SOME EFFECTS OF RADIUM RADIATIONS ON WHITE MICE.

By KANEMATSU SUGIURA AND GIOACCHINO FAILLA.

(*From the Harriman Research Laboratory, The Roosevelt Hospital, and Memorial Hospital, New York.*)

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It is well known that radium rays, as well as x-rays, are capable of affecting the living cell. Bergonié and Tribondeau[1] from extensive experimental studies made the generalization[2,3] that: "Immature cells and cells in an active state of division are more sensitive to the x-rays than are cells which have already acquired their fixed adult, morphological or physiological characters."[2] Subsequent observations have resulted in the extension of this generalization to the effect of radium radiations as well. For this reason the mice of the experiments described in the present paper were exposed to radium rays soon after birth and during the period of rapid growth.

Experimental.

The body weight of mice at birth and their subsequent rate of growth are influenced by many factors. It is essential, therefore, that control experiments be carried out with animals as nearly as possible like those treated; and that external conditions be identical for both groups during the period of observation. The age, physical condition, and body weight of the mother, the length of the gestation period, the size of the litter, and its position in the litter series affect the inital weight of mice and their growth. Accordingly, a litter of young was divided into two nearly equal groups of both sexes; one to be exposed to radiation and the other to be used as control. The method devised

[1] Bergonié, J., and Tribondeau, L., *Compt. rend. Acad.*, 1906, cxliii, 983.

[2] Colwell, H. A., and Russ, S., Radium, x-rays, and the living cell, London 1915, 253.

[3] Béclère, M., Cottentot, P., and Laborde, S., Radiologie and radiumtherapie, Paris, 1921, 223.

by Jackson[4] for albino rats was used to determine the sex of the new born mice. Any underdeveloped animals were discarded, but otherwise the litter was divided into the two groups at random. In this way individual inequalities would be divided fairly evenly between the two groups. It was found, in fact, that the average weight of the mice of one group from day to day was very nearly the same as for the other group, when neither was treated with radium.

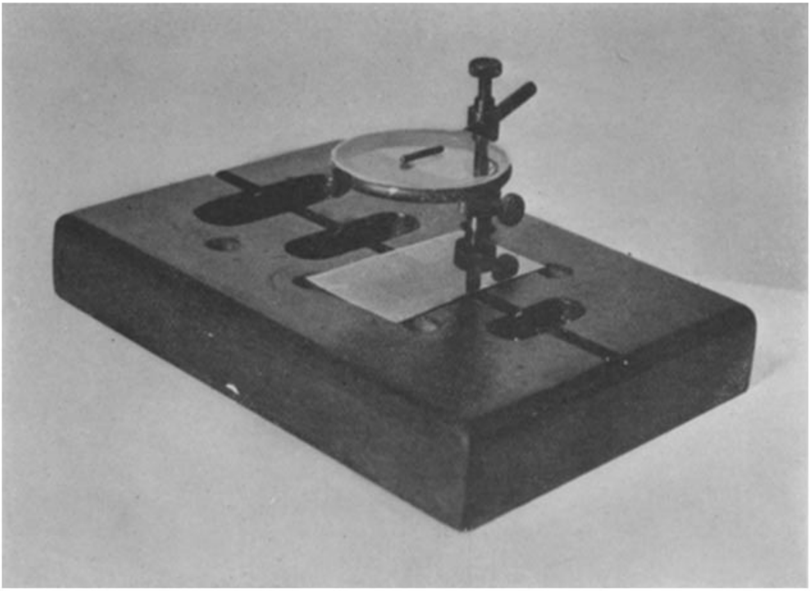

FIG. 1.

The apparatus of Fig. 1 was used to irradiate the mice, one at a time. The new born animal was placed in a cavity slightly larger than itself and was covered with a very thin sheet of mica. In this manner the mouse remained in a fixed position during the treatment, with its back touching the mica. A capillary glass tube 10 to 14 mm. in length, and about 0.5 mm. in external diameter, containing radium emanation, was placed on another sheet of mica which was rigidly supported at a distance of 2 cm. from the animal. The holder was

[4] Jackson, C. M., *Biol. Bull.*, 1912, xxiii, 171.

TABLE I.

Experimental conditions, and doses of radiation.

