. Scheme showing the privileged glucose respiratory pathway of the HNO cells (heavy lines) and the relationships with the non-photosynthetic CO₂ fixation to contribute, together with ammonia detoxification, to the overproduction of polyamines. MAL : malate; OAA : oxaloacetic acid; PPP : hexose monophosphate and pentose phosphate pathway; PYR : pyruvate, (adapted from Gaspar et al. [62]).

Table 7. Glucose, fructose and sucrose levels (mg [g FW]⁻¹) and enzyme activities (nmol NAD(P)H reduced or oxidised min⁻¹ [mg prot]⁻¹) involved in the HMP pathway and glycolysis of N and HNO calli after 14 days of culture. Mean values, n=3. From Bisbis et al. [24] and Kevers et al. [97].

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>HNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.1</td>
<td>12.4</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.8</td>
<td>30.7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>8.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Hexose phosphate isomerase</td>
<td>34.6</td>
<td>16.6</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>3.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>10.0</td>
<td>19.2</td>
</tr>
<tr>
<td>6-phosphogluconate dehydrogenase</td>
<td>11.8</td>
<td>16.6</td>
</tr>
</tbody>
</table>
d) Operational GABA-shunt through glutamate and PAs degradation

Notwithstanding the above anaplerotic pathway of the Krebs cycle through nonphotosynthetic CO$_2$ fixation, there was still a gap between α-KG and malate. Unpublished results indeed show deficiency of the HNO cells in α-ketoglutarate dehydrogenase complex that converts α-KG to succinyl-CoA and in succinyl-CoA synthetase that allows the synthesis of succinate from succinyl-CoA. It is possible that the Kreb's cycle could be provided with succinate through what is called the GABA (γ-aminobutyric acid) shunt. GABA synthesis results predominantly from the decarboxylation of L-glutamate in a reaction catalysed by glutamate decarboxylase. GABA can enter the Kreb's cycle after transamination by a GABA-transaminase to succinic semialdehyde which in turn is oxidized to succinate by a succinic semialdehyde dehydrogenase [30,132]. The GABA level in plant tissues often increases in response to various stresses [141,152]. The GABA shunt operates in stressed tissues, especially under hypoxia [145]. The HNO cells examined here have been shown to be hyperhydric (cells enveloped by a layer of water [44,69] and to be under permanent stress [98,119].

GABA can also occur from pyrroline via the degradation of PAs [146]. Diamine- and polyamine-oxidase activities were highest in the HNO callus as compared with the normal one [96] (Kevers et al., 1999), as were the intermediary enzymes (Δ1-pyrroline dehydrogenase, GABA-transaminase and succinic semialdehyde dehydrogenase) of the GABA shunt from PAs [20]. This GABA shunt therefore constitutes a second TCA replenishing pathway as schematized in Figure 3.

The entry of GABA in the TCA cycle is supported by an increase in O$_2$ consumption and CO$_2$ evolution [38]. This is in good agreement with a higher O$_2$ uptake observed in HNO cells as compared to the cells [25,35]. Although the HNO cells evolved less CO$_2$ than did the N cells [35], this small net respiratory loss is due to the fact that part of the CO$_2$ evolved is refixed by the active PEPc to reenter the TCA cycle [14].

e) A higher alternative respiratory pathway

A comparison was made of the respiration of cells and of isolated mitochondria of the HNO and the N calli [25]. The measurements of oxygen uptake in the presence of potassium cyanide (CN) or benzohydroxamate (BHAM) by the cells showed that the total O$_2$ uptake (total respiration) and the alternative pathway (CN-resistant) were higher in the HNO cells as compared to the N cells. The cytochrome pathway (BHAM-resistant) still operated in the HNO cells. The relative (Valt)(Vcyt) ratios from HNO and N cells were about 1.7 and 0.6 respectively.

The measurements of O$_2$ uptake from isolated HNO and N mitochondria showed that addition of substrates (NADH, succinate, malate) resulted in a higher activity of the alternative pathway of mitochondria from the HNO callus. Maximum activity was obtained when all the substrates were combined for the two calli.

