La vésicule séminale d'un individu castré pèse statistiquement moins que celle d'un témoin mais le poids ne diffère pas entre castrat partiel et total. Même très longtemps après la castration totale le poids de la vésicule séminale n'est pas négligeable, car il y subsiste encore des spermatozoïdes. Par ailleurs, la vésicule séminale des castrats partiels contient moins de spermatozoïdes que celle des témoins (la gonade des opérés est moins grosse que chez les témoins et produit moins de spermatozoïdes).

Les autres portions des tractus génitaux après 140 jours ont un poids frais et sec qui n'est manifestement pas influencé par la présence ou l'absence de l'ovotestis. L'hypertrophie des portions glandulaires, particulièrement au niveau de la partie femelle, a été mise en évidence chez *B. contortus, T. centimetralis* et *P. corneus*[3]. Rien de comparable n'apparaît chez *Biomphalaria glabrata,* que la castration ait été partielle ou totale. Longtemps après la castration, le poids des différentes parties des tractus génitaux ne dépend pas d'une éventuelle action de la gonade.

L'absence totale de gonade n'empêche pas le dépôt de pontes semblables à celles des témoins mais stériles. Le nombre de ces pontes, le nombre de sphères d'albumine produites, sont inférieurs à ceux observés chez les témoins. La présence d'une gonade même profondément amputée suffit pour que s'établisse un rythme d'oviposition comparable aux témoins. Qualitativement, l'oviposition est donc indépendante de l'ovotestis. Cependant, l'intensité du phénomène dépend quantitativement de la présence d'une gonade fonctionelle. Les tractus génitaux, même en l'absence de gonade, ont un fonctionnement qui conserve une intensité liminaire et la présence de l'ovotestis l'augmente nettement.

1 H. W. Harry, Trans. Am. microsc. Soc. *84,* 157 (1965).
2 M. Vianey-Liaud, Bull. Soc. zool. Fr. *97,* 675 (1972).
3 P. Brisson, Ann. Embryol. Morph. Fr. *4,* 189 (1971).
4 H.H. Boer, A.M. Mohamed, J. van Minnen et M. de Jong-Brink, Neth. J. Zool. *26,* 94 (1976).
5 A.S.M. Saleuddin, non publié, communication personnelle.

DNA-repair in mammary tumor cell lines with different X-ray sensitivities

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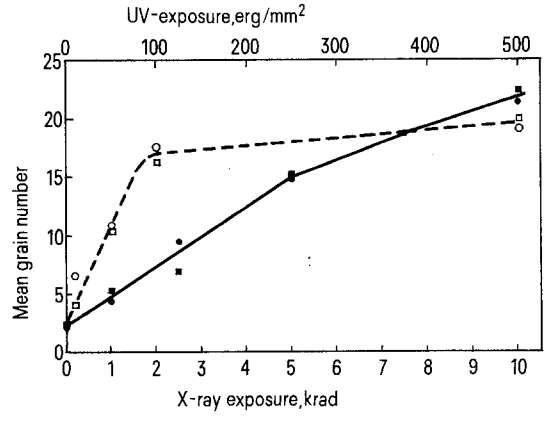
Summary. 2 murine mammary tumor cell lines with different radiosensitivities (D_0 = 65 and 92 rad) showed no significant differences in levels of unscheduled DNA-synthesis following X-ray or UV-exposure.

Mammalian cells not in the normal DNA synthetic (S) phase may carry out a DNA-repair synthesis following exposure to ionizing and UV-radiation[2] or certain chemicals[3]. This DNA-repair synthesis is called unscheduled DNA-synthesis (UDS) when assayed autoradiographically or repair replication if assayed by density gradient centrifugation techniques[4].

A deficiency in UDS following UV-exposure in xeroderma pigmentosum is correlated with carcinogenesis and UV-radiation sensitivity in this disease[5] and this has prompted examination of levels of UDS in other diseases and tumors[6,7].

