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Drosophila-Chinese hamster hybrid cells from cell lines adapted to grow in the same environmental conditions

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SUMMARY — Evidence is given concerning the possible production of *Drosophila melanogaster*-Chinese hamster hybrid cells, after PEG mediated fusion experiments between cell lines adapted to grow in the same environmental conditions: 31° C and a modified mammalian medium. However, autoradiograpy failed to detect viable complementation in *Drosophila*-Chinese hamster TK^- heterokaryons, and this is consistent with the failure to obtain proliferating hybrids in a semiselective HAT medium.

INTRODUCTION

Previous PEG-mediated interspecific fusion experiments with *Drosophila* and mammalian cells (human and rat) essentially demonstrated the formation of viable heterokaryons, with the presence of mitotic figures of both types of nuclei in fused heterophasic cells, and also the phenomenon of premature chromosome condensation (PCC) induced in *Drosophila* nuclei as well as, exceptionally, in mammalian nuclei (HALFER *et al.* 1987; HALFER and VALLANZASCA 1987). However, mitotic hybrid cells, containing chromosomes of both *Drosophila* and mammals, have not yet been observed. In fact, it should be borne in mind that, in interspecific cell fusion between such distant phyla, not only the physiological and genetic differences between the parental nuclei, but also a persistent mitotic asynchrony, due to the different nuclear sizes, and the considerable differences in the environmental conditions required for culturing mammalian and insect cells, may be responsible for the inability of heterokaryons to produce viable and proliferating hybrids. The present work was undertaken to ascertain whether viable synkaryons could be produced in a semiselective HAT medium i) employing a mammalian cell system with a lower chromosome number, ii) establishing environmental conditions which could permit the growth of both cellular types.

MATERIALS AND METHODS

Cell lines and culture techniques. — A diploid permanent epithelial Chinese hamster cell line: *B 14-150 TK*$^-$ — (Flow Laboratories) and a *Drosophila melanogaster* polyploid (*5n-6n*) embryonic cell line: *11 P 102* were used (HALFER *et al.* 1980; HALFER *et al.* 1987).

The Chinese hamster cell line was routinely cultured in 25 cm^2 Falcon flasks at 37° C in a CO_2 humidified incubator, in Mc Coy's 5A medium (Gibco Life Technologies Inc.) at pH 7.4, supplemented with 10% foetal bovine serum (Flow Laboratories), antibiotics (75 I.U./ml penicillin, 75 g/ml streptomycin) and 2 mM L-glutamine (Flow Laboratories). The *Drosophila* cells were maintained at 26° C in a normal atmosphere incubator, in D225 medium (ECHALIER and OHANESSIAN 1970) at pH 6.5, supplemented with 15% heat-inactivated bovine serum.

Adaptation of Drosophila and Chinese hamster cell line to a common environment.

As previously revealed, *Drosophila* and mammalian cells, co-cultivated at an intermediate temperature of 31° C and in mixed media, are capable only of a poor growth and for a limited span of time (HALFER *et al.* 1987; HALFER and VALLANZASCA 1987). Therefore, in order to promote an active and continuous proliferation of both cell types, and consequently to favour the development of hybrid cells, use was made, for PEG mediated cell fusion, of cell lines previously adapted to grow in a common environment and of two different culture media: 1) mixed media Mc Coy's 5A and D225 in different ratios, and 2) a modified Mc Coy's 5A medium.

A) *Adaptation of Drosophila cells to mixed media.* — *Drosophila* cells were gradually adapted to grow in mixed media containing Mc Coy's 5A and D225 in three different proportions: 1:4, 1:1, and 3:2 respectively, with a progressive decrement of the D225 medium.

Initially the cells were maintained at the optimal growth temperature of 26° C, and only after the cells had achieved a successful proliferation in each of the mixed media at 26° C was the temperature increased to 31° C. The mixed media were always supplemented wih 15% heat inactivated bovine serum and with the usual concentration of antibiotics and L-glutamine.

