

females do not experience any sensory or motor deficiency in the light that disrupts their mating behavior. Previous studies in *D. melanogaster* report that both male and female pigment-deficient flies exhibit a deficit in their optomotor response. Therefore, pigment-deficient *Drosophila* females are most likely not affected by light in their mating behavior due to their passive role in courtship activity.⁵ The light dependent disruption in mating behavior of both white-eye *D. pseudoobscura* and *D. auraria* males is extreme when compared to the light-dependent disruption reported for *D. melanogaster*. Nevertheless, the inhibitory response shown by white-eye Arrowhead *D. pseudoobscura*

males in the light may also be relevant in previous studies using pigment-deficient *D. melanogaster* mutants. If so, the contrast perception deficiency exhibited by *D. melanogaster* mutants in the light might be due to a neurobehavioral disruption produced by faulty visual input.

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Insulin can completely replace serum in *Drosophila melanogaster* cell cultures in vitro

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Summary. D225 medium for *Drosophila* cell lines contains some chemically undefined compounds. An attempt was made to replace them with chemically defined substances. It was revealed that insulin can replace foetal calf serum in supporting *D. melanogaster* cell growth.

D225 medium² used for *Drosophila* cell lines contains some chemically undefined compounds: lactalbumin hydrolysate, yeast extract and foetal calf serum. Their presence in the medium causes difficulties in many physiological and genetical studies. Therefore efforts have been made to replace them with chemically defined substances. Lactalbumin hydrolysate supplies amino acids. Wyss and Bachmann³ found that 2 *D. melanogaster* lines were able to grow in D22 medium in which lactalbumin hydrolysate had been replaced by a synthetic amino acid mixture. Yeast extract is a source of B-complex vitamins and purines. Replacing this compound with either adenosine or inosine, Marunouchi and Miyake⁴ obtained growth of 2 lines of *D. melanogaster*. It is believed that the main role of serum, present in all cell culture media, is to provide hormones. In fact, when a mixture of hormones replaces serum in medium some mammalian cell lines grow at the same rate as in serum-supplemented medium^{5,6}.

This report describes an attempt to develop a completely synthetic medium, taking advantage of the findings of earlier works. 7 kinds of media were prepared (table 1): media 1,2 and 3 in which yeast extract (YE), lactalbumin hydrolysate (LH) and foetal calf serum (FCS) were replaced respectively by inosine, an amino acid mixture and insulin; media 4, 5 and 6 in which 2 of the 3 chemically undefined substances were replaced and medium 7 which was completely synthetic. Inosine was used at a concentration of 0.003 g/l. The amino acid mixture was the same as that used by Wyss and Bachmann in ZD medium³. Insulin crystalline bovine (BDH, approximately 25 IU/mg, Zn 0.33%, SO₃ 1.5%) was used at a concentration of 0.001 g/l. These media were tested on 4 *D. melanogaster* established cell lines of embryonic origin: 1XII, 0.57, 1.56, C1 82, the last kindly provided by Prof. G. Echalier, Paris. The others were obtained from the wild stock Varese of *D. melanogaster*.

Table 1. Culture media tested for their ability to support growth of *D. melanogaster* cell lines in vitro

Medium D225 ^a	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5	Medium 6	Medium 7
YE ^b 1.3 g/l	Inosine 0.003 g/l	YE ^b 1.3 g/l	YE ^b 1.3 g/l	Inosine 0.003 g/l	Inosine 0.003 g/l	YE ^b 1.3 g/l	Inosine 0.003 g/l
LH ^c 13 g/l	LH ^c 13 g/l	Aa mixture	LH ^c 13 g/l	Aa mixture	LH ^c 13 g/l	Aa mixture	Aa mixture
FCS ^d 15%	FCS ^d 15%	FCS ^d 15%	Insulin 0.001 g/l	FCS ^d 15%	Insulin 0.001 g/l	Insulin 0.001 g/l	Insulin 0.001 g/l

^a Standard medium used for *D. melanogaster* cell lines in vitro. ^b yeast extract; ^c lactalbumin hydrolysate; ^d foetal calf serum.

Table 2. Growth of the 4 *D. melanogaster* lines in the different media tested

Cell lines	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5	Medium 6	Medium 7
1XII	Standard	None	Standard	Slow	Slow	Slow	None
1.56	Standard	None	Standard	Slow	Slow	Slow	None
0.57	Standard	None	Standard	Slow	Slow	Slow	None
C182	Standard	None	Standard	Slow	Slow	Slow	None

*ter*⁷ according to the method of growing cells in vitro of Echalié and Ohanessian².

2 cultures were set up for each line, while 2 control cultures per line were maintained in D225 medium.

