corresponding increase of the yield of isochromatid breaks (i). The opposite effect, i.e., an increase in the yield of chromatid translocations accompanied by a decrease in the yield of isochromatid breaks, was found after combined treatment with urea and X-rays. All results summarized in the Table are significant at the 0.1% level (χ-square test). Similar i:t ratio shifts have been observed for all recovery times tested (see Table) and also after prolonged urea pretreatments (6 and 8 h).

Three conclusions may be drawn from the present results: 1. Urea pre- or combination treatments remain without influence on the patterns of intrachromosomal distribution of chromatid aberrations induced by the clastogens mentioned above. 2. Urea exerts a differential clastogen-dependent (EA, MI, MH and ^{3}HT on the one hand, X-rays on the other) influence on the i:t ratios found after treatment with the clastogens: The quantitative change of the yield of one of the two aberration types (i or t) was found to be regularly accompanied by an opposite change of the frequency of the other type which completely compensated for the first. 3. In the case of treatment with EA, MI and ^{3}HT, urea is without effect on the total yield of induced chromatid aberrations. The yield of X-ray induced chromatid aberrations becomes increased with urea pretreatment; a decreased yield of MH-induced chromatid aberrations is obtained after urea pretreatment.

We are presently unable to explain these mutagen-specific effects of urea. Very similar results have, however, been obtained by GEBHART[10] after combined treatments of human lymphocytes with Trenimon and various amino acids. We hope that further experiments will provide a more detailed insight into the mechanisms underlying the differential, mutagen-specific modifications by urea of aberration yield and aberration spectrum.

[10] E. GEBHART, Humangenetik *18*, 237 (1973).

Stimulation of Growth by Insulin in *Drosophila* Embryonic Cells in vitro

GIULIANA MOSNA and C. BARIGOZZI

Istituto di Genetica dell'Università, Via Celoria 10, I-20133 Milano (Italy), 24 December 1975.

Summary. For obtaining a better yield of established lines of embryonic *Drosophila* cells, insulin proved to be a useful substance to be added to the culture medium. 10% of lines became established, showing a predominantly diploid chromosome number.

The establishment of cell lines of *Drosophila* in vitro is obviously an important step for the study of several problems of genetics such as cell differentiation and gene action; therefore the possibility of obtaining a sufficiently high proportion of cell lines in a relatively short time is of fundamental importance.

Sor far the establishment of lines from embryonic cells obtained in the same conditions as ours has been very irregular[1–5] and the number of lines obtained from primary cultures is still low. Moreover, it is still impossible to predict (even roughly) the time needed by primary cultures to start growing continuously and the frequency of continuous lines. To improve the production of continuous lines from primary cultures, we took advantage of a paper by SEECOF and DEWHURST[6], in which it is briefly mentioned that insulin added to the medium seems to facilitate the initiation of continuous cell lines. The aim of the present work was to increase this frequency by supplementing the medium with different doses of insulin. The experiment consisted in setting up 80 primary cultures from the wild stock Varese of *D. melanogaster* according to the method of growing cells in vitro of ECHALIER and OHANESSIAN[2]. Thus 20 primary cultures were set in a medium without insulin, and 60 cultures in a medium supplemented with insulin in the following doses: 1.85 mU/ml, 3.70 mU/ml, 7.4 mU/ml, each dose being tested on cultures.

Insulin was obtained from BDH (bovine, crystalline, 24.4 U/mg). The three doses were established with reference to the dose used by SEECOF and DEWHURST[6]. As cell lines we considered those primary cultures which required to be subcultured owing to the excessively high cell concentration. Within a fixed period (3 months), we counted the lines which required subculturing. Those which failed to show any growth at the end of the 3rd month were discarded. To date, the majority of the lines have undergone several passages.

To obtain preliminary information on the caryotype, slides of each line were prepared and conventionally stained with orcein. The experiment was performed twice.

The effect of insulin on growth of *D. melanogaster* embryonic cells in vitro

Insulin concentration (mU/ml)	No. primary cultures	No. continuous lines	Continuous lines (%)
1st experiment started in November 1974			
0.002[a]	20	1	5
1.85	20	5	25
3.70	20	–	–
7.40	20	2	10
2nd Experiment started in May 1975			
0.002[a]	20	–	–
1.85	20	2	10
3.70	20	2	10
7.40	20	2	10

[a] In unsupplemented medium traces of insulin may derive from fetal calf serum.

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[2] G. ECHALIER and A. OHANESSIAN, In Vitro *6*, 162 (1970).
[3] V. T. KAKPAKOV, V. A. GVOZDEV, T. P. PLATOVA and L. G. POLUKAROVA, Genetika, USSR *5*, 67 (1969).
[4] I. SCHNEIDER, Drosoph. Inf. Ser. *46*, 111 (1971).
[5] G. MOSNA and S. DOLFINI, Chromosoma *38*, 1 (1972).
[6] R. L. SEECOF and S. DEWHURST, Cell Different. *3*, 63 (1974).

