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COMMUNICATIONS

THE EFFECT OF GAMMA RAYS ON CELL DIVISION IN THE DEVELOPING RAT RETINA*

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Introduction

It has been shown that the first recognisable result of an effective gamma or X-radiation is a diminution in cell division. A dose far too small to produce any skin erythema or to have any effect on blood vessels can temporarily reduce mitosis to less than half its normal value. This has been demonstrated for chick fibroblasts in tissue culture by Strangeways and Oakeley (1923) who used X-radiation and by Spear (1935) who used gamma rays. The chief value of tissue culture experiments is the precision which the method allows both on the physical and on the biological side consequent upon the small mass of tissue which is exposed to radiation, and the simplification of experimental conditions which

* From the Strangeways Research Laboratory, Cambridge.

the material permits. But, to quote Gladstone and Colwell (1933), although "research by the methods of tissue culture occupies a deservedly prominent place at the present time yet we think that experiments on the whole chick have considerable value. The tissues are irradiated as parts of the living organism existing under natural conditions, with an established circulatory system and growing in interdependence with each other. The two methods are complementary the one to the other, and either method may afford confirmatory evidence of results obtained by the other."

Experiments on whole chicks by Gladstone and Colwell (1933) and by workers at the Strangeways Laboratory (Wilson and others 1935) have given results surprisingly similar to those obtained with tissue cultures. All these observers found that the variation in mitosis following radiation of avian tissue *in vivo* closely resembles that previously seen with tissue cultures *in vitro*.

The present paper represents a first attempt to determine whether the biological response, as measured by alteration in the mitotic count, changes when mammalian is substituted for avian tissue. Is the difference, if any, in the response of mammalian and avian cells to gamma radiation one of degree or one of kind? What is the ultimate fate of cells which have been affected by radiation?

Certain more permanent results of irradiating the rat eye, including diminution in the size of the eyeball, the appearance of retinal rosettes and an extra nerve fibre layer dividing the inner nuclear layer into two have been described in detail elsewhere (Glücksman and Tansley, 1936).

Object of Experiments

The experiments described here were undertaken:—

- (1) To determine the precise effect of gamma radiation on cell division in mammalian tissue.
- (2) To compare the results with those previously obtained with chick tissue.
- (3) To determine quantitatively the alteration in cell division and the production of cell degeneration after different doses of radiation.
- (4) To investigate the relationship, if any, between the mitotic changes and the degeneration which follow radiation.

Method

The rat retina was chosen as the tissue for this investigation because mitotic frequency is still high during the first few days after birth. The eye has the further advantage that being a surface organ its position can be accurately adjusted in relation to

the source of radiation thus allowing any given dose to be administered with reasonable precision.

Serial experiments were done on 2-day old rats, the left eye of each animal being irradiated and examined.

The source of radiation was 302.1 mgm. radium element, in the form of sulphate, put up in a cylindrical container, 13 mm. in diameter and 2.5 mm. deep. This was mounted for ease of manipulation in a flattened brass ring to form a plaque 4.75 cm. in diameter. The total filtration was 0.5 mm. platinum plus a thin sheet of mica, and the distance between plaque and eye-lids (left eye) was 0.75 cms. The eye thus received only gamma rays at an intensity on the skin surface of approximately 40 r/min or 300 Sievert units.* The exposures varied from $\frac{1}{2}$ minute up to 30 minutes, the smallest dose used being only a fraction of the human skin erythema dose.

The technique of exposure was as follows:—a litter of rats with their mother was brought in a cage to the radium room and one of the litter was removed and killed as a control. The remaining young rats were then exposed in turn, each being suitably marked for purposes of subsequent identification. To restrict movement during irradiation the rat was wrapped in a blanket of cotton wool and then placed on its right side with its head on a pillow in a wooden cradle fitted with a heating device to prevent any chilling during irradiation. A sheet of mica, mounted on a stand, was then fixed over the cradle so that cross-lines marked on the mica lay over the centre of the left eye of the rat. The mica exercised a very slight pressure on the closed eye-lids. The radium plaque, also centred with respect to the mica cross-lines, was then placed in position by hand and the exposure timed by stop-watch (Fig. 1).

Exposures varied from $\frac{1}{2}$ minute up to 30 minutes. After exposure the rats were either dealt with immediately or returned to the mother and killed at given intervals from 30 minutes up to 3 days; they consequently suffered the least possible disturbance during the course of an experiment. The left (irradiated) eye of each rat killed was fixed in Bouin's solution, sectioned and stained with haematoxylin and eosin. A section was selected in the central area of the eye and counts were made of all the mitotic figures present, the results being recorded as follows:—All the early changes in the nucleus up to the appearance of discrete chromosomes were classed as prophase; from the appearance of chromosomes to the

* One Sievert unit, or intensity millicurie (Imc.) is defined as the intensity due to a point source of 1 mgm. radium acting at a distance of 1 cm.; the rays being filtered through 0.5 mm. platinum.

FIG. 1a.

Apparatus for the irradiation of the rat eye, shown in section and plan. P, radium plaque. S, lead screen with C counterweight. R, rat. M, mica disc with cross lines. H, water-filled heater incorporated in rat carrier.

FIG. 1b.

Rat carrier in perspective.

beginning of the journey to the poles, the cells were reckoned as being in metaphase; the remaining stages of division were classed as telophase (*i.e.*, under this term was included anaphase+telophase as commonly understood). Counts were also made of the normal and of the degenerate cells present in small samples of the undifferentiated region (outer nuclear layer and outer part of inner nuclear layer) of the same section. Control rats subjected to the same pressure under the mica sheet but without radiation showed no recognisable changes in the eye.

