groups modified by *p*CMB and NEM are probably not identical, or that additional groups are modified by *p*CMB. NEM-reactive 40S proteins from human 80S ribosomes have been recently identified by two-dimensional polyacrylamide gel electrophoresis[12]. Work is now in progress to identify the *p*CMB-reactive proteins from the 40S particle. *p*CMB has been shown to stimulate non-enzymatic polypeptide synthesis respectively translocation on *E. coli* ribosomes by interaction with the ribosomal protein S12 from the 30S subunit[14]. A similiar mechanism has thus far not been observed on the eucaryotic ribosome.

[14] L. P. GAVRILOVA, V. E. KOTELIANSKY and A. S. SPIRIN, FEBS Lett. *45*, 324 (1974).

Juvenile Hormone Analogue Counteracts Growth Stimulation and Inhibition by Ecdysones in Clonal *Drosophila* Cell Line

C. WYSS[1]

Institute for Cell Biology, Swiss Federal Institute of Technology, CH-8093 Zürich (Switzerland), 19 May 1976.

Summary. Depending on concentration, ecdysones either stimulate or inhibit proliferation of a clonal *Drosophila* cell line. Both effects are counteracted by ethyl dichlorofarnesoate, a juvenile hormone analogue, which by itself is growth inhibitory. Qualitatively no difference was seen between α- and β-ecdysone.

The balanced interplay of ecdysones and juvenile hormones, essential for the regulation of normal development in insects, has been the object of extensive investigations. Besides a large number of studies using whole animals (review ref. [2]), a considerable effort has also been made to develop more manageable in vitro systems. Explanted imaginal discs[3,4], ovaries[5] and salivary glands[6] have so far been the most responsive targets. In all these cases, the limited quantity and especially the limited homogeneity of the starting material, has been a serious problem. It was of interest, therefore, to develop culture systems of continuous cell lines capable of responding both to ecdysones and juvenile hormones.

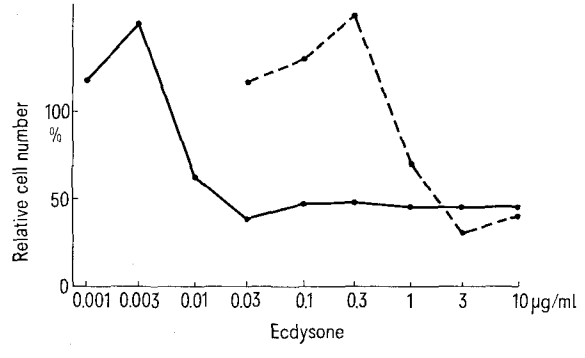

Fig. 1. Effects of ecdysone concentration on proliferation of KcC7 Drosophila cell line. 72-h growth response to α-ecdysone (●---●) and β-ecdysone (●—●) Ordinate: Relative cell number expressed in percents of control (=cultures without hormones). Abscissa: Concentration of ecdysone. Initial cell density was 2.3 10^5 cells/ml. Exponentially growing KcC7 cells were washed and inoculated into media with the hormone concentrations indicated. 1 ml cultures were set up in 17×100 ml Falcon culture tubes and incubated at 25 °C in air. Cell numbers were determined, after dilution of whole 1 ml cultures with saline, in a Coulter counter ZBl. All points represent means of duplicate cultures which varied by less than 5%. In parallel, 4 ml cultures were set up in 25 cm^2 Falcon flasks. α-Ecdysone (Fluka) and β-ecdysone (Rohto) were dissolved in culture medium at 100µg/ml, lower concentrations were obtained by dilution with medium. EDCF (gift from Hofmann-La Roche) was added to culture medium at 1 µl per ml. After shaking at 25 °C for 4 h, this mixture was filter-sterilized and taken to be a 20 µg/ml solution of EDCF. All lower concentrations were obtained by dilution with medium.

Reports on such systems published so far[7-9] indicate only limited success, however, particularly in respect to the combined action of the two classes of hormones.

I report here on the growth response of a hypotetraploid clonal cell line from *Drosophila melanogaster* to the supplementation of culture medium with a juvenile hormone analogue, ethyl dichlorofarnesoate (EDCF) and/or ecdysone (α- and/or β-). Low doses of ecdysones stimulate cell proliferation, whereas high concentrations inhibit it. EDCF, alone or in combination with low doses of ecdysone, produces a dose-dependent growth inhibition. In combination, however, EDCF and high concentrations of ecdysone no longer inhibit, but can even stimulate cell proliferation. Apart from a 100-fold difference in effective concentrations, the two ecdysones tested give identical results.

