CH-3873
Health and Biology
General

METALLURGICAL LABORATORY

University of Chicago

THE HISTOLOGICAL EFFECTS OF X RAYS ON CHICKENS WITH SPECIAL REFERENCE TO THE PERIPHERAL BLOOD AND HEMOPOIETIC ORGANS

by

Raymond Murray, Mila Pierce, and Leon O. Jacobson

Based on work done prior to July 1946

Biology Division

March 2, 1948

Contract W-7401-eng-37

Contents

THE HISTOLOGICAL EFFECTS OF X RAYS ON CHICKENS WITH SPECIAL REFERENCE TO THE PERIPHERAL BLOOD AND HEMOPOIETIC ORGANS

By

Raymond Murray, Nila Pierce, and Leon O. Jacobson*

ABSTRACT

Chickens, 3 to 11 weeks old, were treated with doses of 200 kv X rays, ranging from 2 r to 1200 r. The median lethal dose for 3-week-old chickens appeared to lie between 400 r and 800 r. Histopathologic changes in the 11-week chickens paralleled those reported for mammals of comparable maturity, with the exception of damage to neurilemma sheath cells in the chickens. The 3-week-old chickens suffered additional damage to immature structures, but the threshold of observable damage to the lymphatic tissue (25 r) was comparable to that reported for mammals.

By application of a slight modification of Kyes' method for peripheral blood counting of fowl, dramatic decreases in blood constituents, paralleling those reported elsewhere for mammals, were seen in 3-week-old and 5-week-old chickens after treatment with 600 r and 800 r, respectively. Numerous degenerating cells were observed in dry smears made during the first week after irradiation, in contrast to the scarcity of such findings in smears of mammalian blood at the same period. Although the normal values for leukocytes of chicken blood were found to be several times those of mammalian

---

* The authors are indebted to Matthew H. Block who contributed to the early phases of the investigation and to Mrs. Lorine Newman for her technical assistance.

blood, this difference could account only in part for the greater degeneration observed in the chickens.

THE HISTOLOGICAL EFFECTS OF X RAYS ON CHICKENS WITH SPECIAL REFERENCE TO THE PERIPHERAL BLOOD AND HEMOPOIETIC ORGANS

By

Raymond Murray, Mila Pierce, and Leon O. Jacobson*

I. Introduction

The acute effects of 200 kv X-ray treatment on chickens were investigated in a series of 200 animals exposed to doses of 2 r to 1200 r. An additional series of 100 chickens served as the control. Several features rendered the chick particularly useful as the experimental subject in the Metallurgical Laboratory, notably; the ease of handling, the uniformity of development, and especially the adaptability of chick's anatomical structure to a study of certain blood-cell-forming processes. Since erythropoiesis in the bone marrow of the chick is intravascular while myelopoiesis is extravascular, and since the bone marrow contains true lymph nodules, radiation effects upon the separate marrow constituents are more readily apparent than in mammals. Although lymph nodes, as such, are not present in the chick, lymphatic nodules are found in almost all of the organs; extra-medullary myelopoiesis, especially granulocytopoiesis, occurs in the same organs identified with this activity as in rodents, but also occurs in the heart, intestine, loose connective tissue, and elsewhere. In addition,

---

* The authors are indebted to Matthew H. Block who contributed to the early phases of the investigation and to Mrs. Lorine Newman for her technical assistance.

observation of the effect of radiation on still-developing organs, as for instance upon the metanephrogenic tubules, is permitted by the use of 3-week-old chicks.

This report is so arranged that the effects of 800 r, serve as the basis of criteria for describing the changes due to other dose levels of radiation in each organ. Results obtained with 1000 r are then presented, followed by those with 400 r and the lower dose levels in turn. A comparison is offered of the effects of the base dose of 800 r upon the individual organs of a series of 3-week-old and 11-week-old chicks. A summary and discussion follow. No detailed report is made here for the animals receiving 1200 r, since few survived for serial sacrifice, and since the pattern of damage observed was very similar to that seen after 1000 r. The number of animals sacrificed at the various doses and intervals is detailed in Table 1.

The chickens for both the blood and histological studies were exposed, in groups of three in plastic boxes, to 200 kv X radiation. Characteristics of the radiation and dosimetry are described in detail in another report[1]. The animals were sacrificed by decapitation, and the tissues were fixed in Zenker-formol solution, imbedded and sectioned in nitrocellulose, and stained with hematoxylin-eosin-azure II.

There were no specific studies made to determine the median lethal dose or time of maximum mortality among these animals, but the record of deaths suggests an LD_{50} between 400 r and 800 r. With doses of 1000 r or more, most of the 3-week chickens died within the first day, although a few survived for a week. After 800 r many died early, but about half survived for a week and a few for at least 6 weeks. With 400 r or less, there were a very few deaths just under 2 weeks.