Results

(a) *Consideration of Material.* At birth the normal rat eye is still immature. In the retina the optic nerve fibres, the ganglion cell layer, the inner fibre layer and part of the inner nuclear layer

FIG. 2.

Section through the eye of a normal 2-day rat. The optic nerve fibres, ganglion cells and inner fibre layer are differentiated. (Bouin, haematoxylin and eosin, $\times 160$)

sc, sclera. ch, choroid. p, pigment epithelium. ud, undifferentiated part of the retina. d, differentiated part of retina. m, mitosis. ifl, inner fibre layer. gcl, ganglion cell layer. opfl, optic nerve fibre layer.

FIG. 3.

Part of a similar section to Fig. 2. Mitosis is present only on the outer edge of the retina. (Bouin, haematoxylin and eosin, $\times 410$)

are already differentiated (Glücksmann and Tansley, 1936). Differentiation proceeds little further during the first two days of life (Fig. 2) but throughout this period mitosis is abundant. Cell division only occurs, however, along the outer edge of the retina (Fig. 3). A few degenerate cells are always present in the undifferentiated part of the retina at this stage, but these normally represent less than one per cent. of the total (undifferentiated) cell count.

Before describing the results it is necessary to consider the variability of the biological material and the way in which this affects the accuracy of our results. If mitotic counts are made on a series of normal 2-day rats the individual variation is considerable (Table 1), but the ratio of the different phases of mitosis is remarkably constant and very nearly in the proportion of 50:100:50 for prophase (P.): metaphase (M.); telophase (T.). Whenever possible each irradiated individual was related to a control taken from its own litter. This course was followed in the majority of results recorded. But since individual variation within the litter is nearly as high as the variation between different litters, it seemed permissible, if a litter control was not available, to use as a "standard control" the mean count of fifteen normal 2-day rats taken from eleven different litters. The "standard control" count was taken as 123:250:110 for prophase, metaphase and telophase, giving a P.M.T. ratio of 49:100:44. There was some evidence (where large litters enabled many points of one graph to be determined on the same rat family) that certain litters tended to react more slowly or less vigorously to radiation than others. Natural variation in the mitotic count can in part be accounted for by differences in nutrition since the results of some early experiments in which rats were isolated in an incubator after irradiation were consistently at variance with those obtained when the rats were returned to the litter cage. It was our invariable rule in the present investigation, therefore, to put the rats back with the mother after exposure until required for examination. Even so it was obviously impossible to ensure that each rat received adequate maternal care. It is our belief that inequality in this respect may account for some of the irregularities observed in the counts after irradiation.*

In view of the variations found in the normal material (Table 1) it was to be expected that the counts in the irradiated specimens would show fluctuations at least as wide, especially under those

* Some collateral experiments on the effect of irradiation on tadpoles at different temperatures have demonstrated that temperature, as well as nutrition, is significant in determining the final result of the exposure. As it was not possible to keep our rat cages constantly at body temperature, any young rats which strayed from the nest were liable to suffer considerable temperature variations.

conditions where the mitotic count was changing most rapidly. The variation was in fact greatest for the metaphase counts over the period 3-15 hours after a 4-minute exposure. The figures in Table 2 (col. 6 M. and Fig. 4) represent the actual metaphase counts expressed as a percentage of the litter control metaphase count, two or three readings being taken for each point.

FIG. 4.

Showing the variation in metaphase counts over a period of 20 hours following a four minute exposure.

Ordinates show the actual metaphase count expressed as a percentage of the litter control metaphase counts.

Abscissae show time in hours after exposure.

(b) *Results of Radiation.* The doses of radiation used by us in these experiments affected only those parts of the retina which were undifferentiated at the time of exposure. We are therefore concerned here only with undifferentiated and proliferating cells. With the shortest exposure ($\frac{1}{2}$ minute) no significant effect on cell division was observed. Exposures of one minute and upwards invariably produced a fall in cell division which depended in amount and duration on the dose given. If the dose was sufficient, mitosis disappeared altogether but later on it might return and sometimes temporarily exceeded the normal. The initial fall in

FIG. 5.

Showing the mitotic phase count and the degenerate cell count over a 3-day period following different lengths of exposure.

Prophase indicated by continuous line, metaphase by dash line, telophase by dot-and-dash line. Degenerate cell count indicated by stippled area.

Ordinates represent mean values for mitotic counts expressed as percentage of control metaphase count, and degenerate cell count as percentage of total undifferentiated cell count.

Abscissae represent time in hours after exposure.

a, control. *b*, 2-minute exposure. *c*, 4-minute exposure. *d*, 6-minute exposure. *e*, 10-minute exposure. *f*, 12-minute exposure. *g*, 30-minute exposure.

mitosis was followed by scattered degeneration among the undifferentiated cells of the retina. The amount of this degeneration also depended on the size of the dose. Complete details of a single experiment are given in Table 2. The typical results of the whole series are summarised in Table 3 and expressed as graphs in Fig. 5 (a-g).

The mitosis curves shown were obtained by taking the mean value of the readings at each time interval (shown in Table 4), and plotting a graph. Besides dividing cells, degenerate cells were also counted and the result expressed as a percentage of the

FIG. 6.

Showing the effect of individual variation in radiosensitivity. The upper graph is a reproduction of Fig. 5b, the lower graph gives corresponding figures obtained from litter 60 (see Table 5). Ordinates and abscissae as in Fig. 5.

total undifferentiated cell count for each specimen examined. Where more than one reading was taken the mean value was used in tabulation.

$\frac{1}{2}$ -minute exposure produced no significant effect on cell division and caused no degeneration.

