Reports

Cultures of Gonads of Mammalian Embryos

Since the explanation of sex reversal in the freemartin was given by Lillie (*1*), there have been a number of attempts to investigate sex reversal experimentally, but, until recently, results with mammalian forms have been rather meager. Grafts of embryonic mammalian gonads to adult hosts of the opposite sex (*2*) have given negative or nearly negative results. Hormones of adults, when given to pregnant animals, have produced marked effects on the fetal reproductive tracts but inconsistent effects on the gonads. Recently MacIntyre (*3*), by grafting embryonic rat gonads in pairs to castrated adult hosts, has demonstrated that, when an ovary is grown with an embryonic testis, differentiation of the ovary is suppressed, while the testis is unaffected. Similar experiments on the rabbit by one of us (*4*) have produced the same result.

Wolff and his coworkers (*5*) have cultured embryonic duck gonads together in male-female pairs. In these experiments the ovary proved to be the dominant gonad, and the testis was suppressed.

It was felt that such cultures of embryonic mammalian gonads might be of some value as a control on the grafting experiments, inasmuch as cultures provide an environment more nearly free of extraneous hormones than adult hosts.

We therefore undertook a series of cultures of embryonic mammalian gonads. Both rabbit and rat embryos were used. The cultures were grown in hollow ground slides. We used plasma clots and embryonic extract as a culture medium after attempts with synthetic media failed. The embryonic extract was prepared from the anterior (gonad-free) halves of 11-day chick embryos, the plasma from heparinized blood of castrated rabbits. Gonads for culturing were obtained from embryos of the desired age and were grown in cultures for 4 to 18 days.

The 56 cultures were distributed as follows: male-female combinations, 17; male-male, 8; female-female, 10; single male, 9; single female, 8. There were also four cultures of gonads recovered at indifferent stages. Rabbits and rats gave the same results. No heteroplastic combinations were attempted.

Embryonic testes usually grew and differentiated well in all combinations. The seminiferous tubules were well formed and contained spermatogonia, some of which were degenerating, as they normally do. The differentiation was never equal to that occurring in a similar period of normal development but, in most cases, was well advanced over the differentiation that had occurred at the time of explanation.

Embryonic ovaries, on the other hand, only grew and differentiated in 19 of 35 cultures. When cultured alone or in combination with other ovaries, those that did grow produced some cortical differentiation. There were cell nests, occasional structures suggestive of primordial follicles, some proliferation of interstitial cells, and the retention of a cuboidal epithelium, which might be interpreted as a germinal epithelium. When explanted with testes, the ovaries which grew were always retarded in development in comparison with those cultured in the absence of the male gonad. None of them developed cell nests or other indications of cortical structures. In three instances there were indications of a masculinizing effect, as shown by the development of structures suggestive of testicular cords in medullary portions of the cultures. MacIntyre has suggested that such cords may in fact be "converted follicles of cortical or secondary sex cord origin." In either case, differentiation of the ovary was altered when the ovary was cultured with a developing testis. The differentiation of the testis, on the other hand, was not affected by the presence of the ovary.

These experiments (*6*) appear to support the findings obtained by grafting embryonic gonads. As the embryonic ovary is not affected in its development when grafted to the adult male, even if grown in the host testis (*7*), we suggest that it is probable that the embryonic testis produces a substance or "hormone" capable of modifying ovarian development and that this substance is not identical with the adult testicular hormone.

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Ultraviolet Mitigation of X-ray Lethality in Dividing Yeast Cells

Abstract. The lethal effect of x-rays on dividing yeast cells can be decreased by small ultraviolet exposures delivered before or after x-ray exposure. This mitigating action can be decreased by exposure to visible light concomitant with photoreactivation of ultraviolet lethality. The results suggest considerable overlap between x-ray and ultraviolet lethality sites in dividing cells.

In the course of a study of the molecular and anatomical nature of the sites sensitive to lethal irradiation in dividing yeast cells, different combinations and permutations of x-rays, ultraviolet (UV), and visible light (VL) were employed. Depending upon the radiation and the sequence of administration, both coupling and uncoupling effects were observed. One such effect is the ability of UV either to protect or to reactivate x-rayed dividing yeast cells (that is, cells in division when irradiated).

Although the results to be described were obtained with a nonrespiring strain of haploid *Saccharomyces cerevisiae,* SC-7(ρ), qualitatively similar results were obtained with the parental, respiring diploid strain, SC-6, and the respiring haploid strain SC-7 from which SC-7(ρ) was derived. The mitigating effects were largest in SC-7(ρ), comparable in SC-6, and small but present in

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

SC-7. Cells exposed to x-rays (55 kv peak; unfiltered emission from a 1 mm Be window tube; dose rate 30.2 krad/min) were spread on YED agar surfaces (0.5 percent Difco yeast extract, 1 percent dextrose, 2 percent agar); the same technique was used for UV exposure (three low-pressure, 8-w germicidal Hg lamps; UV flux, 21 erg/mm² sec) or the cells were exposed while being agitated in 0.05*M* KH_2PO_4 buffer solution. Photoreactivating VL exposures were performed in the setup previously used for photolethality studies with SC-7(ρ) (*1, 2*), except that the cell suspension was maintained at 30°C, and a 500-w projector was used. Although strain SC-7 is also sensitive to visible light, appropriate precautions were observed to avoid both incidental photolethality (*1*) or photoreactivation during the course of the experiments, or both. The criterion of survival was formation of visible colonies after 5 days' incubation at 30°C.