The data provided by the 2 experiments are given separately in the Table, which clearly reveals a positive result. The homogeneity between the two sets of data is remarkable, as also the similar response to all 3 insulin doses. The number of cell lines obtained is $^{13}/_{120}$, i.e. 10.83%. The only cell line obtained withlout insulin (i.e. $^{1}/_{40}$ or 2.5%) corresponds to expectation, since it conforms to the results obtained by the authors already quoted. At the present date (end of October 1975), 9 lines are on their way to becoming established, i.e. to reach the conventional number of passages (70). 4 further lines are in an earlier stage. A preliminary survey to determine the caryotype of the lines obtained indicates that all of them are predominantly diploid. The individual chromosomes look normal or close to normality.

As a general conclusion it seems that insulin increases significantly continuous growth in primary cultures of embryonic cells of *Drosophila*. The action mechanism of insulin remains to be ascertained, as also the optimal dose. The lack of difference between the results of the 3 doses employed may signify that all were far above the minimum required for influencing cell growth.

Nuclear Projections in Tumour Cells and Large Chromosome Markers

G. L. CASTOLDI, G. D. GRUSOVIN, M. GUALANDI and G. L. SCAPOLI

Clinica Medica Generale, Università di Ferrara, I-44100-Ferrara (Italy), 24 December 1975.

Summary. The application of banding techniques on cytological smears from pleural effusion in a case of histiocytic sarcoma has provided direct evidence for correspondence between nuclear projections in tumor cells and extra large chromosome markers observed in the neoplastic karyotype obtained by direct preparations.

Several authors[1-4] have reported the presence of nuclear projections in the interphase nuclei of various tumours in association with large chromosome markers. These protrusions are readily observed in well flattened neoplastic cells of the chromosome preparations as well as in cytological smears, histological sections[5], and even in blocks prepared from neoplastic pleural effusions[6].The size of the chromosome markers and the nuclear volume appear to influence significantly the observation of the projections: in fact abnormally long chromosomes and diploid or hypodiploid sets seem to favour the detection of these nuclear extrusions[6].

In this connection we should like to add direct evidence that the nuclear projections do in fact correspond to the presence of extremely long chromosome markers.

Direct chromosome preparations from a pleural effusion in a patient affected by histiocytic sarcoma showed in 94% of the metaphases the presence of a pseudodiploid karyotype characterized by a giant submetacentric chromosome (Figure 1) whose length was approximately 2,5 times the length of the long arm of the A_1 chromosome. Banding techniques with quinacrine[7] and trypsin[8] demonstrated that this abnormal chromosome was constituted by a whole A_1 chromosome joined with a large

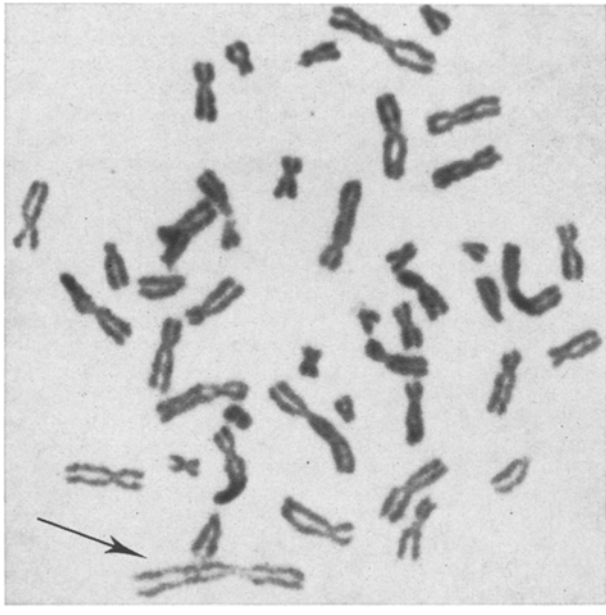

Fig. 1. Pseudodiploid metaphase showing a giant submetacentric chromosome marker (arrow). Direct preparation from pleural effusion.

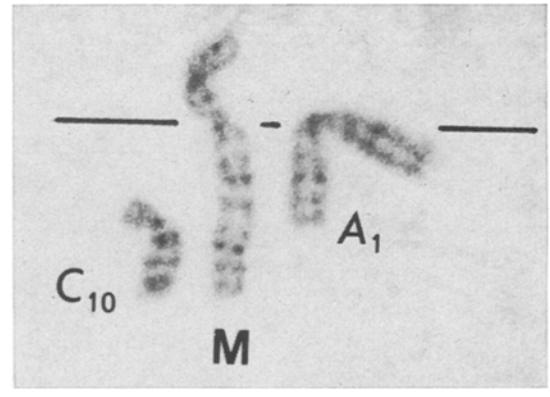

Fig. 2. Structural constitution of the marker chromosome demonstrating the involvement of a whole A_1 chromosome and a large segment of the q arm of a C_{10} chromosome (trypsin banding technique).