From the data in Figure 3, it can again be seen that EDCF enhances cell proliferation when added to cultures with inhibitory concentrations of ecdysones, and that its inhibitory effect upon growth is more pronounced in cultures stimulated by ecdysones than in controls.

In the experiments reported here, no evidence was obtained for qualitatively different effects of α- or β-ecdysone, as have been reported for several in vitro systems[11,13–15]: including the *Drosophila* cell line Kc (parent line to KcC7 used here). Also a dependence on mammalian serum proteins for ecdysone to be effective[10] could not be found (data not shown). Most of these differences can probably be explained either by differing experimental conditions and/or the limited hormone concentration ranges tested. However, the critical variables remain to be identified. The same holds for the action of juvenile hormones or their analogues.

For β-ecdysone the concentrations effective in this system fall within the low range of those actually measured in vivo[16]. The much lower activity of α-ecdysone compared to β-ecdysone has been seen in many other systems (References in[17]). For EDCF significant effects on cell proliferation were observed at concentration lower than needed to affect adult *Drosophila* development[18]. So by these criteria, the reactions observed are not unphysiological. However, since the tissue of origin of these cells is not known[10], it cannot be said what their physiological reaction to hormones would be. Certainly natural juvenile hormones and other juvenile hormone analogues will have to be tested in this system before it can be considered established as a model for the investigation of hormone action and interaction, or possibly used as a bioassay during the isolation of the 'true' *Drosophila* juvenile hormone.

Since in this study only the increase in the total cell population was measured, no information could be obtained on the mechanism of action of the added hormones. In particular it remains unknown whether ecdysone and EDCF have to be present simultaneously and continuously to produce an antagonistic effect, and whether this effect is the result of antagonistic changes in cellular physiology (e.g., opposite effects on membrane permeability) or of the induction of changes in hormone metabolism (e.g., EDCF-induced ecdysone inactivation).

Note added in proof: When tested at a concentration of 10 μg/ml, singly or in combination with 1 μg/ml α-ecdysone, C-18 juvenile hormone, epoxygeranylsesamole and ZR-515 gave a similar result as EDCF, whereas the in vivo inactive analogue methylepoxyhexadecanoate was without effect on cell proliferation.

[13] E. P. MARKS, Gen. comp. Endocr. *21*, 472 (1973).
[14] U. CLEVER, I. CLEVR, I. STORBECK and N. L. YOUNG, Devl Biol. *31*, 47 (1973).
[15] P. MANDARON, Devl Biol. *31*, 101 (1973).
[16] D. W. BORST, W. E. BOLLENBACHER, J. D. O'CONNOR, D. S. KING and J. W. FRISTROM, Devl Biol. *39*, 308 (1974).
[17] M. J. MILNER and J. H. SANG, Cell *3*, 141 (1974).
[18] J. H. POSTLETHWAIT, Biol. Bull. *147*, 119 (1974).

The Interaction of Indol-3-Acetic Acid with the Uncoupler and Non-Uncoupler Herbicides

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Summary. Auxin was shown to be able to cancel the growth inhibitory effect imposed by those herbicides which are not known as strong uncouplers of oxidative phosphorylation, while it was unable to do so with regards to the uncoupler herbicides.

Although a great deal of research has been carried out during the past 20 years concerning the mode of action of the herbicides, our information concerning the mechanism of the action of most herbicides in the molecular and cellular level is not well known[1,2]. At the present time, the general mechanism through which herbicides interfer with the growth of the susceptible plants are assumed to be by their interference with the energy metabolism of the cell due to the interruption of the respiratory electron transfer chain of the mitochondria, or the possibility of the combination of the herbicides with an intermediate of energy coupling chain which stops oxidative phosphorylation[1,2]. Also the interference of some herbicides with the photoreactions of photosynthesis, e.g. the Hill reactions or the light-induced electron transfer and photophosphorylation, are well reported[2,3]. In the past few years some work on the effect of herbicides on the protein synthesis and nucleic acid metabolism have also been reported[4–6], but it was rather difficult to demonstrate whether inhibitory effects were a direct result of the herbicides action on protein synthesis, or whether it was merely due to a secondary effect caused by the drop in the ATP level and or the inhibition of the active uptake of the growth elements by cells.

The purpose of this study is to show a simple way of differentiation between those herbicides which are not known to be involved in the energy metabolism of the cell, and those which are known to have mild or strong uncoupling effect on oxidative phosphorylation and hence affect the ATP synthesizing systems. The interaction between the growth hormones and some selected herbicides, are being carried out and the results obtained indicate the possibility of using this method to differentiate between the two groups of herbicides. We also used interaction of abscisic acid (ABA) which has no direct effect on energy metabolism and ATP level (7, 8) with groth hormones as a reference for comparative purpose.

[1] J. L. HILTON, L. L. JANSEN and H. M. HULL, A. Rev. Plant Physiol. *14*, 353 (1963).
[2] D. E. MORELAND, A. Rev. Plant Physiol. *18*, 365 (1967).
[3] D. E. MORELAND, Progr. photosynth. Res. *3*, 1693 (1969).
[4] J. D. MANN, L. S. JORDAN and B. E. DAY, Plant Physiol. *40*, 840 (1965).
[5] E. H. SHOKRAII and D. E. MORELAND, Abstr. Weed Soc. Am. Meeting St. Louis (1966), p. 32.
[6] D. E. MORELAND, S. S. MALHORTA, R. D. GRUENHAGEN and E. H. SHOKRAII, Weed Sci. *17*, 556 (1969).

