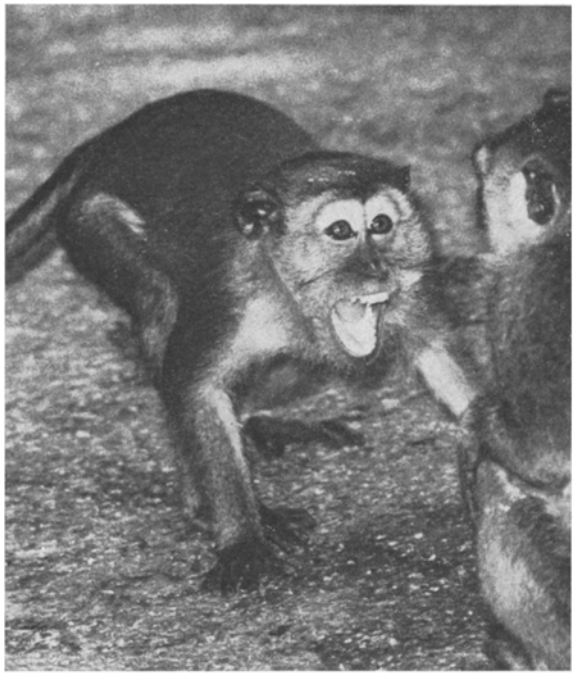

Fig. 2. Open-mouth face by adolescent female.

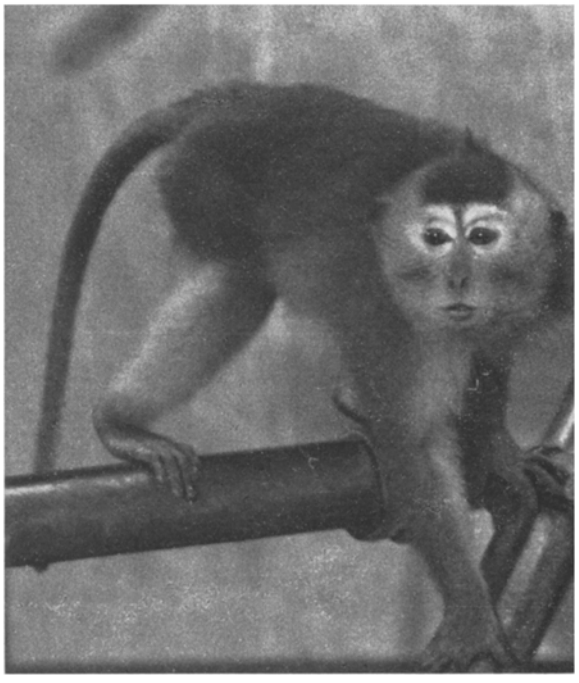

Fig. 3. Pointing face by juvenile male.

out of consideration.) Every pair of elements for which this q-value reached higher than $+2$ or lower than -2 (i.e. corresponding with a X^2-value of at least 4) received a connecting line on the positive (i.e. right) respectively negative (i.e. left) side in Figure 1. After this, the agonistic elements were arranged into subgroups in such a way as to maximize the number of positive linkages between elements belonging to the same subcategory and to minimize the number of negative linkages between these.

Results (II). This procedure resulted in 3 subcategories of agonistic behaviour: only 6 of the 101 positive linkages and all 14 negative linkages ran between elements belonging to different subcategories. The 3 clusters of elements, which have been rendered in Figure 1, clearly correspond with the subcategories previously indicated by us[4,5]: 1. straight-aggression (Figure 1, upper), 2. appeal-aggression and sub-directed behaviour (middle) and 3. fear-behaviour (lower).

It may finally be noted that the two types of aggressive behaviour which have been distinguished are easily recognizable as the facial expressions characterizing them differ strikingly: i.e. *open-mouth* (straight-aggression) and *pointing* (appeal-aggression); see Figures 2 and 3[10].

[10] Photographs made by H. VAN BEEK.

The Effect of Sulfhydryl Reagents upon the Activity of 40S Ribosomal Subunits

D. TIRYAKI[1], U. ÜÇER[2] and E. BERMEK[1,2]

Biofizik Kürsüsü, Tip Fakültesi, Istanbul Universitesi, Istanbul (Turkey); and Abteilung für Molekulare Genetik, Max-Planck-Institut für Experimentelle Medizin, Göttingen (German Federal Republic, BRD), 16 March 1976.

Summary. *p*-Chloromercuribenzoate inhibited the poly (U)-dependent binding of Phe-tRNA to the 40S ribosomal subunit but displayed no inhibitory effect on the binding of poly (U) to the ribosome. Other sulfhydryl reagents tested, like *N*-ethylmaleimide and iodoacetamide, did not affect the binding of Phe-tRNA to the small ribosomal subunit.

