TABLE I. The Renotrophic and Androgenic Effect of Orally Administered Androgens.*

	Mice No.	Body wt — Initial, g	Body wt — Change, g	Food intake, g/day	Thymus, mg	Kidney, mg	SVPr, mg	Iner.† K/SVPr, mg/mg
		.1 mg steroid/g food, 30 days						
Control (castrated)	8	31.2	4.4	3.7	64±5	355±13	16±1	—
17-Methyltestosterone	7	28.6	5.1	4.2	36±6	478±36	112±8	1.28
17-Methyl-Δ^5-androstene-3β,17β-diol	7	30.4	4.7	4	38±4	452±27	42±6	3.73
17-Methylandrostan-17β-ol	4	29.9	3.7	3.8	34±9	446±29	40±7	3.80
Testosterone	6	29.4	5.5	4.1	52±4	426±26	51±10	2.32
17-Methylandrostane-3α,17β-diol	6	28.6	5.6	3.9	34±4	412±21	57±9	1.43
3β,17β-diol	5	29.6	5.5	3.8	39±5	399±20	34±8	2.45
17-Methylandrostan-17β-ol,3-one	4	27.2	3.3	3.7	29±8	395±23	59±9	.93
Androstane-3α,17β-diol	6	30.2	4.7	3.9	50±5	394±20	25±4	4.33
Δ^4-Androstene-3,17-dione	6	30.4	5.2	3.8	52±6	379±27	17±2	—
		.5 mg steroid/g food, 30 days						
Control (castrated)	8	29.7	4.1	4	47±5	367±18	15±3	—
17-Methyltestosterone	6	31.4	4.1	4	6±1	565±36	307±26	.68
17-Methylandrostan-17β-ol‡	4	31.4	7	3.9	37±5	523±26	100±16	1.84
17-Methylandrostane-3α,17β-diol	6	30.8	4.5	4.3	8±3	514±14	232±27	.68
17-Methylandrostan-17β-ol,3-one	4	30.2	4.1	4.3	4±3	488±29	226±26	.45
17-Methyl-Δ^5-androstene-3β,17β-diol	6	31.7	6	3.9	23±8	482±16	189±48	.66
Androstane-3α,17β-diol	7	31.8	7.3	4	35±3	455±32	90±18	1.49
17-Methylandrostane-3β,17β-diol	8	31.4	3.5	4	27±5	451±15	137±20	.70
Testosterone	6	31	4.8	3.9	30±8	420±26	160±19	.36
Δ^4-Androstene-3,17-dione	6	31.3	6.3	4	44±6	398±23	36±6	1.43

* The values for the organ weights are given with the stand. error of the mean $\sqrt{\frac{\overline{X^2}}{N} - \overline{X}^2} \Big/ \sqrt{N-1}$.

† Increase in kidney wt divided by increase in seminal vesicles and prostates wt.

‡ This steroid was at only .2 mg/g of food because of unavailability of further material.

absence of a functional group in the 3 position, *e.g.*, 17-methylandrostan-17β-ol resulted in a marked decrease in androgenic, but not renotrophic, activity.

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Fetal X-Irradiation and Fertility.* (19634)

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It is important to determine, with experimental animals, the effect on subsequent fertility of high but sub-lethal exposures of the fetus. For this study the Swiss mouse was used, the pregnant female receiving chronic exposures for 6 days prior to delivery. The offspring were then tested at 6 months (the height of normal reproductive activity) for fertility. There have been numerous reports of the effect on the mammalian gonad of di-

