STUDIES ON X-RAY EFFECTS.

X. The Biological Action of Small Doses of Low Frequency X-Rays.

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Plates 32 and 33.

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Attempts have been made in this laboratory to compare the biological action of the soft or low frequency and the hard or high frequency x-rays, but the impossibility of comparing doses of the two types of rays with our present apparatus and measuring devices has rendered the results of these studies of little value. However, by varying the doses of the soft and hard rays there is one point which seems definite; namely, that with the softer rays it is possible to induce an apparent stimulation of the lymphoid cells which is preceded by only a very short and transitory period of depression.[1–4] With the harder rays the stimulation phase is less pronounced, if present at all in the dosages employed by us. Russ, Chambers, Scott, and Mottram[5] have succeeded in bringing about an increase in the circulating lymphocytes of rats by repeated small exposures to x-rays described by them as being of "medium" or "medium soft" quality. Our first satisfactory results were obtained with the tube operated at a spark-gap of about 1½ inches. Later experiments with a spark-gap of about ⅞ inch gave a more uniform and pronounced reaction.

The present study has to do with the effect of still softer x-rays than those used in previous experiments. For the generation of these

[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[2] Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75.

[3] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 83.

[4] Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1920, xxxi, 13.

[5] Russ, S., Chambers, H., Scott, G. M., and Mottram, J. C., *Lancet*, 1919, i, 692.

rays a special water-cooled tube has been devised with a window of thin glass which will allow the passage of rays usually held back by the thicker glass of the standard tubes.[6]

Production of Lymphocytosis.

The following experiments were planned to determine the time of exposure to the soft rays necessary for the production of a maximum stimulation of the lymphoid system of mice.

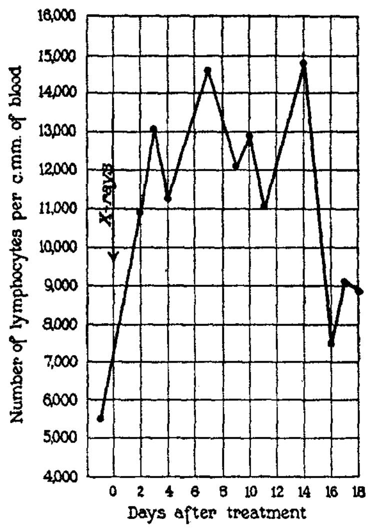
Experiment 1.—Forty-five white mice were divided into nine groups of five mice each. The groups were exposed to x-rays for ½, 1, 2½, 4, 5, 10, 20, 30, or 60 minutes respectively. The special tube described above was used with ½ inch spark-gap, 11 milliamperes, and 6 inches distance, for the treatment. A cardboard was placed over the animal container for protection against the heat. Blood counts were made on all of the animals before and 1 week after the treatment. The mice given the 1 minute exposure showed a definite lymphocytosis, but no consistent change was noted in the blood picture of the other groups.

Experiment 2.—Blood counts were made on thirty normal white mice and they were then exposed to x-rays given by the special tube with the governing factors the same as in Experiment 1, the length of the exposure being 1 minute. Counts were made at intervals afterwards. The changes in the lymphocytes are shown in Text-fig. 1 by a composite curve and the results for the individual animals in Table I.