1-minute exposure* caused a marked variation in the mitotic count and produced a very few degenerate cells which were seen 15 hours after exposure.

2-minute exposure:—The initial fall in cell division was followed by a transitory rise which exceeded the normal count. The minimum prophase count occurred 1 hour and the maximum about 6 hours after the end of exposure. The trough values for metaphase and telophase were, approximately, 2 hours and 3 hours respectively after radiation; and the corresponding peak values

* The actual counts obtained for exposures of 1 and $8\frac{1}{2}$ minutes have been omitted from Tables and Graphs as these results resembled so closely those following exposures of 2 and 10 minutes respectively.

were seen about 7 and $8\frac{1}{2}$ hours after removal from the radium. It should be noted (Fig. 5 b) that the peak value for telophase is unexpectedly high in relation to the prophase count.

Degenerate cells were first observed 6 hours after the end of exposure and reached a maximum 6 hours later (*i.e.*, 12 hours from exposure), when 12 per cent. of the undifferentiated cells were degenerate. Degeneration gradually diminished over the next 24-hour period and had disappeared by the 36th hour after irradiation.

The case of litter 60 (Table 5, Fig. 6) should be noted for its bearing on the question of individual variation in radiosensitivity. It was found in making the 2-minute curve that individuals of this litter always gave an aberrant count which did not fit on the general graph. When, however, a separate graph was constructed for this litter alone, it was seen that while its general shape conformed to type, the peaks and troughs of both mitosis and degeneration curves were shifted to the right and corresponded to a different radiation dosage, suggesting an altered radiosensitivity throughout the litter.

4-minute exposure:—The general shape of the curves was very similar to that obtained after 2 minutes exposure except for the telophase count. The minimum prophase count was seen $1\frac{1}{3}$ hours, and the maximum prophase count 6-7 hours, after the end of exposure. The trough values for metaphase and telophase were (approximately) $1\frac{2}{3}$ and $2\frac{1}{2}$ hours after radiation respectively; and the corresponding peak values occurred about $7\frac{1}{2}$ and 9 hours after removal from the radium.

Numbers of degenerate cells were observed 6 hours after the end of exposure though a few were seen as early as $\frac{1}{3}$ hour after irradiation. They reached a maximum 15 hours from exposure, when 17 per cent. of the total undifferentiated cells were degenerate. Degeneration gradually diminished over the next 20-hour period and had disappeared by the 35th hour after radiation.

6-minute exposure:—The initial fall in mitosis was followed by a rise in the count which just exceeded the normal for prophase and telophase. The metaphase count did not reach normal level and its peak value was much lower than would be expected from the prophase count. Degeneration was first seen 4 hours after exposure to radium and lasted over a period of 31 hours. The degenerate cell count was maximum about 12 hours after radiation, when it reached a value of 25 per cent.

$8\frac{1}{2}$ -minute exposure:—These results were very similar to those obtained with the 6-minute exposure except that the times taken to reach the peak values both for cell division and for degeneration were longer and the proportions of degenerate cells were greater.

TABLE 1

Variation in normal counts in 26 rats from different litters.

Litter number	Mitotic phase counts			Mitotic phase counts as per cent. of metaphase		
	P	M	T	P	M	T
19	143	307	119	47	100	39
26	105	204	93	51	100	46
27	86	175	82	48	100	47
41	113	255	88	44	100	35
45	128	267	120	48	100	45
46	89	184	91	48	100	49
51	94	218	116	40	100	62
52	120	221	111	52	100	50
57	161	320	172	50	100	54
58	178	350	149	51	100	43
65	83	148	109	56	100	54
66	88	166	81	53	100	49
67	98	157	76	62	100	48
68	109	165	96	63	100	58
69	114	236	112	48	100	47
70	81	163	97	50	100	60
72	108	227	95	48	100	42
73	85	194	116	44	100	60
75	83	163	103	51	100	63
79	93	206	97	45	100	47
80	89	187	100	48	100	53
81	100	206	102	47	100	48
83	80	187	105	43	100	56
84	80	180	107	44	100	59
85	113	203	106	56	100	52
90	100	202	109	50	100	54

TABLE 2

Table showing complete results for the 4 minute exposure.