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rect x-irradiation (Bergonie and Tribondeau (1), Eschenbrenner *et al.*(2-5),) and in the earlier reports the primary spermatocytes were regarded as particularly radiosensitive but Regaud and Blanc(6), Schintz and Slotopolsky(7), Wigoder(8), Metz(9), and Lacassagne and Gricouroff(10), claim that the spermatogonia are the more radiosensitive. In general, the ovary was regarded as less sensitive (Blass)(11) until recent studies, possibly for the reason that the histological changes in the ovary are less obvious. Nevertheless, histological and physiological changes in the adult ovary have been described following direct x-irradiation (Regaud and Lacassagne(12), Brambell *et al.*(13), Goldstein (14), Murray(15), Rock *et al.*(16), Peck *et al.*(17), van Eck-Vermande(18), Halberstaedter and Ickowica(19)). Brambell and Parkes(20), found a compensatory hypertrophy of the untreated ovary in unilateral x-ray sterilization and recently Furth(21) and Gardner(22), described the induction of ovarian tumors by x-rays. X-irradiation has been used to produce temporary or permanent sterility in experimental animals and in human subjects (Dantchakoff and Lacassagne(23), Glucksmann(24), Mason and Shaver(25), Shaver and Mason(26), Russell(27), Kaplan (28), and Metcalf and Inda(29)). Kaplan claims that x-irradiation sterilization of the human female need not be permanent, and that females may be temporarily sterilized and yet subsequently become pregnant and deliver what he terms as normal offspring (Kaplan)(28). He did not consider the genotypic implications as does Muller(30). Other studies on offspring of x-irradiated parents have been made (Doderlein(31), Murphy (32), Snell and Ames(33), Henson(34), and Nicolle(35)). Curiously, in the face of these studies, x-irradiation has been used in a few instances to promote ovulation (Haman)(36).

In this study it was decided to give the mouse fetus 6 daily exposures, each equivalent to a substantial fluoroscopic examination (50 r) and totalling a dose which was known to be tolerable (300 r). It was further decided to test the mice for fertility at one time only, namely 6 months, in order to avoid the possible stimulation of the gonads by prior reproductive activity. The results of this study are presented below.

Materials and method. Ten pregnant Swiss mice were given whole body x-irradiation to 50 r for each of 6 consecutive days beginning on the 14th day of gestation. (50 r is comparable to an extended fluoroscopic examination). This meant a total accumulated exposure of 300 r before parturition, a dose which was known to have a slight stunting effect on the offspring if given at one time late in pregnancy. By fractionating the dose in this manner apparently normal litters were produced without any visible effects on size or viability. Ten similarly pregnant female Swiss mice were maintained as controls. At the normal time of weaning (4 weeks of age) the males and females were separated into groups of 5 per cage, all similarly treated, and were maintained for 6 months on the standard laboratory diet. At the end of the 6 months period, at a time when one expects a normal mouse to be at the height of its reproductive activity, all surviving mice were tested for fertility. A total of 45 experimental (x-irradiated) and 58 control offspring were so segregated at weaning and maintained. The test for fertility for each male was made against 2 normal females, each of which was known to have had a previous litter by a normal male. The test for the female was exposure to 2 males, at successive 5-day intervals, each male having been previously used to produce a normal litter with a normal female. Since the mouse has a 4½ day estrous cycle, 2 males successively available to the female over a 10-day period should certainly have tested its fertility. The x-irradiation facilities were the Westinghouse Therapy machine of this department, regulated to 210 kv and 15 a, at a target distance of 50 cm and output of 96.2 r per minute with filtration of 0.28 mm Cu and 0.50 mm Al.

Experimental data and observations. All mice, controls and experimental, maintained for 6 months will show some mortality. All x-irradiated pregnant females were thereby made subsequently sterile. Of the 58 control offspring 51 or 88% survived. Of the 45 x-irradiated offspring 40 or 89% survived. Irradiation under these conditions did not