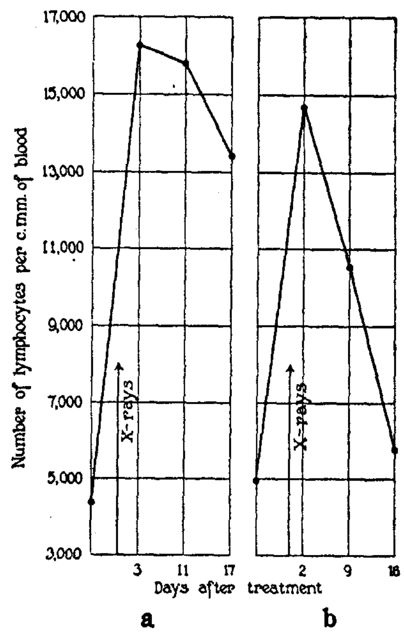
The counts showed that there was no definite alteration in the absolute number of polymorphonuclear leucocytes, but the lymphocytes were increased to a level considerably above that of the initial count. By the 2nd day these latter cells had almost doubled their number and continued to increase with some variations until the 14th day, after which they subsided but were still above their initial level on the 18th day. There was considerable variation in the extent and duration of the reaction in individual mice, as shown in Table I and in Text-fig. 2. In only two animals was there a drop in the lymphocytes at the time of the first count after the x-ray treatment. One of these, however, showed a reaction later with an increase of over 30 per cent, but in the other no stimulation was observed. It should be noted here that none of these counts were made soon enough after the

[6] We wish to acknowledge our indebtedness to the Research Laboratory of the General Electric Company for the design and construction of this tube.

x-ray treatment to demonstrate the amount of depression preceding the stimulation, for this point has been adequately dealt with in previous publications.[7] The increase in number of circulating lymphocytes after this dose of x-rays is of greater magnitude and more constant than that previously induced by this agent.



TEXT-FIG. 1.



TEXT-FIG. 2.

TEXT-FIG. 1. Composite curve of lymphocyte counts on thirty-five mice before and after an exposure to low frequency x-rays.

TEXT-FIG. 2, *a* and *b*. (*a*) Curve of lymphocyte counts on Mouse 23 in Table I. (*b*) Curve of lymphocyte counts on Mouse 12 in the same table.

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[7] In a recent article Mottram and Russ (Mottram, J. C., and Russ, S., *J. Exp. Med.*, 1921, xxxiv, 271) have objected to our use of the term "stimulating" doses of x-rays in describing the effect of this agent on the lymphoid tissue. They object to this term on the basis of the fact that the stimulation is preceded by a short period of depression. This point was noted in our first publication on the subject[1] and has been repeatedly referred to in subsequent articles.[4,9] The fact that with a suitable small dose of x-rays a stimulation of the lymphoid tissue is produced with only a slight and transitory depression, while with larger doses a more marked and lasting depression is induced with no subsequent stimulation, seems to us to justify the use of the expression.

TABLE I.

Mouse No.	Count before treatment.	Count after treatment. No. of days after x-ray treatment.										
		2	3	4	7	9	10	11	14	16	17	18
1	6,018				15,908				13,250			
2	6,541				11,105				17,713			8,430
3	5,897				14,455							
4	5,899				16,210				14,446			
5	5,526				15,463				14,744			
6	5,823	11,041				12,252				7,091		
7	4,617	7,658										
8	4,336	12,070				16,532				11,645		
9	6,585	17,070				10,603				8,455		
10	4,239	10,972				13,650				9,423		
11	4,288	7,604				11,766				5,471		
12	4,998	14,734				10,567				5,816		
13	7,104	7,070				12,786				7,005		
14	5,477	7,406				10,472				6,808		
15	5,185	12,878				10,332				6,520		
16	6,110		16,122				12,800				8,888	
17	6,113		12,775				16,861				7,1[illegible]3	
18	4,215		9,435				12,890				6,008	
19	4,909		12,002				7,338				5,360	
20	4,790		7,007				9,495				9,231	
21	3,714		12,064				9,304				8,232	
22	4,145		10,989				12,543				9,382	
23	4,425		16,261				15,814				13,209	

24	6,203		18,252				15,681				12,124	
25	7,155		15,352				17,055				12,836	
26	3,594			7,527				9,303				
27	9,313			7,027				8,975				9,897
28	4,428			8,815				7,196				6,675
29	5,264			3,767				7,986				6,878
30	4,404			12,452				12,870				10,178
31	3,974			15,300				15,082				
32	8,330			13,167				10,277				8,571
33	7,329			16,480				17,274				14,796
34	6,175			16,787				12,894				9,893
35	5,635			11,851				8,518				5,092
Average.........	5,507	10,850	13,026	11,317	14,628	12,105	12,978	11,037	15,038	7,581	9,245	8,934

The counts before treatment were made a number of days in advance so that no animal had more than one count in a week. For example, on the group of mice counted 2 days after treatment, the pretreatment count was made 5 days before, thus allowing a week between counts.