Table 1

Doses and intervals

	3-week Chicks (BB)										11-week Chicks (BC)	
Doses:	1200 r	1000 r		800 r		400 r	100 r		25 r	6 r	2 r		800 r	
Intervals		Exp.	Cont.	Exp.	Cont.	Exp.	Exp.	Cont.	Exp.	Exp.	Exp.	Cont.	Exp.	Cont.
	Number of Animals													
30 min.						2	2	2		2	2	2		
45 min.				2	1				2					
1 hour		2	2										2	4
90 min.									2	2	2	2		
2 hours		2	2			2	2	2						
2 hrs., 15 min.				2	1									
3 hours													2	3
3 hrs., 45 min.	3													
4 hours		2	2			2	2	2	2	2	2	4		
5 hours				1	1									
6½ hours	1													
7 hours		2	1			2	2	2						
8 hours				1	1									
10 hours									2	2	2	2		
11 hours		2	1											
12 hours													2	2
13 hours						2	2	2						
14 hours				2	1									
15 hours									2			2		
16 hours		2	1										2	2
18 hours						2	2	2						

Table 1 (Continued)

Doses: / Intervals	3-week Chicks (BB)										11-week Chicks (BC)	
	1200 r	1000 r Exp.	1000 r Cont.	800 r Exp.	800 r Cont.	400 r Exp.	100 r Exp.	100 r Cont.	25 r Exp.	6 r Exp.	2 r Exp.	2 r Cont.	800 r Exp.	800 r Cont.
	Number of Animals													
24 hours		2	1	3	3				2	2	2	2	2	2
30 hours						2	2	2						
36 hours		2	1											
48 hours		2	2	2	1	2	2	2	2			2	2	3
72 hours		2	1	2	1	2	2	3	2	2	2	2		
4 days		2	2	2	1								2	3
5 days				1		2	2	2	2	2				
6 days				1	1								2	2
7 days				2										
8 days				1	1	2	2	2						
9 days				1	2				2	2	2	2	1	
12 days						2	2	2					2	
14 days									2	2		2		
17 days				(		2	2	3						

After treatment the chickens often grew lethargic, and showed signs of weakness, especially in their legs. Those that survived the irradiation for any time were stunted in growth. The feathers of many were ruffled, and the combs of the males did not develop normally. At autopsy the lymphatic organs were found to be small, and the intestine often contained hemorrhagic areas; no other significant changes could be discerned on gross examination.

2. Peripheral Blood

2.1 Materials and Methods: The methods used for counting mammalian blood cells cannot be used for counting the blood cells of chickens for several reasons. The diluting fluids used for the study of mammals cause, in chickens, hemolysis and clumping of the erythrocytes, and hence, spurious counts of red cells. "Nucleated stromata" from the hemolysed nucleated erythrocytes may introduce, furthermore, a large, variable error in the leucocyte count. Any method that depends on complete hemolysis of the erythrocytes in order to count the leucocytes cannot be applied to the blood of chickens, in which almost of all the erythrocytes are nucleated.

Wetmore's[2] modification of the Blain method of differential staining of the leucocytes with cresyl blue and pyronin was tried without satisfactory results. The relatively faint staining did not add materially to the morphologic criteria for distinguishing between the cell types, and clumping of erythrocytes frequently occurred so rapidly that counts were extremely unreliable.

In attempts to find a diluting agent that would fix the cells quickly before clumping occurred, 1 per cent acetic acid, 0.1 per cent picric acid,

and 0.2 per cent mercuric chloride were used. In each case hemolysis or agglutination made counting impossible. With 50 per cent propylene glycol, to which azure II dye had been added, the cells were discrete and well-stained, but difficult to see because of the similarity in refractive index of the cells and the diluting medium.

Because osmic acid has been used by Kyes[3] with satisfactory results, 1 per cent and 2 per cent osmic were tried, with and without dyes. It was found that the dyes did not appreciably aid in the differentiation and seemed to increase the likelihood of erythrocyte agglutination. The 2 per cent osmic diluent at room temperature, rather than at 43°C, as used by Kyes, was satisfactory for distinguishing all the cell types if the count was made at 500 X magnification. The thrombocytes could be distinguished from the lymphocytes by their oval or spindle shape and polar granules, and the granulocytes, by their specific granulation. No "nucleated stromata" or partially hemolysed erythrocytes were seen.