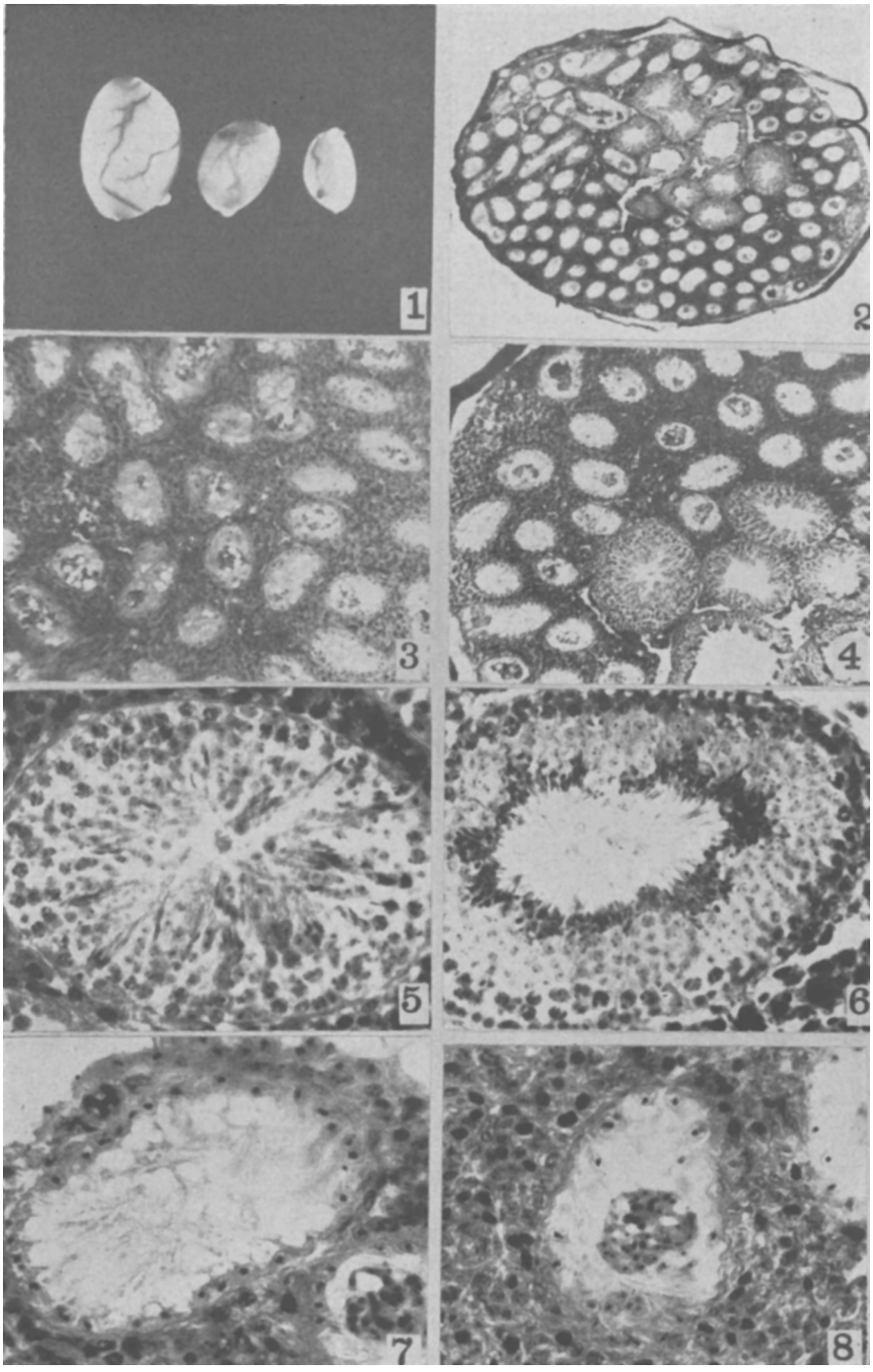

FIG. 1. Whole testes excised and photographed fresh. Control on the left, x-irradiated but fertile in center, and x-irradiated but sterile to the right.

FIG. 2. Central section of sterile testes showing about 10% of the tubules containing maturation stages. Note amount of interstitial tissue in sterile and non-sterile portions of the section. Hyperplasia of the interstitial tissue is absolute but the impression is accentuated because of large size of the non-sterile tubules as compared with those that are completely sterile.

FIG. 3. Completely sterile portion of x-irradiated testes.