Histological Changes.

The material for this study was collected from twenty-six mice which were carried as a parallel series to the above experiment in which blood changes were observed. After exposure to x-rays (special tube, spark-gap ½ inch, milliamperes 11, distance 6 inches, time 1 minute) these animals were killed in groups at intervals of 24 hours, 4 days, 7 days, 10 days, and 14 days. The spleen, lymph nodes, bone marrow, thymus, thyroid, liver, kidney, suprarenal, ovary, and testis were taken for examination. Carnoy 6–3–1 solution was used for fixation and the sections were stained with Heidenhain's iron-hematoxylin for mitotic figures or with Ehrlich's acid hematoxylin and eosin.

Lymphoid Organs.—The changes in the spleen and lymph nodes were practically identical and will be described together. 24 hours after treatment there was no demonstrable change in the lymphoid organs. At the 4 day period numerous mitotic figures were found in all of these organs, a condition which our previous work indicates is associated with the increased production of lymphoid cells. This condition was found equally as pronounced in the animals killed on the 7th day after treatment. The organs from eleven mice were examined for these two periods and without exception this increase in proliferation was observed. The animals killed on the 10th and 14th days after treatment showed that the proliferative activity had subsided to about the normal rate.

Suprarenal Glands.—The suprarenals were found at autopsy to be distinctly reddish in color and somewhat enlarged. Microscopic examination showed the sinus-like spaces between the cortex and medulla to be much distended with blood, and this dilatation extended to the capillaries between the cortical cell columns, separating these columns by a wide margin (Figs. 1 and 2). The sinusoids of the medulla showed little if any modification. There was no sign of hemorrhage or of necrosis of the suprarenal tissue in any of the specimens examined. This vascular change was observed in varying degrees in all of the mice examined except those killed 24 hours after the treatment. In addition to this engorgement of the capillaries, a proportion of the animals killed between 7 and 14 days after the x-ray treatment showed a pronounced perivascular infiltration in the cortex along the fibrous capsule and often extending down to the zona reticularis along the cortical cell columns.

Other Organs.—There was no histological evidence of changes in the other organs examined after the x-ray treatment. Even the germ cells of the fourteen ovaries and twelve testes examined, cells well known to be extremely sensitive, showed no damage which could be detected microscopically (Figs. 3 and 4). In a few instances the interstitial tissue of the testis was found to be hypertrophied. Distinct perivascular lymphoid infiltration was observed in a number of the livers and kidneys examined, but these findings were not uniform enough to be of great importance. This condition is also found occasionally in normal animals.

Effect on Resistance to Transplantable Cancer.

In previous publications it has been shown that a small dose of x-rays sufficient to stimulate the lymphocytes will increase the resistance of mice to transplanted cancer.[1,8,9,10] Russ, Chambers, Scott, and Mottram[5] have shown that the same condition holds for rats. As noted above, a more pronounced reaction of the lymphoid tissue is induced by the treatment outlined here than by the dosage previously used. It is of interest, therefore, to determine the effect of the very soft rays on the resistance to cancer.

Experiments 3 to 9.—Normal white mice of about the same age and size were exposed to a dose of x-rays governed by the same factors as those used in Experiment 2, with the special x-ray tube described above. These animals were inoculated in groups from 3 to 14 days after the treatment, with a graft of a transplantable tumor (Bashford Adenocarcinoma No. 63) along with a suitable number of controls. The results of seven such experiments are given in Table II.