The activity of cytochrome c oxidase (complex IV in the mitochondrial electron transfer chain) was also measured in the isolated mitochondria. The activity in the N callus was double that of the HNO callus. This would explain partly the higher alternative pathway in the HNO cells, and this is probably related to the low content of
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Figure 3. Scheme of privileged metabolisms (heavy lines) of the HNO callus in relation to the replenishment of the TCA cycle, and the contribution of the pentose phosphate pathway, (adapted from Gaspar et al. [62]).

cytochromes since the HNO cells are deficient in tetrapyrrolic compounds [10,84,101]. The high alternative oxidase activity may also be explained by the availability of TCA substrates (succinate, malate and NADH) [156].

Additionally, it is known that the alternative respiratory pathway is induced by free radicals and H$_2$O$_2$ accumulation may arise in the HNO cells from PAs degradation (see above), and is favoured by a high superoxide dismutase activity [81] and its non-detoxification by catalase which is at a low level in the HNO cells [81,84]. Figure 4 illustrates how all the above described changed metabolisms contribute to induce the
alternative respiratory pathway in the HNO cells. Reciprocally, the alternative pathway, through the regeneration of NAD⁺, feeds the functioning of the TCA cycle.

It must be noticed that exogenous PAs as well as inhibitors of their biosynthesis applied to the HNO callus modified both the endogenous PAs level and growth (Table 8), demonstrating a great sensitivity of these habituated cells to this category of regulators.

Table 8. Effect of polyamines and polyamine biosynthesis inhibitors applied during the 14 days of culture on growth index and on total free polyamines of habituated non-organogenic callus, (according to Kevers et al., [96]).

<table>
<thead>
<tr>
<th></th>
<th>Growth index(% fresh weight)</th>
<th>Polyamine content (nM/g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>14 days</td>
</tr>
<tr>
<td>Control</td>
<td>41.4 ± 4.1</td>
<td>126.0 ± 13.1</td>
</tr>
<tr>
<td>PUT (10⁻⁶M)</td>
<td>37.5 ± 6.2</td>
<td>152.2 ± 20.2</td>
</tr>
<tr>
<td>PUT (10⁻⁵M)</td>
<td>32.8 ± 3.5</td>
<td>48.5 ± 5.1</td>
</tr>
<tr>
<td>SPD (10⁻⁶M)</td>
<td>47.3 ± 6.8</td>
<td>176.9 ± 26.5</td>
</tr>
<tr>
<td>SPD (10⁻⁵M)</td>
<td>47.0 ± 2.4</td>
<td>59.8 ± 3.0</td>
</tr>
<tr>
<td>DFMA (10⁻⁶M)</td>
<td>48.6 ± 5.6</td>
<td>162.2 ± 14.6</td>
</tr>
<tr>
<td>DFMO (10⁻⁶M)</td>
<td>33.7 ± 4.0</td>
<td>192.3 ± 16.9</td>
</tr>
<tr>
<td>DFMO + DFMA (10⁻⁶M)</td>
<td>32.9 ± 4.63</td>
<td>155.1 ± 1.09</td>
</tr>
<tr>
<td>MGBG (10⁻⁵ M)</td>
<td>33.3 ± 2.7</td>
<td>53.3 ± 4.3</td>
</tr>
<tr>
<td>CHA (10⁻⁵M)</td>
<td>43.3 ± 3.9</td>
<td>138.0 ± 15.4</td>
</tr>
<tr>
<td>MGBG + CHA (10⁻⁵M)</td>
<td>23.5 ± 1.9</td>
<td>53.5 ± 6.6</td>
</tr>
</tbody>
</table>

Factors and metabolisms contributing to deficiency of tetrapyrrole-containing compounds, particularly peroxidases