FIG. 4. Enlarged view of sterile and non-sterile portions of Fig. 2 testes.

FIG. 5 and 6. Seminiferous tubules containing maturation stages in x-irradiated but fertile testes.

FIG. 7 and 8. Completely sterile seminiferous tubules in x-irradiated testes showing intact Sertoli cells and interstitial tissue, the latter being increased in amount. In Fig. 8 note accumulation within the lumen of a few loosened Sertoli cells and probably some abortive early maturation stages.

alter survival. Of the controls, 27 out of 30 males and 24 out of 28 females survived while among the experimentals all of the males and 13 of the 18 females survived. Male survival among the x-irradiated offspring was therefore somewhat better than female survival, but this probably has no significance because of the small numbers. The surviving control mice were slightly heavier than the x-irradiated experimentals.

The fertility tests resulted as follows:

Controls: 51—all fertile			
X-irradiated:			
Males 27	Females 13		
Fertile 5	Fertile 9	(2 with abnormal litters)	
Sterile 22	Sterile 4		

The gonads of all experimental animals and 12 controls (6 males and 6 females) were excised and fixed for histological analysis.

It was noted immediately that there was a wide range of sizes among the testes of the x-irradiated males (Fig. 1), but no such size variable was seen in the ovaries. The smallest testes were invariably found in the sterile x-irradiated males (Fig. 3, 7, 8) while the fertile but x-irradiated males had testes of intermediate size between those that were sterile and the controls (Fig. 1). There was always some size reduction in all x-irradiated testes. It was estimated that the volume of the completely sterile testes was roughly 25% that of the controls, with the maximum length being reduced from some 10 mm to 3 mm (Fig. 1). The size variation among the testes of x-irradiated males was correlated with the degree of histological integrity, the larger ones being the more normal. One could say that if the testis was reduced to below 40% of its original (normal) size, it would be sterile even though it could produce some spermatozoa (Fig. 2). If the testis is somewhat above 40% the volume of the control testis, it would, in all probability, be able to produce a sufficient number of viable spermatozoa to be considered as fertile (Fig. 5 and 6).

Histological analysis. Control testis of the mouse at 6 months is perfectly normal for the mammalian gonad at the height of its activity. It contains approximately 100 seminiferous tubules in any central cross section, each showing maturation stages and most of them showing all stages from spermatogonia to mature spermatozoa. The tubules are approximately of the same size in cross section, and are clearly delineated from each other so that in fixed material they appear to be separated by spaces. This is, in part, because of the relative sparsity of the interstitial tissue in the controls. In the testes of mice found to be sterile 6 months after x-irradiation *in utero,* never more than 10% of the seminiferous tubules could in any way be compared with the controls with respect to cellular constituents (Fig. 2 and 4). When one counts the completely sterile tubules (*i.e.,* devoid of maturation stages of any sort) and compares them with the tubules which contained recognizable maturation stages, one gets a range of ratios from 2/103 to 10/91 or approximately 2 to 10%. The few normal appearing tubules are generally bunched together, often toward the center of the testes (Fig. 4).

Most of the completely sterile tubules are devoid of all maturation stages but do retain their Sertoli cells in which the nuclei appear as (generally 3) beads linearly joined (Fig. 3, 7, and 8). The cytoplasm is clear and the cell is rounded rather than elongate as in the control tubules. A few of the tubules in the sterile testes may be completely devoid of the Sertoli cells and contain only a fibrous debris. There is sometimes a coagulum within the lumen of some of these sterile tubules, the coagulum consisting of some loosened Sertoli cells and unrecognizable cells which may be abortive maturation stages (Fig. 8). The