Mammalian ribosomes have been previously shown to possess sulfhydryl (SH-) groups essential for their activities in the coded binding of AA-tRNA[3-7] and in the EF II-dependent binding of GTP[7,8]. Experiments done with *N*-ethylmaleimide (NEM) and iodoacetamide (IAA) suggested that SH-groupes required for these activities must reside on the large ribosomal subunit[6-10]. 40S ribosomal subunits appeared to be resistent to inhibition by SH-reagents on account of the results obtained with NEM[6,7]. Considering the different modes of action of different SH-reagents, we decided to study the effect of some other SH-reagents as well, before excluding the presence of SH-groupes essential for the activity of the 40S particle.

Materials and methods. Ribosomes and ribosomal subunits were prepared from human tonsillar lymphatic tissue as described[5,6]. tRNA (*E. coli*) was obtained from Schwarz Bioresearch and charged with [^{3}H] Phe as described[11]. [^{3}H] poly(U), specific activity 10,4 Ci/mole, was obtained from Schwarz/Mann, NEM from Serva (Heidelberg), IAA and *p*-chloromercuribenzoate (*p*CMB) from Merck (Darmstadt). poly(U)- dependent (non-enzymatic)

binding of Phe-tRNA to 40S subunits was assayed as previously described[6]. [^{3}H] poly(U) binding to 40S or 80S ribosomes was assayed in 100 μl reaction mixtures for 15 min at 37 °C. 1,6 nCi [^{3}H] poly(U) was incubated with 0.2 A_{260} units ($\simeq$ 13 pmoles) 40 S or 0,6 A_{260} units ($\simeq$ 12 pmoles) 80S ribosomes in the salt medium of non-enzymatic binding of Phe-tRNA[5]. After incubation, reaction mixtures were washed onto nitrocellulose filters which had been preincubated with 0.1 *N* KOH for 5 min at 37 °C and then equilibrated in ice cold wash buffer[5]. The radioactivity bound to the filter was determined in 2 ml toluene containing 0.4% 2,5-diphenyl-oxazole in a Packard liquid scintillation counter (Tricarb).

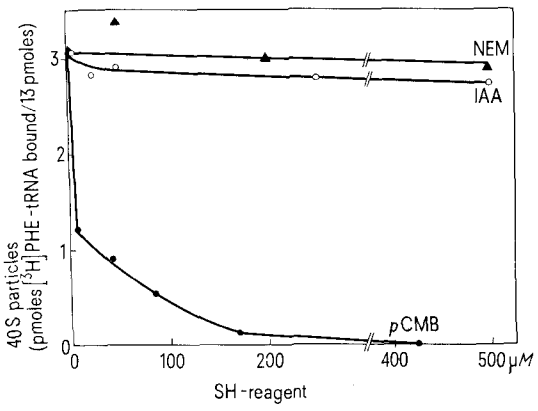

Fig. 1. Effect of concentration of different sulfhydrylreagents on the poly(U)-dependent binding of Phe-tRNA to the 40S particle.

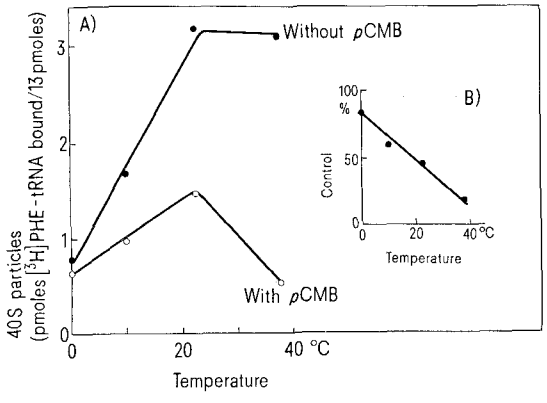

Fig. 2. A) Effect of different incubation temperatures on the *p*CMB-caused inhibition of Phe-tRNA binding to the 40S ribosomal subunit. B) Evaluation of the data from A. PLOT of the values obtained with *p*CMB as percent of the values obtained without *p*CMB.

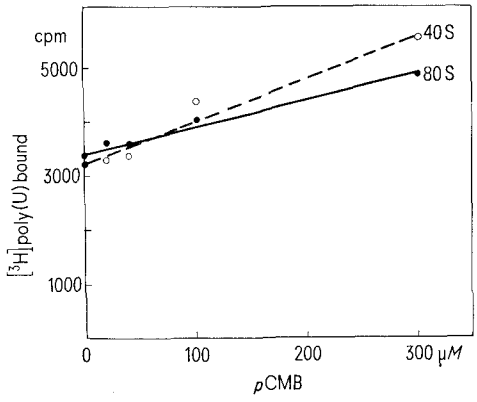

Fig. 3. Effect of *p*CMB concentration on the binding of [^{3}H] poly(U) to 40S or 80S ribosomes.

