positive genetic response than for tests relying on smaller genetic targets, e. g. mouse specific locus test[12,13].

Since most phenotypes in sperm are under diploid control, we assume that at least some of the enzyme inactivity resulted from dominant mutations[14]. However, since several sperm proteins are translated from mRNA first transcribed in haploid cells[15], it is possible that at least some of the enzyme activity loss in individual sperm was caused by recessive mutations.

A practical strength of the heritable sperm enzyme test (HSET) is that it can be performed simultaneously with other mammalian transmission genetic tests that keep F_1 progeny alive until sexual maturity.

The HSET allows progeny testing of F_1 males either by mating before killing for sperm examination or after examination of sperm obtained by semi-vasectomy or semi-castration. With the latter approach only variant males need to be progeny tested. Sperm from F_1 or later progeny could be obtained by ejaculation in some species of mammals.

It remains to be seen whether or not the HSET in conjuction with the heritable sperm morphology assay[10] and other sperm tests[16–18] will prove to be practical tests for detection of mutations in humans. A possible application of these tests may be with sperm of males contemplating parenthood and who may be concerned with receiving mutations from their parents who were exposed to known or suspected mutagens. Undoubtedly more developmental and validating work needs to be done in animals with progeny sperm tests before testing human sperm for parentally induced mutations could be recommended.

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Sex chromatin analysis of unstained mouse amniotic cells using brightfield microscopy

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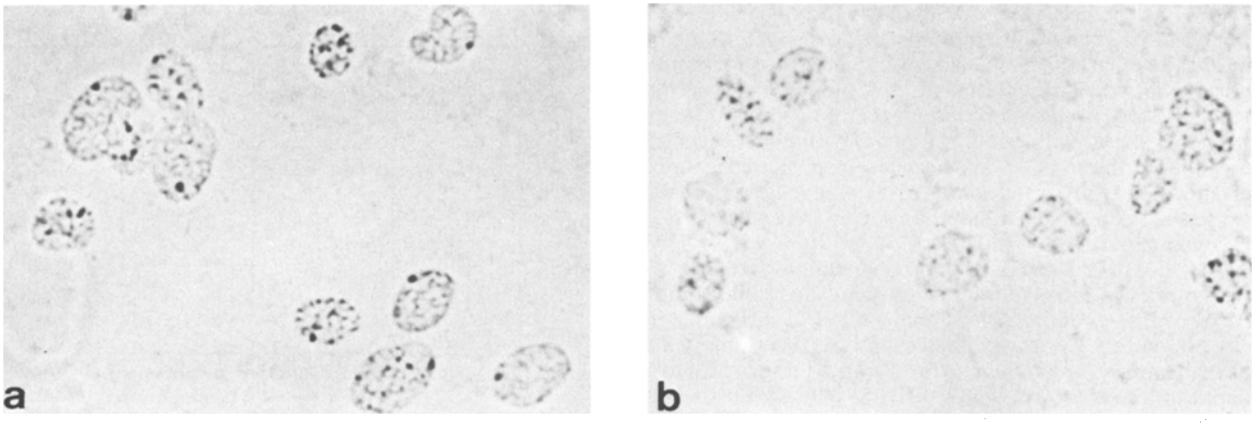
Summary. Sex chromatin of unstained mouse amniotic cells was identifiable using ordinary brightfield microscopy with optimal setting of the illumination.
Key words. Sex chromatin; mouse amniotic cells; unstained examination.

Our laboratory is at present involved in improving the techniques for sex chromatin analysis of rodent embryos at midgestation. During the study, we found that mouse sex chromatin is easily and quickly identifiable with unstained slides under ordinary light (brightfield) microscopy if we optimize the illumination setting. This was unexpected, since several reports have described the difficulty of identifying mouse sex chromatin even with stained preparations[1,2]. Therefore we describe the method here.

The extra-embryonic membranes were removed from the mouse conceptuses. This is possible as early as the 9th day of gestation. The amniotic membrane was separated and immediately placed in a fixative (60% glacial acetic acid) at room temperature for 30 min (20 min at least). Then, the specimens were gently touched on the surface of the slide using sharpened forceps, and dissociated amniotic cells were attached to the surface in a small spot (5 mm in diameter). About 10–15 spots (enough for the embryos from a single litter) can be taken on a single standard size (75 × 25 mm) glass slide.

