enzymes in adults are higher than in larvae. Sex of larvae has little if any significant influence on enzyme activities whereas male adults consistently maintain higher enzyme activities as compared to females. Armstrong et al.[9] also noted sex dimorphism in activity of PER in adult flies with the greatest difference being observed in the 21–26 days age group.

We examined the sensitivity of developing *Drosophila* to H_2O_2 and AT (a noncompetitive inhibitor of CAT resulting in the irreversible destruction of CAT without interferring with the rate of new synthesis). Results of these studies are presented in figure 2. In each case (1.5%, 3% H_2O_2 1%, 0.5% AT) younger organisms are more sensitive to H_2O_2 or AT than older ones. These results are similar to those of Lubinsky and Bewley[16] who found 1st instar larvae most sensitive to AT (LD_{50} of 0.65 mM) and adults capable of injesting a solution of up to 10 mM for 8 days. The sensitivity pattern for H_2O_2 or AT does not follow that of O_2[7] perhaps reflecting different modes of toxicity. In addition, survivors of the H_2O_2 or AT treatment were free of the developmental abnormalities noted for the eyes of O_2-treated pupae. Because our preliminary studies (unpublished) included a range of H_2O_2 concentrations up to 30% in which no eye abnormalities were observed, we conclude that elevated levels of O_2 specifically alter eye pigmentation in *Drosophila.* Attempts to subject developing *Drosophila* to elevated levels of the superoxide anion failed due to toxicity of components of the generating system.

The relationship of SOD, CAT, and PER to O_2-provoked toxicity is uncertain. The results reported here show that increased sensitivity to O_2 and H_2O_2 is greatest when activities of SOD, CAT, and PER are relatively low. However, in our study and others[9], adult males have significantly higher levels of these 3 enzymes per mg protein than females, yet Kloek[5] found adult males more susceptable than females to elevated O_2. Age related changes in O_2 sensitivity and activity levels of SOD, CAT, and PER have been observed in other adult systems[5,6] and are suspected as being related to natural senescence and aging phenomena[17].

1 Supported by a Hewlett Foundation grant of Research Corporation.
2 Halliwell, B., Cell Biol. int. Rep. *2* (1978) 113.
3 Fee, J.A., in: Metal ion activation of dioxygen. Ed. T.G. Spico. Wiley, New York 1980.
4 Koppenol, W.H., Bull. eur. Physiopath. Resp. *17* (1981) 85.
5 Kloek, G.P., Ralin, D.B., and Ridgel, G.C., Aviat. Space envir. Med. *47* (1976) 272.
6 Hoffmann, M., Stevens, J.B., and Antor, A.P., Toxicology *16* (1980) 215.
7 Nickla, H., Fugiwara, D., and Fried, R., Experientia *38* (1982) 114.
8 Lindsley, D., and Grell, E., Genetic variations of *Drosophila melanogaster.* Carnegie Inst. Wash. Publ. No. 627, Washington, D.C. 1968.
9 Armstrong, D., Rinehart, R., Dixon, L., and Reigh, D., Age *1* (1978) 8.
10 Luck, H., in: Methods of enzymatic analysis. Ed. H. Bergemeyer. Academic Press, New York 1965.
11 Rapp, U., Adams, W.C., and Miller, R.W., Can. J. Biochem. *51* (1973) 158.
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
13 Massie, H.R., Aiello, V.R., and Williams, T.R., Mech. Ageing Dev. *12* (1980) 279.
14 Fernandez-Sousa, J., and Michelson, A., Biochem. biophys. Res. Commun. *73* (1976) 217.
15 Baird, M.B., Samis, H.V., and Massie, H.R., Drosoph. Inf. Serv. *47* (1971) 81.
16 Lubinsky, S., and Bewley, G.C., Genetics *91* (1979) 723.
17 Harman, D., Age *3* (1980) 64.