From these experiments it is seen that no immunity is evident when the inoculation is made as early as 3 days after the x-ray treatment, a result which corresponds with the earlier experiments in which the standard Coolidge tube was used. Among 86 mice inoculated from 7

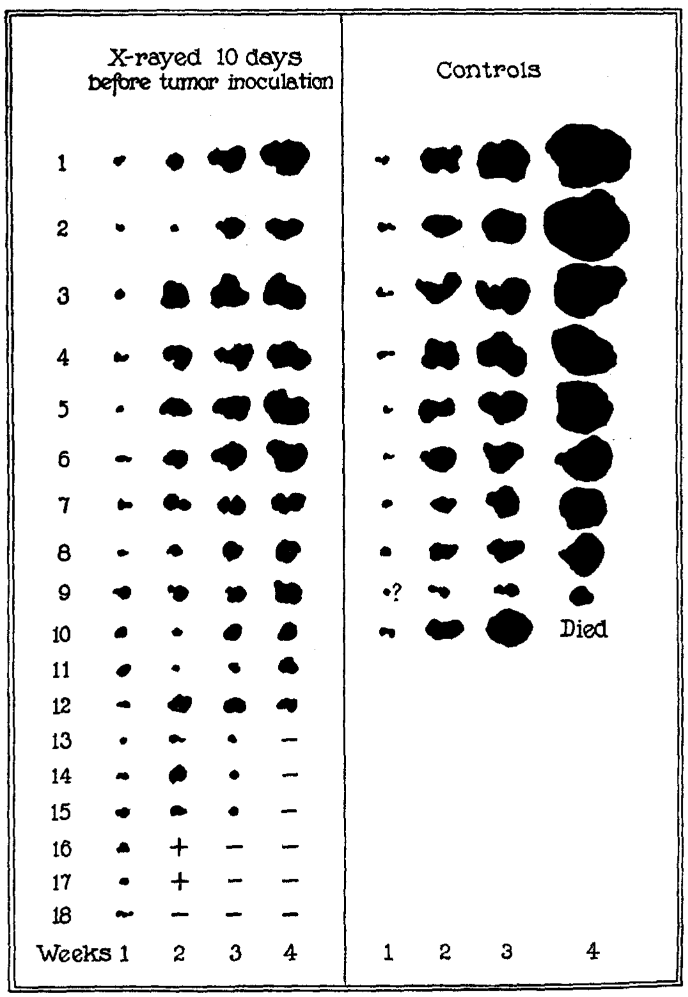
[8] Murphy, Jas. B., *Proc. Nat. Acad. Sc.*, 1920, vi, 35.

[9] Murphy, Jas. B., Nakahara, W., and Sturm, E., *J. Exp. Med.*, 1921, xxxiii, 423.

[10] Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1921, xxxiii, 429.

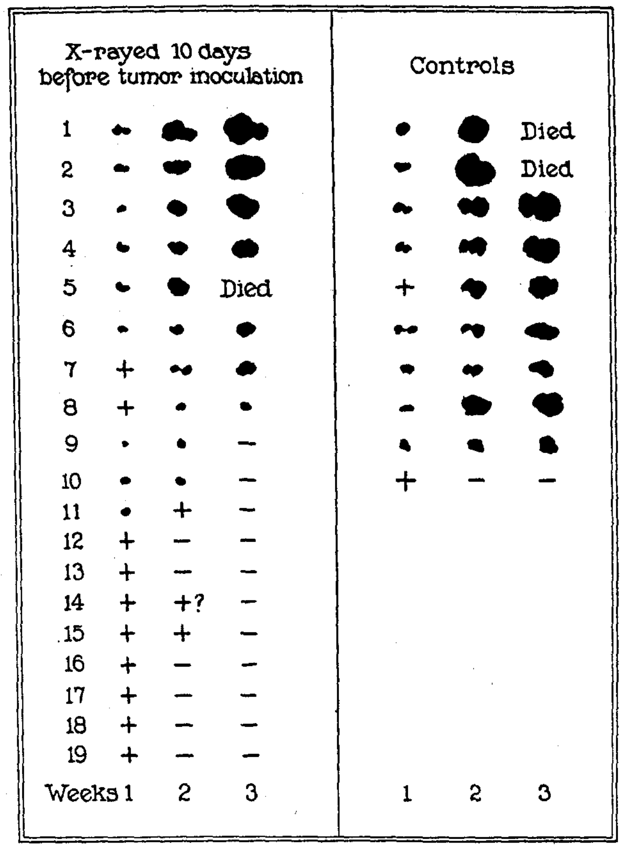
TABLE II.