B) *Adaptation of Chinese hamster cells to mixed media.* — The Chinese hamster cells were also gradually adapted to mixed media in three different proportions of Mc Coy's 5A and D225: 4:1, 3:2, and 1:1 respectively, the amount of Mc Coy's 5A medium being progressively diminished. Here again, the mixed media were supplemented with 15% inactiveted foetal bovine serum and the usual concentration of antibiotics and L-glutamine. At first the cells were maintained at 37° C in mixed medium of Mc Coy's 5A and D225 in the ratio of 4:1 respectively. Only when the cells became able to grow in a 4:1 mixed medium, were they brought to 31° C and then in two successive steps, transferred to the other two mixed media (3:2 and 1:1).

C) *Adaptation of Drosophila and Chinese hamster cell lines to modified mammalian Mc Coy's 5A medium.* — Mammalian and insect cells require considerably different

environmental conditions in terms of temperature, pH and composition of culture medium. Neverteless, the main differences we considered related to: i) the presence in the mammalian media of sodium bicarbonate (2.2 g/l) as buffering system in an atmosphere of 5% CO_2, while the insect media are usually buffered with phosphates in a normal atmosphere; ii) the presence in D225 *Drosophila* medium of a notable amount of lactalbumin hydrolysate (15 g/l) and of inactivated foetal bovine serum. Hence, the modified mammalian medium devised for adapting both cell lines consisted of a complete Mc Coy's 5A medium with a low concentration of $NaHCO_3$ (0.35 g/l) to which 15 g/l of lactalbumin hydrolysate (Difco laboratories) and inactivated foetal bovine serum were added. Here again the adaptation of *Drosophila* cells was carried out first at 26° C and then at 31° C.

In the case of the Chinese hamster cells, the adaptation was accomplished starting with cells already adapted to grow at 31° C in 3:2 mixed medium Mc Coy's 5A and D225 respectively, as well as with cells growing in the standard medium at 37° C.

In all the media tested a range of variations of pH values (6,9-7.2) and of osmotic pressure (310-285) mOsm), proved to be suitable for a normal growth of both cell types.

Cell fusion method. — Fusion experiments were performed in monolayer in 25 cm^2 Falcon flasks, essentially according to the method described by DAVIDSON *et al.* (1976), using PEG Merk (p.m. 1000) and PEG Eastman Kodak (p.m. 400 and 1450). The Chinese hamster and *Drosophila* cells (adapted to grow in mixed media or in modified Mc Coy's 5A medium at 31° C), were mixed in a ratio of 1:24 (5×10^5 Chinese hamster and 12×10^6 *Drosophila* cells) 4-16 or 24h before fusion. Interspecific cell fusion was accomplished by exposure of the cells for 30'' to 1' to 50% PEG, or to 45% PEG supplemented with 10% dimethyl sulfoxide (DMSO) as described by NORWOOD *et al.* (1976).

Autoradiography. — In order to learn from the heterokaryons whether the *Drosophila* nuclei had produced a sufficient amount of thymidine-kinase to achieve a viable complementation, ^{3}HTdR (Amersham spe. act. 3 Ci/Mm) was added in the proportion of 0.5 μCi/ml of culture medium for 24h, 24 or 48h after the fusion. The slides were coated with Kodak stripping film, exposed for about 20 days, developed and fixed for subsequent autoradiographic detection of nuclear ^{3}HTdR in the cells.

Cytological preparations. — For the cytological preparations, the standard air drying technique was usually employed, after exposure of the cell culture to 0.05 μg/ml colcemid for 4h or 16h. The harvested cells were then treated with a hypotonic solution (1% sodium citrate or 0.56% KCl) for 5'-15', fixed in 3:1 absolute methanol and acetic acid for 30' and stained with acetic orcein for 30'. In order to improve both the yield and the detection of the fusion products, a modified fixation procedure, described by KORTHOF (1986) was adopted. This method, which consists in only a brief washing of the fused cells in the hypotonic solution and fixation with 10:1 mixture of methanol-acetic acid for 30', gives good chromosome spreading but prevents cell membrane disruption.

Selective system for hybrid cells. — In some cultures, 2-5 days after fusion, the medium was replaced by selective HAT medium supplemented with 15% bovine

serum. In the present case the system functioned as a half selective system. In fact, the Chinese hamster cells, because of their enzyme deficiency, cannot grow in HAT medium, while the *Drosophila* cells, which are unable to utilize exogenous hypoxanthine because of the complete absence of HGPRT enzyme (BECKER 1874), represent the unmarked parent cells, and cannot grow owing to the presence of the non-inactivated bovine serum.

