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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Fisiologia vegetale. — *Fusicoccin as a tool for the analysis of auxin action* (*). Nota di ERASMO MARRÈ, PIERA LADO, FRANCA RASI CALDOGNO e ROBERTA COLOMBO, presentata (**) dal Corrisp. E. MARRÈ.

RIASSUNTO. — La fusicoccina, una tossina vegetale, stimola la crescita per distensione in modo simile all'auxina, ed inoltre induce variazioni a livello metabolico qualitativamente simili a quelle osservate con auxina, ma quantitativamente assai superiori. Ciò ha consentito di riesaminare alcune componenti dell'effetto auxinico e di formulare alcune ipotesi per quanto riguarda almeno una parte del meccanismo di azione dell'ormone.

Two of the more conspicuous effects of auxin at cellular level are the increase of the plasticity of the cell wall (resulting in the well known effect of cell enlargement) and the stimulation of respiration. No satisfactory theory is as yet available as far as the biochemical mechanism of either of these effects is concerned. Most of the work in this field is centered along the following lines: *a*) the attempt to elucidate the primary step of the hormone action (presumably the binding to some macromolecular species [1, 2, 3, 4, 5]; *b*) the understanding of the nature of the changes of cell wall structure, leading to the increase of deformability [6, 7, 8, 9, 10, 11, 12]; *c*) the analysis of the metabolic changes involved in the chain of interrelated steps running from the response of the primary acceptor to the final responses at cell wall as well as at respiration level.

An advantage of the third type of approach is that although it may be of little help in solving the auxin problem, it should usefully contribute to our understanding of the basic relationship between energetic and biosynthetic metabolism, growth and differentiation. Working along this line, a series of investigations carried on in this laboratory led to the following conclusions:

I. *Gas exchanges.* – Auxin increases Q_{O_2} and Q_{CO_2}, this effect being suppressed by low concentrations of CO [13]; auxin decreases the ratio between the rate of dissimilation of 1–C and that of 6–C of ^{14}C labeled glucose (C_1/C_6 ratio) [14, 15, 16, 17].

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II. *Metabolite and cofactor levels.* – Auxin increases the level of glycolytic intermediates, from glucose–6–P to pyruvate [18]. No decrease of ATP, but rather an increase of its level is observed under the conditions of growth stimulation by the hormone [19, 20].

III. *Oxidation–reduction systems.* – A shift in favour of the reduced form of the NADP, glutathione and ascorbate systems has been reported [21, 22, 23, 24]. Treatments inducing a shift of the same systems in the opposite direction have been shown to inhibit both cell enlargement and respiration.

The magnitude of most of these responses is relatively modest, ranging from a +20 % in the case of the effect on Q_{O_2} to +30 % in that on the NADPH level. It is clear that any amplification of the above mentioned metabolic effects would be of great value for any attempt to understand their mutual relationship and their significance as far as the final effects on growth and respiration are concerned.

Recent work carried out in this and other laboratories seems to open some new experimental possibilities in this direction. Fusicoccin, the active agent produced by *Fusicoccum amygdali* [25, 26], has been shown to induce on the excised pea internode test (a well known material for studies on the mechanism of auxin action) a series of responses of both metabolism and cell enlargement qualitatively very similar, but quantitatively much larger than those induced by the natural or by the synthetic auxins so far investigated.

In fact, with F.C., instead of IAA, used as a growth promoting agent at optimal concentration and in short time (2 h) experiments, the effects on cell enlargement, Q_{O_2}, Q_{CO_2}, the C_1/C_6 ratio and glucose–6–P concentration are amplified by ca. 80–120 % and the effect on pyruvate concentration even much more (by ca. 250 %). F.C.-induced cell enlargement (just as that induced by the natural auxin IAA), is accompanied by unreversible stretching of the cell wall; moreover, at concentrations of the two compounds inducing the same effect on longitudinal growth, also the effects on transverse growth are identical [27, 28, 29, 30, 31, 15]. Finally, experiments on the interaction between F.C. and IAA showed that the effects of the two substances on cell enlargement are strictly additive when they are fed at very low, suboptimal concentrations, while at optimal concentrations of F.C. IAA becomes ineffective [31].