In practice, the method used was as follows: A free flowing drop of blood from the leg or wing vein was drawn to the 1 mark on a standard red pipette, and diluted immediately (speed is of great importance) to the 101 mark with 2 per cent osmic acid, giving a dilution factor of 100. Lymphocytes, granulocytes, and thrombocytes in the four large squares of the chamber were counted separately, and the totals multiplied by 250 to give the total number of each cell type per cubic mm. Counting the cells required individual morphological identification of each cell under the 43 X objective, but the tedium of this procedure was partially compensated by the fact that a differential as well as an absolute leucocyte count were obtained in

this way. The differential counts thus obtained checked well with others made from dry smears.

The total leucocyte count, exclusive of thrombocytes, was found by this method to range from about 15,000 to 50,000 or more, in a variety of chickens from various sources and of varying ages. Lower values were found in chicks 1 to 2 weeks of age, the highest in 6-week chicks, and intermediate values in mature (3-month to 12-month) hens (Tables 2, 3, and 4). Although the variations were great, all values were relatively high compared to those of mammals, and in no case could we confirm Kyes' finding of leucocyte values similar to those of mammalian blood. The increase in total leucocytes with increasing age appeared to be almost entirely an increase in granulocytes, as appears in the accompanying tables. The 3-week chickens used for the present experiment, chosen to correspond in age to the histological material, had leucocyte counts more like the 6-week chicks than like the 1-week chicks.

Some indication of the variations encountered in repeated counts of a single subject can be seen in Table 5. The wide variations in the first few samplings may represent technical errors resulting from unfamiliarity with the methods. On the last two days the variations are much less, but still perhaps somewhat greater than usually encountered in the counting of mammalian blood.

Reticulocyte counts were made on smears prepared in the standard manner with brilliant cresyl blue stain. Wright stained dry smears were examined for morphologic changes in the circulating cells. Hemoglobin determinations were made in a photoelectric hemoglobinometer calibrated for human blood and the values given are uncorrected.

Table 2

Leucocyte counts on chickens 4 days to 12 days of age*

Chick No.	Date	Age in Days	Total	Leuco-cytes	Lympho-cytes	Granulo-cytes	Thrombo-cytes
401	10-5-44	4	67,700	24,250	20,500	3,750	43,500
402	10-6	5	66,250	20,250	14,750	5,500	46,000
403	10-6	5	68,000	23,000	12,750	10,250	45,000
404	10-9	8	61,000	21,500	16,000	5,500	39,500
405	10-9	8	52,750	16,750	14,250	2,500	36,000
406	10-9	8	66,000	15,500	13,500	2,000	50,500
407	10-9	8	70,000	23,250	14,250	9,000	46,750
408	10-9	8	51,500	17,750	14,500	3,250	33,750
409	10-9	8	46,250	13,750	12,500	1,250	32,500
410	10-11	10	65,000	19,500	14,500	5,000	45,500
411	10-11	10	55,250	22,750	18,750	4,000	32,500
412	10-11	10	69,750	25,750	19,750	6,000	44,000
413	10-11	10	55,000	21,750	17,750	4,000	33,250
414	10-11	10	63,750	25,750	18,500	7,250	38,000
415	10-11	10	62,750	24,000	15,500	8,500	38,750
416	10-11	10	62,500	24,500	18,250	6,250	38,000
417	10-12	11	71,000	26,250	14,500	11,750	45,750
418	10-12	11	95,750	27,500	21,000	6,500	68,250
419	10-12	11	80,750	31,000	19,750	11,250	49,250
420	10-12	11	63,500	20,250	16,500	3,750	43,250
421	10-13	12	57,250	22,750	14,500	8,250	34,500
422	10-13	12	56,500	18,000	15,500	2,500	38,500
423	10-13	12	54,000	19,500	16,000	3,500	34,500
424	10-13	12	60,750	13,000	10,500	2,500	47,500
425	10-13	12	56,750	29,000	22,250	6,750	27,750

* Each count represents a different chicken, but all were from a group of 30 hatched on the same day (10-1-44).

Table 3

Leucocyte counts on 6-week-old chicks

Chick No.	Date	Total	Leuco-cytes	Lympho-cytes	Granulo-cytes	Thrombo-cytes
I	9-16-44	84,000	34,250	20,750	13,500	49,750
	9-19	81,500	39,250	22,000	17,250	42,250
	9-22	77,000	28,750	13,250	15,500	48,250
II	9-16	86,250	47,750	31,750	16,000	38,500
	9-19	62,500	30,000	24,500	5,500	32,500
	9-22	94,250	43,750	27,000	16,750	50,500
	9-26	93,500	47,500	31,250	16,250	46,000
III	9-16	78,250	39,250	25,500	13,750	39,000
	9-19	140,750	61,000	26,000	35,000	79,750
	9-22	111,000	78,250	33,250	45,000	33,000
	9-26	116,000	55,750	25,000	30,750	60,250
IV	9-15	59,000	26,500	20,000	6,500	32,500
	9-20	94,250	52,750	18,250	33,500	41,500
	9-25	103,500	59,250	27,000	32,250	43,250
V	9-15	88,500	42,750	26,500	16,250	45,750
	9-20	94,250	34,500	22,750	11,750	59,750
	9-25	77,250	33,750	18,500	15,250	43,500
VI	9-15	54,000	27,500	15,750	11,750	26,500
	9-20	59,000	30,250	14,250	16,000	28,750
	9-25	85,000	46,500	25,000	21,500	38,500

