Percentages (±SD) of ^{3}H-thymidine incorporation (Inc. ^{3}H), the mitotic index (MI) and the labeling index (LI) of the epidermis (Ep) and the mesenchyme (Me) from denervated blastemas with respect to the controls (blastemas with ganglia) for different culture times (T). An asterisk indicates that the difference between the denervated blastemas and the controls is significant ($p < 0.05$). In parentheses: number of cases

	T	Ep	Me
Inc. ^{3}H	72 h	(10) 102 ± 35	(10) 47* ± 40
	96 h	(9) 74 ± 33	(9) 48* ± 32
MI	96 h	(3) 136 ± 31	(4) 62* ± 21
LI	96 h	(3) 96 ± 31	(3) 89 ± 27

and fourth days of culture. Following the labeling period the blastemas were prepared for autoradiography. The total number of cells was scored. A minimum of 5000 cells were counted for each blastema. The labeling index was then calculated by finding the percentage of marked cells.

Results and discussion. Cell proliferation in denervated blastemas is expressed as a percentage of that in innervated blastemas for all three of the measurements (table): ^{3}H-thymidine incorporation (Inc ^{3}H), mitotic index (MI) and labeling index (LI).

The results show that after the 3rd day, denervation causes a decrease of ^{3}H-thymidine incorporation close to 50% for the mesenchyme. Similarly, the mitotic index of the mesenchyme drops 40% with denervation. Neither of these parameters has significantly changed in the epidermis.

The difference in labeling indices for both the mesenchyme and the epidermis between the denervated blastemas and blastemas associated with ganglia is not significant. After 4 days of culture, this index is close to 24% in both cases in the epidermis. Therefore, ¾ of the epidermal cells can be considered to be non-cycling. However, the labeling index of the mesenchyme is between 67% (denervated situation) and 75% (innervated situation). Only ¼ of the mesenchymal cells can, therefore, be considered non-cycling (figs. 1, 2, 3).

After 4 days of culture, in vitro denervation essentially affects the proliferation of mesenchymal cells. They show a decrease of 40% (mitotic index) to 50% (^{3}H-thymidine incorporation). The proliferation rate of the epidermis is not significantly influenced by the denervation under these conditions. However, the effects caused by the denervation procedure are weaker in vitro than in vivo. In vivo 4 days after denervation[1] there is a 70–75% diminution of mesenchymal cell proliferation, and the proliferation of the epidermal cells falls 50–60%. It is likely that the presence of a significant amount of insulin (0.035 U/ml) somewhat masks the effects of denervation in vitro, and explains the discrepancy between the results obtained in vivo and in vitro.

Nevertheless, the results obtained in vitro concerning the influence of denervation on the proliferation of mesenchymal cells and the relative independence of the epidermis agree with those observed in vivo[1,5].

We have shown[6] by cytophotometry that, in vivo, the diminution of proliferation which follows denervation results from an accumulation of blastemal cells in a cell cycle phase with a DNA content equal to the 2C amount. This behavior is due to both a lengthening of the G1 phase and an exiting from the cell cycle[2]. This anticipated exiting from the cell cycle is not provoked by denervation in vitro, as is shown by the nearly identical labeling indices for blastemas cultured with and without ganglia. The labeling index for the mesenchyme (67–75%) is about three times the one measured in the epidermis (24%). This difference in the percentage of cycling cells in the two tissues may account for the weaker sensitivity of the epidermis to denervation as seen in vivo[1].

Even though denervation in vitro does not provoke an exiting from the cell cycle by the regenerating cells, it is clear that the observed fall of the proliferation indices indicates a lengthening of the cycle time for mesenchymal cells. It remains to be determined whether the G1 phase is the affected phase in vitro as it is in vivo.

In vivo denervation causes lengthening of the G1 phase which then terminates in exiting from the cycle into G_{0-1} [review in Rothstein[7]].

It seems that in vitro only the first part of this phenomenon, the lengthening of G1, takes place without the cells exiting from the cycle. Perhaps the culture conditions (addition of a growth factor like insulin) and/or the short duration of the culture (4 days) do not permit the cells to reach an exiting from the cell cycle as seen after denervation under in vivo conditions.

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H-Y antigen expression in heterogametic males (XY) and females (ZW): a factor in reproductive strategy?

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Summary. The cells of heterogametic females with ZW sex chromosomes express H-Y or H-W antigen. A hypothesis is formulated to explain why these animals are capable of 'practicing' *amphigonia retardata*, i.e., delay in actual fertilization of eggs by retaining viable sperm within the oviduct for a considerable time (several months).

Key words. H-Y antigen; H-W antigen; heterogametic females (ZW); amphigonia retardata.