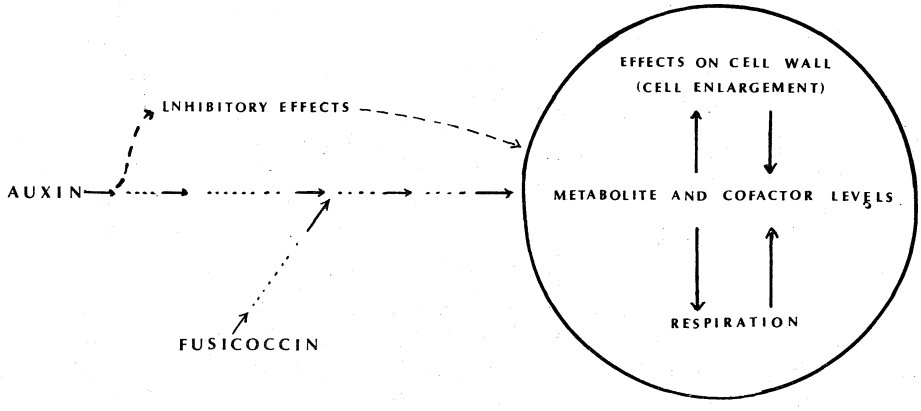
These results strongly suggest that at least several steps of chain of reactions leading to final responses of growth and respiration are in common for the two substances, IAA and F.C. This hypothesis is supported by the finding that the same metabolic inhibitors which repress auxin-induced stimulation of cell enlargement are also effective in the case of F.C. treatment [27]. This has been found true for inhibitors of glycolysis (NaF); of electron transfer-linked phosphorylation (2–4 dinitrophenol), of RNA synthesis (actinomycin D) of protein synthesis (cycloheximide, puromycin).

It seems thus ascertained that both the F.C.-induced and the IAA-induced effects on cell enlargement depend at least at a considerable extent

on the functionality of the protein synthesis and the oxidative-phosphorylative systems.

On the other hand, there are good reasons to believe that the primary site of action of F.C. is not the same as that of natural or synthetic auxins. In fact, the molecule of F.C. does not show the well known structural features typical of the compounds of the auxin group [26]. Moreover, the shape of the dose/response curve of F.C. is different from that of IAA and of the other auxins, inasmuch as the typical inhibitory effect at relatively high concentrations (up to 10^{-3}M) is lacking [31]. Finally, the organ specificities of the two substances (on the basis of the cell enlargement effect) are completely different. F.C. is almost ineffective in inhibiting root elongation, while it markedly stimulates cell enlargement in leaf disks; auxins strongly inhibit root elongation while they do not influence the leaf disks [30].

In conclusion, the present evidence suggests a working hypothesis, according to which only the initial part of the chain of events mediating the effects of F.C. and respectively of IAA on growth and respiration would be specific of each compound, while the final part of this chain, including a considerable number of metabolic steps, would be common, as represented in the following scheme



If this hypothesis were confirmed, F.C. should be considered as a very interesting tool to understand at least a large fraction of the hormone-induced metabolic and growth responses, inasmuch as these appear amplified by a factor of about 2 fold in the case of F.C.

As an example of application of this view, some preliminary observations on the relation between protein synthesis activity, respiration and cell enlargement can be mentioned.

Recent work by Nooden and Thimann [4] and other authors [1, 3] led to an interpretation according to which the effects of auxin are mediated by the stimulation of the synthesis of some protein species. This interpretation is mainly based on the finding that a number of inhibitors of RNA

and protein synthesis inhibit also auxin-stimulated cell enlargement. On the other hand, several experiments carried out in this laboratory on the effects of protein synthesis inhibitors on protein synthesis, respiration and cell enlargement led to an alternative interpretation, namely that even if normal protein synthesis activity (as well as ATP availability) are required for cell enlargement, however a specific action of the hormone at protein synthesis level is not a necessary step in its action mechanism. In fact, conditions were found under which it was possible to obtain a decrease of overall protein synthesis together with a still significant increase of auxin-induced respiration and cell enlargement [32]. Here again, however, the relatively small size of the responses investigated made the evidence in favour of this latter view too weak for a definite conclusion.

When the same problem was taken up again with F.C., instead of IAA, as a growth promoting and respiration stimulating agent, it appeared quite clear that one can still observe a very marked effect on respiration (stimulation by ca. 30 %) and a still significant effect on cell enlargement, even under conditions of practically complete inhibition of labeled leucine incorporation into proteins.

This suggests that F.C. influences growth and respiration rather through changes of activity of preformed proteins than by acting at transcription or translation level. If this interpretation is correct, and if it is true that the mechanism of action of F.C. is fundamentally similar to that of auxin, then the whole sector of nucleic acid and protein metabolism would appear of minor relevance for the investigation of the final steps of the mechanism of auxin action on respiratory metabolism and cell wall structure.

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