Table 4

Leucocyte counts on mature chickens from another laboratory

Chick No.	Total	Leuco-cytes	Lympho-cytes	Granulo-cytes	Thrombo-cytes
W 1 yr. ♀	58,500	26,250	21,500	7,750	32,250
R 1 yr. ♀	72,500	43,000	39,250	3,750	29,500
(1) 3 mo. ♀	76,250	38,000	35,000	3,000	38,250
(2) 3 mo. ♀	90,750	47,500	40,500	7,000	43,250

Table 5

Repeated leucocyte counts on a single mature hen

Date		Total	Leuco-cytes	Lympho-cytes	Granulo-cytes	Thrombo-cytes
9-6-44	(1)	54,250	25,750	-	-	28,500
	(2)	52,500	28,250	-	-	24,250
	(3)	70,500	25,000	-	-	45,500
9-7	(1)	61,000	26,250	20,000	6,250	34,750
	(2)	71,000	29,000	21,250	7,750	42,000
	(3)	110,500	33,000	23,000	10,000	77,500
	(4)	151,000	51,000	37,000	14,000	100,000
9-9	(1)	106,500	39,500	33,250	6,250	71,000
	(2)	86,250	31,250	21,750	9,500	55,000
	(3)	116,250	41,000	24,500	16,500	75,250
9-11	(1)	89,500	39,500	26,000	13,500	50,000
	(2)	94,250	40,750	21,500	19,250	53,500
	(3)	103,250	39,750	23,500	16,250	63,500
9-13	(1)	90,000	41,750	21,250	20,500	49,250
	(2)	81,500	39,000	23,250	15,750	42,500

Two groups of six chicks each were treated with 600 r (23-day-old animals) and 800 r (37-day-old animals), respectively. A comparable group of controls was used for each experimental group. Four of the six chickens receiving 600 r survived four weeks of the blood sampling period, but all those receiving 800 r died within 12 days. The number of chicks counted at each interval is shown in Table 6.

Table 6

	1 hr	3 hrs	10 hrs	1 day	2 days	4 days	7 days	8 days	14 days	28 days
600 r	-	6	6	5	5	5	-	5	4	4
800 r	6	6	5	4	4	4	4	-	-	-

2.2 Hemoglobin and cell counts: (a) Hemoglobin in g per 100 cc of blood and erythrocytes per mm^3: Single-dose, total-body X radiation had only a moderate effect on hemoglobin and erythrocyte values (Figures 1-4). The rise in hemoglobin at 1 day is probably significant only after 800 r, but there was an appreciable depression in this function at 4 days with both 600 r and 800 r. The apparent elevation of hemoglobin 4 weeks after 600 r might be more significant if more than 4 chicks had survived and if intervals intermediate between 4 and 28 days had been sampled.

The sharp early rise in erythrocytes, seen only after 800 r, possibly reflected changes in the volume of blood, rather than an absolute increase in the number of erythrocytes; the first sampling of the 600 r series was too late to detect this change if it did occur. At 4 to 8 days there was a moderate depression in the number of erythrocytes, though after 600 r this depression was less marked and a full recovery was effected at 4 weeks.

Figure 1. Effect of 800 r X irradiation on the hemoglobin values of 37-day chicks.

Figure 2. Effect of 600 r X irradiation on the hemoglobin values of 23-day chicks.

