monomagnesium complexes of nucleotides and of pyrophosphate are the true substrates for the enzyme[12]. Mg-ion concentration and the pH-value does not affect the apparent equilibrium constant since the acid dissociation and Mg stability constants of IMP have almost the same values as those of GMP[11]. Taking into account that temperature, pH-value, magnesium, and pyrophosphate concentration do not appreciably affect the apparent equilibrium constant of the reaction, this value could be employed to discuss the role of the HGPRT-catalyzed IMP-GMP exchange within living cells.

The HGPRT-catalyzed IMP-GMP exchange is the 1st reaction of the interconversion pathway of these nucleotides so far described[2-4] which does not appear to be irreversible. Through this reaction, the reversible conversion of IMP to GMP does not depend on the availability of ATP, NAD, NADPH, and glutamine. Intracellular concentrations of IMP and GMP have been determined in different cells and vary both with the type of cell considered and with the composition of the medium in which cells are living[13-18]. The concentrations of the 2 nucleotides appear to be in most instances of the same order of magnitude. Hypoxanthine concentration has been determined in many intra- and extracellular fluids[19-21]. The concentration of guanine appears to be very low[19] and almost unassayable[22]. Although the HGPRT-catalyzed IMP-GMP exchange is reversible, the equilibrium favors the formation of hypoxanthine and GMP from IMP and guanine. It is possible that this reaction has an important role in determining and/or maintaining the cellular concentration of IMP, GMP, and their respective purine bases.

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Effect of an ecdysteroid on DNA synthesis, DNA polymerase α and thymidine kinase activities of *Drosophila melanogaster* cells in vitro

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Summary. 10^{-7}M 20-OH-ecdysone treatment of a diploid *Drosophila* clone results in an inhibition of 60% of the DNA synthesis from 18 h of treatment on. After 48 h of hormone treatment the thymidine kinase activity is 70% inhibited; concomitantly a 60–70% lowering of the acid-soluble specific activity is observed. In the meantime the DNA polymerase α activity is reduced.

Cell proliferation and differentiation in vivo result in a complex balance between distinct but interrelated controls. Interactions between cells and diffusible factors such as hormones or nutrients are one of these controls. Despite numerous investigations, how and why cells start growing, or differentiate, are not cleary understood. In this perspective our purpose was to study the mechanism of action of an insect steroid hormone, 20-OH-ecdysone, on in vitro cultures of a clonal *Drosophila melanogaster* cell line. It has already been shown that ecdysteroid hormone treatment causes morphological transformation of *Drosophila* cell lines in vitro, and also agglutination[1], amplification of specific protein synthesis[2-4] and enzymatic induction[5,6]. The inhibition of cell proliferation of 20-OH-ecdysone treated *Drosophila* cell lines has been observed earlier[7,8].

To obtain further insight into the mechanism of inhibition of cell growth and cell proliferation by 20-OH-ecdysone we have studied the effect of this hormone on DNA synthesis and enzymes involved in DNA replication, such as DNA polymerase α and thymidine kinase, of a *Drosophila melanogaster* diploid cell clone.

Material and methods. The diploid (XX haplo IV) 5284 clone derived from the Kc cell line[9] of *Drosophila melanogaster* was grown in D22 medium supplemented with 10% fetal calf serum, at 23 °C. The cells were incubated for various times with 20-hydroxyecdysone (Rhoto, Osaka, Japan) added 2 h after plating. Tritiated thymidine [(^{3}H) TdR] (23 Ci/mM) from the Radiochemical Centre (Amersham, England) was added to control and treated cells at a final concentration of 0.5 μCi/ml 1 h before the end of the incubation.

After labelling, cells were washed thrice in cold glutamate-glycine-phosphate buffer (pH 7, 360 mosm), scraped away and adjusted to 10^8 cells/ml with 10 mM Tris-HCl (pH 7.1). The nucleic acids were separated[10] after elimination of the acid-soluble fraction (0.2 N perchloric acid), lipid extraction at 0 °C[11] and alkaline hydrolysis (0.3 N NaOH 1 h at 60 °C). DNA and acid-soluble fraction of the various samples were measured after their phosphorus content[12]. The radioactivity incorporated into DNA and the acid-soluble fraction was counted on aliquots mixed with Lumagel (Lumac, Basel) in a liquid scintillation spectrometer (Intertechnique, Plaisir, France). The specific activity of DNA or acid-soluble fraction is expressed, after quenching correction, as dpm/µg DNA-phosphorus or dpm/µg acid-soluble phosphorus.