Experiment No.	No. of mice radiated.		No. of controls.		Part of body radiated.	Distance between animal and tube.	Duration of daily exposure.	Strength of emanation tube used.												Total dose.
	Male.	Female.	Male.	Female.				1st day.	2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.	12th day.	
						cm.	*min.*													*millicurie hours*
1	1	2	2	2	Back.	2	5	4.3	6.0	5.2	4.0	3.8								1.9
2	2	2	2	2	"	2	5	5.4	4.5	6.7	6.7	5.6								2.4
3	2	2	2	1	"	2	5	5.0	6.0	5.0	5.0	4.5	7.5	4.8	5.0	4.0	3.4	2.9	6.0	4.9
4	1	2	1	1	Head.	2	5	10.5	9.0	7.6	6.4	5.4	8.0	6.7	8.0	6.9	7.5	6.3		6.8
5	4	2	2	3	Back.	2	5	14.5	12.2	14.2	11.0	11.0	11.0	10.5	11.0	10.0	10.0	9.0	12.0	11.5
6	1	1	1	3	Head.	2	5	27	23	29	30	32	33	31	26	32				21.9
7	1	3	1	3	"	2	5	30	33	28	30	33	31	28	30	28	27	23		26.8
8	2	2	1	1	Back.	2	5	48	48	48	53	45	38	54	45					31.6
9	2	2	1	2	"	2	5	65	40	34	29	50	50	50	62	58				36.5

adjusted so that the tube was either directly over the central dorsal region or over the head of the animal. The time of exposure was always 5 minutes. The amounts of emanation employed and the number of treatments given on successive days are shown in Table I.

To identify the controls from the radiated mice in the same litter a small piece of tail was clipped off from the specimens of one group. The mice of each group were weighed together for the first time within 12 hours of the time of birth, and then every 24 hours until the 21st day, when the young were suckled. From this time on the mice were weighed individually about once a week. For any group the average weight for one mouse was calculated. A careful record was kept of the physical condition, body weight, and hair development for the different mice; also the time when they opened their eyes or reached sexual maturity, and the duration of life.

The biological effect of radium radiations is related to the amount of radiant energy absorbed by the tissue. This depends on various factors, of which the quality of radiation used and the distance of application are the most important ones. At the present time there is no satisfactory unit in which to express the dose of radiation administered. In these experiments radium emanation enclosed in capillary glass tubes of a definite size was used as the source. The quality of the radiation employed, mainly β-rays, therefore, was always the same, and the animal was placed at a distance of 2 cm. from the emanation tube for every treatment. Under these conditions the dose depends only on the amount of emanation in the tube and the duration of the application. The former is given in millicuries and the latter can be expressed in hours; the product of the two (millicurie hours) in this case may be taken to represent the relative doses. It should be noted in this connection that in each case the millicurie hour dose appearing in Table I is the total administered during the whole period of treatment in fractional daily doses.

The typical effects of different doses are shown graphically in Fig. 2. Here the body weights of the radiated mice and their respective controls are given for a period of 8 weeks, this being the time during which the maximum radium reaction is likely to occur. It will be seen that a dose of 2.4 millicurie hours, fractionally applied, in the manner previously described, accelerated the growth of the mice.

