Comparative Observations on the Behaviour *in vitro* of Cephalic Ganglia of Wild Homozygous and Heterozygous Drosophilae

During the last few years the progress made in the field of insect tissue cultures has made possible the application of this technique to problems of pharmacology, physiology and particularly of pathogenesis (AIZAWA and VAGO[1], TRAGER[2], MARAMOROSCH[3]).

As regards *Drosophila*, although so far only a long survival of organs and of cells spontaneously released from these has been obtained, it has already been possible to detect genotypical differences between wild stocks, as different responses to identical culturing conditions (CASTIGLIONI and REZZONICO RAIMONDI[4,5]).

In this note, the authors analyse the behaviour *in vitro* of larval cephalic ganglia belonging to wild inbred stocks Varese, Aspra, Santa Maria and to heterozygous genotypes Varese × Santa Maria and Varese × Aspra. The medium used was that of KURODA and TAMURA[6] (pH 7,2) and the cultures were set up according to the technique of CASTIGLIONI and REZZONICO RAIMONDI[4,5]. The explants (from third instar larvae) were cultured for 4 weeks and at the end of each week the ganglia were observed under phase contrast microscope and transferred into fresh medium. The residue drop (i.e. without ganglion) was stained with May-Grünwald Giemsa and the cells adhering to the coverslip were counted. At the end of the first and second weeks, some cultured ganglia belonging to the same homozygous and heterozygous genotypes were squashed and stained with acetolactic orcein of OSTER and BALABAN[7].

In comparing the homozygotes with the heterozygotes, three points were considered: (1) the general behaviour of ganglia during cultivation, (2) the conditions of squashes of cultivated ganglia and the presence of mitoses, (3) the number of cells released and adhering to the coverslip during the 4 weeks of culture.

(1) The heterozygous ganglia Varese × Santa Maria differ from both homozygous ganglia in their diffuse and greater disintegration; on the other hand, the genotype Varese × Aspra shows a behaviour similar to that of the Varese genotype.

(2) As regards the squashed ganglia, in the heterozygous Varese × Santa Maria it was noted that the staining was better than that of both parent stocks, whilst Varese × Aspra shows the same stainability as Varese. Scarce mitoses have been detected only in the heterozygotes. The degree of stainability is considered as a test for maintenance *in vitro* of same of the characteristics of living cells.

(3) The mean number of cells released and attaching to the coverslip for 4 weeks by Varese is very similar to that of heterozygous Varese × Aspra, whilst in Varese × Santa Maria it is higher than in the parent stocks (Table I).

The statistical analysis of the mean number of cells released from the homozygous stocks Varese, Aspra and Santa Maria, according to the Bartlett test[8], showed a significant difference between the stocks. Each heterozygous genotype was compared separately with both parent stocks (Table II). It results that Varese × Santa Maria differs from both parent stocks, whilst Varese × Aspra is closer to Varese.

We may conclude, therefore, that heterozygotes can stand the culture conditions better than inbreds.

To interpret genetically these results we propose the hypothesis that the stocks studied differ in the genic systems controlling the phenomena described above. So, in the case of heterozygous genotype Varese × Aspra, there seems to exist a dominance of one parental stock (Varese) over the other one (Aspra) for all characters considered, whilst in the case of the genotype Varese × Santa Maria the highest release of cells observed seems to prove an interaction between the parental stocks.

Given the high variability between ganglia cultures within the same homozygous stock, a decrease in variability of the heterozygotes was expected, owing to a higher buffering of the heterozygotes. This expectation was not confirmed by statistical analysis. Although at present the detection of genotypical differences in tissue cultures is still in the early stages, its possibilities are clear. By way of general comment, since only a very small number of mitoses occurred, our results may be interpreted as long survivals rather than as true cultures, but even so they show that long survival *in vitro* has a biological significance.

Finally, we wish to recall the results obtained by SANG[9] growing Drosophilae on synthetic media. In this case also the heterozygotes prove to use certain nutrients more efficiently than inbreds, as probably occurred in cultivated cells[10].

Table I. Mean number of cells attaching to the coverslip for 4 weeks

	No. of cultures	$\bar{x}$	s.e.
Varese	30	1157.26	247.77
Aspra	30	505.30	91.32
Santa Maria	30	649.20	174.48
Varese × Santa Maria	9	4509.67	1233.42
Varese × Aspra	8	1517.25	402.35

Table II. Analysis of variance between heterozygotes and homozygotes

Varese × Santa Maria and Varese	$F = 7.31$ (d.f. 8.29); $P < 0.001$
Varese × Santa Maria and Santa Maria	$F = 14.69$ (d.f. 8.29); $P < 0.001$
Varese and Varese × Aspra	$F = 1.42$ (d.f. 29.7); $P > 0.20$
Varese × Aspra and Aspra	$F = 5.71$ (d.f. 7.29); $P < 0.001$

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Riassunto. Gli autori analizzano il comportamento in cultura di gangli cefalici larvali di Drosofile appartenenti a 3 ceppi inbred, confrontandolo con quello dei 2 genotipi eterozigoti da questi ottenuti. Gli eterozigoti sembrano utilizzare meglio certe sostanze nutritizie del terreno, ed inoltre uno di essi rivela col suo comportamento in cultura, analizzato sotto diversi aspetti, la dominanza di un ceppo parentale, l'altro invece una interazione tra i genotipi parentali.

G. Rezzonico Raimondi,
C. Ghini, and S. Dolfini

Istituto di Genetica, Università di Milano (Italy), May 11, 1964.