1	2	3			4			5			6			7		
Time after irradiation	Litter No.	Actual litter control count			Control count as per cent. of metaphase			Actual experimental count			Experimental count as per cent. of litter control metaphase			Mean values from last column		
Hours		P	M	T	P	M	T	P	M	T	P	M	T	P	M	T
1	72	108	227	95	48	100	42	36	176	104	16	77	46	16	99	64
	75	83	163	103	51	100	63	24	198	132	15	121	81			
1	73	85	194	116	44	100	60	10	97	94	5	50	48	4	36	42
	75	83	163	103	51	100	63	5	36	54	3	22	33			
1½	51	94	218	116	40	100	62	4	8	3	2	4	3	1	9	24
	75	83	163	103	51	100	63	0	21	72	0	13	44			
1½	73	85	194	116	44	100	60	7	6	24	4	3	12	—	—	—
2	73	85	194	116	44	100	60	44	28	7	22	14	4	14	8	3
	75	83	163	103	51	100	63	8	2	4	5	1	2			
3	72	108	227	95	48	100	42	130	116	28	57	51	12	39	30	7
	75	83	163	103	51	100	63	34	13	3	21	8	2			
4	73	85	194	116	44	100	60	145	167	57	75	86	29	54	63	22
	75	83	163	103	51	100	63	52	62	23	32	40	14			
6	51	94	218	116	40	100	62	209	231	101	96	104	46	82	124	56
	73	85	194	116	44	100	60	197	351	171	102	181	88			
	79	93	206	97	45	100	47	99	178	73	48	86	35			
9	51	94	218	116	40	100	62	162	305	220	74	140	101	78	111	77
	72	108	227	95	48	100	42	122	173	113	54	76	50			
	75	83	163	103	51	100	63	141	193	128	87	118	79			
12	51	94	218	116	40	100	62	101	170	143	46	78	66	36	78	52
	72	108	227	95	48	100	42	72	171	82	32	75	36			
	79	93	206	97	45	100	47	61	166	109	30	81	53			
15	51	94	218	116	40	100	62	48	89	98	22	41	45	32	70	41
	79	93	206	97	45	100	47	86	202	103	42	100	50			
	79	93	206	97	45	100	47	66	141	55	32	68	27			
18	72	108	227	95	48	100	42	63	120	68	28	53	30	31	55	33
	80	89	187	100	48	100	53	61	104	67	33	56	36			
19	52	120	221	111	52	100	50	82	169	107	37	76	50	—	—	—
21	72	108	227	95	48	100	42	40	102	59	18	45	26	—	—	—
24	52	120	221	111	52	100	50	48	100	59	21	45	22	23	64	34
	73	85	194	116	44	100	60	71	170	93	37	88	48			
	80	89	187	100	48	100	53	21	110	61	11	59	33			
30	73	85	194	116	44	100	60	42	93	71	22	48	37	—	—	—
36	51	94	218	116	40	100	62	57	128	92	26	67	42	20	62	36
	80	89	187	100	48	100	53	21	90	54	11	48	29			
	80	89	187	100	48	100	53	42	134	69	22	72	37			
48	52	120	221	111	52	100	50	94	206	116	43	93	52	22	58	45
	73	85	194	116	44	100	60	36	114	119	19	59	61			
	80	89	187	100	48	100	53	9	40	39	5	21	21			
72	52	120	221	111	52	100	50	41	129	67	19	58	30	13	47	36
	75	85	194	116	44	100	60	13	69	82	7	36	42			

TABLE 2—continued

Table showing complete results for the 4 minute exposure.

	8	9	10	11	12	13	14	15				
Time after irradiation.	Experimental count as per cent. of standard control metaphase (250)*			Mean values from last column			Total cell divisions	Total cell divisions as per cent. of litter control total	Mean values from last column	Per cent. of undifferentiated cells degenerate	Mean values from last column	Remarks
Hours	P	M	T	P	M	T						
½	15	72	43	13	77	49	316	73	87	0	0	Skull depression
	10	81	54				352	101		0		
1	4	40	39	3	28	31	201	51	39	0	0	
	2	15	22				95	27		0		
1½	2	4	1	1	7	16	15	4	16	8	4	
0	9	30	93				27	0				
1½	3	2	10	—	—	—	37	9	—	0	—	
2	18	12	3	11	7	3	79	20	12	<1	<1	
	4	1	2				14	4		0		
3	54	48	12	35	27	7	274	64	39	<1	<1	
	15	5	1				50	14		0		
4	60	69	23	41	48	17	369	93	67	<1	<1	
	21	26	10				137	40		<1		
6	85	95	41	69	104	47	541	126	132	14	11	
	80	144	70				719	182		6		
	41	73	30				350	88		14		
9	67	126	91	58	92	64	487	114	110	19	10	
	50	70	47				408	95		8		
	58	79	53				462	122		4		
12	41	70	59	32	69	46	414	97	86	25	14	
	30	70	34				325	76		6		
	25	68	45				336	85		12		
15	19	37	41	27	59	36	235	55	73	24	17	
	35	82	43				391	99		10		
	27	58	23				262	66		17		
18	26	49	28	26	46	28	251	58	60	10	11	
	25	43	28				232	62		12		
19	34	69	44	—	—	—	358	79	—	13	—	
21	16	42	24	—	—	—	201	47	—	8	—	
24	20	41	24	19	52	29	207	46	61	7	4	
	29	70	38				334	85		1		
	9	45	25				192	51		5		
30	17	38	29	—	—	—	206	52	—	1	—	
	23	53	38				277	65		1		
36	9	37	22	16	48	29	165	44	58	<1	<1	
	17	55	28				245	65		0		
	39	85	48				19	49		34		426
15	47	49	269	68	0							
4	16	16	88	23	0							
72	17	53	28	7	41	31	237	53	48	0	0	
	5	28	34				164	42		0		

* "Standard control." Actual counts:—prophase 123, metaphase 250, telophase 110.
P.M.T. ratio = 49, 100, 44.