FIG. 1

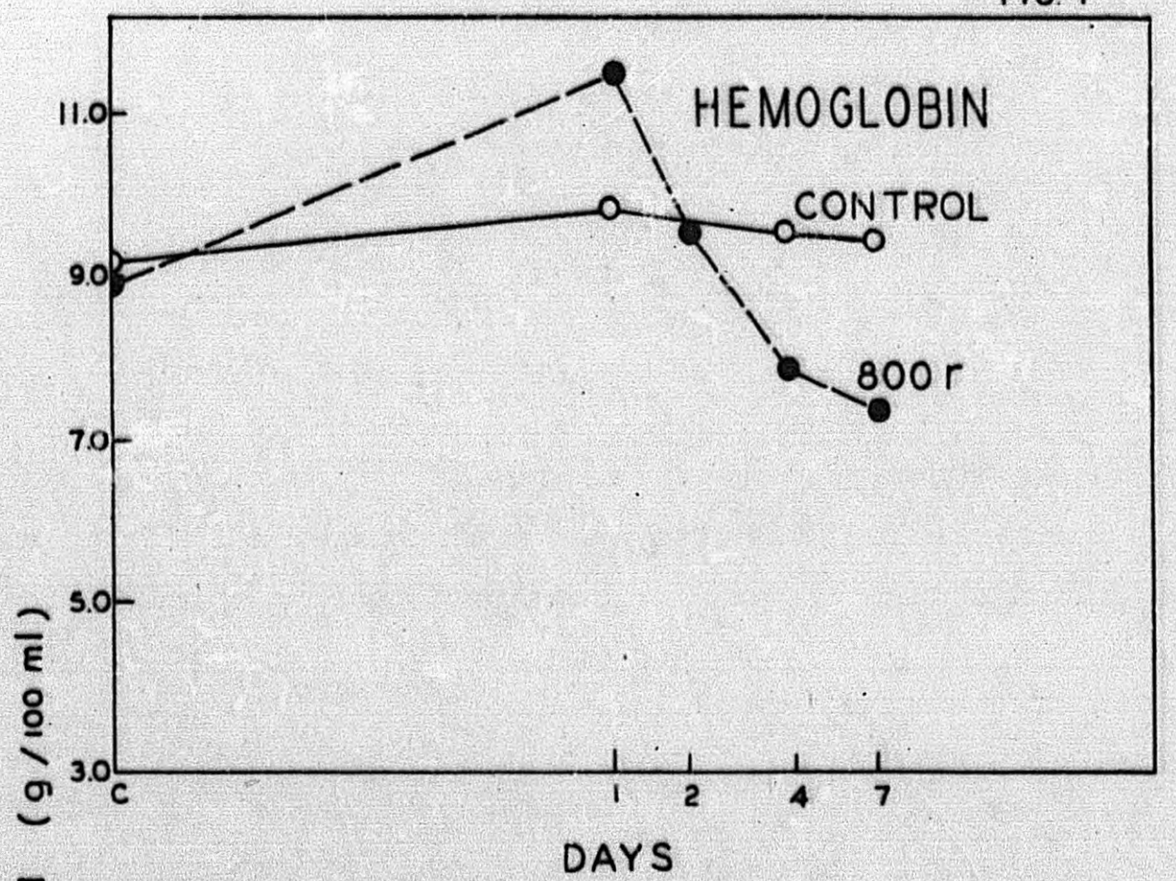

HEMOGLOBIN
CONTROL
800 r
11.0
9.0
7.0
5.0
3.0
C
1
2
4
7
DAYS
HEMOGLOBIN (g/100 ml)

