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*The Effect of Gamma Irradiation on Cell Division in Tissue Culture in Vitro.—Part II.*

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In a previous paper (1) the effects of the gamma rays of radium upon the number of cells in mitosis in tissue cultures has been described, the cultures being examined 80 minutes after irradiation. Under the conditions of the experiment it was shown that there was a threshold of intensity below which no diminution in the number of cells in mitosis was apparent, and also a threshold of time for each intensity which must be exceeded before diminution could be observed.

Gilman and Baetjer (2) showed that there was an acceleration in the development of the eggs of *Amblystoma* after irradiation by X-rays. Hastings, Beckton and Wedd (4) showed an increase in the rate of hatching out of silkworm eggs which had been irradiated by X-rays; and Lazarus-Barlow and Beckton (3) showed that small intensities of beta-rays acting for a long period were followed by a greater rate of cell division in the eggs of *Ascaris Megalocephala*. In the case of tissue cultures Canti and Donaldson (5) described an experiment in which cessation of mitosis having been caused by exposure to the gamma rays of radium a return of mitosis was observed after removal of the radium. Since the completion of the present experiments it has been shown by Spear (6) that by lowering the temperature of tissue cultures to 0° C. for 4 hours and subsequently incubating for various periods there is a fall in the number of cells in mitosis followed by a return in increased numbers which is compensatory.

The experiments described in the present paper were carried out in order to study the rate of falling off and the rate of subsequent return of mitosis in tissue cultures exposed to the gamma-rays of radium, the intensity and the duration of exposure remaining constant in any one series of determinations.

*Technique.*—The tissue used was obtained from the choroid and sclerotic of the chick embryo and the cultures were in the form of hanging drop preparations

as previously described (1). The radium plaque containing 100 mgs. of radium element filtered with 0.5 mm. of platinum was employed in the radium lantern. The details of these have already been given (1). The intensity of irradiation remained constant throughout all the experiments, the exposures being made at a distance of 0.5 cm. The cultures were kept at 37° C. and care was taken to prevent their being chilled during manipulation.

Four series of determinations were made with different durations of exposure for each series, namely, 1½ minutes, 2½ minutes, 9 minutes and 30 minutes. The determinations in any one series were made at various intervals of time up to 9 hours after irradiation had ceased. In order to arrive at any one determination in a series, sets of four test cultures and four control cultures of the same batch were taken. The test cultures were exposed to radium and after the desired period of incubation were fixed and stained. The controls were fixed at the time at which irradiation of the corresponding test specimens was commenced. The number of cells in mitosis in the test cultures was then counted and expressed as a percentage of those present in the controls. In certain instances a second set of control cultures was fixed and stained at the end of the experiment to act as a check on the selected controls.

Tables I to IV show the results obtained. These are plotted as curves in the accompanying figure.

Table I.—Cultures exposed to 100 mg. radium for 1½ minutes at a distance of 0.5 cm. and examined after incubation at 37° C. for the period indicated.

Period of incubation.	Examined immediately.		40 minutes.		80 minutes.		2 hours.		3 hours.	
Number of cells in mitosis	<i>30</i>	31	<i>30</i>	20	<i>29</i>	43	<i>43</i>	28	<i>43</i>	22
	<i>24</i>	25	<i>24</i>	33	<i>33</i>	36	<i>31</i>	47	<i>31</i>	26
	<i>46</i>	47	<i>46</i>	38	<i>31</i>	31	<i>36</i>	29	<i>36</i>	49
	<i>36</i>	30	<i>36</i>	36	<i>29</i>	23	<i>26</i>	31	<i>26</i>	33
Totals ....	<i>136</i>	133	<i>136</i>	127	<i>132</i>	133	<i>136</i>	135	<i>136</i>	130
Average per slide ....	<i>34</i>	33.3	<i>34</i>	31.8	<i>33</i>	33.3	<i>34</i>	33.8	<i>34</i>	32.5
Per cent.....	97.9		93.5		100.9		99.4		95.6	

Figures in roman represent number of cells in mitosis in irradiated cultures. Figures in italics represent number of cells in mitosis in control cultures.



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Table II.—Irradiation 2½ minutes. Examined after interval indicated.