Experiment No.	Interval between x-ray exposure and tumor inoculation.	Immunity in x-rayed animals.	Immunity in control animals.
	days		
3	3	0.0 per cent (9 mice).	0.0 per cent (10 mice).
4	7	30.0 " " (10 ").	11.1 " " (9 ").
5	7	28.5 " " (17 ").	9.9 " " (11 ").
6	10	37.5 " " (8 ").	0.0 " " (10 ").
7	10	33.3 " " (18 ").	0.0 " " (10 ").
8	10	57.8 " " (19 ").	10.0 " " (10 ").
9	14	20.0 " " (10 ").	0.0 " " (7 ").



TEXT-FIG. 3. Experiment 7. The rate of growth of Bashford Adenocarcinoma No. 63 in mice given a small dose of low frequency x-rays 10 days before inoculation, contrasted with the rate of growth in untreated mice.

to 14 days after the treatment, 34.4 per cent were immune, while in 57 control mice inoculated with the same tumor there were only 5.1 per cent immune. The largest proportion of resistant mice was found among the animals treated 10 days after inoculation, with 42.8 per



TEXT-FIG. 4. Experiment 8. The rate of growth of Bashford Adenocarcinoma No. 63 in mice given a small dose of low frequency x-rays 10 days before inoculation, contrasted with the rate of growth in untreated mice.

cent in 45 mice against 3.3 per cent in 30 control mice. Text-figs. 3 and 4 show that the rate of growth of the tumor is slower in the treated animals than in the controls, and that in a proportion of the treated mice tumors after a period of growth are absorbed, a condition which rarely occurs with this tumor in normal mice.

The Absorption Coefficient of X-Rays Produced by a Voltage Measured by a ½ Inch Spark-Gap.[11]

It is of considerable interest in the light of the above experiments to arrive at some idea of the amount of penetration of the soft rays. The very soft rays produced by a voltage measured by a ½ inch point spark-gap were found to be practically homogeneous with a mass absorption coefficient equal to 3.4 for water. This value includes the mass-scattering coefficient usually taken as 0.2.

The value was measured photographically. A series of small areas of a single film was exposed to the rays for various times. A second series on the same film was exposed for various times to rays which had passed through layers of water of various thicknesses. After development the areas of the second series were matched for blackness against those of the first series and the relative intensities of the rays transmitted by the water thus discovered. A preliminary experiment gave the value for the absorption coefficient which was used to calculate the exposures through various depths of water necessary to give equal degrees of blackening. Another film was made accordingly and the exposed areas were found to be of uniform density. It will be noticed that, according to the quantum law, the wave-lengths produced by a ½ inch gap are in the neighborhood of the K characteristic absorption discontinuities of the silver and bromine of the plate. In spite of this it can be shown that the constant value found for the absorption coefficient is a proof of homogeneity.

The mass absorption coefficient of these rays in animal tissues may be assumed to be somewhat smaller than for water. A mouse skin weighing 0.2 gm. per cm. should stop less than one-half of the rays.

The relative amount penetrating various depths of water when this water-cooled tube is operated at ½ inch spark-gap is as follows:

Depth.	Intensity.
cm.	
0	1.0 (taken as standard).
¼	0.43
½	0.18
¾	0.076
1	0.032
1¼	0.0133
1½	0.0056

[11] We are indebted to Dr. Harry Clark for the measurements recorded.

DISCUSSION.