RESULTS

Adaptation of Drosophila cell line (11 P102) in mixed media Mc Coy's 5A and D225.

The mixed media containing Mc Coy's 5A and D225 in three different proportions: 1:4, 1:1, and 3:2 respectively, were capable of supporting the growth of the established *Drosophila* cell line 11 P102 at 26° C. The adaptation of the *Drosophila* cells in the 1:4 mixed medium appeared to be very rapid, whereas in the other two media the adaptation took 1-2 months. On the other hand, the *Drosophila* cells could not be maintained in the mixed medium with Mc Coy's 5A and D225 in the ratio of 4:1 respectively. When, finally, the temperature was raised to 31° C, the adapted cells exhibited a moderate growth, sometimes for only 5-6 passages.

Adaptation of Chinese hamster cell line B 14-150 in mixed Mc Coy's 5A and D225 media.

The Chinese hamster cells differed in behaviour from the *Drosophila* cells in adaptation to the mixed media. In fact, the cells incubated at first at37° C in 4:1 mixture of Mc Coy's 5A and D225 media respectively, immediately revealed little or no growth, with survival limited to a few days. However, in one of the several cultures maintained in the mixed medium for a period of 10 days, 3 clones appeared, from which it was possible to obtain a cell line capable of growing in the mixed medium 4:1.

Successively, such cells, at 31° C, revealed no growth but once again some clones were obtained which gave rise to a cell line capable of growing at 31° C and in mixed medium 4:1.

This cell line was then transferred to the mixed medium Mc Coy's 5A and D225 in the proportion of 3:2 respectively at 31° C. This time the cells succeeded in growing but the adaptation to this new cultural condition was slow and progressive. In fact, only after nine months did the cells begin to grow vigorously, showing a perfect adaptation to the mixed medium 3:2 at 31° C.

Since the attempt to grow such cells in the mixed medium containing equal parts of Mc Coy's 5A and D225 met with little success, further experiments were abandoned.

Adaptation of Drosophila cell line to Mc Coy's 5A modified medium.

Drosophila cells maintained at 26° C in the modified Mc Coy's 5A medium immediately revealed a fairly good adaptation to this medium. However, at 31° C, the cells revealed a progressively less active proliferation.

Adaptation of Chinese hamster cell line to Mc Coy's 5A modified medium.

For this adaptation experiments, use was made of the original cell line as well as of the cell line adapted to grow in the mixed medium 3:2 at 31° C. The cells of the original cell line (cultured at 37° C) appeared sensitive to the lactalbumin hydrolysate and therefore did not survive in this modified medium. On the contrary, the cells adapted to mixed medium (3:2) and incubated at 31° C, showed a good and immediate adaptation to the modified mammalian medium.

Fusion experiments between Chinese hamster and Drosophila cells.

Interspecific PEG mediated cell fusion experiments were carried out between *Drosophila* and Chinese hamster cells in three different conditions: 1) between cells non-adapted to grow in the same environment, 2) between cells adapted to grow in mixed media, 3) between cells adapted to grow in Mc Coy's 5A modified mammalian medium. In all these series of experiments, in order to determine the most suitable conditions for fusion of these cells in different cultural environments, various parameters were considered: the time of co-cultivation of the mixed cells before fusion, different PEGs, grades and concentrations in the solvent, presence of absence of DMSO supplement, and the duration of exposure to the fusogen. PEG treated cells were then either exposed to HAT medium for selection of hybrids or fixed and stained for cytological analysis.