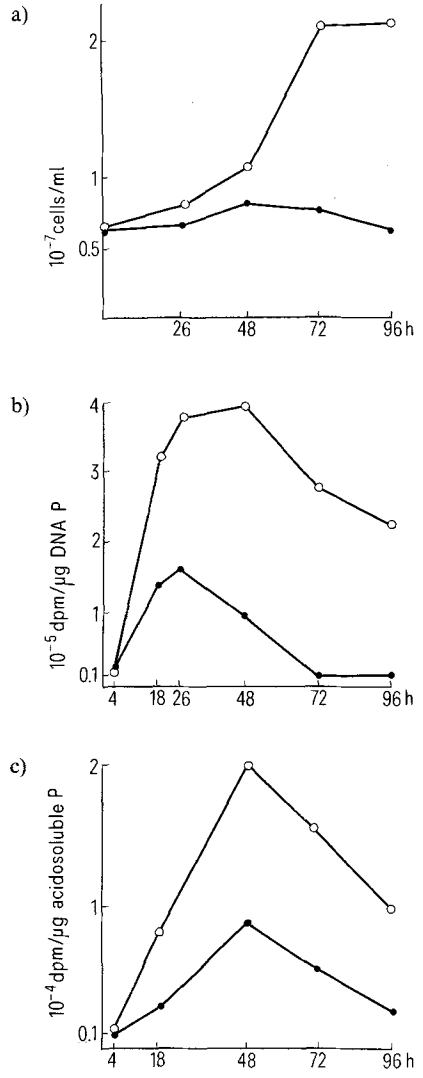

Figure 1.*a* Growth of *Drosophila melanogaster* 5284 diploid clone ●——● in the presence of 10^{-7}M 20-hydroxyecdysone added 2 h after plating of the cells or O——O in its absence at different times (h) after seeding the cells ($7 \cdot 10^6$ cells/ml). *b* DNA specific activity of *Drosophila melanogaster* 5284 diploid cone ●——● in the presence of 10^{-7}M 20-hydroxyecdysone or O——O in its absence at different times (h) after seeding the cells ($7 \cdot 10^6$ cells/ml). The DNA specific activity which measures DNA synthesis is expressed in dpm/µg DNA phosphorus as described in 'Material and methods'. *c* Acid-soluble specific activity of *Drosophila melanogaster* 5284 diploid cell clone ●——● in the presence of 10^{-7}M 20-hydroxyecdysone or O——O in its absence at different times (h) after seeding the cells. The acid-soluble specific activity is expressed in dpm/µg acid-soluble phosphorus as described in 'Material and methods'.

Results. Effect of 20-OH-ecdysone on DNA synthesis. If we consider the DNA synthesis of *Drosophila melanogaster* clone cells, the DNA specific activity of control cells, which measures the DNA synthesis of these cells, is maximum between days 1 and 2 (fig. 1, b), just before the clonal population enters exponential growth (fig. 1, a). The plateau phase is reached 72 h after plating when the cell population has multiplied 3.3 times and is thus in its 3rd or 4th cell cycle. After treatment with 20-OH-ecdysone the growth curve of the clone (fig. 1, a) reaches its maximum at 48 h and the population has multiplied only 0.2 times. DNA synthesis increases only slightly up to 26 h and is 60% inhibited from 18 h on (fig. 1, b).

As shown in figure 1, c, it is noticeable that the specific activity of the acid-soluble fraction of the control cells increases up to day 2 like the DNA specific activity (fig. 1, b). For the 20-OH-ecdysone treated cells we also observe up to day 2 an increase in the specific activity of the acid-soluble fraction. Nevertheless this specific activity remains from 18 h on after 20-OH-ecdysone addition, 60–70% lower than for the controls. These results and the following have been confirmed by experiments differing in the cell lines or in the incubation times with 20-OH-ecdysone.

Effect of 20-OH-ecdysone on DNA polymerase α and thymidine kinase. Figure 2, a represents the α polymerase activity of the cells cultivated in the presence or absence of 20-OH-ecdysone. In the control cells, as for the DNA synthesis previously observed (fig. 1, b), the α polymerase activity reaches a maximum of 14.9 units/10^8 cells on day 2; it then remains quite stable until day 4. A similar phenomenon is observed for the α polymerase of 20-OH-ecdysone treated cells with the restriction that the measured activities are

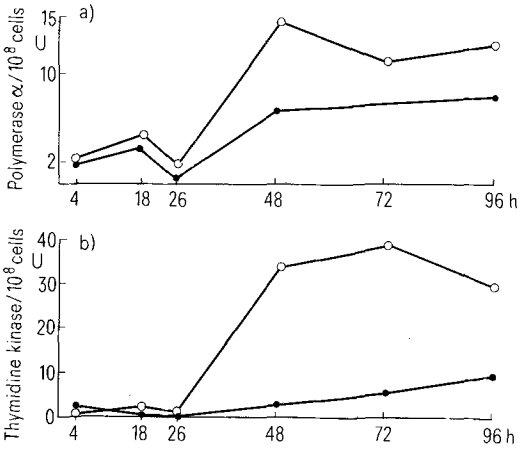

Figure 2.*a* DNA polymerase α activity of *Drosophila melanogaster* 5284 diploid clone ●——● in the presence of 10^{-7}M 20-hydroxyecdysone or O——O in its absence. The results are expressed in α polymerase units per 10^8 cells. The abscissa represents the time (h) after seeding the cells ($7 \cdot 10^6$ cells/ml). *b* Thymidine kinase activity of *Drosophila melanogaster* 5284 diploid clone ●——● in the presence of 10^{-7}M 20-hydroxyecdysone or O——O in its absence. The results are expressed in thymidine kinase units per 10^8 cells. The abscissa represents the time (h) after seeding the cells ($7 \cdot 10^6$ cells/ml).

respectively 76, 54 and 38% lower than in the control cells on days 1, 2 and 4.