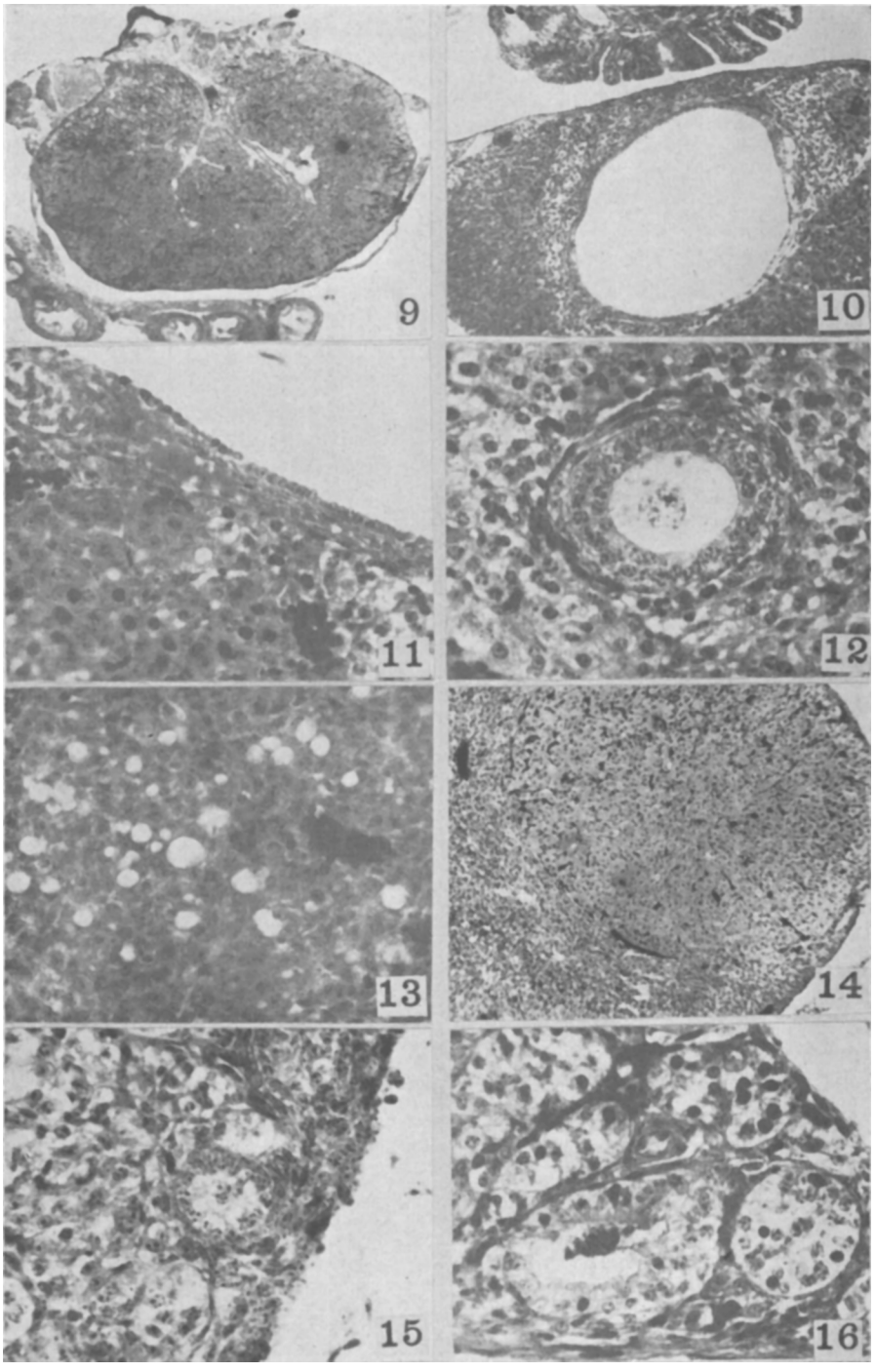

FIG. 9. Low power view of central section through completely sterile ovary.

FIG. 10. Enlarged view of cyst found in sterile ovary.

FIG. 11. Marginal germinal epithelium of x-irradiated ovary appears normal but the underlying tissue does not contain germinal nests.

FIG. 12. Partially developed but abnormal follicle containing ovum in x-irradiated mouse from whom 2 aborted and abnormal fetuses were delivered following mating with normal male.