The established *Drosophila* cell lines Ca and Kc were kindly provided by Prof. G. ÉCHALIER of Paris and maintained in D22 medium supplemented with 10% heat inactivated fetal calf serum (GIBCO)[10]. Both lines, when tested after 3 passages in this laboratory, had a hypotetraploid karyotype. All data shown are obtained with a hypotetraploid clonal subline of Kc, designated KcC7, isolated in semisolid agar medium and since cultured in a modified D22 medium: Lactalbumin hydrolysate replaced by a defined mixture of amino acid; 360 mOsm; pH 6.8 supplemented with 3% heat inactivated horse serum (FLOW) (C. WYSS and G. BACHMANN, in preparation). Similar results were obtained with the uncloned lines Ca and Kc in D22 medium.

[1] Supported by the Swiss National Science Foundation, grant No. 3.8640.72. Parts of this work were presented in preliminary form at the May, 1974, meeting of the Union of Swiss Societies for Experimental Biology in Lausanne (C. WYSS, Experientia *30*, 750, 1974). I thank Prof. H. EPPENBERGER and Drs. M. LEZZI and D. TURNER for help with the manuscript.

[2] K. SLAMA, J. Insect Physiol. *21*, 921 (1975).

[3] C. J. CHIHARA and J. W. FRISTROM, Devl. Biol. *35*, 36 (1973).

[4] D. L. SILHACEK and H. OBERLANDER, J. Insect Physiol. *21*, 153 (1975).

[5] A.-M. LAVERDURE, Gen. comp. Endocr. *17*, 467 (1971).

[6] M. LEZZI, Molec. cell. Endocr. *1*, 189 (1974).

[7] J. MITSUHASHI and T. D. C. GRACE, Appl. Ent. Zool. *5*, 182 (1970).

[8] A.-M. COURGEON, C. r. Acad. Sci., Paris *280*, 2563 (1975).

[9] E. COHEN and L. I. GILBERT, J. Insect Physiol. *18*, 1061 (1972).

[10] G. ÉCHALIER and A. OHANESSIAN, In Vitro *6*, 162 (1970).

Figures 1 and 2 give the results of one large experiment, which have been confirmed by numerous less extensive experiments. Figure 1 shows the bimodal response of line KcC7 to the 2 ecdysones, with β-ecdysone being 100-fold more active than α-ecdysone: Low ecdysone concentrations (Optima: 3 ng/ml for β-ecdysone and 300 ng/ml for α-ecdysone) stimulate proliferation whereas higher concentrations inhibit. Figure 2 shows the influence of EDCF, alone or in combination with various doses of ecdysone, on cell proliferation. With increasing concentrations of EDCF, cell proliferation is progressively inhibited. This inhibition is even more pronounced in cultures containing 1 µg/ml α-ecdysone, whereas in the absence of ecdysones, EDCF inhibition is significant only