Period of incubation.	Examined immediately.		35 minutes.		80 minutes.		2 hours.		2½ hours.		3 hours.	
Number of cells in mitosis	21	22	21	17	28	11	29	29	38	41	50	76
	30	27	30	14	31	12	50	25	34	31	62	67
	22	22	22	22	24	11	76	39	35	25	60	53
	21	26	21	14	20	6	33	21	42	26	51	46
Totals	94	97	94	67	103	40	188	114	149	123	223	242
Average per slide	23.5	24.3	23.5	16.8	25.8	10	47	28.5	37.3	30.8	55.8	60.5
Per cent.	103.4		71.5		38.8		60.6		82.6		108.4	

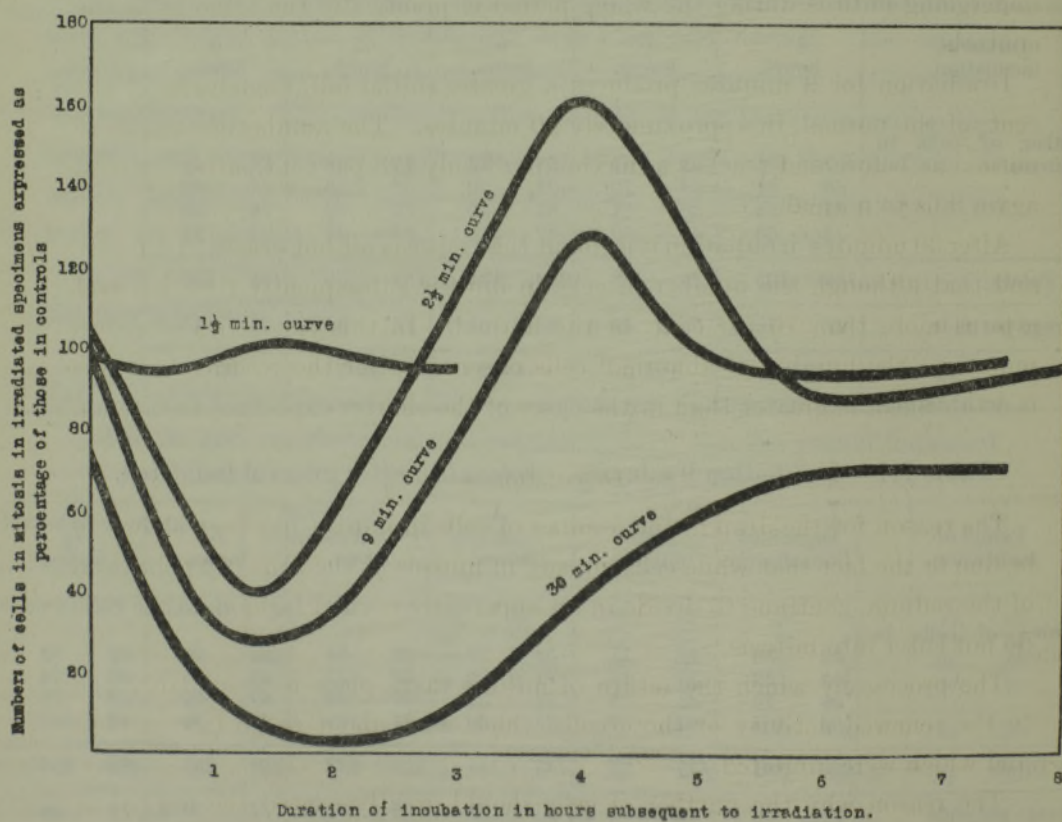
Period of incubation.	4 hours.		5 hours.		6 hours.		7 hours.		9 hours.	
Number of cells in mitosis	29	101	38	47	38	43	29	28	29	42
	50	84	34	30	34	29	50	56	50	52
	76	54	35	51	35	49	76	52	76	53
	33	66	42	50	42	10	33	42	33	33
Totals	188	305	149	178	149	131	188	178	188	180
Average per slide	47	76.3	37.3	44.5	37.3	32.8	47	44.5	47	45
Per cent.	161.7		119.4		88.2		94.7		95.7	

Table III.—Irradiation 9 minutes. Examined after interval indicated.