1) *Interspecific cell fusion between cells non-adapted to grow in the same environment.*

Thirteen fusion experiments were carried out between *Drosophila* and Chinese hamster cell line grown in the standard conditions. The co-cultures were maintained for 4 or 16-24h in mixed medium Mc Coy's 5A and D225 in the proportion of 4:1 respectively at 31° C. After the fusion, ten of these co-cultures were used for detection in HAT medium of eventual hybrid colonies. In this case, the culture temperature was raised to 37° C in order to favour the Chinese hamster cells as host cells of *Drosophila* nuclei. On the other hand, three co-cultures were used for cytological preparations at 24h or 48h after cell fusion. Irrespective of the various parameters considered in different experiments, the preparations revealed, as usual, bi- and multinucleated heterokar-

yons (Fig. 1), the presence of mitotic figures at different stages of both types of nuclei in fused heterophasic cells, the mammalian nucleus prevalently in mitosis and the *Drosophila* nucleus in interphase (Fig. 2 a, b), and the phenomenon of premature chromosome condensation (PCC) induced in *Drosophila* nuclei (Fig. 3 a, b), but no hybrid cells were observed.

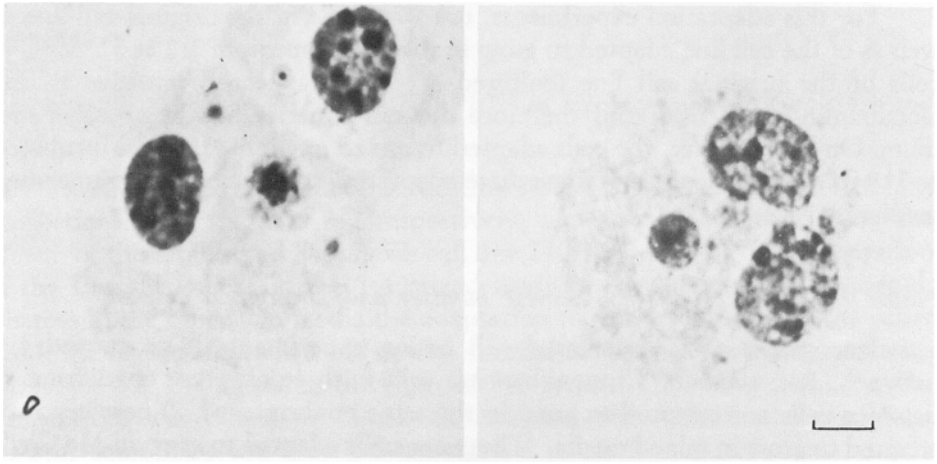
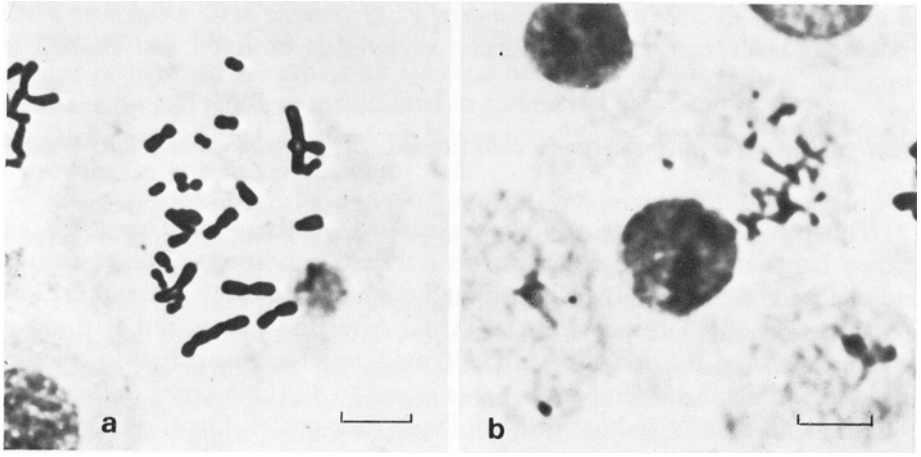
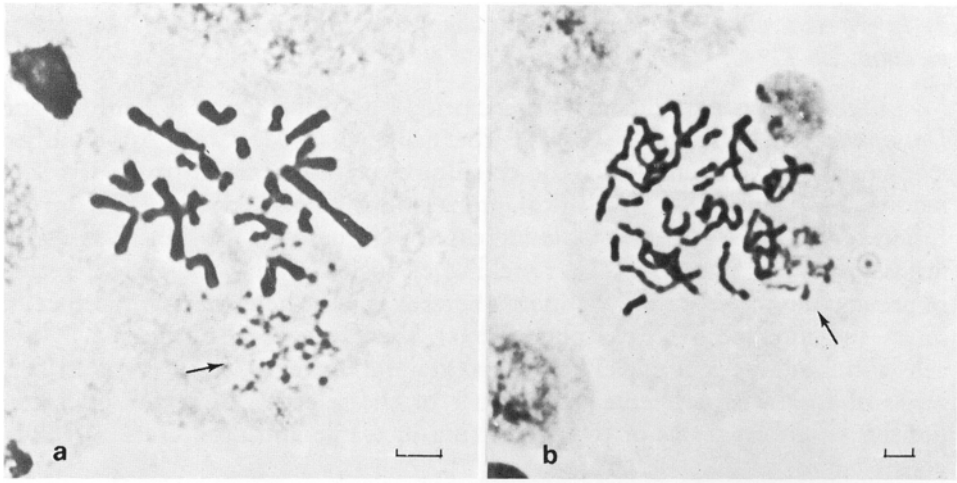