TABLE 3
Summarising results of all experiments

Time after irradiation Hours	Litter No.	2 minute exposure						4 minute exposure						6 minute exposure					
		Litter control count			Experimental count			Litter control count			Experimental count			Litter control count			Experimental count		
		P	M	T	P	M	T	P	M	T	P	M	T	P	M	T	P	M	T
		Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.	Litter No.
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
½	58	178	350	149	76	279	174	72	108	227	95	36	176	104	137	94	17	137	94
	59†	49	234	38	38	450	271	75	75	83	163	24	198	132	65	65	66	133	101
1	57	161	320	172	39	162	128	73	85	194	116	10	97	94	65	65	12	55	85
	60*	—	—	—	54	171	252	75	83	163	103	5	36	54	69	69	10	14	34
1½	58	178	350	149	104	56	123	51	94	218	116	4	8	3	41	113	2	0	1
	59†	49	234	38	153	188	157	73	85	194	116	7	6	24	62†	62†	12	60	91
2	57	161	320	172	112	131	118	73	85	194	116	44	28	7	65	65	7	41	66
	60*	—	—	—	44	31	39	75	83	163	103	8	2	4	69	69	5	4	56
3	58	178	350	149	119	60	45	72	108	227	95	130	116	28	62†	62†	10	108	35
	59†	49	234	38	—	—	—	75	83	163	103	34	13	3	65	65	28	8	3
4	57	161	320	172	137	134	60	73	85	194	116	145	167	57	65	65	0	0	0
	59†	49	234	38	130	313	154	75	83	163	103	52	62	23	69	69	72	159	102
4½	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	58	178	350	149	177	480	269	51	94	218	116	209	231	101	41	113	96	101	23
	60*	—	—	—	69	56	61	73	85	194	116	197	351	171	62†	62†	48	31	6
9	—	—	—	—	—	—	—	79	93	206	97	99	178	73	65	65	42	26	8
	—	—	—	—	—	—	—	51	94	218	116	162	305	220	41	113	164	250	86
	—	—	—	—	—	—	—	72	108	227	95	122	175	113	65	65	101	131	91
	—	—	—	—	—	—	—	75	83	163	103	141	193	128	69	69	93	177	115
10	57	161	320	172	161	388	270	—	—	—	—	—	—	—	—	—	—	—	—
	60*	—	—	—	143	314	164	—	—	—	—	—	—	—	—	—	—	—	—

12	58	178	350	149	106	206	155	51	94	218	116	101	170	143	41	113	255	88	160	273	137
	59†	49	234	38	86	198	97	79	108	227	95	72	171	82	62†	—	—	—	76	143	78
	—	—	—	—	—	—	—	—	93	206	97	61	166	109	65	83	148	109	71	101	85
15	58	178	350	149	119	242	169	51	94	218	116	48	89	98	65	83	148	109	85	105	121
	60†	—	—	—	—	407	250	79	93	206	97	86	202	103	70	81	163	97	55	91	69
	—	—	—	—	—	—	—	—	93	206	97	66	141	55	—	—	—	—	—	—	—
18	58	178	350	149	142	241	181	72	108	227	95	63	120	68	66	88	166	81	82	112	109
	59†	49	234	38	104	193	105	80	89	187	100	61	104	67	70	81	163	97	54	52	71
19	57	161	320	172	111	218	155	52	120	221	111	82	169	107	—	—	—	—	—	—	—
	60*	—	—	—	160	258	149	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21	58	178	350	149	141	280	157	72	108	227	95	40	102	59	66	88	166	81	62	85	65
	59†	49	234	38	97	184	94	—	—	—	—	—	—	—	66	88	166	81	57	99	74
24	58	178	350	149	72	189	78	52	120	221	111	48	100	59	41	113	255	88	91	152	109
	60†	—	—	—	96	250	120	73	85	194	116	71	170	93	66	88	166	81	28	73	39
	—	—	—	—	—	—	—	80	89	187	100	21	110	61	66	88	166	81	41	96	47
27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
30	58	178	350	149	93	218	135	73	85	194	116	42	93	71	66	88	166	81	17	38	24
	59†	49	234	38	135	450	232	—	—	—	—	—	—	—	66	88	166	81	35	80	53
32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
36	58	178	350	149	120	263	148	51	94	218	116	57	128	92	66	88	166	81	27	67	35
	60*	—	—	—	138	351	239	80	89	187	100	21	90	54	66	88	166	81	25	80	45
	—	—	—	—	—	—	—	80	89	187	100	42	134	69	—	—	—	—	—	—	—
39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
48	—	—	—	—	—	—	—	52	120	221	111	94	206	116	71	88	102	67	61	98	79
	—	—	—	—	—	—	—	73	85	194	116	9	40	39	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	80	89	187	100	36	114	119	—	—	—	—	—	—	—
72	60*	—	—	—	—	123	164	52	120	221	111	41	129	67	71	88	102	67	15	43	31*
	—	—	—	—	—	—	—	73	85	194	116	13	69	82	—	—	—	—	—	—	—

† Litter 59. Abnormal P:M:T proportions in control. Rather undernourished litter. Results not used in plotting curves.

* Litter 60. Curves plotted in Fig. (6), see p. (281). No control count so curves plotted to standard control.

† Litter 62. No control. Figures not used in plotting curves.
* Very bad section, difficult to get accurate count.

TABLE 3—continued

[illegible]

TABLE 4
Mean values calculated from results given in Table III and used in plotting graphs in Fig. 5