FIG. 2

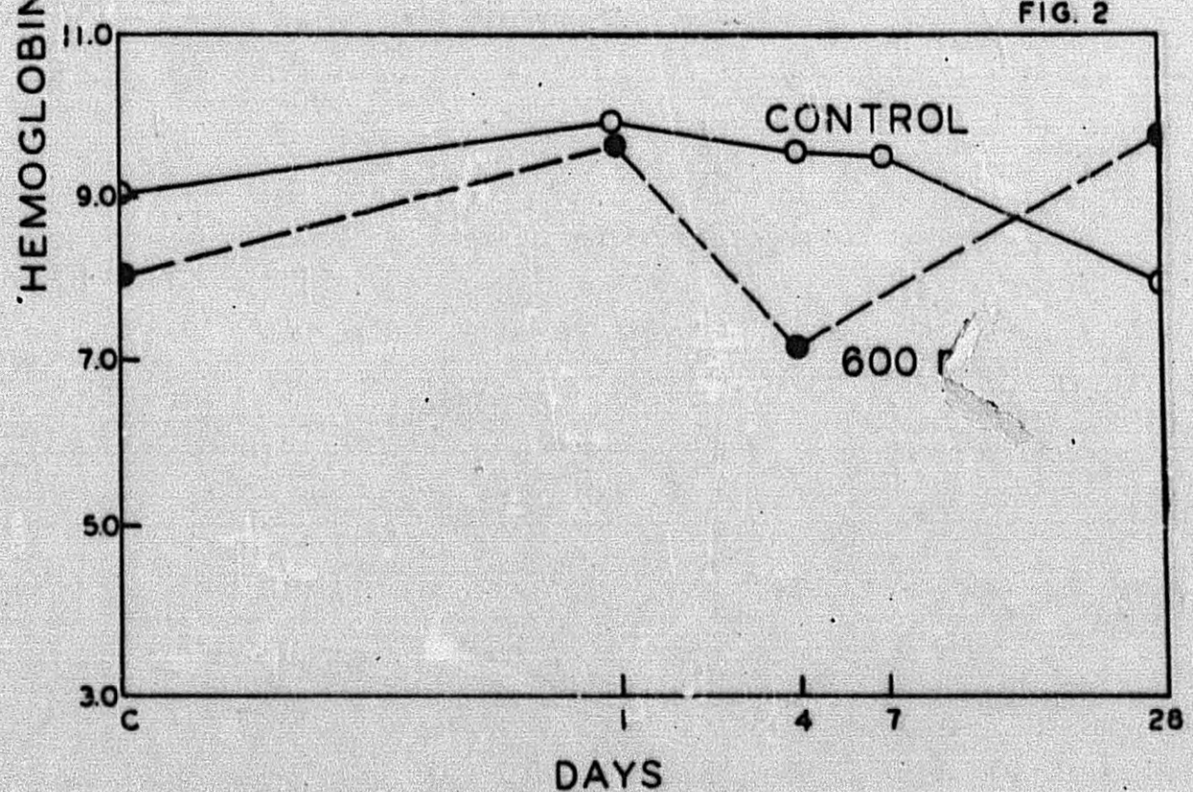
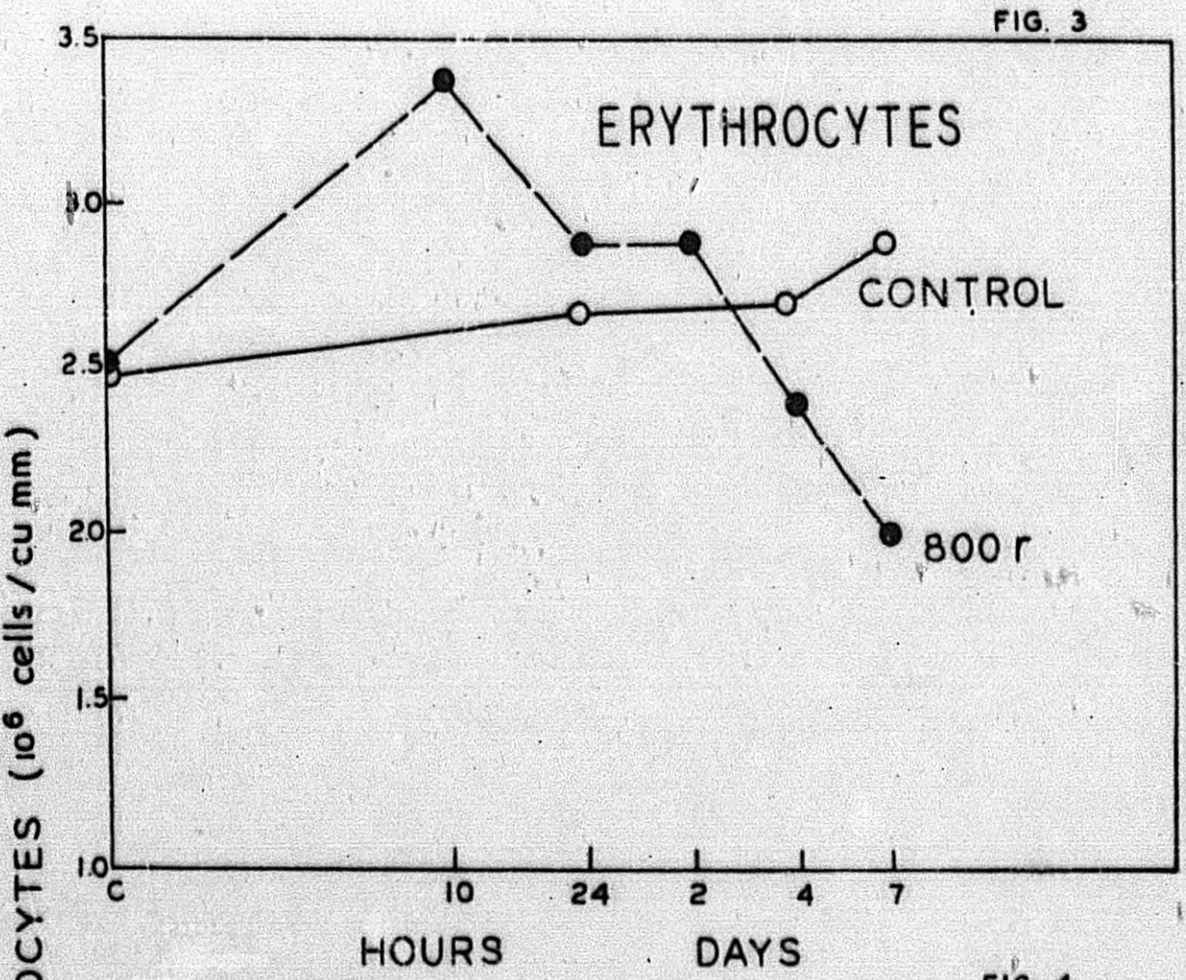

CONTROL
600 r
11.0
9.0
7.0
5.0
3.0
C
1
4
7
28
DAYS
HEMOGLOBIN

Figure 3. Effect of 800 r X irradiation on the erythrocyte values of 37-day chicks.