Cells from primary N (hormone-dependent) calli, which can be considered as teratological neoformations entering a neoplastic progression towards true cancer cells [63], are always richer in peroxidase(s) than cells of tissues or organs from which they derive [5,9,46,54,86,111,116,138] Such an increase in peroxidase activity has most often been interpreted as a result of stresses resulting from tissue excision and the culture medium. Secondary calli from the subculture of the primary callus progressively show a lower peroxidase activity [11,29,54]. This gradual loss of activity of total peroxidase and of specific isoperoxidases corresponds to a progressive decline in organogenic capacity in long-term cultures [40,133]. Cells from the HNO callus, at the end of their neoplastic progression, show a very low peroxidase activity and a very low capacity to secrete isoperoxidases compared to normal auxin- and cytokinin-requiring cells [44,71,72,78,101,109,110,118,122,136]. A low peroxidase activity is a general characteristic of habituated calli [28,87,113,114,115]. Some animal hormone-independent tumours similarly have significantly lower peroxidase levels than
Figure 4. Illustration of the favoured metabolisms (heavy lines) and their contributions in the induction of the alternative pathway activity in the HNO callus. 6-PGDH: glucose-6-phosphate dehydrogenase; PEPc: PEP-carboxylase; GDH: glutamate dehydrogenase; GDC: glutamate decarboxylase; GABA-T: GABA-transaminase; SSDH: succinic semialdehyde dehydrogenase; \( \Delta' \)-pyrDH: \( \Delta' \)-pyrroline dehydrogenase; CoA: coenzyme A (according to Bisbis et al. [25]).
hormone-dependent ones [137]. It must be noticed, however, that certain compartments of an HNO callus (purified plasma membrane, for instance) exhibit a peroxidase activity as high as that of the normal callus [83], and that the transfer of a light-cultured HNO callus to darkness increases enzyme activity [10,98]. The latter regulation could be mediated through soluble effectors that act as potential peroxidase inhibitors and/or by differential expression of peroxidase isoenzyme patterns [10]. In any case, peroxidase is not the only porphyrinic compound deficient in habituated calli. Other tetrapyrrole-containing compounds such as chlorophylls [17,44,93,149], cytochromes and catalase [84] are also present in low amounts in habituated tissues.

Figure 5. Beale and Shemin pathways for the biosynthesis of tetrapyrrole-containing compounds (including peroxidase) via aminolevulinic acid; (according to Bisbis et al. [22]).
Aminolevulinic acid (ALA) is an obligatory intermediate in the biosynthesis of tetrapyrrole-containing compounds. It is synthesised through the plastidial Beale pathway from oxoglutarate and glutamate, or through the mitochondrial Shemin pathway from succinate and glycine [22] (Fig. 5). The former commonly operates in algae and higher plants while the latter is generally found in many bacteria, fungi and animals. The accumulation of ALA, haems and chlorophylls, and the activities of peroxidase and catalase were compared in normal green and in achlorophyllous white HNO sugarbeet calli, in light and under darkness in the presence of precursors of the Beale or Shemin pathways, with or without inhibitors of the Beale pathway [21,22]. The results indicated the co-existence of both pathways in normal callus, with increased participation of the Shemin pathway under conditions that reduced the Beale pathway (darkness, inhibitors). These results confirmed the few existing results in the literature about the functioning (although at a limited rate) of the Shemin pathway in higher plants. Additional results also indicated the unique functioning of the Shemin pathway in the HNO callus [23]. This discovery confirms the ontogenetic retrogradation of these cancerous cells as very primitive cells. Furthermore, the HNO callus preferentially accumulates benzoic derivatives contrary to the normal callus, which synthesizes cinnamic derivatives [49]. It was shown that most benzoic

![Figure 6. GABA-shunt, through glutamate and through polyamines, in the TCA cycle, with the indications of the Beale (from glutamate) and Shemin (from succinate) pathways of aminolevulinic acid (ALA) and porphyrin (including peroxidase) biosynthesis.](image)
derivatives inhibit 5-aminolevulinate dehydratase, the enzyme which converts ALA to porphobilinogen, in contrast to most cinnamic derivatives, except ferulic and caffeic acids [122]. Thus both the disturbance in phenolic metabolism and the Shemin pathway might lead to the reduction of the porphyrin pathway and especially of haemoprotein synthesis in the HNO callus. Indeed, the HNO cells also appeared to be deficient in the α-ketoglutarate (α-KG) dehydrogenase complex that converts α-KG to succinyl-CoA and in succinyl-CoA synthetase that synthesizes succinate from succinyl-CoA. However a minimum of succinate necessary for the Shemin pathway can be provided via the GABA shunt as shown in Figure 6. As this model indicates, the Shemin pathway only supports the synthesis of heme proteins (at a limited rate), while the Beale pathway supports chlorophyll synthesis, of course when chloroplasts are present, which cannot be the case for the HNO cells.