Cells from males but not females of all mammalian species tested, including human, have been shown, directly or indirectly, to express H-Y or a cross-reactive cell surface antigen[1,2]. Apparently H-Y antigen[3] is evolutionarily highly conserved and the sex-specific antigen is found in the cells of the heterogametic sex of all vertebrate species[1–4]. It has been proposed that H-Y antigen directs the initially indifferent embryonic gonad to become a testis in male heterogametic species such as the mouse and an ovary in female heterogametic species such as the chicken[5–7]. In chickens, the female-specific histocompatibility locus coded antigen, H-W, which cross-reacts with H-Y is present on cells from female members of the species[8–10]. Female cells from other

oviparous and viviparous animals with ZW sex chromosomes such as snakes and reptiles are expected to have the H-W antigen expressed on their cell surface.

In viviparous animals, many reproductive tissues, such as early embryos and most placental trophoblast cells, do not express major histocompatibility complex (MHC) antigens probably because foreign paternal MHC antigens are potential elicitors of the immunologic rejection of embryonic tissues in the maternal organism[11]. Similarly, lack of expression of such antigens by sperm may also help sperm to escape immunologic destruction in genetically disparate hosts[12].

In contrast, sperm from heterogametic males (XY) express H-Y antigen and are killed by H-Y antibodies produced by male-sensitized female mice[13,14]. This potential vulnerability is reflected in part by the fact that fertilization takes place only within days (6–144 h[15]) of mating in mammals. This compromise may be a weighted advantage if we consider that males are generally more accessible in social animals, such as mammals, in general.

In lower vertebrates, the lack of expression of H-W antigen in homogametic (ZZ) males and the presence of the antigen in heterogametic females (ZW) may be one of the means which facilitate sperm storage thus rendering the female partially independent of the male sexual cycle or availability. For example, females of numerous birds and reptiles, all of which are of ZW heterogametic sex chromosome type, and of snakes, many of which are ZW, are known to retain viable sperm within the oviduct for a considerable time before actual fertilization of the egg. This delay in actual fertilization of eggs, as distinct from the mating process, was termed 'amphigonia retardata' by Kopstein in 1938[16]. Mating occurs in snakes several months before ovulation and live sperm have been recovered from the uterus after 3–8 months[16,17] and young were reported to be born from isolated females of Pope's pit viper, *Trimeresurus popeorum*, at least a year after mating[18]. In turtles, a high percentage of fertile eggs have been shown to be obtained from female *Malaclemys* for 2 years without annual copulation, although fertility levels tend to fall later[19]. In birds, live sperm were found in oviducts for many weeks and fertile eggs were obtained for 30–32 days after removal of the male[20,21]. Rahn[22] suggested that the survival of sperm in the uterus might be a safety factor enabling eggs to be fertilized in the absence of males at the time of ovulation or when ecologic conditions in some way prevent preovulatory mating[23].

The presence of H-W antigen in females rather than males of heterogametic ZW animals such as snakes, reptiles, and birds suggests an immunologic basis of reproduction without constant availability of males in these desert dwelling, 'non-social', or polygamic animals.

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Dominance for enzyme activity in *Drosophila melanogaster*

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Summary. A regulatory element tightly linked to the *Gpdh* locus in *Drosophila melanogaster* has been isolated from a natural population. Flies homozygous for second chromosomes bearing the element, *H31*, have half the GPDH activity of normal homozygotes. Heterozygotes between *H31* and *F* or *S* alleles exhibit dominance in GPDH activity. Heterozygotes between *H31*, *F* or *S* and *Df(2L)GdhA* have half the diploid level. The contribution of the *S* allele to the activity in *S/H31* heterozygotes is more than four times that of *H31*. The regulatory element distinguishing *H31* is tightly linked to the *Gpdh*$^+$ locus.
Key words. Drosophila; enzyme; sn-glycerol-3-phosphate; dehydrogenase; dominance; *trans*, regulation.

Heterozygotes for allozyme variants that differ in the amount of enzyme protein they produce generally have an intermediate level of enzyme activity. The activity variation is additive and the expression of the alleles is co-dominant[1,2]. Preferential expression of one allele over another, giving rise to non-additivity in enzyme levels, has been reported in a number of eukaryotes, usually in interspecific hybrids[3–5], but also within species[6–8]. In *D. melanogaster* examples of differential gene expression have been found to be due to *cis* acting regulatory elements[8,9], although both *cis* and *trans* acting modifiers of enzyme levels have been described[10–12]. We describe here a *trans* acting element affecting the steady state level of soluble sn-glycerol-3-phosphate dehydrogenase (GPDH; NAD$^+$ oxidoreductase; EC 1.1.1.8) in *D. melanogaster* which, in heterozygotes, results in dominance in GPDH activity.

The second chromosome bearing the variant was discovered in a survey of GPDH activity in a Huonville, Tasmanian population of *D. melanogaster*. This chromosome, designated *H31*, was