FIG. 13. Fatty degeneration in x-irradiated ovarian stroma.

FIG. 14. Ill-defined corpus luteum in ovary from which abnormal fetuses were delivered.
FIG. 15. Anovular rings and irregular germinal epithelium on x-irradiated ovary, completely sterile.
FIG. 16. Germinal nests which do not develop further in x-irradiated ovary, incompletely sterile.

cells of the interstitial tissue appear to be undamaged in x-irradiated testes so that one might suppose that the conditioned secondary sexual characters of these sterile mice are normal. The interstitial tissue of the sterile testes is increased in amount relative to the other tissues of the testis as determined by the Chalkley method(37). This observation is accentuated by the closer proximity of the acellular and collapsed tubules. The Chalkley method allows one to utilize the micrometer eye piece, taking each of the 10 figure numbers as points, and the ratio of interstitial to all other types of tissue in the testes can be determined by random sampling. This was done for 25 samples from each testis examined. In the controls this amounted to approximately 15% while in the completely sterile regions of the sterile testes, the interstitial tissue amounted to 44%. This is most clearly illustrated in the sterile testes in which there are clustered tubules containing maturation stages among sterile tubules (Fig. 2 and 4). Eschenbrenner *et al.*(3) found a similar increase in the relative amount of interstitial tissue in testes of adult mice exposed to low dose chronic x-irradiation, but no absolute increase in the interstitial tissue. Whether the cells are all of the same original type (and function) would be difficult to determine, but at least it is all interstitial and not fibrous.

The testes of the x-irradiated but fertile mice can always be recognized in sections by the presence of some tubules completely devoid of maturation stages, generally located at the periphery of the organ. Actual counts showed that the number of sterile over the number of fertile tubules in random centrally located sections of such testes ranged from 9/97 to 52/42 or approximately 9 to 53% sterile tubules. In the latter testes the general impression is of a rather normal organ. The cellular tubules are large and centrally placed while the sterile tubules are very small and located around the periphery, for the most part. One might predict, therefore, the (x-irradiation produced) sterility in these mice will obtain when the number of fertile tubules (*i.e.*, tubules containing recognizable maturation stages) does not go much above 10% and that fertility may be expected from those testes in which something over 40% of the tubules contain maturation figures. Of course, mammalian fertility is somehow related to the production of large numbers of viable spermatozoa, even though the litter may be small, so that a single normal tubule in a testis, producing hundreds of apparently normal spermatozoa, is not sufficient (quantitatively) to insure fertility. This study of the testes casts no light on the matter of specific sensitivity of the various maturation stages because the animal was exposed to x-irradiation during its fetal life when the germ cells were undifferentiated. In most of the sections, even those of sterile mice, there were some tubules containing all maturation stages and this presumes only incomplete inhibition of the normal process of maturation even though a functional number of sperm were not produced.

The ovaries. Size variations in control and x-irradiated ovaries suggest that the sterile ovaries are the smaller, but this would be difficult to quantitate. In the sectioned material the control ovaries at median level showed approximately 14 developing follicles, and 4 to 8 corpora lutea. In contrast, the sterile ovaries of mice x-irradiated *in utero* and examined at 6 months of age contained no follicles of any sort (Fig. 9), and only occasionally small nests of cells that might be arrested follicles (Fig. 15). The sterile ovary consists of a condensed ovarian stroma, interspersed with evidences of fatty degeneration (Fig. 13) and an occasional enlarged cyst (Fig. 10). There is germinal epithelium (Fig. 11), but the usual picture of subepithelial germinal nests is completely abnormal (Fig. 11). In the ovaries of x-irradiated mice which proved to be partially fertile in that they produced fetuses which were de-

formed and dead at birth, there were ill-defined and arrested follicles (Fig. 12), some sub-epithelial germinal nests (Fig. 16), and some slight evidences of corpora lutea (Fig. 14). The fertile but x-irradiated ovaries appeared quite normal except for the apparent reduction in the number of developing follicles with ova.