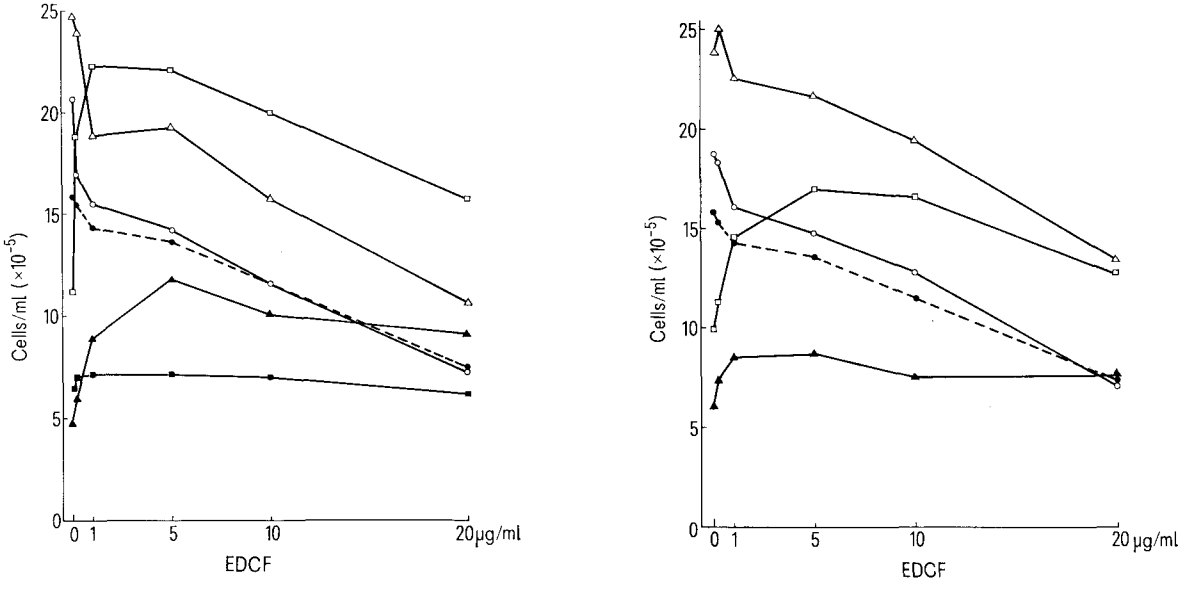

Fig. 2. Effects of increasing EDCF-concentration on proliferation of KcC7 *Drosophila* cell line in media containing varying amounts of ecdysone. A) α-ecdysone: ●–––●, 0 µg/ml; ○—○, 0.1 µg/ml; △—△, 0.3 µg/ml; □—□, 1.0 µg/ml; × — ×, 3.0 µg/ml; ■—■, 10.0 µg/ml. B) β-ecdysone: ●–––●, 0 µg/ml; ○—, 0.001 µg/ml; △—△, 0.003 µg/ml; □—□, 0.01 µg/ml; × — ×, 0.03 µg/ml. Data from the same experiment as Figure 1. 72-h growth response: Ordinate: cell density; Abscissa: EDCF concentration.

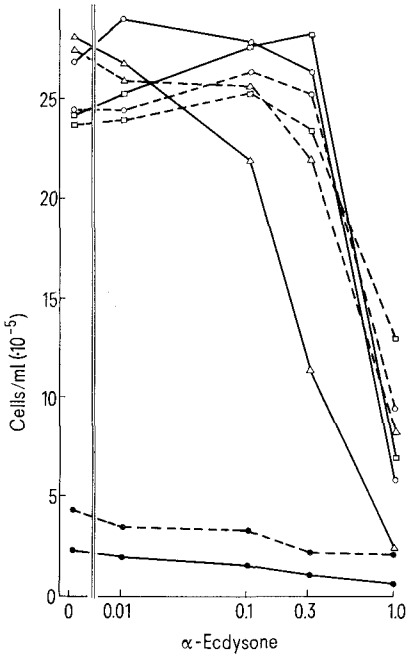

Fig. 3. Effects of various mixtures of α- and β-ecdysones on proliferation of KcC7 *Drosophila* cell line in the presence or absence of EDCF. 6 day growth response to α-ecdysone. Ordinate: cell density; Abscissa: concentration of α-ecdysone. Solid lines: no EDCF; Dashed lines: 2 µg/ml EDCF. □, without β-ecdysone; 0,1 ng/ml β-ecdysone; △, 3 ng/ml β-ecdysone; ●, 10 ng/ml β-ecdysone.

above 5 µg/ml (Figure 2A) (significant at 0.01 level, Students *t*-test). On the other hand, EDCF turns out to be stimulatory in cultures containing high concentrations of ecdysone: 0.2 µg/ml EDCF significantly stimulates cultures with 1 µg/ml α-ecdysone (Figure 2A). The morphology of cells treated with ecdysones was as described by COURGEON[11,12]. Cells inhibited by EDCF tend to increase in size but remain spherical. In cultures stimulated by EDCF to grow inspite of high concentrations of ecdysones, a large proportion of all cells are in aggregates and often spindleshaped.

To investigate whether α- and β-ecdysone act by independent mechanisms, cultures were set up with various mixtures of the 2 ecdysones, either alone or in combination with 2 µg/ml EDCF. The results presented in Figure 3 suggest a common site of action for the 2 ecdysones: If they are added to cultures in concentrations at which each of them alone gives its optimal growth stimulation, a growth inhibition is observed which again can be counteracted by EDCF. At no concentration tested, could one of the ecdysones stimulate a culture containing inhibitory concentrations of the other; there was always an accentuation of inhibition. On the other hand, maximal stimulation can be achieved by a mixture of the 2 ecdysones, in which each is present in lower concentrations than needed to give optimal stimulation by itself.

[11] A.-M. COURGEON, Nature New Biol. *238*, 250 (1972).
[12] A.-M. COURGEON, Expl Cell Res. *74*, 327 (1972).

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

E. HOSSEINI SHOKRAII and M. MORADI

Biology Department, Faculty of Science, University of Tehran, Tehran 14 (Iran), 12 February 1976.

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).