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Evidence for post-zygotic lag in *Chlamydomonas moewusii* (Chlorophyta; Volvocales)

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Summary. A wild-type characteristic may be transmitted through heterozygotes and manifest itself initially in haploid mutant progeny. Evidence for this is adduced from experiments with a paralyzed pf^- mutant of *Chlamydomonas.*

Although ordinarily the phenotype of cells, and hence of tissues, organs, plants or animals, is an expression of their nuclear genotype, this is not always so. A newly mutated gene, for instance, does not immediately manifest its presence: if it arose in a wild-type cell, then for a while – often for several cell generations – the phenotypic effects of its antecedent wild-type allele continue to be manifest. This phenomenon, as observed in various micro-organisms[2–4], has been called phenomic lag.

Another circumstance where phenotype may not immediately reflect genotype is in cells immediately following meiosis. A heterozygous diploid cell, with the phenotype of a specified dominant gene, typically cleaves into 4 meiotic products of which 2 bear nuclei with the dominant allele and 2 bear nuclei with the recessive allele; but all 4 inherit from the zygote cytoplasmic factors which had been produced by the dominant gene. In microbial genetics the phenotype is not usually examined until after several post-meiotic divisions have given rise to colonies or clones, by which time such residual products of parental dominant genes have been diluted out or otherwise caused to disappear. However, in some cases even individual post-meiotic cells can be seen to manifest certain genetic characteristics. This is true, for instance, for motility of algal flagellates such as *Chlamydomonas.*

We present here evidence for what we propose to call post-zygotic lag, in which certain vegetative (haploid) cells of *C. moewusii* known to carry a gene for flagellar paralysis (pf^-) nevertheless can swim normally. Germinating zygotes from a cross $mt^+ \cdot pf^-$ (mutant strain M.1002)[5] $\times$ $mt^- \cdot pf^+$ (wild type) give rise initially to 4 haploid cells which can be grown to produce sub-cultures of 2 kinds, with cells respectively motile (pf^+) and paralyzed (pf^-), in equal numbers (2:2 segregation). However examination of the behavior of those first 4 meiotic products reveals that they all can swim normally. One simply immerses germinating zygotes singly

Motility of genetically pf^- cells, 1 to 3 mitoses after meiosis of $pf^+ \cdot pf^-$ heterozygotes

Zygote	Meiotic progeny clone			
	a	b	c	d
A	8/8	6/8	3/4	0/4
B	4/4	4/4	2/2	1/2
C	4/4	3/4	2/2	2/2
D	3/4	3/4	1/1	1/1
E	2/4	2/2	1/1	0/1
F	9/8+?	8/8	6/8	5/8
G	8/8	5/8	4/4	2/4
H	7/8	7/8	2/2	2/2

a and b clones presumably pf^+, since the cells had divided faster than c and d clones - presumably pf^-. Swimming cells (counted)/total cells (estimated; in water droplets under oil it was hard to distinguish immotile cells from dust or oil droplets).

in small droplets of water or nutrient medium under mineral oil on non-wettable glass slides, keeps them in a moist chamber, and watches them under a magnification of $\times 100$ to see how many of the emergent cells swim. If allowed to undergo 2 or 3 post-meiotic mitoses, each of the 4 gives rise to 4 or 8 cells all still capable of swimming. (Data from one such cross are shown in the table). It is only after several divisions that the paralysis phenotype becomes manifest in 2 out of the 4 original meiotic progeny clones, presumably because in these cells, genetically pf^-, cytoplasmic factors required for normal motility have been degraded or diluted to a level, concentration or number below the lower threshold required for motility. Whether these postulated factors are immediate gene products, perhaps messenger RNA (mRNA), or some other kind of metabolite remains to be established. We know only that they must be relatively long-lived, since they demonstrably persist in pf^- cells for several days.