Fig. 1. — *Drosophila*-Chinese hamster heterokaryons. Orcein staining. Bar represents 10 μm.



Figs. 2 a, b. — Heterophasic heterokaryons. a) Chinese hamster mitotic chromosomes and *Drosophila* interphase nucleus. b) *Drosophila* mitotic chromosomes and Chinese hamster interphase nucleus. Bar represents 10 μm.



Figs. 3 a, b. — Premature chromosome condensation (PCC) of *Drosophila* nucleus (arrow) in heterophasic binucleate cells. a) S type PCC of *Drosophila*. b) G1 type of *Drosophila*. Bar represents 5 μm.

Likewise, in none of the cultures exposed to HAT medium did hybrid colonies develop.

2) *Interspecific cell fusion between cells adapted to grow in mixed media at 31° C.*

A series of 23 interspecific fusion experiments were carried out using cells adapted to grow in Mc Coy's 5A and D225 mixed medium in the proportion of 4:1 respectively, while another series of 83 experiments were made with cells adapted to grow in the 3:2 mixed medium, at 31° C. The co-cultures, before fusion, were maintained for 4-24h (only in some cases for some days) in the respective mixed medium. Here again, after the fusion, a part of the cultures was utilized for cytological analysis while another part was exposed to the selective HAT medium. In neither series of fusion experiments did the chromosome preparations reveal hybrid cells, and even the cultures exposed to HAT medium were always negative.

After the fusion between cells adapted to 4:1 mixed medium, the cytological preparations made at 48h after the fusion appeared to be of inferior quality and revealed a much more lower interspecific fusion efficiency. Even more negative results were obtained from interspecific fusion experiments between cells adapted to grow in 3:2 mixed medium. In fact, in most of the experiments, the cells already appeared to be badly damaged or completely disintegrated immediately after the fusion. For this reason it was not possible to utilize the cytological preparations for detection of fusion products.

3) *Interspecific cell fusion between cells adapted to grow in Mc Coy's 5A modified medium, at 31° C.*

Eight fusion experiments were carried out using Chinese hamster and *Drosophila* cells adapted to grow in the mammalian Mc Coy's 5A modified medium at 31° C. Here again, the co-cultures were performed from 4 to 24h before cell fusion. The cytological preparations examined 48-72h after cell fusion revealed, besides a notable increase of Chinese hamster and *Drosophila* mitoses and a significantly higher yield of all fusion products already detected in previous fusion experiments (homo-heterokaryons, heterophasic fused cells, intra- and interspecific PCC phenomena), the presence also of some hybrid cells with both types of nuclei in mitosis (Fig. 4). However, not even in this last series of fusion experiments did the use of the selective HAT medium give positive results in terms of the production of viable and proliferating synkaryons.

Analysis of the complementation between Drosophila cells (TK^+) and thymidine-kinase deficient Chinese hamster cells.