These data illustrate the interdependency of PAs biosynthesis and degradation (see above), and the biosynthesis of tetrapyrrole-containing compounds.

Factors and metabolisms contributing to low ethylene production in HNO cells

The HNO callus contains more auxins and less cytokinins than the N one. These results have been discussed in relation with habituation [16,99] but they are recalled here because they may be causal relationships with ethylene production (see [70], part I of this paper series).

The HNO sugarbeet callus indeed emits and retains much lower ethylene quantities than the normal callus [18,71,79,108]. A low rate of ethylene production may well be a general characteristic of habituated cell lines since it has also been observed in habituated tobacco [112,150] and periwinkle (J. Créche, University of Tours, France, personal communication), and in hormone-autonomous radiation-induced tumours of Arabidopsis thaliana [34]. "Does ethylene play a role in the habituation" was the question posed by Köves and Szabo [112]. That low ethylene production resulted from the habituation process rather than causing it was examined by Bisbis et al. [18]. It is difficult to provide a definitive answer. First of all, it is difficult to credit the low level of ethylene production by habituated cells to the absence of auxins and cytokinins in the culture media, even if these growth regulators are known to modulate ethylene biosynthesis [6,68,158]. No treatment could enhance the ethylene production of the HNO callus to the level of the normal callus [18]. Szabo et al. [150] did show this, but the difference between the tissue used in these two studies was that the former had reached an irreversible state [63]. In both cases, however, low ethylene production was related to deficient cell wall differentiation [100]. Low ethylene production, resulting from a retro-inhibition of synthesis, may be responsible for hypolignification of vitreous tissues [100]. The HNO callus can be considered vitreous because it is hyperhydric [44] and because it contains no lignin [78].

As reported and discussed above, accumulation of PAs is another characteristic of habituated and other neoplastic tissues. SAM is involved in both polyamines, spermidine and spermine, and ethylene formation (Figure 7) and thus both pathways should be in competition [50]. Often indeed ethylene and polyamines negatively affect
the accumulation of each other [153]. The experiments designed to determine the interactive effects of these compounds have given contradictory results, which has been related with the differences in developmental stages or physiological states of the tissues [147]. To understand how ethylene and polyamine interact in the HNO callus, the callus was treated with exogenous polyamines, polyamine biosynthesis inhibitors, and titers of polyamines and ethylene production were determined [19]. The data showed that treatment of the callus with exogenous putrescine or spermidine reduced polyamine titers and the ethylene production. The decrease in polyamine accumulation could be explained by an autoregulatory mechanism of polyamine biosynthetic pathway or by a rapid degradation or conjugation of polyamines. Exogenous polyamines could induce an inhibitory signal of the enzymes involved in polyamine biosynthesis. The observed effect on ethylene biosynthesis could be a direct inhibitory effect of exogenous putrescine and spermidine through the inhibition of the enzymes involved in ethylene biosynthetic pathway. Surprisingly, lower concentrations of Put and Spd (10^{-6} \text{mol/L}) induced a more inhibitory effect on HNO growth than the higher ones (10^{-5} \text{mol/L}). This could be attributed to the time course of polyamine uptake.