Period of incubation.	Examined immediately.		80 minutes.		3 hours.		4 hours.		5 hours.		7½ hours.	
Number of cells in mitosis	62	50	42	11	34	20	50	71	62	66	62	62
	60	53	35	11	41	29	62	75	60	65	60	74
	69	70	35	8	21	32	60	68	69	60	69	71
	93	108	23	7	47	30	51	—	93	92	93	—
Totals	284	281	135	37	143	111	223	214	284	283	284	207
Average per slide	71	70.3	33.8	9.3	35.8	27.8	55.8	71.3	71	70.8	71	69
Per cent.	99		27.8		77.6		127.8		99.7		97.2	

Table IV.—Irradiation 30 minutes. Examined after interval indicated.

Period of incubation.	Examined immediately.		80 minutes.		3 hours.		4½ hours.		6 hours.		7½ hours.	
Number of cells in mitosis ....	48	38	37	4	44	7	44	22	48	29	44	—
	24	22	34	2	47	3	47	20	24	25	47	33
	39	15	30	2	48	4	48	22	39	21	48	22
	22	23	22	1	30	4	30	—	22	19	30	35
Totals ....	133	98	123	9	169	18	169	64	133	94	169	90
Average per slide ....	33.3	24.5	30.8	2.3	42.3	4.5	42.3	21.3	33.3	23.5	42.3	30
Per cent. ....	73.9		7.5		10.6		50.4		70.6		70.9	



*Explanation of Curves.*—Irradiation for 1½ minutes showed no significant fall in the number of cells in mitosis during the three hours over which they were observed subsequent to irradiation. This is in accordance with the experiment previously described (1) in which under the same conditions of experiment, the time threshold was not reached.



When however the period of irradiation is increased to  $2\frac{1}{2}$  minutes the number of cells in mitosis rapidly falls off till a minimum of 40 per cent. is reached in 80 minutes. After this the number increases and reaches the normal in about  $2\frac{3}{4}$  hours. This increase continues until the end of the fourth hour, when the number of cells in mitosis reaches a maximum, which is 160 per cent. of the normal. The numbers now fall off and again reach the normal during the sixth hour, after which they remain constant. It is thus seen that when the threshold of time for the particular intensity pertaining to 0.5 cm. distance from the radium is just passed, the diminution in the number of cells in mitosis is followed by an increase; further this increase is compensatory, as shown by the regular S shape of the curve and the fact that the total number of cells undergoing mitosis during the whole period is practically the same as in the controls.

Irradiation for 9 minutes produces a greater initial fall, namely, to 27 per cent. of the normal, in approximately 80 minutes. The number then rises to normal as before and reaches a maximum of only 128 per cent., after which it again falls to normal.

After 30 minutes' irradiation it is found that mitosis all but ceases in 2 hours, and that although the number of cells in mitosis subsequently rises it never attains more than 70 per cent. of the normal. In this last series of determinations the number of abnormal cells observed after the return of mitosis is established, is greater than in the cases of the shorter exposures to radium.

#### *Discussion.*

The reason for the drop in the number of cells in mitosis has been shown to be due to the fact that while cells already in mitosis at the time of application of the radium, continue to divide in an apparently normal fashion, other cells do not enter into mitosis.

The process by which the return of mitosis takes place is presumably due to the renewed activity of those cells which were about to go into mitosis and which were inhibited.

The reason why the number of cells should greatly exceed the normal in the case of the  $2\frac{1}{2}$ -minute series is not evident. The possibilities to be considered are: first, the number of cells entering mitosis per unit of time is increased, or, secondly, the time occupied for the process of mitosis to take place is increased, or, thirdly, a combination of both of these.

Some evidence obtained by direct observation suggests that the latter may be the cause, but no quantitative experiments have so far been carried out.

It would appear in the case of the longer exposures that one or more of these factors is still acting, but that more permanent damage has been done to the cells, which, in the case of the 30-minute exposure, prevents mitosis from again reaching the normal level.

*Summary.*

1. In cultures irradiated with gamma-rays under the conditions of the experiment it was found that the fall in the number of cells undergoing mitosis was followed by a rise.

2. With a certain duration of exposure and with the intensity of irradiation employed in the experiment this rise was compensatory to the fall.

3. With longer exposures, though there was a tendency to rise, the number of cells undergoing mitosis never reached the normal.

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