experimentally in human or animal semen by acids, alkalis, carbon dioxide, salts of heavy metals, dyes, extracts of eggs, bacteria and antisera developed by injecting spermatozoa of the same or different species(5). None of these agents appears to be involved in the cases reported here.

Summary. The presence of sperm agglutinins in the seminal plasma and blood serum of 2 sterile men whose spermatozoa agglutinate in the ejaculate is reported. The sera have antibody titers of 1:80 and 1:20, respectively. Head, tail, and mixed varieties of agglutination are seen in the early stages, but tail agglutination predominates in the larger clumps. Agglutination occurs only in the presence of motile spermatozoa and none is seen in the fresh ejaculate until liquefaction begins and active sperm movement develops. Temperature influences agglutination by its effect on sperm motility. The motility of the clumped spermatozoa is impaired with consequent loss of the ability to penetrate cervical mucus. The clumps can be completely dispersed by mechanical agitation after which the spermatozoa reagglutinate although not quite to the original extent. Complement inactivation does not influence agglutination. The antibody can be removed by absorption.

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Combined Effects of X-Irradiation and Testosterone Propionate on Accessory Sex Organs. (20983)

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A temporary inhibition of cell division appears to be a general action of radiation, having been demonstrated in a great variety of cells. If the cells are prevented from dividing, but continue to grow at the normal rate, then the size of the cells will increase instead of their number. When division is delayed by irradiation it is of interest to determine at which stage or stages the retardation occurs. The answer to these questions can most readily be obtained in the case of materials in which the cells develop synchronously, so that all the cells can be irradiated at the same stage. It is obvious, that this experimental condition cannot as a rule be fulfilled in growing tissues as they are composed of cells at different stages of cell division, so that it is not possible to choose a single stage to be irradiated(1). Experiments in which a single stage was irradiated were performed by Henshaw(2), who treated unfertilized eggs of the sea urchin, *Arbacia punctulata*, with x-rays and studied the delay of first cleavage following subsequent fertilization. In these experiments all cells were in the resting stage during irradiation and cell division was induced subsequently by fertilization.

It occurred to us that irradiation of the secondary sex organs (seminal vesicles and prostate) of the castrate male rat and subsequent stimulation by an androgen offers a unique opportunity for an analogous study on the effect of radiation on mammalian cells.

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TABLE I. Effect of Combined Irradiation and Treatment with TP. All rats received colchicine 7½ hours before death.

Group	No. of rats	TP, hr	X-ray, hr	Accessories, wt in mg Mean	S.D.	Access. hyper-trophy	Access. mitoses	Pelvic skin mitoses	Head skin mitoses
A	7	—	—	24.8	7.0	0	0	0	+
B	12	48	—	80.7	27.2	+	++	+	+
C	9	48	48½	65.4	13.8	+	0	0	+
D	7	48	168	72.0	6.5	+	++	+	+

Statistical comparison of weights

Groups compared	P
A-B	<.01
A-C	"
A-D	"
B-C	.1
C-D	.3

The accessory sex organs of the castrate male rat do not grow unless stimulated by androgens. Treatment of the castrate male rat with androgens is followed by hyperplasia and mitotic activity of the cells of the accessories. The abundance of mitotic figures in the stimulated accessories is in striking contrast to their absence in the resting organs. This effect on mitosis can be emphasized by the use of colchicine(3-5). A preliminary study showed that the weight response of the accessories of the castrate male rat to testosterone propionate (TP) is not inhibited by irradiation of the accessories with x-ray. Even if doses of 10000 r are used the accessories respond with an increase in weight to subsequent treatment with TP(6).