The two inhibitors of putrescine biosynthesis, DFMO for ODC and DFMA for
ADC, used separately, induced an inhibition of polyamine accumulation but also a decrease of ethylene production. Based on these results, it could not be concluded about a possible competition between the two metabolites. Since the two enzymes are involved in putrescine synthesis, the use of one or the other inhibitor couldn't completely block putrescine accumulation. When applying DFMO and DFMA in combination, free polyamine contents were highly reduced compared to the control. Not only a reduction of putrescine content was observed in this case but also a decrease in spermidine and spermine levels. In these conditions only, an increase of ethylene production was observed. If an interactive effect between ethylene and polyamines is a metabolic competition for SAM as substrate, inhibition of spermidine and spermine biosynthesis by inhibiting the transfer of aminopropyl groups into polyamines by MGBG would increase the flow of SAM into ethylene biosynthesis, decrease the spermidine and spermine synthesis, and simultaneously increase putrescine accumulation. The data showed not only a decrease in the levels of polyamines in the presence of MGBG but also a decrease in ethylene production. This result could mean that the ethylene and polyamine interactions in HNO callus are not necessarily based only on a metabolic competition. If a metabolic competition exists, it is necessarily located at spermidine and spermine levels. For this reason, the HNO callus was treated with an inhibitor of spermidine synthase (CHA). In the presence of CHA, one could expect an increase of ethylene synthesis, a decrease in spermidine and spermine content, and then an accumulation of putrescine. This was not the case: ethylene synthesis and polyamine levels decreased in the presence of CHA. The situation where both SAM decarboxylase and spermidine synthase were inhibited was also tested using MGBG and CHA in combination. In such conditions, the HNO callus increased by three times its ethylene emission but decreased its free polyamine contents. The inhibition of polyamine contents, especially putrescine content in this case, could be explained by several metabolic actions. First, the feed-back regulation due to the accumulation of putrescine which could induce an inhibitory effect on the biosynthetic pathway of putrescine (via ODC or ADC). Second, putrescine could be metabolised rapidly or conjugated with other molecules. This has precisely been shown in the HNO callus [96]. In this last situation, the increase of ethylene production could be directly related to the inhibition of polyamine synthesis.

The results concerning the application of polyamines and inhibitors of polyamine biosynthesis showed that the interactions between ethylene and polyamines were not based only on a metabolic competition for SAM as it was shown by the combination of DFMO and DFMA or MGBG and CHA. The interactive effect between the two metabolites concerned the direct inhibitory effect of polyamines on ethylene biosynthetic pathway.

The effect of growth regulators (auxin and cytokinin) on growth of HNO callus showed that the callus was sensitive to the exogenously applied hormones. Their effect on ethylene production was followed to determine if the growth regulators still exerted some control on the ethylene biosynthetic pathway in the HNO callus. The results showed that the ethylene emission was increased in the presence of auxin and cytokinin as compared to the callus grown in the absence of exogenous plant hormones in the medium. That means that the lower ethylene production by the HNO callus is
partly due to absence of the hormonal control, particularly, the auxin/cytokinin balance.

In conclusion from these results, two explanations could be given to the lower ethylene production of the HNO callus. The first concerns the absence of the usual exogenous hormonal balance and the second refers to polyamine accumulation. The polyamines are known to have antisenescent properties acting by their ionic interactions with membranes and by their function as free radical scavengers [48,147]. The last function of polyamines could affect the ethylene biosynthesis since ACC-oxidase involved in the conversion of ACC to ethylene needs free radicals for its activity [159]. Furthermore, Apelbaum et al. [2] have shown that polyamines, Spd and Spm, inhibit ethylene biosynthesis; these two polyamines precisely accumulate in the HNO callus. On the other hand, it has been shown that auxin applied to plant tissues stimulates the ethylene production [112]. This stimulus may generate signals that regulate the synthesis or the activity of ACC-synthase [103]. If we summarise the results, ethylene biosynthesis in HNO callus is controlled negatively at two levels. First, it is controlled at the ACC-synthase level due to the absence of exogenous growth regulators, especially auxins/cytokinins, preventing the synthesis of ACC. It was indeed shown that HNO callus increased its ethylene production in the presence of exogenous ACC [18]. Second, it is controlled at ACC-oxidase level due to the higher accumulation of polyamines which inhibit the conversion of ACC to ethylene.

Table 9. Effects of inhibitors of ethylene synthesis (AOA and AIB), ethylene action (NRD and STS), ethylene precursor (ACC), exogenously applied ethylene, ethylene trapper and darkness on ethylene production and growth of the normal and habituated non-organogenic sugarbeet calli, (according to Bisbis et al. [18]).