Results. Figure 1 shows the effect of different SH-reagents on the binding of Phe-tRNA to 40S particles. The binding of Phe-tRNA was very strongly inhibited by pCMB: 6 μM *p*CMB caused a 50% inhibition of the binding. On the other hand, the binding was not affected by NEM or IAA at concentrations as high as 500 μM. As shown in Figure 2, the inhibition amounted to 18% at 0 °C and to 82% at 37 °C. An almost proportional increase in the inhibition was observed with increasing incubation temperatures. Figure 3 shows that the observed inhibition of Phe-tRNA binding was not due to a primary inhibition of poly(U) binding to the 40S particle: even at concentrations as high as 300 μM, *p*CMB failed to exert an inhibitory effect on the binding of poly(U) to 40S or 80S ribosomes. Increasing concentrations of the reagent even stimulated the binding of poly(U) to 40S ribosomes by 40% and that to 80S ribosomes by 30%.

Discussion. The present report shows that 40S particles can be specifically inactivated by the sulfhydryl reagent *p*CMB. The finding that NEM and IAA fail to display a similar inhibitory effect attest to significant differences in the mode of reaction of these reagents. The results, furthermore, suggest that the inhibition of the binding of Phe-tRNA does not represent the result of the inhibition of poly(U) binding, as the binding of poly(U) to the 40S subunit is not inhibited by *p*CMB.

The *p*CMB-caused inhibition of the binding is temperature-dependent: the extent of inhibition increases in proportion to the elevation of incubation temperature. The inhibition observed at higher temperatures might be due to the modifications of the groups inaccessible to the reagent at low temperatures. These groups are likely to be involved in the mechanism of Phe-tRNA binding to the 40S particle. Alternatively, the inhibition might solely reflect the conformational changes and consequent destabilization of the *p*CMB-treated ribosomes at elevated temperatures. A similar temperature-dependent inactivation of 80S or 60S ribosomes by NEM has been previously observed[7,12]. 40S subunits incubated with 100 μM [^{14}C] NEM revealed 18 labelled groups[13]. That NEM fails to inhibit the binding reaction implicates that

[1] Biofizik Kürsüsü, Tip Fakültesi, Istanbul Üniversitesi, Istanbul, Turkey.
[2] Abteilung Molekulare Genetik, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Federal Republic of Germany.

[3] R. L. HEINTZ, H. C. MCALLISTER, R. ARLINGHAUS and R. S. SCHWEET, Cold Spring Harbor Symp. quant. Biol. *31*, 633 (1966).
[4] H. C. MCALLISTER and R. S. SCHWEET, J. molec. Biol. *34*, 519 (1968).
[5] E. BERMEK, W. KRÄMER, H. MÖNKEMEYER and H. MATTHAEI, Biochem. biophys. Res. Commun. *40*, 1311 (1970).
[6] E. BERMEK, H. MÖNKEMEYER and R. BERG, Biochem. biophys. Res. Commun. *45*, 1294 (1971).
[7] H. MÖNKEMEYER and E. BERMEK, Hoppe-Seyler's Z. physiol. Chem. *355*, 26 (1974).
[8] B. S. BALIGA and H. N. MUNRO, Nature New Biol. *233*, 257 (1971).
[9] T. HULTIN, Biochim. biophys. Acta *269*, 118 (1972).
[10] T. C. CHENG and H. C. MCALLISTER, J. molec. Biol. *78*, 123 (1973).
[11] H. MATTHAEI, G. HELLER, H. P. VOIGT, R. NETH, G. SCHÖCH and H. KÜBLER, in FEBS Symp. Genetic Elements Warsaw (Ed. D. SHUGAR; Polish Scientific Publishers Warsaw; Academic Press London 1967), p. 233.
[12] U. ÜÇER, H. MÖNKEMEYER and E. BERMEK, Biochim. biophys. Acta *402*, 206 (1975).
[13] R. D. NOLAN and P. TAILLEUR, Biochim. biophys. Acta *374*, 52 (1974).

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.

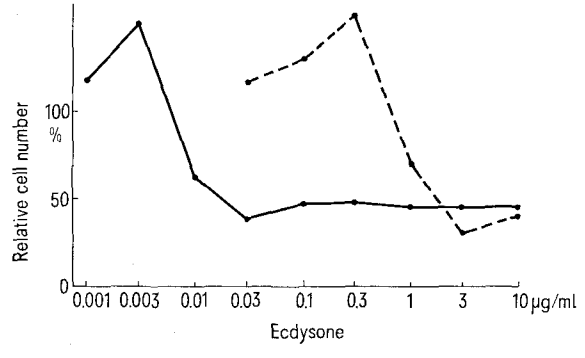

Fig. 1. Effects of ecdysone concentration on proliferation of KcC7 Drosophila cell line. 72-h growth response to α-ecdysone (●---●) and β-ecdysone (●—●) Ordinate: Relative cell number expressed in percents of control (=cultures without hormones). Abscissa: Concentration of ecdysone. Initial cell density 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).