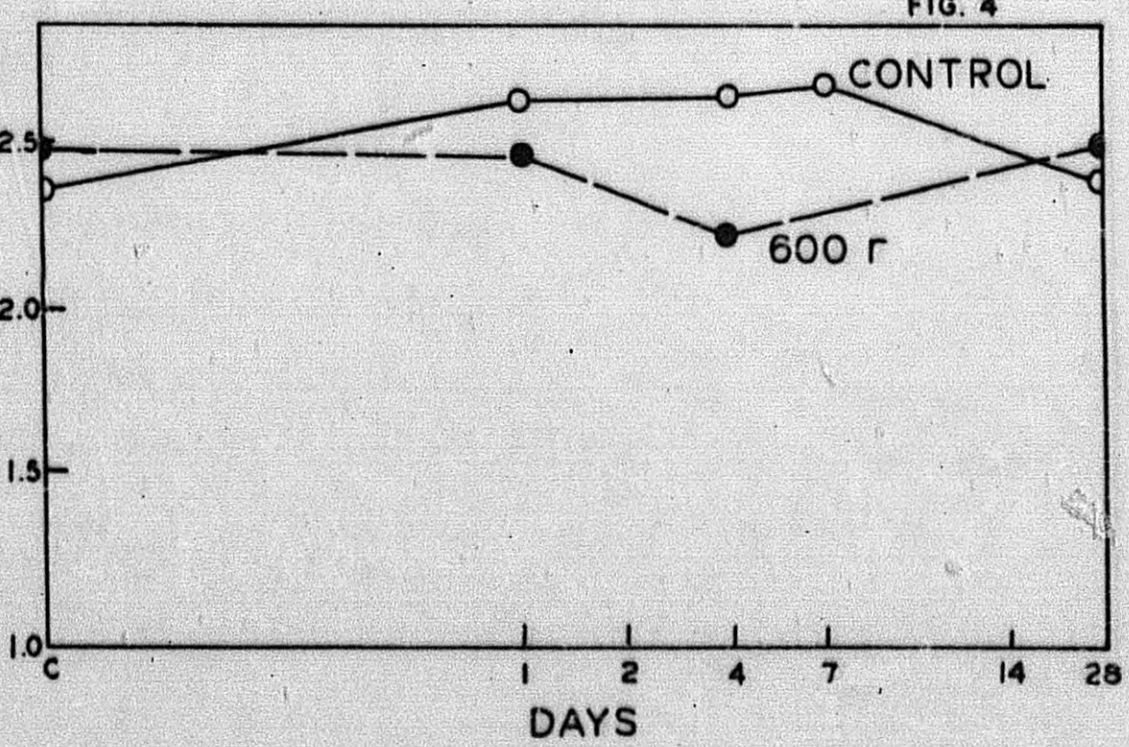
Figure 4. Effect of 600 r X irradiation on the erythrocyte values of 23-day chicks.

FIG. 3



ERYTHROCYTES (10^6 cells / cu mm)

FIG. 4



(b) Reticulocytes, increase in percentage of erythrocytes: After doses of both 800 r and 600 r (Figures 5 and 6), the reticulocytes were depressed at 10 hours or earlier, elevated at 24 hours, and again depressed at 2-4 days (800 r) or 8 days (600 r). Thereafter, the value remained low until death with 800 r, but was well above the level of the control animals 4 weeks after 600 r.

(c) Thrombocytes per mm^3: The precipitous fall in the number of thrombocytes from 45,000 to 2,000 or less in a week was almost identical in the 600 r and 800 r series (Figures 7 and 8). The value in the surviving 600 r chickens had recovered substantially at 14 days but at 4 weeks was still definitely below that of the controls.

(d) Leucocytes per mm^3: After doses of 600 r and 800 r the leucocyte value (Figures 9 and 10) fell approximately 50 per cent by 3 hours and rose abruptly again by 10 hours to values more than 25 per cent above the initial or control values. Thereafter, a drastic reduction was apparent, reaching a maximum at 4 days, and still prevailing at 7 days. The chickens surviving 600 r showed a substantial recovery in the number of leucocytes at 2 weeks but very little additional increase at 4 weeks. The curves for leucocytes and thrombocytes are thus very similar except for the early rise in the former.