Fucosidases du suc digestif d'*Helix pomatia*

Le suc digestif d'*Helix pomatia* est d'une très grande richesse enzymatique. Il contient, entre autres, plusieurs osidases, parmi lesquelles un enzyme qui hydrolyse le *o*-nitrophényl-β-D-galactoside[1]. Or, la β-galactosidase d'*Escherichia coli* possède également une action sur les β-fucosides[2]. Il a donc paru intéressant de rechercher, dans ce suc, la présence d'une β-D-fucosidase. De plus, la possibilité d'une activité α-L-fucosidasique a été envisagée.

Les substrats utilisés sont le *p*-nitrophényl-β-D-fucoside et le *p*-nitrophényl-α-L-fucoside. Les méthodes de dosage sont celles préconisées par Levvy et McAllan[3,4]. Les caractéristiques de la β-D-galactosidase et de la -N-acétyl-β-D-glucosaminidase, qui sont également rapportées, ont été déterminées sur le *o*-nitrophényl-β-D-galactoside et sur le *p*-nitrophényl-N-acétyl-β-D-glucosaminide. Les préparations enzymatiques sont des dilutions du suc effectuées extemporanément.

Le pH optimum de l'α-L-fucosidase (3,2) est nettement plus acide que ceux de la β-D-fucosidase (5,5) et de la N-acétyl-β-D-glucosaminidase (6,0), qui sont assez voisins.

Les activités spécifiques de ces enzymes d'*Helix pomatia* exprimées en μg de nitrophénol libéré par ml de suc, en 1 h, à 37°, sont les suivantes: β-D-fucosidase 2 500 000; α-L-fucosidase 1800; N-acétyl-β-D-glucosaminidase 65 000; β-D-galactosidase 1 500 000[1]. L'α-L-fucosidase est donc différente de la β-D-fucosidase, elle-même voisine, par l'importance de son activité et par son pH optimum, de la β-D-galactosidase (pH optimum 5,3)[1].

L'action de la température sur les activités a été également étudiée. L'α-L-fucosidase se distingue par une température optimum élevée (59°) (β-D-fucosidase 50°; β-D-galactosidase 46°; N-acétyl-β-D-glucosaminidase 48°). Ces résultats ont été complétés par l'étude de l'inactivation par la chaleur: le suc est incubé à diverses températures, au pH optimum de chaque activité enzymatique; les activités résiduelles sont comparées à celles du suc non traité. La β-D-fucosidase, conservant intégralement son activité pendant 1 h à 50°, se conduit comme un enzyme différent de la β-D-galactosidase et de la N-acétyl-β-D-glucosaminidase, qui perdent respectivement 50% et 80% de leur activité après 30 min d'incubation à la même température (Figure a). A 52°, la β-D-fucosidase perd 50% de son activité et la β-D-galactosidase 85%, après 20 min d'incubation (Figure b). Quant à l'α-L-fucosidase, il faut atteindre des températures supérieures à 60° pour commencer à l'inactiver (Figure c).

Le suc d'*Helix pomatia* possède donc deux activités fucosidasiques distinctes. La β-D-fucosidase est particulièrement active; elle l'est beaucoup plus que celle de *Patella vulgata* (2 500 000 μg de *p*-nitrophénol par ml de suc au lieu de 58 000 par g de tissu).

Les caractéristiques rapportées dans cette étude, semblent prouver que cet enzyme est différent de la β-D-galactosidase; Levvy et McAllan avaient déjà démontré que la β-D-fucosidase de *Patella vulgata* était distincte de la β-D-galactosidase de même origine[3].

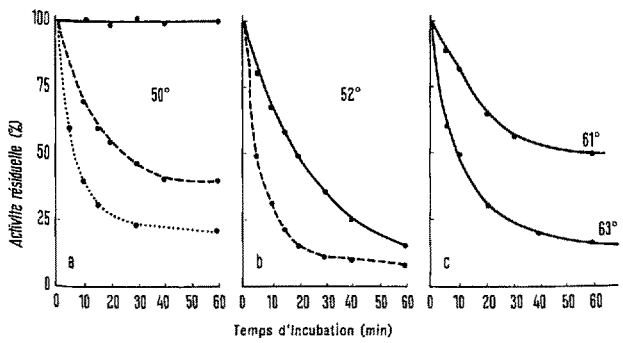
Un fractionnement du suc digestif d'*Helix pomatia* est en cours: la caractérisation des préparations purifiées et l'application à ces fractions de méthodes de discrimination, comme l'action d'inhibiteurs, devrait permettre de conclure avec certitude.

Enfin, l'α-L-fucosidase est peu active, mais elle se distingue sans ambiguité des autres osidases d'*Helix pomatia*[5].

Summary. The β-D-fucosidase and α-L-fucosidase activities of digestive juice of *Helix pomatia* have been studied. β-D-fucosidase can be separated from β-D-galactosidase by heat inactivation.

Annette Marnay, R. Got
et P. Jarrige

Laboratoire de Biochimie, Faculté de Médecine, Paris (France), le 2 mars 1964.



Activité résiduelle dans le suc digestif d'*Helix pomatia* après incubation durant des temps variables. a, 50°. ——β-D-fucosidase, pH 5,5; – – – β-D-galactosidase, pH 5,3; .... N-acétylglucosaminidase, pH 6. b, 52°. —— β-D-fucosidase, pH 5,5; – – – β-D-galactosidase, pH 5,3. c, —— α-L-fucosidase, pH 3,1; 61° et 63°.

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