Summary and conclusions. 1. Mice x-irradiated *in utero* to 300 r accumulated in 6 exposures of 50 r each on 6 consecutive days beginning on the 14th day of gestation, reveal various degrees of sterility at 6 months, the period of peak reproductive activity in the mouse. The 10 pregnant females, producing these litters, were all made sterile by this x-irradiation. 2. Within any single litter, there were variations in response of fetuses indicating, possibly, a slight spread in the developmental stage of the gonad primordia at the instant of x-irradiation. Certainly one can hardly visualize a dosimetry sufficiently different within a fetal litter to account for the variations in response. 3. Testes sterility was correlated with the relative number of seminiferous tubules in which maturation stages occur. In those testes where 40% or more of the tubules contain maturation figures, the male was fertile. The completely sterile testes contained 10% or less of tubules having maturation stages. 4. The precursors of the Sertoli cells, interstitial tissue and ovarian stroma are radioresistant. The primordia of the maturation stages and ovarian germinal nests are definitely radiosensitive. It must be emphasized again that at the time of x-irradiation these stages were not yet differentiated so that the sensitivity is related to the primordia of these various cell types, and not to the fully formed cell types. 5. The testes are reduced in size to a degree correlated with the failure of the seminiferous tubules to develop maturation stages. Consequently there is sterility. This failure to develop is accompanied by an apparent relative increase in the interstitial tissue within the testis from some 15% in the controls to 44% in the completely sterile testes. 6. A sub-lethal exposure of the mouse fetus to 300 r will produce sterility in roughly 2/3 of all offspring, with the slightly greater sterility among the males but a greater number of first generation teratologies among the females, mated to control animals. The genetic sequelae of such x-irradiation exposure, while undoubtedly of serious moment, are not considered in this study.

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Lack of Correlation Between Dietary Level of Manganese and Growth Rate of Induced Fibrosarcomas.*† (19635)

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Recently we have demonstrated an effect of the level of dietary manganese on growth in young rats(1). Earlier Maxwell and Bischoff(2) reported that "no effect on tumor growth was noted" in 7 carcinoma-bearing rats that were given intraperitoneal doses of 8-10 mg/kg of Mn $(C_2H_3O_2)_2$ at 3- to 4-day intervals until 45-50 mg Mn/kg had been given. These authors observed, however, that there was less liquefaction of the tumors in the "dosed" animals in the later stages, and that "the occurrence of regressions was also somewhat higher in the dosed series. . . ." Ferguson, Templeton, and Patras(3) reported that "spindle cell sarcoma" transplants grew only about 75% as rapidly in rats on a diet from which the usual "salt-mixture" was omitted. This salt mixture contained small amounts of $MnSO_4$. Popoff (1941)(4) found that minute amounts of Mn, Li, and I_2 added together to the diets of tumor-bearing mice decreased the growth-rate of the tumors to 1/3 or 1/4 of the usual rate. Thus we have one group of investigators(2) reporting "no effect" on tumor-growth from the intraperitoneal administration of extra manganese to tumor bearing rats; a second group (3) reporting a decrease in tumor-growth when the salt-mixture containing manganese is *omitted* from the diet; and a third group (4) reporting that minute amounts of Mn, Li, and I_2 *added* to the diet cause a decrease in the growth-rate of tumors in mice. In all except the first of these experiments, other factors in addition to Mn were varied.

In view of the inconclusive nature of these reports with respect to the growth of tumors, and in view of our demonstration(1) of the effect of the dietary level of Mn on the growth-rate of normal tissue, we have thought it worthwhile to investigate the growth-rate of methylcholanthrene induced-sarcomas of the thigh muscle of the rat with respect to the dietary Mn level.

Experimental. The rats used in these experiments were 30-60-day-old females from a strain that has been inbred at this institution for more than 20 years. In numerous earlier experiments we have shown that the injection of single doses of methylcholanthrene into the posterior muscles of the thigh by our technic produces fibrosarcomas in more than

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