Autoradiographyc preparations of fused cells, incubated with ^{3}HTdR for 24h, at 24 or 48h after the fusion, were analysed in order to determine the ability of the two types of nuclei to synthetize DNA in heterokaryons. As shown in the Fig. 5, the presence of a uniform and heavy labelling of only *Drosophila* nuclei in the fusion products, as well as in the unfused cells,

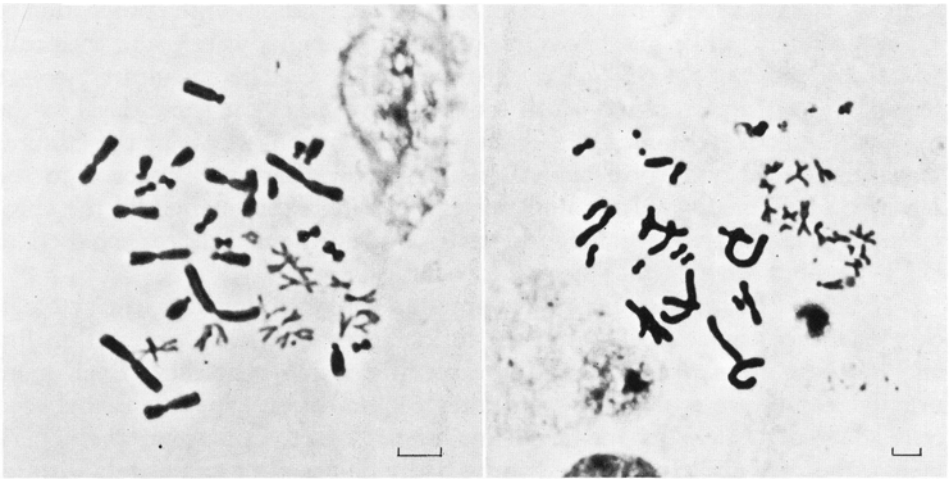
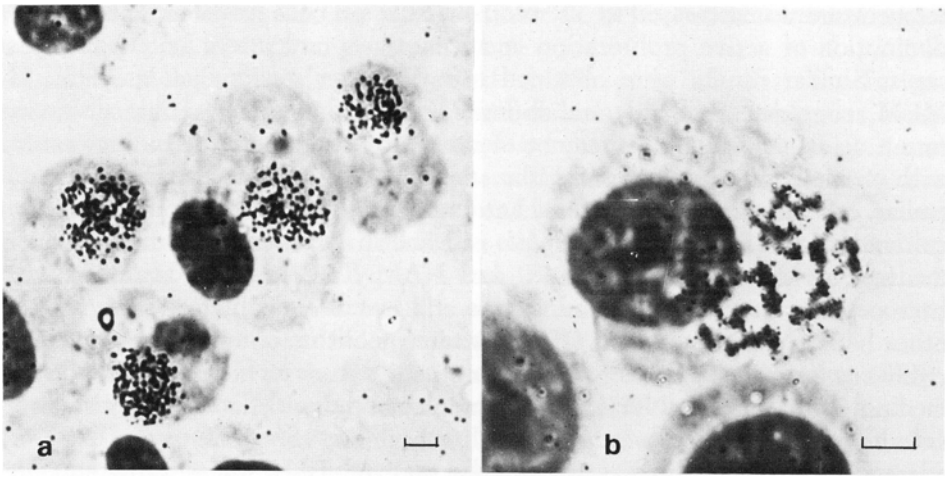


Fig. 4. — *Drosophila*-Chinese hamster hybrid cells, 64 h after cell fusion between cell lines adapted to grow in mammalian Mc Coy's 5A modified medium, at 31° C. Bar represents 5 μm.



Figs. 5 a, b. — Autoradiography of *Drosophila*-Chinese hamster fused cells incubated with ^{3}HTdR for 24h. Only the *Drosophila* nuclei appear heavily labelled. a) Heterocarions and *Drosophila* unfused cells. b) Heterophasic fused cells showing a Chinese hamster interphase nucleus and grains only over *Drosophila* metaphase chromosomes. Bar represents 5 μm.

provides evidence of absence of complementation and also of a metabolic cooperation between the *Drosophila* and Chinese hamster cells.

DISCUSSION

The present report relates to fusion experiments between a Chinese hamster cell line ($2n = 22$) and a polyploid *Drosophila* cell line ($5n = 19$), adapted to grow in the same environmental conditions, aiming to favour the development of hybrid cells. For this purpose, in view of some peculiar differences characteristic of mammalian and insect cultural media, essentially 2 cultural environments were devised to which to adapt both cell lines at an intermediate temperature of 31° C.