After air-drying, the unmounted slides were examined under medium power (dry × 40 objective and × 10 ocular) with a brightfield microscope (Olympus BH-2) while finely focusing up and down with low illumination. For optimal viewing, it is essential that, as with a conventional analysis of unstained urinary sediments, illumination intensity be low enough. For this, the condenser was placed almost in its lowest position, the size of the diaphragm aperture was diminished, and also the voltage of illumination lamp was set at about one half of the maximum (12 V). With this setting, scattering refraction of the light within the nucleoplasm may produce phase contrast-like effect to reveal the structural contours of the nuclei more distinctly. As shown in figure a, the sex chromatin mass is easily identifiable as a dark mass adjacent to the inner nuclear membrane in amniotic cells from female embryos. Such a mass was observed very seldom in male amniotic cells (fig. b). In female cells, more than 50% of the cells had a sex chromatin mass, whereas such a mass could be seen in less than 2–3% of the male cells. Analysis of aged slides (more than two months) produced the same results. In more than 30 embryos at near term, sex diagnosis by this method coincided with morphological diagnosis by internal sex organs without a single exception. It was also found that carbol fuchsin staining of the slides after un-



Interphase nuclei of unstained amniotic cells from female (a) and male (b) mouse embryos under brightfield microscopy. A dark mass adjacent to the inner nuclear membrane is identifiable in the cells from female mouse embryos, whereas no such mass can be seen in the male cells.

stained examination demonstrated consistently a clearly identifiable sex chromatin in the same position as that in which the dark mass had been seen.

As expected, by using a phase-contrast microscope with dark (positive) contrast (Olympus, PLL; absorbance rate 60%), we could obtain similar results. In retrospect, we found several early reports[3-7] that mentioned the possibility of visualizing sex chromatin of cultured cells under phase-contrast microscopy. Miles[4] has seen sex chromatin 'clumps' in cultured human amniotic cells. Unstained amniotic cells may be suitable specimens for nuclear sex diagnosis. Previously three groups of investigators[8-11] described the techniques for sex chromatin preparations of rodent amniotic cells and yolk sac. However, no mention was made of the possible use of unstained specimens.

Our preliminary attempts with rat embryos suggest that this method is also applicable. The method presented here may be useful for quick sex diagnosis of murine embryos for various purposes in experimental embryology.

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Differential expression of isozymes in relation to organogenesis in two closely related species[1]

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Summary. Two closely related species of Gentianaceae were established in tissue culture and transferred to a regeneration medium containing 2,4-D. Even though the age of cultures and all the environmental conditions were identical, only one of them expressed a clear morphogenic potential, and the differences are expressive in the isozyme banding patterns observed for creatine phosphokinase, lactate dehydrogenase and indoleacetaldehyde dehydrogenase. No differences in alkaline phosphatase isozymes were detected.
Key words. Gentianaceae; morphogenesis; caulogenesis; isozymes.

Exacum affine Balf. (German violet) and *Eustoma grandiflorum* (Raf.) Shinn (prairie gentian) are two closely related species belonging to the family Gentianaceae. The former is a herbaceous mesophyte and the latter is a succulent xerophyte. Evidence is given hereby showing that their morphogenic potential is different under identical cultural conditions, the difference being distinctly identifiable from their isozyme banding patterns for three of the four different enzymes studied.

Exacum affine and *Eustoma grandiflorum* were established in tissue culture using MS medium supplemented with 1.0 mg/l of 2,4-D and 0.1 mg/l of kinetin. Transfers were made to a regeneration caulogenic MS medium supplemented with 0.03 mg/l of 2,4-D and 1.0 mg/l of kinetin. The explants were never exposed to exogenous IAA. The pH of the regulation medium was 5.8 upon establishment of the cultures and remained constant throughout the course of the experiment. Sequential manipulation has proved to be successful in the induction of various levels of morphogenesis[2, 3], but the isozyme differential in relation to morphogenesis in such closely related species has not been shown before. Within four weeks in the regeneration medium, the friable callus of