Mean values (as % of normal metaphase) for Mitotic Counts after an exposure of :—															Percentage of Degenerate cells after an exposure of :—															
Time after exposure in hrs.	½ min.			2 min.			4 min.			6 min.			10 min.			12 min.			30 min.			½ min.	2 min.	4 min.	6 min.	10 min.	12 min.	30 min.		
	P	M	T	P	M	T	P	M	T	P	M	T	P	M	T	P	M	T	P	M	T									
0	—	—	—	—	—	—	52	97	52	—	—	—	—	—	—	40	84	25	29	64	36	—	—	—	—	—	—	—	0	0
½	—	—	—	22	80	50	16	99	64	45	90	68	16	57	59	8	39	34	3	10	20	—	—	0	0	0	0	0	0	0
1	—	—	—	12	51	40	4	36	42	7	11	36	<1	10	23	1	3	6	10	23	15	—	—	0	0	0	0	—	—	0
1½	46	86	48	30	16	35	1	9	24	3	14	23	1	3	10	3	2	3	0	1	1	0	0	0	4	0	0	0	0	0
2	—	—	—	35	41	37	14	8	3	7	5	20	<1	1	2	—	—	—	0	2	0	—	—	0	<1	0	0	—	—	—
3	46	92	58	34	17	13	39	30	7	16	5	2	<1	<1	<1	0	0	0	0	3	0	0	0	0	<1	0	<1	0	<1	—
4	—	—	—	43	42	19	54	63	22	16	34	21	27	12	8	<1	<1	0	<1	1	<1	—	—	<1	<1	<1	0	8	0	8
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	<1	0	—	—	—	—	—	—	—	—	0
6	46	88	58	51	137	77	82	124	56	33	29	7	70	57	24	0	1	1	0	1	<1	0	0	4	11	4	5	9	7	7
9	—	—	—	50	121	84	78	111	77	59	88	48	53	77	38	18	21	4	—	—	—	—	—	10	10	12	12	20	—	—
12	—	—	—	30	59	44	36	78	52	55	88	56	36	69	36	26	30	8	2	5	1	—	—	13	14	25	11	42	3	3
15	—	—	—	34	69	48	32	70	41	46	64	62	19	43	19	19	21	10	0	0	0	—	—	9	17	23	13	41	6	6
18	—	—	—	55	69	52	31	55	33	41	50	55	20	28	19	—	—	—	—	—	—	—	—	3	11	16	12	—	—	—
19	—	—	—	35	68	48	37	76	50	—	—	—	—	—	—	21	23	7	0	0	0	—	—	10	13	—	—	41	32	—
21	—	—	—	40	80	45	18	45	26	36	56	42	13	31	14	—	—	—	—	—	—	—	—	4	8	16	13	—	—	—
24	—	—	—	21	54	22	23	64	34	26	54	32	17	30	15	3	10	3	<1	1	0	—	—	2	4	19	16	47	62	—
27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	<1	2	<1	—	—	—	—	—	—	—	—	64
30	—	—	—	27	62	39	22	48	37	16	36	23	12	25	13	—	—	—	—	—	—	—	—	7	1	5	8	—	—	—
32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	<1	2	11	—	—	—	—	—	—	—	—	66
36	—	—	—	34	75	42	20	62	36	16	44	24	6	22	11	3	8	7	1	2	10	—	—	<1	1	4	44	81	—	—
39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	2	<1	—	—	—	—	—	—	—	—	89
48	—	—	—	—	—	—	22	58	45	—	—	—	14	25	18	5	7	3	3	3	2	—	—	—	0	0	<1	—	—	50
72	—	—	—	—	—	—	13	47	36	—	—	—	8	37	15	3	8	4	2	4	4	—	—	—	0	0	0	—	—	0

TABLE 5

Giving the data concerning litter 60 from which Fig. 6 was constructed

Time after exposure in hours.	Mitotic phase and degenerate cell counts			
	Prophase	Metaphase	Telophase	Degenerations
1	22	70	101	0
2	18	12	16	0
6	28	22	24	1
9	57	126	66	5
15	80	163	100	5
19	64	103	60	9
24	38	100	48	13
36	55	140	96	1
72	49	102	66	3

10-minute exposure:—The initial fall in the prophase count was followed by a rise which exceeded the normal, but the metaphase count showed an increase to a maximum only slightly higher than the prophase while the telophase count did not reach normal. Degenerate cells were first seen 2 hours after exposure; although the degenerate cell count never exceeded 16 per cent., it remained at about this figure for 16 hours (9 to 25 hours after exposure).

12-minute exposure:—Mitosis was reduced to zero for 6 hours, then it reappeared but never reached the normal value. Metaphases and prophases were about equal instead of being in the usual ratio of 2:1; and the number of cells in telophase was much below the number in prophase instead of being about equal to it. The peak values for prophase and metaphase occurred 12 hours after exposure. Degeneration was first seen within 6 hours of exposure, rose rapidly in amount and was maximum (47 per cent.) 24 hours after radiation. It persisted for over 48 hours.

Counts made immediately after exposure showed that a slight reduction of mitosis had occurred during the irradiation.

30-minute exposure:—Mitosis was reduced during exposure and came to zero within one hour and, except for a small wave 12 hours after radiation, it was not observed again during the experiment. Degenerate cells appeared within 4 hours of exposure

and reached a maximum 35 hours later when 89 per cent. of the undifferentiated cells were dead. At 48 hours degeneration was still high (50 per cent. of the total) but it had disappeared 72 hours after exposure when the experiment concluded.

Discussion

It seems probable that the reaction of any actively proliferating tissue to radiation is of the same general type. The biological response to an effective exposure is certainly very much the same whether the material used is rat retina *in vivo*, chick neural tissue *in ovo* (Wilson and others, 1935), or embryonic fibroblasts, mammalian or avian, *in vitro* (Spear, 1935). In all these tissues the first effect to be observed after quite small doses of radiation is a fall in cell division. With doses just above the minimal threshold,

FIG. 7.

Showing the effect of radiation on cell division in tissue cultures (left) and that obtained with rats in the present experiments (right). Ordinates represent total cells in division as a percentage of the controls. Abscissae represent time in hours after irradiation. The tissue cultures were exposed to 100 mg. radium at 0.5 cm. distance; the rats to 300 mg. at 0.75 cm. distance.

Note difference in time scale in the two graphs.

the initial fall is followed, in each case, by a wave of mitotic activity which temporarily exceeds the normal. In Fig. 7 the results obtained with mammalian tissue are compared with those previously observed in tissue cultures of avian fibroblasts.

As the dose is further increased, mitotic recovery becomes progressively less marked, until, having passed through an intermediate stage where it fails to reach normal again, a dose is reached which causes complete disappearance of mitosis. In the case of rat tissue *in vivo* once this dose is passed mitosis tends to remain at zero for a longer time than is the case with tissue

cultures of avian fibroblasts, *i.e.*, recovery appears to be less in the rat than in the tissue cultures. This is partly due to the fact that in the retina, at this stage, many of the cells which have survived irradiation do not proceed to further divisions, but differentiate instead, thus removing themselves from the field of action, like the croquet implements provided by the Queen for Alice in Wonderland ("a very difficult game indeed").