For thymidine kinase (fig. 2,b), as for polymerase α, the control cells present an increasing activity especially between the 1st and the 3rd day. This activity equals 1 unit/10^8 cells at 4 h and reaches 39.6 units/10^8 cells on day 3. The thymidine kinase activity of the 20-OH-ecdysone treated cells, which is not detectable before 26 h of culture, increases slowly afterwards but remains at least 70% lower than in the control cells.

Discussion. In the present paper we have described the effect of 20-OH-ecdysone on the DNA synthesis and on the enzymatic activities of DNA polymerase α and thymidine kinase of a diploid clonal *Drosophila melanogaster* cell line.

The growth curve of the control clonal population shows a long lag phase followed by a very short growth phase (fig. 1,a). For the treated cells a growth inhibition is observed. An inhibition of growth initiation seems also to be involved; indeed, from 18 h on the DNA synthesis of the 20-OH-ecdysone treated cells is inhibited by 60% (fig. 1,b), An even earlier inhibition has been described for a Kc_0 *Drosophila* cell line[14]. Moreover our growth curves show that the treated cells do not go through their 1st cell cycle. This agrees with recent results showing an arrest of cell division in the G2 phase, 12 h after 20-OH-ecdysone treatment of *Drosophila* Kc_0 cells[15].

With respect to the major enzymes involved in DNA replication, DNA polymerase α[16,17] and thymidine kinase which catalyses the phosphorylation of thymidine to thymidine 5′-monophosphate[18], at 26 h of culture we measure low enzymatic activities while DNA synthesis is already high. Later on, up to 48 h the time course of the DNA synthesis in the control cells corresponds to the time course of the enzymatic variations. Afterwards, while the DNA synthesis declines, the DNA polymerase α and thymidine kinase activities remain high. Similar variations of DNA polymerase α activities occur in 20-OH-ecdysone treated cells, but these activities are at least 40% lower than in the control cells. Thymidine kinase activity remains 70% lower than in the control cells. These results suggest that TdR kinase, a component contributing to the value of DNA specific activity, and involving the salvage pathway, is unlikely to be the single limiting factor of DNA synthesis in our system as its activity remains very low at least up to 26 h while DNA synthesis takes place. It seems that de novo synthesis of pyrimidine nucleotides, involving important enzymes, like thymidilate synthetase, will be involved in the modifications of DNA synthesis observed. Salvage activities may also be affected as suggested by various authors, linking variations in TdR kinase activities and variations in DNA synthesis[19-21].

Besides, for the 20-OH-ecdysone treated cells, the specific activities of the acid-soluble fraction, which correspond in part to the (^{3}H) thymidine uptake across the cell membrane, are strongly inhibited (70%). It is to be noticed that the extent of thymidine uptake has been correlated in hamster cells with the level of thymidine kinase[22].

In conclusion, a lower acid-soluble specific activity, and a lower DNA polymerase α activity associated with a lowered DNA synthesis, 20-OH-ecdysone dependent, are compatible with an arrest in cell division which has been shown to take place in the G2 phase of the cell cycle[15].

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Glutamine synthetase activity during mouse brain development

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Summary. The specific activity of glutamine synthetase (GS) in mouse brain was 2-fold higher in the olfactory bulbs than in other regions. After birth, the specific activity of GS increased more rapidly in medulla oblongata and in olfactory bulbs, than in cerebral and cerebellar cortex. The activity of GS in primary cultures of brain hemispheres increased more slowly than in homogenates of whole brains. However, when astroblasts were treated in vitro with glucocorticoids or mouse brain extracts, GS activity reached 4 times the level measured in the homogenate of an adult mouse brain. We conclude that levels of GS activity may relate to the maturation of astrocytes, and propose that GS may be used as a marker of astrocytic maturation.

It is now well-estabished that glutamine synthetase (EC 6312) is implicated in the metabolism of glutamic acid, an excitatory transmitter. L-Glutamate ammonia ligase (ATP forming) also seems to play a role in the detoxification of brain ammonia. Its presence in glial cells has been demonstrated by immuno-histochemical procedures[1]. Using similar techniques, Norenberg has assigned the astrocyte glutamine synthetase (GS) location to the hippocam-