Curves *a* and *b* of Fig. 1 are the x-ray and UV survival curves, respectively, for a population of SC-7(ρ) harvested after overnight growth on YED agar. As was previously shown (*3*), in general the survival curve resulting from ionizing irradiation is the sum of an exponential and a sigmoid curve, the former reflecting the survival of interdivisional cells and the latter corresponding to dividing cells. It is noted that the UV survival curve in Fig. 1 has an inflection point at a survival level which appears to correspond to the inflection point in the x-ray survival curve, suggesting that dividing cells are less sensitive to UV, as well as to x-rays, than interdivisional cells.

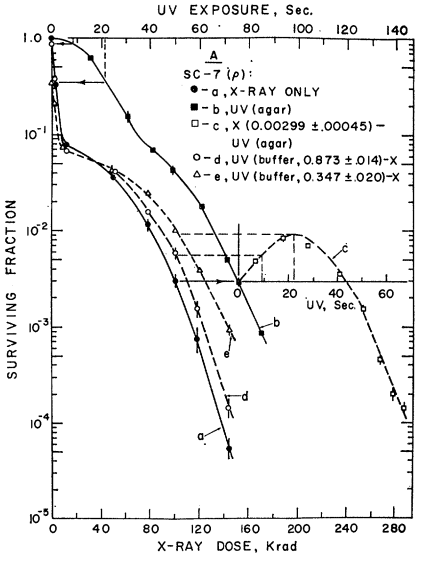

Fig. 1. Equivalence between pre- or post-UV exposure in increasing the survival of x-rayed dividing haploid yeast cells. A typical description of a curve like that for *c* means that a pre-x-ray exposure reduced the survival to 0.003 and that this was followed by a graded series of UV exposures. Standard errors are shown where larger than the plotted points (also in Fig. 2).

There is conflicting information in the literature relative to the compound nature of the UV survival curve (*4–6*) except in those instances in which the preparation of the cells was such as to insure a homogeneous, interdivisional cell population (*7, 8*). Recently we have shown (*9*) that the inflection in the UV survival curve results from the presence of the same moieties displayed in the case of x-rays, although the inflection in the former case is not as pronounced because the curves for both moieties are sigmoid. In addition, we have proved (*9*) that the lesser UV sensitivity of dividing yeast cells results from basic changes in site sensitivity attendant with division and are not due, for instance, to increased scattering or increased specific but non-active absorption of the 254-mμ irradiation. Curve *c* resulted from a single x-ray exposure which reduced the survival to 0.003 followed by a graded series of UV exposures. Since the pre-x-ray dose was large enough to kill essentially all the interdivisional cells, *c* suggests that dividing cells are first reactivated by 3X before additional inactivation is produced by the UV. (The UV exposure sequence of *c*, when repeated with plate covers left on, produced neither additional killing nor reactivation.)

That this mitigating action of UV is independent of the sequence of administration is evidenced by curves *d* and *e* Fig. 1, and by curve *e*, Fig. 2. For the first two curves, small UV exposures were followed by graded x-ray exposures; each curve clearly shows that its initial portion lies below the x-ray-only curve while the reverse is true for its final portion. Further, there is quantitative agreement between the protection afforded by pre-UV and the reactivation resulting from post-UV exposures. Curve *e* of Fig. 2 shows that the mitigating UV exposure can be given during the x-ray exposures with results equivalent to those of the preceding cases.

In Fig. 2, curve *b* resulted from a maximally protective UV dose and is functionally similar to *a*, the dose-reduction factor being 0.77. Since in the case of VL photoreversal of UV lethality in yeast, dose-reduction factors of from 0.4 to 0.65 have been reported (*5, 8*), the value of 0.8 observed in this case is significant particularly in view of the general injurious nature of UV exposure.

That UV protection is also photoreversible by VL is shown by curve *c* in Fig. 2. In this case, the pre-UV exposure was followed by a maximally reactivating VL exposure; however, the dividing cell portion of *c* lies below that of *b*, indicating that VL reverses the UV protection of x-ray lethality as well as UV lethality. That UV protection is temperature stable and that curve *c* resulted primarily from the effect of VL is shown by *d*, for which case the exposure and temperature sequence was the same as that for *c* except that VL was omitted.

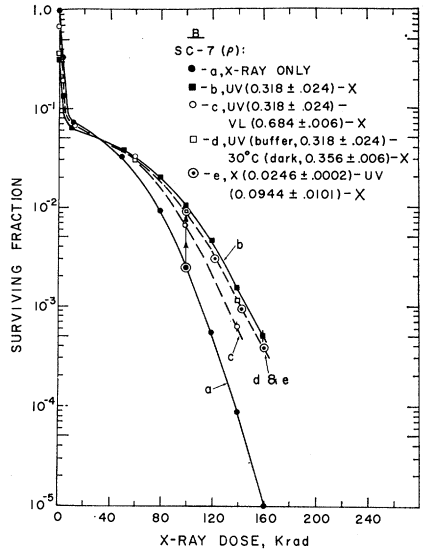

Fig. 2. Photoreversal of the UV protection of x-ray lethality in dividing haploid yeast cells. Descriptions of the treatment sequences are as explained for Fig. 1.

These results are consistent with a number of others (*9*) which support the view that the sites of action of lethal irradiation are chromosomal deoxyribonucleic acid. In addition, this mitigating effect of UV is reminiscent of cytogenetic observations in *Tradescantia* pollen tubes (*10*) and in *Drosophila* sperm (*11*) where it was observed that pre- or post-UV decreased the observable number of x-ray chromosome breaks.

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