The most instructive results from the biological standpoint are obtained with exposures (2-4 mins.) only slightly above the minimum threshold dose required to produce any effect at all. With the relatively long exposures (*e.g.*, 30 min.) some changes have already occurred before the material is removed from the radium resulting from the early part of the exposure. These changes are masked by the added effects of the rest of the exposure and can only be displayed if exposures are progressively reduced until the smallest effective exposure is reached. Further, the character of the material is probably changed as the result of irradiation and the biological response to the latter part of a prolonged exposure may differ from that resulting from the first few minutes of irradiation.

While much information can be obtained from these variations in mitosis, a more precise understanding of the events following irradiation results from an analysis of the total mitotic count into its constituent phases. It is possible in fact to measure the biological effect of an irradiation quantitatively not only in terms of gross diminution in cell division, but also by the distortion of the mitotic phase ratio as compared with the normal.

This distortion is not constant but undergoes variation according to the time which has elapsed since exposure to radiation. For example, soon after the end of a short irradiation prophase reaches its minimal value first, followed by metaphase and, later still, by telophase. Whatever the individual variation of the actual counts, the phase-minima *always* succeed each other in this order. This must mean that the initial effect of radiation is to postpone the onset of (visible) mitosis whilst allowing those cells which have already begun to divide to complete the process in spite of the radiation. To take an actual experiment, 80 minutes after a 4-minute exposure to gamma rays the normal proportion of 49:100:44 for the prophase: metaphase: telophase ratio became 0.9:30, while another rat from the same litter, killed 100 minutes later (*i.e.*, 3 hours after exposure) gave the ratio 15:5:1. (Table II, col. 8). Thus not only is the prophase count the first to reach zero but also it is the first to rise when mitotic activity returns. It reaches its maximal value to be followed *in succession* by the maxima for metaphase and telophase. The fact that telophase does not reach

zero until some time after prophase would seem conclusive evidence that the first effect of radiation is not on a mitotic process already begun but with cells in the pre-mitotic state which are temporarily prevented from entering division. This effect must, however, be clearly distinguished from those associated but delayed, degenerative effects which will now be discussed.

It is to be noted (Fig. 5 *a-g*) that degeneration only appears after mitosis has reached a minimum. The "degeneration curve" rises to a maximum and gradually falls again. The peak value of degeneration is always reached *after* that of mitosis. Further, as the dose is increased and the mitotic peak is lowered, the degeneration peak is raised.

The question arises whether the loss of mitosis accounts for the presence of degenerate cells, *i.e.*, does a cell die because it cannot divide successfully? If it is assumed that the process of division is an ordeal for which irradiation unfits certain cells, do these results supply any evidence as to the stage or stages at which the cell is especially liable to break down?

The changes observed after the shorter exposures of 2 to 10 minutes suggest that irradiated cells first find difficulty in accomplishing telophase, since the counts often give a persistent, high relative value for this stage of mitosis, indicating a slowing up of the process. This, with small doses, is unassociated with any appreciable lethal action but when the exposure reaches 4 minutes and over, the degeneration produced counteracts the effect on the curve of this prolongation of telophase.

Examination of the prophase and metaphase curves in Fig. 5 *f*. (12-min. exposure) shows that when cell division is at its maximum following this higher dose of irradiation, only a small proportion of affected cells have been able to reach prophase (low prophase count) and of these many fail altogether to accomplish the next step to metaphase. Since the normal ratio of prophase to metaphase is 1:2, the fact that the prophase and metaphase counts are approximately equal after this dose, indicates that about half the cells which reach prophase break down during the next stage of mitosis and are accounted for as degenerate cells. While the greatest number of casualties occurs at metaphase the appearance in certain specimens of very abnormal telophases indicates that some cells which have escaped destruction at the beginning of the mitotic process nevertheless succumb at later stages of division. Low telophase counts are due, therefore, in part to the loss of cells in metaphase but also to an actual destruction of cells which have accomplished the greater part of the process of mitosis. Willmer (1935) has observed that cells in tissue cultures may occasionally be held up in metaphase for an hour or more as if this phase at least was one especially difficult to accomplish and it is clear that

a cell irradiated before it begins division may find this part of the mitotic process quite beyond its powers.

Although mitosis remains at zero after the longest exposure (30 min.), degeneration is still seen. This dose is sufficient to prevent any cell entering division over the period of the experiment and causes a greater amount of degeneration than that which follows the shorter exposures, but the maximum is reached considerably later, *i.e.*, the curve of degeneration rises slowly with a relatively long period between exposure and the attainment of its peak value. It would seem that doses of this order render proliferating cells so fragile that any form of activity is likely to have fatal results.

It is important when studying the degenerative changes occurring after irradiation, to follow the process over a prolonged period and not to examine the material at some arbitrarily chosen moment. For instance, if our material had only been examined, say, 10 hours after exposure we should have been faced with the paradox that whereas a 12-minute exposure produces a 30 per cent. degeneration, a 30-minute exposure produces only one-third of this amount.

Dr. Lea (physicist to the Strangeways Laboratory) has kindly given us the following statistical note on the results of our experiments.

Three causes contribute to the variation in mitotic counts found in different animals subjected to the same dose of radiation.

(a) Some variation is inevitable on account of the finite number of cells counted in any individual. When this is the only cause of spread of the counts the standard deviation is $n^{1/2}$, where n is the number counted.