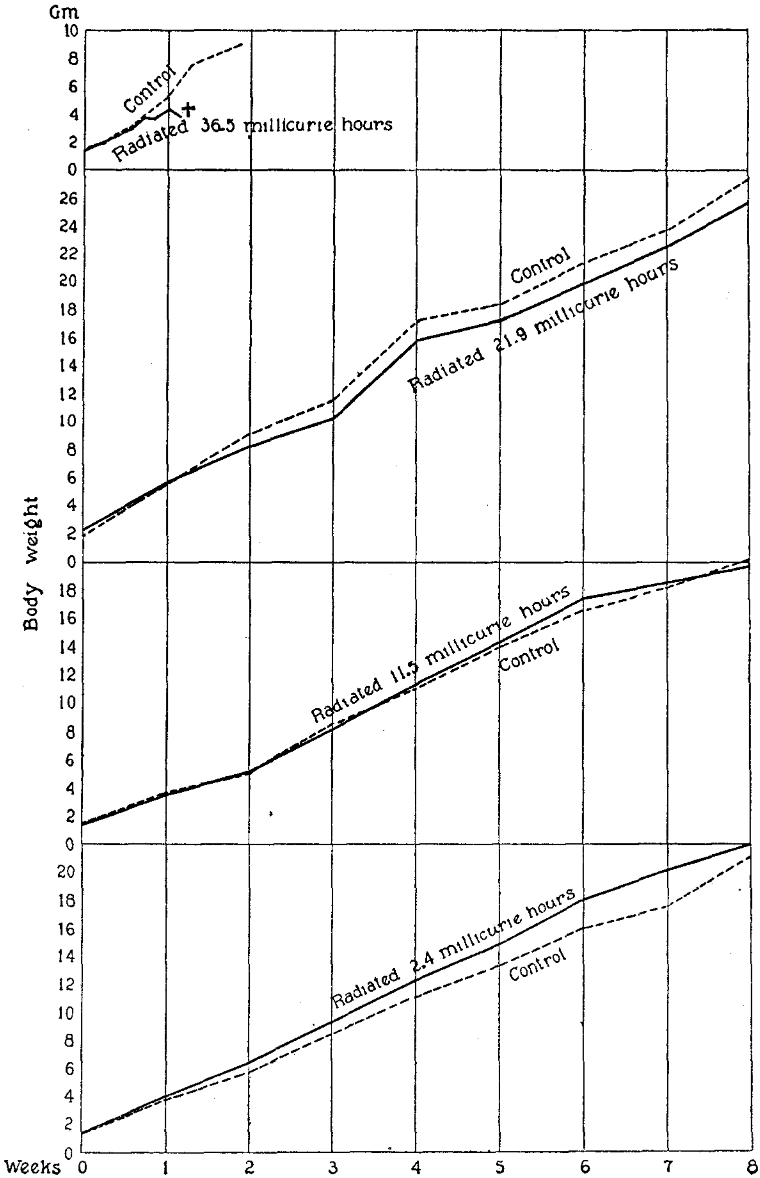

FIG. 2. Charts showing effect of radium radiation on the growth of mice. Source of radiation, one tube of radium emanation, 14 mm. long and 0.5 mm. in diameter. Distance of source, 2 cm. No metallic filter. Divided doses are shown in Table I.

The weight of the radiated mice remained distinctly larger than that of the controls until about the 27th week. After this time the average weight of the two groups was substantially the same. A dose of 11.5 millicurie hours had practically no effect on the body weight of the mice. A dose of 21.9 millicurie hours, however, had a marked influence on the growth of the radiated mice. They remained distinctly smaller than the controls for several months, but finally (in the 45th week) their average weight was the same as that of the control group. The mice which received a dose of 31.6 millicurie hours were affected very seriously by the radiation. Their growth was greatly retarded and they died on the 12th day. The effect of a dose of 36.5 millicurie hours was even more marked, and the mice died on the 9th day.

The results just described may be summarized as follows: (*a*) Sufficiently small doses of radiation accelerate the growth of suckling white mice. (*b*) A larger dose of the proper value will have no influence on the body growth of mice. (*c*) A still larger dose, up to a certain limit, will retard growth, but the animals will eventually attain normal size. (*d*) Still larger doses cause premature death. Similar results have been obtained before in experiments on seeds and plants,[5,6] also on lower forms of animal life exposed to x-rays.[7,8] From these it is commonly assumed that the action of radiation on the living cell follows the same general law[9] which governs the action of all anesthetics, as well as chemical, mechanical, and electrical stimulants; that is, if some form of energy is gradually brought to bear on the cells, at first they may be stimulated to greater activity, then their normal function may be arrested, and finally they may be destroyed.

As a result of the mode of application of the radium emanation adopted in these experiments, the upper part of the body of the animals was exposed to an intense radiation. The lower part received much less radiation, not only because it was farther away from the source, but also because the overlying layers of tissue absorbed most of the

[5] Gager, C. S., Memoirs, New York Botanical Gardens, 1908, iv.

[6] Molisch, H. C., *Sitzungsb. k. Akad. Wissensch. Math-naturw.*, Wien, 1912, cxxi, 121.

[7] Davey, W. P., *J. Exp. Zool.*, 1917, xxii, 573.

[8] X-ray Studies, General Electric Company, Schenectady, 1919, 255, 267.

[9] Christen, Th., *Strahlentherapie*, 1919, lx, 590.

