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.

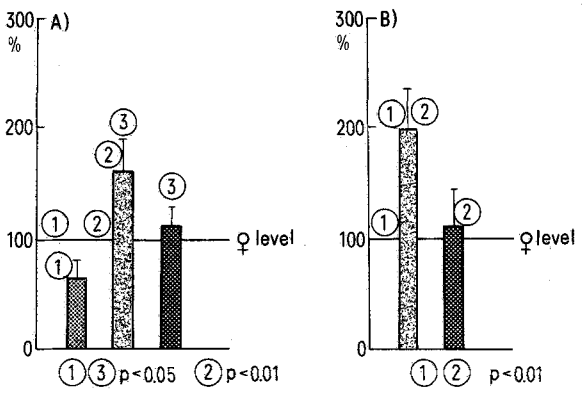
The importance of stroma as an inductor either in a directive or a permissive role in morphogenesis is well known especially in early development[2]. It has been found that in hormone dependent early morphogenesis the primary target structure is the mesenchyme, all changes in epithelium being caused 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



Comparison of levels of testosterone secretion under various conditions. *A* Mouse Leydig cells. *B* Rat Leydig cells. ■, Plastic bottom, □, male, or □, castrated collagen coated bottoms. Each figure represents data, averaged from 5-6 experiments, expressed in percentage (± SD) of the testosterone secretion (ng/μg of DNA) normalized with respect to secretion in female collagen coated plates, which were taken as 100%.

with collagen precoated bottoms. In the latter cases the collagen solution for coating the dishes was prepared from long silver tendons of rat tails[6]. Tendons were taken separately from male and female rat-donors of 10–12 weeks or from males of the same age, castrated at 3 weeks old ('male', 'female', 'castrated' dishes respectively). The collagen solution was maintained at about 1 g per 300 ml in all dishes.

Ham's F12 medium was used, enriched with 10% horse serum and 20% fetal calf serum, containing 0.1 IU/ml insulin, penicillin (100 IU/ml) and streptomycin (100 μg/ml) at 37 °C under 5% CO in air. After 24 h preincubation the medium was aspirated leaving only attached Leydig cells. Fresh medium was added, and these cells were incubated for 48 h. Every plate contained 3 ml of medium.

In all experiments the same amount of pooled Leydig cells were used in plating each of 3 'male' and 3 'female' dishes. In some experiments plain plastic dishes were used and/or 'castrated' dishes. At the end of incubation the medium was stored at −20 °C for subsequent testosterone measurement by radioimmunoassay[7]. The coefficient of variance in replicate samples was + 15%.

Testosterone was indistinguishable in the medium enriched with fetal calf and horse sera before culturing and in the collagen solutions. Gonadotropins were not measured, but all conditions were exactly the same in the plain plates as in those precoated with various kinds of collagen. Leydig cells, attached at the bottom of the dishes, were detached enzymatically (20 min in 0.25 mg/ml collagenase solution at 37 °C), rinsed off and collected by centrifugation for DNA measurement. DNA was determined fluorimetrically using the ethidium bromide method[8]. All results were evaluated by analysis of variance and Duncan's multiple range test.

Results and discussion. The yield of Leydig cells from a mature rat is on average 87 ± 9 million. There is a correlation between DNA concentration and cell number in suspensions. Within the limits examined, the average number of cells which settled in cultured plates (judging from DNA content) appeared independent of the density of pooled Leydig cell suspensions used for plating, of the species, or of collagen plate coating. DNA content was 0.77 ± 0.081 μg/dish for mice and 0.78 ± 0.095 μg/dish for rats.

Hormone secretion of settled cells was, however, dependent on the dish bottom (fig. A and B). It is apparent from the figure A, that mouse Leydig cells, attached to untreated plastic bottoms, produced significantly less testosterone ($p < 0.05$) than the Leydig cells in 'female' collagen coated plates, taken as 100%. On the other hand the cells, attached to 'male' bottoms produced significantly more testosterone ($p < 0.01$) compared to the same 'female' plates. The secretory level in 'castrated' plates was about 100%. However, the difference between the levels in 'castrated' and 'male' plates was also significant ($p < 0.05$).

Rat Leydig cells (fig. B) that attached to collagen coated bottoms showed the same dependence of testosterone secretion on the sex of the collagen donors: the male collagen coated plates exhibited twice the secretory level ($p < 0.01$), while no difference was found between 'castrated' and 'female' plates.

The data obtained suggests that there are sex differences in the collagen structures of rat tail tendons and presumably of other parts of the body[9,10]. Comparably, corticosterone secretion in response to ACTH by organ cultures of male adrenals has been found to be far greater than of female adrenals for 3 strains of rats. In addition, significant strain differences were found in organ cultures. However, in adrenal cell suspensions prepared by collagenase treatment, both - sex-dependent and strain - differences in response to ACTH disappeared[11].

The collagen may serve as an information system, able to bind and to keep hormonal signals. Future research is needed in order to prove this hypothesis.

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2 Cunha, G.P., and Lung Ben, In vitro *15* (1979) 50.
3 Kratochwill, K., and Schwartz, P., Proc. natl Acad. Sci. USA *73* (1976) 4041.
4 Grobstein, C., and Cohen, J., Science *150* (1965) 626.
5 Dufau, M.L., and Catt, K.J., in: Methods in enzymology, vol. 39, p. 252. Eds J.E. Hardman and E.W. O'Malley. Academic Press, New York 1975.
6 Michalopoulus, G., and Pitot, H.C., Exptl Cell Res. *94* (1975) 70.
7 Fujieda, K., Faiman, C., Reyes, F.T., and Winter, J.S.D., J. clin. Endocr. Metab. *53* (1981) 34.
8 Van Duke, K., and Szustkiewich, C., Analyt. Biochem. *23* (1968) 109.
9 Rojkind, M., Gatmaitan, Z., Mackensen, S., Giambrone, M.-A., Ponce, P., and Reid, L.M., J. Cell Biol. *87* (1980) 255.
10 Kleiman, H.K., Klebe, R.J., and Martin, G.R., J. Cell Biol. *88* (1981) 473.
11 Vinson, G.P., Whitehouse, B.J., and Goddard, C., J. Steroid Biochem. *9* (1978) 553.

0014-4754/83/060613-02$1.50 + 0.20/0