Half-life durations of mRNA molecules in the bacterium *Escherichia coli* have been estimated to range between 0.5 and 8 min (at 37 °C)[6]; in the slime mould *Dictyostelium discoideum*, around 4 h[7]; and in cultured cells of the mouse *(Mus musculus)* around 10 h[8]. If wild-type mRNA is responsible for the observed post-zygotic lag, then half-life values for *Chlamydomonas* mRNA could be even longer. And if so, then in mRNA preparations from wild-type cells of this alga one might be able to detect a component which is absent from a paralyzed mutant, and thereby obtain a clue to cytoplasmic factors essential for flagellar motility.

Similar observations have been reported in another algal flagellate, the non-photosynthetic dinoflagellate *Crypthecodinium cohnii*, by Beam et al.[9]. They likewise observed motility in cells known, from the behavior of their subcultured progeny, to bear genes for genetic paralysis.

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2 Tatum, E.L., Cold Spring Harb. Symp. quant. Biol. *11* (1946) 278.
3 Davis, B.D., Proc. natl. Acad. Sci. USA *35* (1949) 1.
4 Wright, D., and Moyer, F., J. exp. Zool. *167* (1968) 197.
5 Lewin, R.A., Phycologia *13* (1974) 45.
6 Blundell, M., Craig, E., and Kennell, D., Nature New Biol. *238* (1972) 46.
7 Margolshee, J.P., and Lodish, H.F., Devl Biol. *74* (1980) 37.
8 Greenberg, J.R., Nature *240* (1972) 102.
9 Beam, C.W.A., Himes, M., Himelfarb, J., Link, C., and Shaw, K., Genetics *87* (1977) 19.

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Testosterone secretion of rat and mouse Leydig cells cultured at plates precoated with collagen taken from male and female rats[1]

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Summary. Testosterone secretion by Leydig cells in vitro was significantly higher on male collagen coated, than on female collagen coated plates. The castration of male rat-donors of collagen demonstrated that 2 months of androgen deprivation eliminated the effect.

The importance of stroma as an inductor either in a directive or a permissive role in morphogenesis is well known especially in early development[2]. It has been found that in hormone dependent early morphogenesis the primary target structure is the mesenchyme, all changes in epithelium being caused by stromal cells (e.g., in the testosterone dependent development of the mammary gland[3]).

It has also been reported that collagen, produced by 'transfilter'-localized mesenchymal cells, has a key role in the morphogenesis of the embryonic salivary gland. Gland formation failed as a result of collagenase treatment, though epithelial growth continued[4].

This work aims to examine the influence of the stroma on secretory activity in mature organisms. An in vitro approach had been chosen in order to manipulate the stromal environment around Leydig cells.

Materials and methods. Testes were aseptically removed from mature mice (Swiss-Webster, b.wt about 25 g) and rats (Sprague-Dawley or Wistar/First, 10-12 weeks old). After decapsulation of the testes a Leydig cell suspension was prepared by first, gentle teasing with eye forceps and followed by enzymatic dispersion[5]. Pooled Leydig cells of 4-8 mice or 2-4 rats were taken per experiment. They were incubated in 7 ml of medium 199 plus Hepes (Gibco) and 0.25 mg/ml collagenase (type 1, Sigma) for 30 min under constant agitation at 30 °C. 20 ml of saline were then added and the cell suspensions were allowed to settle for 10 min. Supernatant was decanted and the tubules were then rinsed twice. Combined suspensions were then filtered through nylon gauze ($\times 60$ µm) and centrifuged at $800 \times g$ for 10 min. The sediment of Leydig cells was resuspended in the cultivation medium.

The viability of the cells was greater than 95% as evaluated by trypan blue dye. Sperm contamination was about 10%, measured by hemocytometer counts. Most cells appeared rounded by light microscopy with several lipid inclusions in their cytoplasm. Cells with the appearance of fibroblasts were less commonly observed. The dispersed cells were plated in Falkon dishes with either plain plastic bottoms or