β-rays. The emanation tube was placed parallel to the length of the animal to obtain a more even distribution of the radiation. Nevertheless it should be expected that the skin directly under the tube would receive more radiation, especially on account of the curvature of the body. Accordingly, when the dose was sufficiently large, marked local effects were produced on the backs or heads of the animals. The results may be summarized as follows: There were no visible changes in the skin and the hair growth of mice exposed to doses of 1.9, 2.4, and 4.7 millicurie hours. A dose of 6.8 millicurie hours retarded the development of lanugo hair directly opposite the emanation tube, and produced a skin erythema. The growth of hair, however, became normal on the 15th day after birth. Larger doses of radiation produced more pronounced effects. The mice irradiated with 11.5 millicurie hours had no hair on their backs on the 8th day. The hairless area was completely filled with new hair on the 14th day, but its retarded growth could be noticed up to the 30th day. The animals exposed to 21.9 millicurie hours showed wrinkled and reddened scalps on the 10th day. The radium reaction on the skin became worse up to the 15th day, when it was at its height. The skin was dry, scaly, wrinkled, and hemorrhagic. The eyes were inflamed and almost closed; the ears red, swollen, and underdeveloped. The healing process began at this time and progressed slowly. The effects from 26.8 millicurie hours were similar to these but more severe. On the 13th day the mice were unable to run normally, but could get about only with great difficulty. However, the animals gradually regained their health and vigor. A narrow strip of hairless skin, over which the emanation tube had been applied at a distance of 2 cm. was still visible at the end of the 616 day. Doses of 31.6 and 36.5 millicurie hours caused the mice to die on the 12th and 9th days respectively. Their hind legs were completely paralyzed the day before death. The viscera were very much undersized, and the brains hemorrhagic and congested.

The age at which the normal mice open their eyes is variable, but generally it is 13 days. Among mice of the same litter, however, there is little irregularity. Female mice often open their eyes a few hours earlier than the males. A record was kept of the age of the individual mice at the time they opened their eyes, and the averages, for each group of controls and radiated mice, are given in Table II. It will be seen that doses below 11.5 millicurie hours had no apparent effect on the time when the radiated mice opened their eyes. But the mice receiving larger doses of radiation opened their eyes from 12 to 24 hours earlier than their respective controls. Similar effects were

TABLE II.

The Effect of Radium Radiations on Opening of Eyes in White Mice.

Experiment No.	Group.	No. of mice radiated.		Average age at which eyes opened.
		Males.	Females.	*days.*
1	Controls.	2	2	13.1
	1.9 millicurie hours.	1	2	13.0
2	Controls.	2	2	13.9
	2.4 millicurie hours.	2	2	14.0
3	Controls.	2	1	13.2
	4.9 millicurie hours.	2	2	13.1
4	Controls.	1	1	12.0
	6.8 millicurie hours.	1	2	12.0
5	Controls.	2	3	13.7
	11.5 millicurie hours.	4	2	13.2
6	Controls.	1	3	13.0
	21.9 millicurie hours.	1	1	12.0
7	Controls.	1	3	12.9
	26.8 millicurie hours.	1	3	12.2

noted by Tribondeau and Belley,[10] who x-rayed the eyes of young kittens and found that the radiated eyes opened always some hours before the controls.

Many experimenters have noted that radium and x-rays can bring about sterility in animals. The literature is abundant,[11] but in many

[10] Tribondeau, L., and Belley, G., *Arch. Med.*, 1907, xv, 907.

[11] Albers-Schönberg, *Münch. med. Woch.* 1903, l, 1859. Frieben, *Münch. med. Woch.*, 1903, l, 2295. Philipp, *Fortschr. Geb. Röntgenstrahlen*, 1904, viii, 114. Halberstædter, L., *Berl. klin. Woch.*, 1905, xlii, 64. Bergonié, J., Tri-

cases the doses of radiation which caused sterility either are not given at all or they are given incompletely. The marked response to radiation which the reproductive cells exhibit can be explained by Bergonié and Tribondeau's generalization, already referred to. In the experiments of this paper, the effect of radiation on the reproductivity of the mice was also investigated. For this purpose the radiated animals were mated with normal mice about the 35th day after birth. One radiated male was mated with two normal females of about the same age. The animals were kept in the same cage until both females had produced at least three litters of young. Two radiated females were mated with two normal males, and they were not separated until the females had reached the age at which they normally cease to breed. In general, the young were separated from the mother soon after birth. In the case of the mice of Experiments 1 and 4 the young of one litter in either experiment were allowed to remain with their mother until weaned. These mice and succeeding generations were kept under observation to determine whether radiation affected the offspring of the radiated mice.