Materials and methods. Male rats of the Wistar strain were castrated at the age of about 30 days and were used not earlier than one month after castration. X-ray was given under amytal sodium anesthesia. Irradiation was given through a portal centered over the pelvis. The diameter of this portal was 3½ cm. The rats received 3000 r (120 KV, 8 ma, filter 1 mm Al; 98 r/min.). Except for the pelvic region the rats were shielded by 2 mm lead. TP (Perandren Ciba) was given dissolved in peanut oil in doses of 3 mg subcutaneously. Colchicine, 3 mg/kg body weight, was given in aqueous solution 7½ hours before termination of the experiment. The animals were sacrificed with amytal sodium. The accessories were dissected out, weighed to the nearest mg and fixed in 10% formalin. Skin from the irradiated suprapubic region and from the shielded head was also fixed. Sections were stained either with dilute Harris hematoxylin or with Nicolle's carbol thionin stain.

Results. The results of the experiments are listed in Table I. From this table the following conclusions can be drawn. Irradiation in the dose given has no or little effect on the growth response of the accessories due to TP under the conditions of the experiment. However, if TP is given immediately after irradiation mitotic activity is suppressed. The cells in group C show hypertrophy without mitosis. The difference in appearance between the non-irradiated and the irradiated tissues is quite striking; the epithelial cells of the non-irradiated accessories show numerous mitoses whereas mitotic figures are completely absent in the irradiated tissues. The contrast can be easily seen by comparing Fig. 1 and 2.

This difference is obvious only in the accessories of rats killed 48 hours after irradiation, followed by TP (Group C). If the rats are killed 24 hours after administration of TP no mitotic figures can be seen. Both the irradiated and the non-irradiated specimens show hypertrophy only. This was to be expected as the onset of mitosis in the prostate of the castrate rat had been determined at about 36 hours after administration of TP (5). On the other hand if the rats are killed 72 hours after administration of TP mitoses are numerous both in the irradiated and the non-irradiated specimens.

If TP is not administered immediately

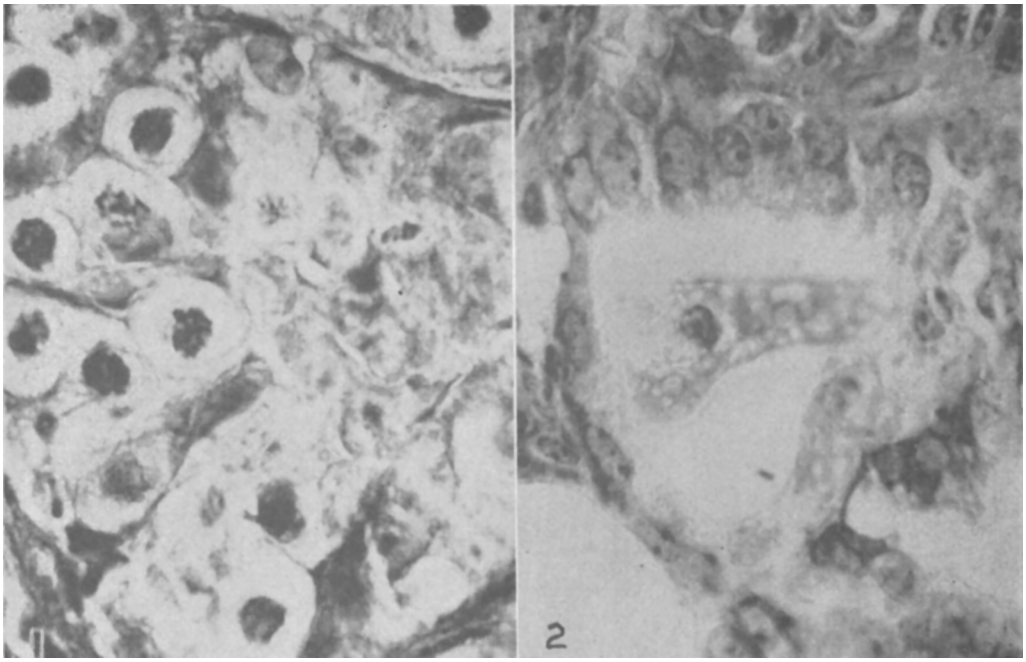

FIG. 1 (left). Seminal vesicle. Rat treated with TP 48 hr before death, with colchicine 7½ hr before death. (Group B.) Fixed in 10% formalin. Stained with carbol thionin. Note numerous typical colchicine mitoses. × 550.