(b) Some variation is due to differences in the mitotic activity of animals before irradiation. This can be estimated by analysing the mitotic counts of the unirradiated controls.

(c) Animals differ in their radiosensitivity. Consequently a group of irradiated animals exhibit a relatively greater variation in their mitotic counts than a group of unirradiated controls.

Controls.—Mitotic counts are available for 47 unirradiated animals drawn from 46 different litters. The mean number of mitotic cells per individual is 413, and the standard deviation (s.d.) of an individual is 71 or 17 per cent. This greatly exceeds the inevitable variation (a, above) which would give a s.d. of 5 per cent. There is thus a variation of mitotic index in the unirradiated animals additional to the inevitable variation due to the finite number of cells counted.

In addition to the 47 controls mentioned, one set of five controls from a single litter (No. 49) was available. The s.d. of an individual calculated from this set of 5 mitotic counts was 16 per

cent. It thus appears that animals from a single litter vary among themselves almost as much as animals from different litters, though the certainty of this conclusion cannot be established with the limited data available.

The relative proportions of prophase (P), metaphase (M) and telophase (T) are remarkably constant in the controls. The P, M and T counts of the 47 controls when averaged and expressed as percentages of the total numbers of cells in mitosis give the ratio $P:M:T=24.3:50.4:25.3$. These figures approximate very closely to the ratio $25:50:25$, and for simplicity the latter ratios are used in the subsequent calculations. The experimentally determined total mitotic counts ($P+M+T$) were divided in the proportions $25:50:25$, and these expected counts compared with the experimental P, M and T counts. The χ^2 method (Fisher, 1936) was used to test whether the differences between the observed and expected P, M and T counts were significantly higher than those to be expected on account of the finite numbers of cells counted. Rejecting 3 controls (from litters Nos. 59, 71 and 93) which yield very high values of χ^2 , the remaining 44 are found to exhibit no greater departure from the expected P, M and T values than is inevitable on account of the finite number of cells counted. It is evident therefore that the P, M and T ratio in the control approximates to $25:50:25$, and that variations of this ratio in different animals are much smaller than variations in the total numbers of mitotic cells. One of the rejected litters (No. 59) had been under suspicion since it was a large one and it was thought that the young rats were probably undernourished.

Irradiated animals.—Plotting mitotic count as a function of time of incubation after exposure, counts from two or three identically treated animals were usually available for each point on the curve. This number is insufficient for satisfactorily estimating the standard deviation of a given point, but an average of the standard deviations of all the points can be made and is found to be 56 per cent. This greatly exceeds the inevitable statistical fluctuation, and also considerably exceeds the variation in the mitotic counts of the controls, making it evident that there is a real difference in radiosensitivity exhibited by different individuals. Differences in radiosensitivity are likely to be exhibited in two ways: (i) as differences in the amplitude of the variations of mitotic count which follow an irradiation, and (ii) as differences in the time scale of the variations. For the first few hours following an irradiation, the variations in mitotic count are rapid, subsequently they are much slower. Thus for the first few hours both (i) and (ii) contribute to the standard deviation, while subsequently only (i) is important. When the mitotic counts are

divided into two groups, the first, slowly varying, group comprising incubation times of 18 hours and more, following 2, 3, 4 or 6 minutes irradiation, and 24 hours and more, following $8\frac{1}{2}$ or 10 minutes irradiation, gives an average standard deviation of 42 per cent. The second, rapidly varying, group comprising the shorter incubation times gives an average standard deviation of 62 per cent. The existence of differences in the time scales of the response to radiation as well as in the amplitude of the response is thus confirmed. Attention has been drawn to the animals of litter 60 which show a time scale in the variation of mitotic count following irradiation markedly different from the other litters which have had the same exposure.

Summary

1. A method for exposing the developing rat eye to fairly accurate doses of gamma radiation is described.

2. It has been found that the normal 50:100:50 value for the prophase:metaphase:telophase ratio is altered as a result of irradiation. The distortion of the ratio varies with the size of the dose and the time-interval between irradiation and examination of material.

3. A relationship has been established between alteration in cell division and the occurrence of degeneration among undifferentiated cells.

4. The results show that cellular degeneration can be produced by very small doses of gamma rays and that this degeneration is accounted for by an effect upon cells which are about to divide which unfits them for the ordeal of mitosis.

5. The quantitative results obtained from this mammalian tissue closely resemble those already provided by experiments on avian fibroblasts cultivated *in vitro*.

6. A statistical analysis has been made of the individual variation of mitotic counts in different rats, both irradiated and un-irradiated.

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"THE ASSOCIATION OF DENDRITIC ULCER OF THE CORNEA AND OF SUPERFICIAL PUNCTATE KERATITIS WITH HERPES FACIALIS"

BY

HUMPHREY NEAME

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THESE combinations, especially that of all three, are rare. In the search through some of the ophthalmological literature of the last ten years for reports of cases such as will be described below, some references to disciform keratitis were found and are included, and a brief summary follows.

Case 1. C. G., aged 49 years.

Dendritic Ulcer with Herpes Febrilis of the Face.

November 22, 1926. Admitted to University College Hospital. "He came in with temperature of 102·6°, oedema of the scalp, herpetiform eruptions on both sides of his face" (House Physician's Notes).

On that date there was considerable dendritic ulceration of the right cornea (see Fig. 1). Dr. A. M. H. Gray diagnosed the lesions on the face as herpes febrilis.

P. H., "Out-patient at Western Ophthalmic Hospital in December, 1924, with nebula of right cornea. Slight ciliary injection; no staining. R.V.=6/36. L.V.=6/9 (partly). History of keratitis some years previously" (from House Surgeon).