The results of the breeding tests were as follows: All the males of Experiments 1 to 7, receiving doses of 1.9 to 26.8 millicurie hours, were fertile. (Those of Experiments 8 and 9 succumbed to doses of radiation larger than 26.8 millicurie hours before they reached sexual maturity.) On the other hand, with the exception of the mice of Experiments 1 to 4, all the radiated females were rendered permanently sterile. Those of Experiments 1 and 4 were fertile. This shows that a dose of 1.9 millicurie hours (Experiment 1) applied as already explained (Table I) was not sufficient to sterilize female white mice. However, with one exception (Experiment 4) larger doses were effective. The apparent anomaly when a dose of 6.8 millicurie hours was used (Experiment 4) is due to the fact that in this case the emanation tubes were placed directly over the heads of the animals (Table I). Thus the ovaries were at a greater distance from the radioactive source, and they also were better protected from the radiation by a greater thickness of tissue in the path of the rays. In Experiment 6, the dose, 21.9 millicurie hours, was sufficient to cause sterilization, even though

bondeau, L., and Récamier, D., *Compt. rend. Soc. biol.*, 1905, xii, pt. 1, 284.
Brown, T., and Osgood, A. T., *Am. J. Surg.*, 1904–05, xviii, 179.

the mode of application was the same as in Experiment 4. The controls of either sex were all fertile. The first litter of young from both radiated and non-radiated female mice was obtained between the 60th and 78th day after birth. The radiated males reached sexual maturity at about the same time as the controls. The young born from the radiated mice grew normally and in turn produced normal

TABLE III.

Effect of Radium Radiations upon Longevity of White Mice.

Experiment No.	Group.	No. of mice used.	Days after birth.	No. of mice alive.	Days after birth.*	No. of mice alive.
1	Control.	4	384	4	587	2
	1.9 millicurie hours.	3		3		1
2	Control.	4	327	0		
	2.4 millicurie hours.	4		0		
3	Control.	3	485	2	602	1
	4.9 millicurie hours.	4		3		1
4	Control.	2	186	2	Discontinued.	
	6.8 millicurie hours.	3		3		
5	Control.	5	502	5	618	3
	11.5 millicurie hours.	6		5		3
6	Control.	4	404	4	607	1
	21.9 millicurie hours.	2		2		2
7	Control.	4	500	2	616	2
	26.8 millicurie hours.	4		3		2

* Final observation made November 20, 1921.

young, judging from their general appearance, weight, and reproductive power.

Davey[7] has reported some experiments with *tribolium confusum* in which by small doses of x-rays he was able to prolong the average life of the beetles. He used a very large number of specimens, and the statistical results are quite reliable. From our experiments, however, no definite conclusions can be reached as to the effect of radiation on the longevity of white mice. The number of animals used was too small to apply statistical methods, and some of the mice are still living. It is interesting to note, however, that even those mice which showed severe local and constitutional effects from the radiation apparently did not have their lives shortened appreciably. Some were a little undersized but otherwise apparently normal on the 618th day. Table III shows the number of mice still living at two different periods in the course of the experiments.

DISCUSSION AND SUMMARY.

It has been estimated[12] that 92 per cent of the total radiation emitted by radium in equilibrium with its subsequent products is given off in the form of α-rays. This, however, cannot be utilized when the source is enclosed in an ordinary container, because the α-rays are absorbed completely by even a small thickness of glass. About 3.2 per cent of the total radiation is emitted in the form of β-rays, and 4.8 per cent as gamma radiation. The effects produced on the radiated mice of these experiments were due mainly to the β-rays, which are easily absorbed by tissue. The γ-rays, being only slightly absorbed by organic matter, probably contributed very little to the observed effects.

It is interesting to correlate the different effects produced by the same dose of radiation. The mice which received a dose of 1.9 millicurie hours showed no local effects on the skin or hair. Neither females nor males were sterilized, and the time at which they opened their eyes or reached sexual maturity was not affected, as far as we could tell. The only difference noted between the radiated animals and the controls was in the body weight. This dose accelerated the growth of the young mice, that is, while initially of the same weight, soon after irradiation they became distinctly bigger than the controls, but finally the animals of each group had substantially the same average weight. That this variation in body weight should be accidental is unlikely, since it was observed also in the animals treated by a slightly larger dose (2.4 millicurie hours). The number of animals

[12] Rutherford, E. Radioactive subtances, and their radiations, Cambridge, 1913, 581.