Materials and methods. Plant material used was 1 cm sections of the hypocotyles of 4-day-old seedlings of sunflower grown under dark conditions. These sections were taken from a region of maximum growth potential but with little or no cell divisional activity. The pure sample of the herbicides used for this study were all in physiological concentration (10^{-4} *M*). The growth response of the tissue toward gibberellic acid (GA) in short period of experiment (6 to 24 h) were not always constant and high, and thus we used solely indol-3-acetic acid (IAA) which gave a good response in our studies. The growth of the sections, and also the rate of protein synthesis as measured by the rate of incorporation of C^{14}-leucine in the newly synthesized protein[6], were measured for this study.

Results and discussion. The results obtained are presented in Tables I, II and III. These results clearly show that the inhibitory effect of ABA can be completely overcome by the growth-enhancing effect of auxin, and this confirms the results obtained by others[7–10]. The data in Table I also shows that the inhibitory effect caused by Ametryne[11] can also be diminished completely by the effect of auxin; and in fact there was no difference between the behavior of ametryne and ABA in this respect. On the other hand, combination of ABA and ametryne did not have a cumulative inhibitory effect, and this is possibly an indication of the similarity of the site of action of the two chemicals. It also suggests the possibility of the occurrence of some sort of competition. The interaction of Diuron and Dichlobenil[11] with auxin are also studied and the results show that auxin completely nullifies the inhibitory effect of Diuron which is not known yet as an uncoupler of oxidative phosphorylation[12,13], while in the case of Dichlobenil, which is known to have strong uncoupling effect[12,14], auxin cannot significantly counteract the inhibitory effect on growth imposed by this chemical (Table III). Among the other uncoupling herbicides, propanil was employed and the result clearly showed the complete inability of auxin to counteract with this herbicide which is a well known uncoupler. The result on protein synthesis was quite similar to that on the growth. The impossiblity of the reversal of growth inhibition caused by the uncoupling herbicides by IAA seems to be quite normal as all the known factors which stimulate the growth processes are all energy-requiring and biosynthetic reactions which annot continue in a condition where the lack or drop of ATP level caused by the herbicide prevails; and in fact there are certain cases where auxin might deteriorate the growth condition which is so caused by an uncoupler herbicide. Very recent studies in this area[15] have also confirmed this view with regard to the interaction of IAA and GA with an inhibitor of ATP synthetizing system. Thus this type of studies could be used as a better and simpler means of differentiating the two major classes of herbicides which are at the present time solely divided on the basis of their effect on ATP synthesis as determind by direct measurement of the ATP level of the tissue which is itself subject to many qualitative and quantitative technical problems; and it may not also be the true indicator of the uncoupling activity of a herbicide.

More elaborate studies of this kind, and specially kinetic type of work, might also lead toward our better understanding of the mechanism through which herbicides as well as the growth hormones work and this paves the way for determining the basic and primary site for the action of the herbicides in the life of the plant cells.

Table I. Interaction of Ametryne[11] and ABA with auxin[a] (IAA) on the growth of sunflower hypocotyles

Treatment	Inhibition or increase of growth (%)
Control	0
Auxin (IAA)	+ 48
Ametryne[b] (10^{-4} *M*)	— 23
Ametryne + IAA	+ 17
ABA (10^{-5} *M*)	— 30
ABA + IAA	+ 12

[a] Results are the average of at least 3 duplicate experiments.
[b] Ametryne was the only member of the triazine group of herbicides which had strong inhibitory effect on the growth of this plant material in physiological concentration.

Table II. Interaction of Ametryne[11] and ABA on the growth responses of sunflower hypocotyles[a]

Treatment	Growth inhibition (%)	
	8 h	24 h
Control	0	0
ABA	— 15	— 30
Ametryne	— 23	— 33
ABA + Ametryne	— 29	— 42

[a] Same conditions as for Table I.

Table III. Interaction of Dichlobenil[11] and auxin on the growth response of sunflower hypocotyles[a]

Treatment	Inhibition or increase of growth (%)
Controle	0
Auxin (IAA)	+ 50
Dichlobenil	— 29
IAA + Dichlobenil	— 11

[a] Conditions of the experiment exactly the same as Table I.

[7] P. E. Pilet, J. exp. Bot. *21*, 446 (1970).
[8] C. Leopold, Plant Physiol. *48*, 531 (1971).
[9] P. E. Pilet and R. Roland, Cytobiologie *4*, 41 (1971).
[10] I. Sussex, M. Clutter and V. Walbot, Plant Physiol. *56*, 575 (1975).
[11] Abbreviations: Ametryne, 2 Ethyl-amino-4-isopropyl-amino-6-methyl thio-s-triazine; Dichlobenil, 2,6-dichlorobenzonitrile; Propanil, 3′,4′-dichloropropionanilide; Diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.
[12] R. D. Gruenhagen and D. E. Moreland, Weed Sci. *19*, 319 (1971).
[13] J. B. St. John, Weed Sci. *19*, 274 (1971).
[14] A. Verloop and W. B. Nimmo, Weed Res. *10*, 65 (1970).
[15] M. Katsumi, Plant Cell Physiol. *17*, 139 (1976).