The comparison of the biological effect of the hard and soft rays is a matter of considerable interest at the present moment but no really satisfactory standard of measurement is available. Such comparisons, therefore, must await a further understanding of the complex nature of the biological changes and the underlying factors bringing about these changes.

As far as our present knowledge extends the only known physical or chemical change induced by x-rays depends on the power of this agent to ionize. If ionization be the underlying factor responsible for the biological changes, it is necessary to determine why some types of animal cells are so profoundly affected in the absence of demonstrable changes in other cells. However, the solution of such problems as this must await further development in biophysics.

In this communication evidence is presented of a biological change induced by a small dose of the very soft x-rays; namely, a stimulation of the lymphoid cells preceded in all probability by a small amount of destruction. We have not been able to induce a reaction of this nature with the harder rays given in varying dosage. Until it is determined that the dosage is the same it will not be possible to say whether this apparent difference in biological action of the soft and hard rays is a real one.

Considering the smallness of the dose, the fact that 57 per cent of the x-rays used here is absorbed in the first $\frac{1}{4}$ cm. of tissue and that only 3.2 per cent penetrated to the depth of 1 cm. strongly suggests that the changes in the lymphoid organs are not the result of the direct action of the x-rays. The fact also that the deeper nodes react as much as the more superficial ones strengthens this idea. The significance of the changes in the suprarenals can only be determined by further study. It is noteworthy that the testicle and ovary, organs supposed to be extremely sensitive, were unaffected by this treatment.

The virulence of the strain of tumor used to test the resistance of the mice was such that the so called natural resistance was almost completely obviated, a fact which renders the result of the inoculation of the treated mice the more striking.

SUMMARY.

A study has been made of the biological effect of a small dose of soft x-rays given off by a special water-cooled tube with a window of thin glass, operated at $\frac{1}{2}$ inch spark-gap and 11 milliamperes. Mice exposed for 1 minute show 2 days later in the blood an increase in the number of lymphocytes and in the lymphoid organs an increased number of mitotic figures. There occurs also a marked dilatation of the vessels of the suprarenals, particularly between the cortex and medulla. The latter condition did not appear until after 24 hours and was still present 14 days after the treatment. No change was detected in other organs.

Mice treated in this way showed a high degree of resistance to cancer transplants. The amount of resistance varied with the time of the inoculation after the treatment. The resistance was not increased before 3 days after and was at its highest point 10 days after the treatment.

EXPLANATION OF PLATES.

PLATE 32.

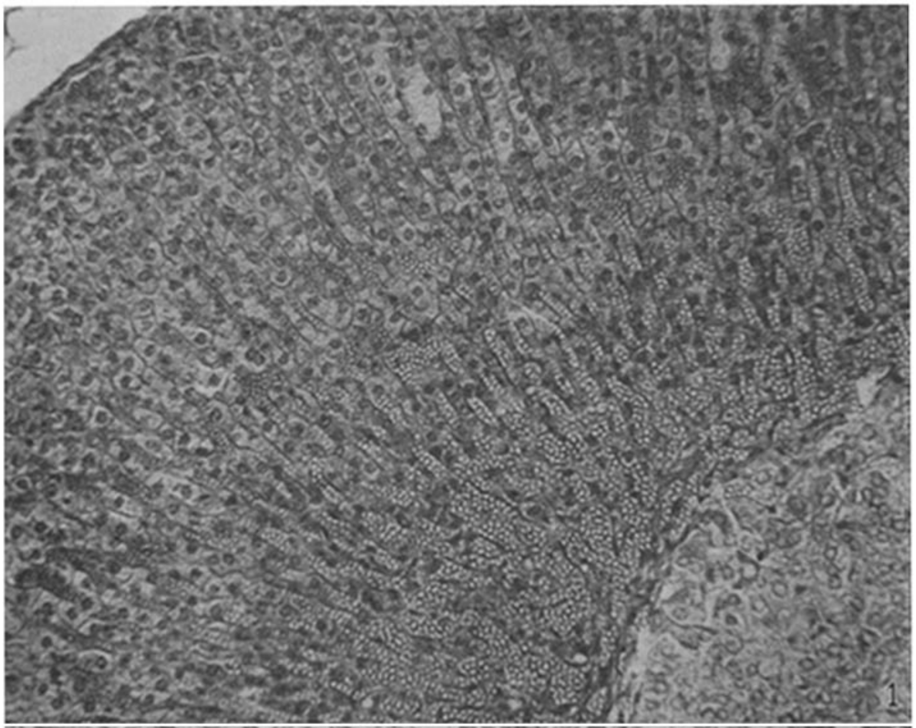
FIG. 1. Suprarenal gland of mouse 7 days after an exposure to low frequency x-rays, showing dilatation and engorgement of capillaries.