(seven) which showed this effect is too small to prove conclusively the accelerating effect of small doses of radiation on the body growth of mice. But considering that similar results have been obtained by radiating plants[6,7] and beetles,[7,8] it is reasonable that the observed increase in weight might be attributed, at least in part, to the effects of radiation. Since this paper was first written Russ, Chambers, and Scott[13] have shown that small doses of x-rays accelerate the body growth of rats. In view of this additional evidence there can be little doubt that the increase in weight observed in our experiments was due to the radiation.

A dose of 2.4 millicurie hours applied over the backs of the animals produced no local skin effects, but it accelerated the growth of the mice as in the previous case. In addition it caused permanent sterilization of all the females. A similar result was obtained with 4.9 millicurie hours, except that the effect on the rate of growth was uncertain. A dose of 6.8 millicurie hours produced a definite but mild skin erythema and retarded the development of lanugo hair. But since in this instance the emanation was applied over the heads of the animals, the dose reaching the ovaries was not sufficient to cause sterilization, as already explained. No other definite effect was noted.

In connection with the sterilization of the females it should be noted that a dose of radiation which produced no visible skin changes was sufficient to cause permanent sterility. On account of the greater distance of the ovaries from the source of radiation as compared with that of the skin directly below the tube, and the depth of tissue which the rays had to traverse to reach the ovaries, the amount of radiation acting on the latter was much smaller than the amount falling on the skin. The radiation emitted by the emanation tube is reduced to about 50 per cent of its initial value after traversing 1 mm. of tissue. Still, while the skin was not visibly affected, the mice were sterilized. This shows that the ovaries are influenced very easily by radiation of this type. We can estimate the amount of radiation reaching the ovaries which is sufficient to cause sterility to be less than 25 per cent of the amount necessary to produce visible skin changes in the mice. It should be noted also that whenever sterility of the female mice was

[13] Russ, S., Chambers, H., and Scott, G. M., *Arch. Radiol. and Elect.*, 1921, xxvi, 128.

induced, it was permanent. Furthermore, those mice which were not rendered sterile by radiation were, as far as the experiments enable us to say, as prolific as the controls. Remembering that a dose of 1.9 millicurie hours had no apparent effect on the ovaries, while a slightly larger dose, 2.4 millicurie hours, caused permanent sterility, it might be concluded that it is not possible to produce temporary sterility by radiation. We know, however, that temporary sterility can be produced, at least when the animals are radiated at a later stage in their development. The mice in our experiments were radiated for the first time soon after birth, and it is not improbable that under these conditions temporary sterility cannot be obtained.

Large sublethal doses produced severe skin burns, retarded the body growth of the animals, but failed to sterilize the males. About one-third of the total skin area of the mice showed marked effects from the radiation. The animals were very sick for a time, and their growth was temporarily stunted. But nevertheless they recovered and finally became apparently normal except for the narrow hairless strip of skin which had been closest to the emanation tube. Only the females were rendered permanently sterile. The males did not show even temporary sterility when the doses of radiation were close to the lethal dose. While the testes of mammals are known to be very easily affected by radiation, still they are more resistant than the ovaries. In addition, in these experiments they were at a greater distance from the source of radiation than the ovaries, and they were better protected by the thicker layer of tissue in the path of the rays. The fact that no sub-lethal dose in these experiments sterilized the males shows that under the conditions of irradiation adopted the amount of radiation reaching the testes was not sufficient to affect them noticeably. If the source of radiation had been applied closer to the reproductive organs of the males, they would have been sterilized by millicurie hour doses much smaller than the lethal dose.

Some of the radiated animals were killed with ether, and macroscopic and microscopic examinations of the reproductive organs were made. The ovaries of the sterile females were generally atrophied and colored yellow. The normal histological structure was altered. The characteristic findings were the destruction of the Graafian follicles, with absence of ovum cells. The testes and the epididymis of the radiated mice of the present experiment appeared macroscopically and histologically normal, with the presence of abundant spermatozoa. Owing to the method adopted for the irradiation of the mice, the testes were too far from the source of radiation, and too well protected by the intervening tissue to be definitely affected by the rays.