Early (6–12th generation) s.c. transplants from a spontaneous CD_2F_1 mammary tumor were highly radioresponsive, exhibiting remarkable tumor shrinkage during fractionated X-ray doses of 2000 rad[8]. Following continued transplantation past the 19th generation, the tumors became refractory to radiation treatment. Cell lines cultured from sensitive and resistant tumors demonstrated a considerable difference in radiosensitivity (D_0 = 65 and 92 rad, respectively)[9]. It is of interest to test the levels of UDS in these cell lines.

Cell lines from the sensitive (12th generation) and resistant (19th generation) tumors were initiated and maintained as previously described[8,9]. For UDS experiments, coverslip cultures in early monolayer were exposed to X-rays or UV-light at room temperature. X-ray exposures utilized a therapy unit operated as follows: 250 kVp, 15 mA, 0.52 mm Cu half-value-thickness, exposure rate of 1000 R/min. UV-irradiations (λ = 254 nm) utilized a Mineralite UV-11 source with an exposure rate of 5 erg/mm^2/sec. Tritiated thymidine (New England Nuclear Corp.) was added to the media within 2 min following irradiation at a concentration of 5 µCi/ml at a specific activity of 18 Ci/mM. After 1 h at 37 °C, the radioactive medium was poured off, and the cultures were washed 3 times with normal saline containing 100 µg/ml unlabeled thymidine. Following overnight fixation in 1:3 acetic ethanol at 4 °C, the coverslip cultures were rinsed in 70% ethanol, cold 4% perchloric acid, and twice in distilled water. Coverslips were then mounted on slides, dipped in Ilford K-5 photographic emulsion, stored



Mean numbers of autoradiographic silver grains over the nuclei of non-S-phase cells cultured from radiosensitive and resistant CD_2F_1 mammary tumors. The dose-dependent increase in mean grain number indicates unscheduled DNA-synthesis. The solid line refers to X-ray exposure; the dashed line refers to UV-exposure. Squares designate the resistant line while circles represent the sensitive line.

1 week at 4 °C, developed in Kodak D-19, stained with hematoxylin and eosin, and mounted with another coverslip.

Slides were scored by counting silver grains over the nuclei of non-S-phase cells, excluding as S-phase cells those with greater than 40 grains per nucleus. Mean grain counts of the non-S-phase cells, corrected for background, were determined on 3 groups of 150 cells at each dose point. The SEM from an average count of 3 such replicate groups of 150 cells at each dose point was 14%. The presence of UDS is demonstrated in the figure by the dose-dependent increase in silver grains over the nuclei of non-S-phase cells, indicating replacement of damaged sites in DNA. For both the X-ray (solid line) and UV (dashed line) curves, there appears to be no significant difference in mean grain count between the resistant (squares) and sensitive (circles) lines. The percentage of cells in the S-phase, i.e., those cells containing >40 grains/nucleus, remained relatively constant ($32.6\pm3.6\%$) in unirradiated and irradiated cultures from both lines.

Positive correlations between radiosensitivity and DNA-repair replication have been made in 2 lines derived from human cervical cancer[10] and 2 L5178Y lines[11]. A similar correlation between radiosensitivity and UDS was not seen in our experiments. One must bear in mind, however, the distinction between apparently 'normal' levels of UDS and the integrity of the repair system. UDS measures only the incorporation of labeled DNA precursors into DNA of non-S-phase cells and not necessarily the correction of radiation-induced lesions. The possibility remains that radiosensitive cells may possess defective repair systems which appear in quantitative terms similar to the functional repair systems possessed by the more radioresistant cells.