FIG. 2 (right). Seminal vesicle. Same treatment as in Fig. 1, except that pelvic region received x-ray immediately preceding treatment with TP. (Group C.) Note absence of mitotic figures. × 550.

but 120 hours (5 days) after irradiation (Group D) mitotic activity appears to be normal as judged from the presence of numerous mitotic figures.

Sections were taken from the irradiated part of the skin to make sure that the pelvic region had received adequate irradiation. Table I shows that in all cases in which mitotic activity was absent in the accessories it was absent also in the pelvic skin. Sections from the skin of the shielded head served as controls. All of these sections showed numerous mitoses.

Discussion. The experiments indicate that the onset of mitosis can be delayed by irradiation of resting tissues. They show furthermore that this inhibiting effect is only transient. Recovery from the inhibiting effect of x-rays on mitosis can take place in the resting tissue as evidenced by the finding of mitoses in Group D. This finding is comparable to results of experiments on the eggs of the sea urchin mentioned above(2). Exposure of eggs of *Arbacia punctulata* to large doses of irradiation leads to delay of the first cleavage after subsequent fertilization. If an interval of time is allowed between the end of treatment and the moment of insemination this delayed effect is lost or reduced as a function of time. In our experiments the mitosis-inhibiting effect due to irradiation is lost or at least reduced if an interval of 5 days is allowed between time of irradiation and of stimulation with TP. It is of interest that the delaying effect of x-irradiation on cell division is lost as a function of time in resting cells. This holds true both for unfertilized ova of invertebrates and for accessory sex organs of castrate vertebrates.

Jadassohn *et al.*(7) studied the combined effect of estrogens and x-irradiation on a target organ. One nipple of the male guinea pig was irradiated and both nipples subsequently treated topically with a solution of an estrogen preparation. Mitotic activity was estimated using the colchicine technic. The action of the estrogen administered after irradiation produced a simple hypertrophy in-

volving both nuclei and cytoplasm associated with inhibition of mitosis. These results are essentially in agreement with those described in this paper.

Our experiments are reported here in a preliminary fashion. As indicated in an earlier note(6) they are part of a project to investigate the influence of x-rays on the formation of chromosomal material. The accessory sex organs of the castrate rat seem to be a suitable object for studies of this kind as their growth response to TP is associated with rapid formation of desoxyribonucleic acid as indicated by tracer studies using P^{32}(8).

Summary. X-irradiation of the accessory sex organs of the castrate male rat and immediate subsequent treatment with testosterone propionate results in cellular hypertrophy with delayed onset of mitosis under the conditions of the experiment outlined above. If an interval of 5 days is allowed between irradiation and administration of testosterone propionate this delaying effect is lost.

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Ion Exchange and Recrystallization in Fixation of Ca^{45} in the Rabbit's Skeleton.*† (20984)

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Ion exchange has been demonstrated to be the principal mechanism for the fixation of radiocalcium and phosphate in bone, both *in vitro*(2,3) and *in vivo*(4-6). Calcium ions, in a solution of an ionized calcium salt, exchange with other calcium ions on the surface of bone crystals(3). Thus, under suitable conditions, it is possible to replace the calcium ions on the crystal surfaces with those of a calcium solution. In the studies herein reported Ca^{45} which remained on the crystal surfaces at various intervals after administration was determined by *in vitro* ion exchange removal in non-radioactive calcium solutions.

The purpose of these studies was to examine directly the role of ion exchange, crystallization and recrystallization in the fixation of calcium in newly formed and mature bone. No attempt will be made to review the concepts of surface chemistry of bone at this time. Recently an excellent review of the field has been made by Neuman(7). If all of a given amount of Ca^{45} were fixed in bone by ion exchange and remained on the crystal surfaces, then nearly all of the Ca^{45} should be removable by ion exchange with non-radioactive calcium solutions. On the other hand, if all of the Ca^{45} fixed in bone was incorporated into newly forming crystals, not more than 50% of the Ca^{45} should be removable by surface ion exchange.‡ The actual process of skeletal calcium fixation in a growing or mature ani-

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