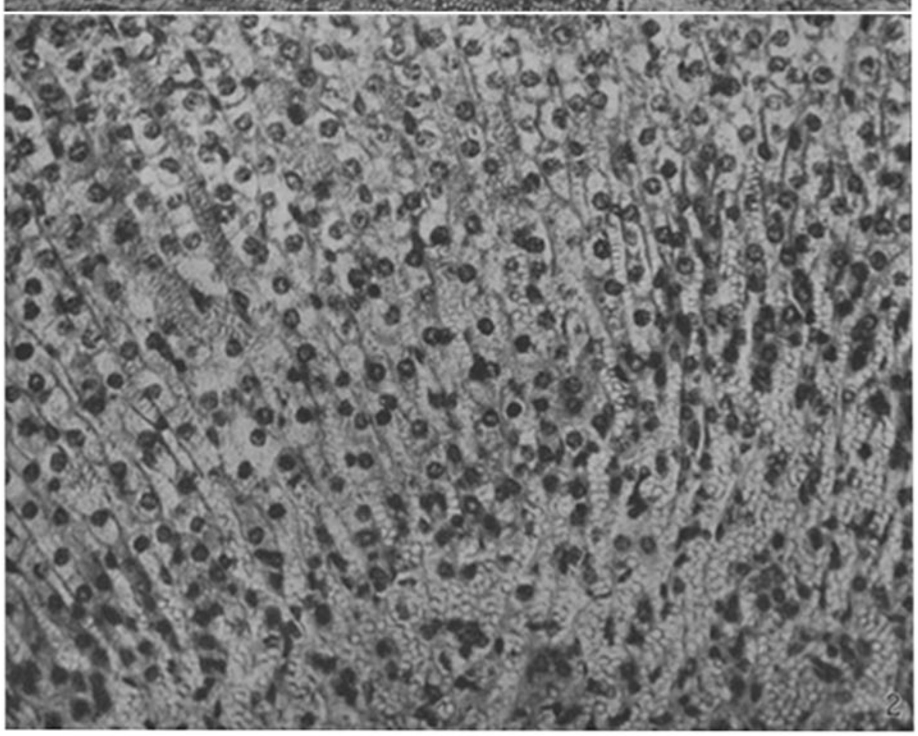
FIG. 2. The same as Fig. 1; higher power view.

PLATE 33.

FIG. 3. Testis of mouse 24 hours after an exposure to low frequency x-rays. Spermatocytes in the periphery of follicles in the so called syncopic stage. In the large follicle to the left are seen several examples of the first spermatocytic division.

FIG. 4. Ovary of mouse 24 hours after an exposure to low frequency x-rays.