<table>
<thead>
<tr>
<th></th>
<th>Ethylene production</th>
<th>Ethylene production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% of the control)</td>
<td>(% of the control)</td>
</tr>
<tr>
<td>Control</td>
<td>N</td>
<td>HNO</td>
</tr>
<tr>
<td>AOA (100µM)</td>
<td>8</td>
<td>65</td>
</tr>
<tr>
<td>AIB (100µM)</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>NRD (4 mM)</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>STS (50 µM)</td>
<td>15</td>
<td>76</td>
</tr>
<tr>
<td>ACC (100 µM)</td>
<td>113</td>
<td>181</td>
</tr>
<tr>
<td>Ethylene (1 p.p.m.)</td>
<td>44</td>
<td>500</td>
</tr>
<tr>
<td>Ethylene (20 p.p.m.)</td>
<td>33</td>
<td>242</td>
</tr>
<tr>
<td>Ethylene trapper</td>
<td>10</td>
<td>461</td>
</tr>
<tr>
<td>Darkness</td>
<td>35</td>
<td>43</td>
</tr>
</tbody>
</table>

Values are means of at least three separate repetitions. The absolute values of the control for the ethylene production are 134.7 ± 9.8 µl. g fresh weight⁻¹ 24 h⁻¹ for the N callus and 2.6 ± 0.4 for the HNO callus. For growth index, the absolute values are 261% ± 21.3 for the N callus and 162% ± 31.8 for the HNO callus.
In addition to its direct involvement as intermediate in polyamine and ethylene biosynthesis, SAM plays other numerous roles in plant cells, as being the major methyl-group donor in transmethylation reactions and methylation of cytosine residues in DNA. This probably constituted part of the difficulties in the interpretations of the results.

Results from Table 9 indicate growth dependency of both the normal and HNO calli upon the level of their endogenously biosynthesized ethylene, based on growth reactions to inhibitors of ethylene biosynthesis and ethylene action, to ACC, and to transfer from light to darkness; the dependency is also upon environmentally retained ethylene, based on growth reactions to exogenously applied ethylene and to trapped ethylene. We have noticed, however that the growth reactions of normal callus to certain additives (aminooxyacetic acid (AOA) and α-aminoisobutyric acid (AIB), for instance) were not always proportional to their inhibiting effect on ethylene biosynthesis. In the same normal callus, we have even shown reduced ethylene production through an auxin-induced growth enhancement [37]. There have been many papers investigating relationships between callus growth and the rate of ethylene production [88,95,112,124,125,130,150,155] and no clear-cut conclusion could be drawn. The general assessment, however, was that some of the endogenous ethylene was active as a growth regulator, although much was simply produced as a consequence of rapid growth [89]. Taking into account the above considerations, the results of Bisbis et al. [18] (Table 9) also clearly demonstrated a response of the HNO callus towards exogenously applied ethylene. The callus responded to ethylene application by increasing its own ethylene production and growth was severely reduced. Bolton and Freebairn [26] have already shown the growth reactions of habituated tissues towards exogenously applied ethylene.

The relationship of low ethylene production in the HNO callus with its low peroxidase activity and with the Shemin pathway is not directly evident, although the direct participation of peroxidases in the conversion of ACC to ethylene has been proposed [100,107]. However, an indirect action through control of the higher IAA level is plausible, as IAA has been shown to influence ACC synthase in the ethylene biosynthetic pathway.

Conclusions and prospects

Taking advantage of our studies on deviations of metabolisms in neoplastic cells and tissues, we came to integrate the metabolisms of at least two hormones, polyamines and ethylene, with primary metabolisms such as those of carbon and nitrogen (see Fig. 4,6,7). We did not succeed yet in explaining the shift of the sensitivities of such cells, compared to normal ones, from auxins and cytokinins, to the other couple polyamines/ethylene. But this means that these problems of sensitivities, linked with hormonal receptors and secondary messenger pathways, also should be if not dependent, at least interrelated with the primary metabolisms. These results, together with those of the preceding paper [70], show the progressive changes of concepts in hormonology.
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