When inosine replaced YE (medium 1), growth rate was lower than in D225 medium for 2 or 3 passages. Afterwards it increased and was comparable to that in D225 medium. At present cells in medium 1 are at the 20th passage. Medium 2, in which an amino acid mixture replaced LH, could not support cell proliferation and cells stopped multiplying after 4 or 5 passages.

When using medium 3 (insulin replacing 15% FCS), cells grew as well as in medium D225. Cells in medium 3 are now at the 24th passage. Media 4, 5 and 6 caused a sudden reduction in growth rate in every line. In fact, the doubling time was almost trebled. Furthermore, subculturing of these cells required an inoculum size 4 times larger than the inoculum size in D225 medium. It is of note that inosine and insulin, when present in the same medium (medium 5) cause a considerable reduction in cell proliferation; on the contrary, when present in different media (medium 1 and 3) they enable cells to grow at the same rate as in D225 medium. Medium 7, completely synthetic, dramatically reduced the growth rate in all 4 lines and cells generally stopped multiplying after 2 passages.

No differences in the behaviour of the 4 lines were noticed when they were grown in the same medium (table 2).

In conclusion, the experiment failed to demonstrate the validity of a chemically defined medium: medium 7 was unable to support cell growth. Nevertheless, one very interesting result was achieved. The findings indicate that insulin can completely replace serum in *D. melanogaster* cell cultures in vitro. This is important for the following reasons: different batches of FCS stimulate cell growth differently and some of them may even be toxic to cells; the cost of FCS is very high and sometimes it is not available. The use of insulin instead of FCS will overcome these difficulties.

- 1 Acknowledgments. The author wishes to thank Prof. C. Barigozzi for critical reading of the manuscript and Mrs A. Bonifazio for her technical assistance. This work was supported by a grant of the Consiglio Nazionale delle Ricerche, Roma.
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Q-banding and A-T rich DNA in *Ornithogalum montanum* (Liliaceae)¹

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Summary. An A-T rich component was detected by CsCl-actinomycin D centrifugation in nuclear DNAs from three natural populations of *Ornithogalum montanum* (Liliaceae). No correlation was found between the Q-banding pattern and the amount of the A-T richest fractions of the genome.

Quinacrine (Q) bands are a common feature of the karyotype in animal species. In plants, although in several species no Q bands can be demonstrated², polymorphism for the banding pattern is frequent within species that do show Q bands³⁻⁵.

Considerable evidence indicates that a base composition of the chromosomal DNA with a preponderance of A-T pairs is the primary determinant of the brightly fluorescing Q⁺ bands⁶⁻⁸; other authors, however, put the emphasis on differential chromosome condensation⁹, on the effects of chromosomal proteins on quinacrine binding¹⁰ or on the pattern of interspersions of G-C between A-T pairs¹¹. In this context it would be of interest to know whether intraspecific variation for the Q-banding pattern is correlated with variation in the base composition of nuclear DNA. In the present study nuclear DNAs from 3 morphs of *Ornithogalum montanum* (Liliaceae) showing extensive variation in the number of Q⁺ bands per diploid genome were characterized by equilibrium centrifugation in CsCl and in CsCl-actinomycin D preparative gradients.

Materials and methods. *Ornithogalum montanum* bulbs were collected by Prof. P. Marchi (Institute of Botany, University of Rome, Italy) from 3 natural populations at 3 widely separated locations in South Italy, i.e. A) Pollino (North Calabria); B) Gargano (Apulia) and C) Aspromonte (South Calabria). As previously reported¹² the number of Q⁺ bands varies from 0 to 3 within population (A); from 11 to 18 within population (B) and from 20 to 25 within population (C). Q-banded karyotypes of the *Ornithogalum montanum* bulbs used for this study were obtained according to Vosa¹³ and kindly provided by Prof. P. Marchi and G. F.

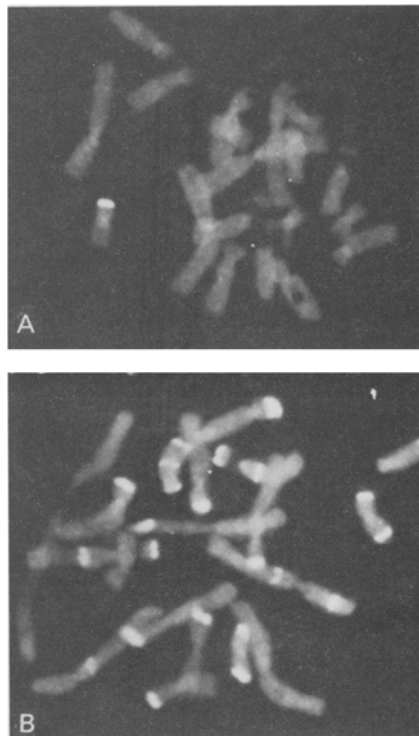


Fig. 1. Q-banded chromosomes from *Ornithogalum montanum* bulbs showing 1 (A) and 24 (B) Q⁺ bands.