The media to which both cell lines were adapted were: i) mixed media Mc Coy's 5A and D225 in different ratios, and ii) a modified Mc Coy's 5A mammalian medium, additioned with lactalbumin hydrolysate and inactivated bovine serum. Both media were also designed for use in an open or closed system by the addition of a low concentration of $NaHCO_3$.

The *Drosophila* cell line, at 26° C, revealed a successful cultivation in the mixed medium Mc Coy's 5A and D225 up to the ratio of 3:2 respectively, in a relatively short period of 1-2 months, while in the mammalian Mc Coy's 5A modified medium, the adaptation was almost immediate. However, when the

temperature was increased to 31° C, these adapted cells revealed a progressive diminution of active proliferation and sometimes underwent only a few passages. Similar results were obtained using another mammalian medium: H-MEM, supplemented with lactalbumin hydrolysate and inactivated bovine serum. In this connection it may be of interest to note that our findings accord with previous reports concerning the adaptation of some insect lines to mammalian cell culture media. In fact, LENGYEL *et al.* (1975) report the successful cultivation of a Schneider *Drosophila* cell line in Dulbecco's modified Eagle's medium, as well as in HAM F 10 and HAM F 12 media, additioned with bactopeptone, non-essential aminoacids and lactalbumin hydrolysate. On the other hand, MCINTOSH *et al.* (1973), after 6 months, obtained adaptation of *Agallia constricta* established cell line to a 1:1 mixture of H-199 and Melnick's medium, both without $NaHCO_3$ and supplemented with lactalbumin hydrolysate and inactivated bovine serum. Furthermore, SPRADLING *et al.* (1975) adapted, over a period of several months, an established line of *Aedes albopictus* to MEM (Joklik-modified) medium, supplemented with non-essential aminoacids and lactalbumin hydrolysate, while CONOVER *et al.* (1971) succeeded in growing *Aedes aegypti* cells in equal parts of MEM and Grace's Insect Tissue Culture media at 26°-28° C.

The evidence presently available indicates that *Drosophila* and other insect cells are capable of growth in several different mammalian modified media, while adaptation to a widely different temperature is rather difficult.

As far as the Chinese hamster cells are concerned, only after a long period of about one year and after a gradual and progressive adaptation did we succeed in obtaining a cell line capable of growing first in the mixed Mc Coy's 5A and D225 medium at a ratio of 3:2 respectively, at 31° C, and then in the Mc Coy's 5A modified medium, whereas the original cell line revealed no growth in this medium. The karyotype analysis of the *Drosophila* cells adapted to grow in the mammalian modified medium, revealed, even after several months, the same chromosome complement, and the typical marker chromosomes. On the other hand, the chromosomes preparations of Chinese hamster cells adapted to the mixed medium (3:2) and to Mc Coy's 5A modified medium, revealed alteration of the genome as well as chromosomal rearrangements. Modal chromosome number, as determined from 50 metaphase counts, was 24 with a frequency of 60%, while about 40% of the cells revealed 25 or 23 chromosomes, and only a very small part of the cell population appeared diploid with 22 chromosomes.

Our findings, which for the first time concerned the adaptation of a mammalian cell line to an atypical cultural environment, thus relate to the co-cultivation of both *Drosophila* and Chinese hamster cell line in condition serving to improve the viability of the heterokaryons and to induce the development of hybrid cells. In fact, our best results were obtained from cell fusion experiments between *Drosophila* and Chinese hamster cells adapted to

grow in the modified Mc Coy's 5A medium at 31° C, where both cell types were capable of an active proliferation.

The cytological analysis performed at 48h and 72h after the fusion, revealed a high frequency of mitoses as well as homo- and heterokaryons, fused heterophasic cells with hamster or *Drosophila* mitotic chromosomes (Figs. 1 and 2), intra- and interspecific PCC phenomena (Fig. 3) and, for the first time, also some hybrid cells with both types of nuclei in mitosis (Fig. 4).