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2 R.E. Rasmussen and R.B. Painter, J. Cell Biol. *29*, 11 (1966).
3 S.R. Ayad, M. Fox and B.W. Fox, Mutat. Res. *8*, 639 (1969).
4 R.B. Painter and J.E. Cleaver, Radiat. Res. *37*, 451 (1969).
5 J.E. Cleaver, Proc. nat. Acad. Sci. USA *63*, 428 (1969).
6 J.E. Cleaver, J. invest. Derm. *54*, 181 (1970).
7 A. Norman, R.E. Ottoman, P. Chan and I. Klisak, Mutat. Res. *15*, 358 (1972).
8 J. Shaeffer, A.M. El-Mahdi and T. Merz, Cancer *29*, 581 (1972).
9 A.M. El-Mahdi, J. Shaeffer and W.C. Constable, Acta Radiol. *13*, 478 (1974).
10 J.E. Byfield, Cancer Chemother. Rep. *58*, 528 (1974).
11 I. Korner, M. Walicka, W. Malz and J.Z. Beer, Stud. Biophys. *61*, 141 (1977).

The formation of disulfide bonds in human protamines during sperm maturation

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Summary. The disulfide contents of human sperm heads, as measured by reduction to the sulfhydryls and subsequent alkylation with ^{14}C-iodoacetamide, increase about 2-fold during the sperm passage from the caput to caudal epididymides. Majority of the increased disulfides reside in the human protamine fractions.

Sperm maturation is a process whereby testicular sperm acquire fertilization potential during passage through the epididymis[2,3]. The maturation process requires the epididymal environments[3] and androgen[4]. In addition to morphological changes, several biochemical changes have been shown to accompany the maturation process including increases in disulfide content, metabolic rate and surface negative charges[5]. In rats, disulfide formation has been shown to occur in the head as well as in the tail of the sperm[6]. Since mammalian protamines have high contents of half-cystine[7,8], they are likely to be involved in this epidymal disulfide formation. This has been confirmed in rats, where the protamines isolated from sperm of the cauda epididymis contain more disulfides per molecule than those obtained from the sperm of the caput epididymis[9]. However, from these studies, it is still not clear whether protamins represent the major class of proteins forming disulfide bonds during sperm maturation. Furthermore, in view of the species specificity of the reproductive process, it is difficult to extrapolate studies in other species to the humans. Studies in our laboratory[7,10] and in others[11] have shown that human protamines consist of several components, which exhibit a different electrophoretic pattern and amino acid composition to the protamines of other species. In this report we have investigated the occurrence of disulfide bond formation in human sperm, with special reference to the relative importance of the protamines in such a process.

Materials and methods. Epididymal sperm were isolated from accident victims within 12 h post mortem following the method of Calvin et al.[6], except that 5 mM iodoacetamide was present throughout. The sperm suspension (in 0.2 M phosphate, 1 mM EDTA, 5 mM iodoacetamide, pH 7.3) was kept in the dark at 4 °C for 1 h to block completely free sulfhydryl groups, and was then sonicated for 30 sec at 70–80% of the maximum output of a Branson Sonicator, model J-17A. Ejaculated sperm were treated in a similar manner and then sonicated for 2 min. The sonicated sperm suspensions were layered on top of 40% sucrose and centrifuged at $700\times$ g for 30 min to pellet sperm heads[10].

The content of unblocked half-cystine, which should be in the disulfide form, was determined by reduction of the above sperm heads is an O_2-free solution containing 5 mM dithiothreitol, 0.1 M Tris. HCl, pH 7.3, 1 M guanidine hydrochloride for 1 h, followed by alkylation with 0.02 M ^{14}C-iodoacetamide (1 μCi/μmole) in the dark at 25 °C for 1 h. The reaction was terminated by adding excess unlabelled iodoacetamide. One portion of the mixture was taken to quantitate the ^{14}C-carboxymethylated products by precipitation with trichloroacetic acid (TCA) and filtration on Whatman GF/A filters, followed by extensive washing with 5% TCA and acidified acetone (39 :1 acetone :1 N HCl). The dried GF/A paper was counted in toluene cocktail with a counting efficiency of 47–52%. Another portion of the ^{14}C-carboxymethylated mixture was extracted with 0.25 N HCl[10] and samples from this acid-soluble