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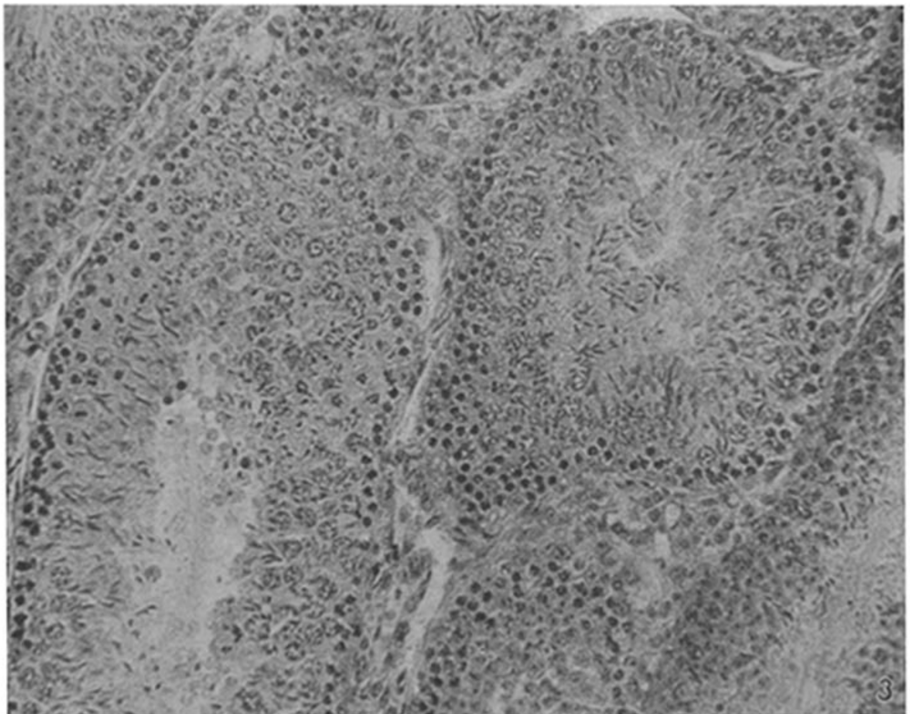

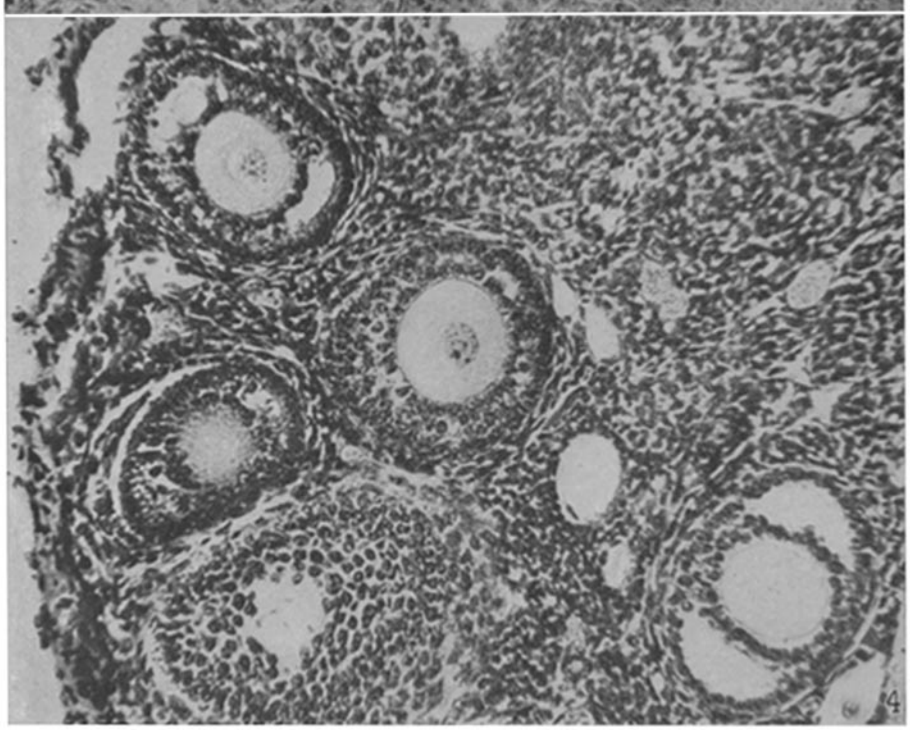

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