By contrast, interspecific cell fusion experiments between cells adapted to grow in mixed media Mc Coy's 5A and D225 (in the ratio of 4:1 and 3:2 respectively) at 31° C, produced the worst results in terms both of fusion efficency and of successful slide preparations. In fact, the cells appeared to be much more sensitive to the fusogen and, owing to a rapid lysis of the cells, quantitation of the fusion products was not feasible.

The failure to recover proliferating hybrids after the exposure of the fused cells to a semiselective HAT medium could be explained by the lack of a viable complementation in *Drosophila* and thymidine kinase-deficient Chinese hamster heterokaryons. In fact, analysis of autoradiographs revealed an active thymidine incorporation, in fused as well as in unfused cells, only in *Drosophila* nuclei, as shown in Fig. 5.

However, since this series of experiments did not give rise to giant multinucleated heterokaryons with a very high number of *Drosophila* nuclei, no evidence is provided concerning a possible dosage effect of the TK enzyme, as demonstrated for PCC induncing factors in *Drosophila*-rat heterokaryons (HALFER and VALLANZASCA 1987).

On the basis of the present observations it can be concluded that it is possible that a lower mammalian chromosome number, as well as the improved environmental conditions, capable of supporting a prolonged and active growth of both cell types, contributed to the formation of the first *Drosophila*-mammalian hybrid cells.

These findings, therefore, offer great encouragement for further studies serving to develop an efficient method for selection of the fusion products and to obtain proliferating mammalian-insect cell hybrids.

REFERENCES

BECKER J.L., 1974. — *Purine metabolism pathways in Drosophila cells grown «in vitro»: Phosphoribosyl transferase activities.* Biochimie, 56: 779-781.

DAVIDSON R.L., O'MALLEY K.A. and WHEELER T.B., 1976. — *Polyethylene glycol induced mammalian cell hybridization: effect of polyethylene glycol molecular weight and concentration.* Somatic Cell Gen., 2: 271-280.

ECHALIER G. and OHANESSIAN A., 1970. — *In vitro culture of Drosophila melanogaster embryonic cells.* In Vitro, 6: 162-172.

HALFER C., PRIVITERA E. and BARIGOZZI C., 1980. — *A study of spontaneous chromosome variations in seven cell lines derived from Drosophila melanogaster stock marked by translocations.* Chromosoma, 76: 201-218.

HALFER C. and VALLANZASCA C., 1987. — *Induction of premature chromosome condensation in Drosophila melanogaster-rat heterokaryons.* Chromosoma, 95: 216-222.

HALFER C., DEL PIN D. and DELL'ORO A., 1987. — *Fusion between human and Drosophila melanogaster cells induced by Polyethylene glycol.* Genetica, 75: 173-187.

KORTHOF G., 1986. — *An improved fixation method for chromosome preparation of Chinese hamster, Chinese hamster-human hybrid, and mouse cell lines.* Cytogenet. Cell Genet., 41: 181-184.

LENGYEL J., SPRADLING A. and PENMAN S., 1975. — *Method with insect cells in suspension culture. II. Drosophila melanogaster.* In: «Methods in cell biology», Vol. X, Prescott D.M. ed., pp. 195-208. Academic Press, New York.

MC INTOSH A.H., MARAMOROSH K. and RECHTORIS C., 1973. — *Adaptation of an insect cell line (Agallia constricta) in a mammalian cell culture medium.* In Vitro, 8: 375-378.

NORWOOD T.H. and ZEIGLER C.J., 1982. — *The use of dimenthyl sulfoxide in mammalian cell fusion.* In: «Techniques in somatic cell genetics», Shaw J.W. ed., pp. 35-45, Plenum Press, New York.

SPRADLING A., SINGER R.H., LENGYEL J. and PENMAN S., 1975. — *Method with insect cells in suspension culture. I. Aedes albopictus.* In: «Method in cell biology», Vol. X, Prescott D.M. ed., pp. 185-194, Academic Press, New York.

ZEPP H.D., CONOVER J.H., HIRSCHHORN K. and HODES H.L., 1971. — *Human-mosquito somatic hybrids induced by ultraviolet inactivated Sendai virus.* Nature, New Biol., 229: 119-121.

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