The peak in the leucocyte curve at 10 hours was caused by a striking rise in the number of heterophils (Figures 11 and 12), more than offsetting a severe concomitant reduction in the number of lymphocytes (Figures 13 and 14). The lowered heterophil values from 4 to 8 days might be due in part to counting as lymphocytes an unknown number of cells which were actually very young heterophil myelocytes but which were indistinguishable as such only

Figure 5. Effect of 300 r X irradiation on the reticulocyte values of 37-day chicks.

Figure 6. Effect of 600 r X irradiation on the reticulocyte values of 23-day chicks.

FIG. 5

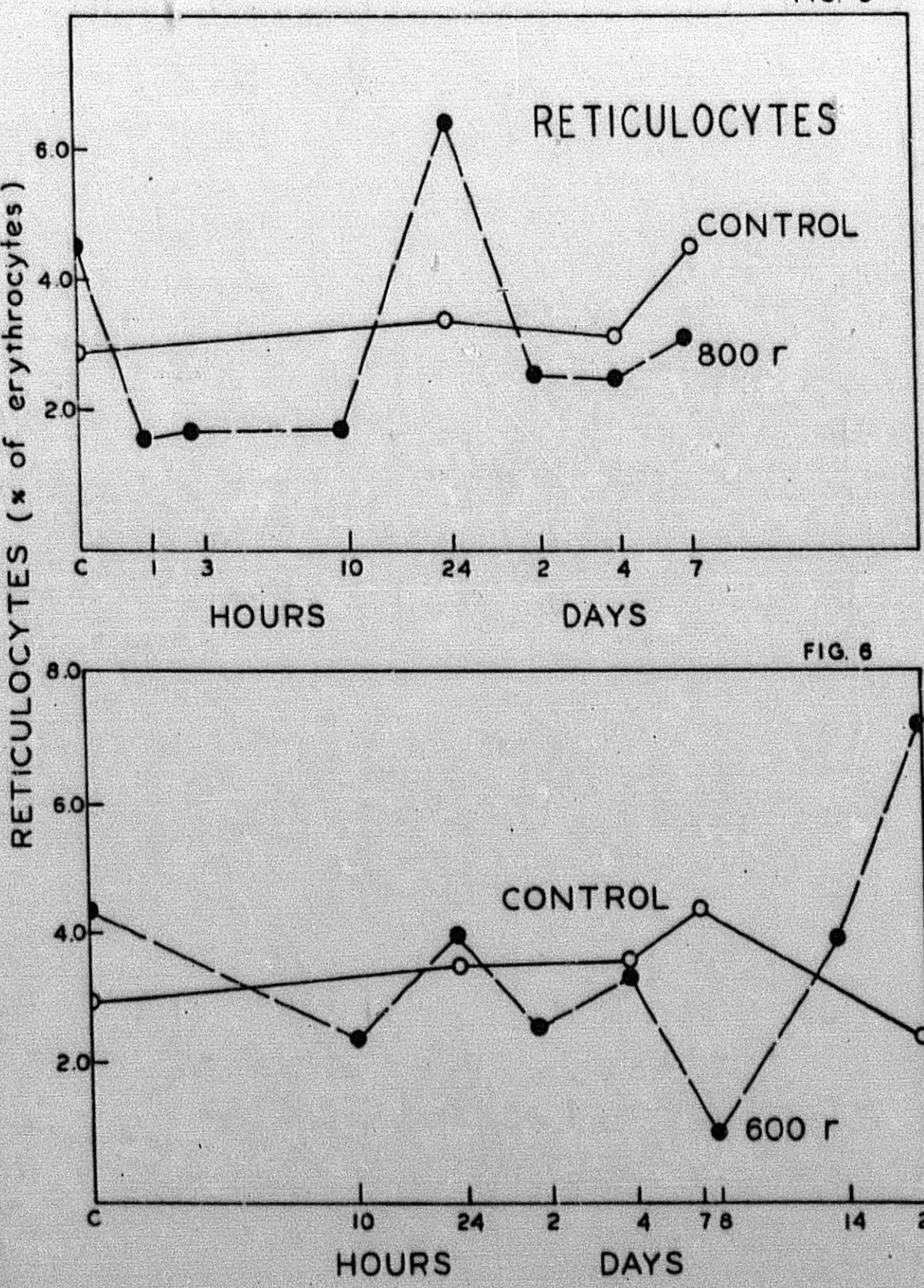

RETICULOCYTES
CONTROL
800 r
6.0
4.0
2.0
RETICULOCYTES (% of erythrocytes)
C
1
3
10
24
2
4
7
HOURS
DAYS

FIG. 6

8.0
6.0
4.0
2.0
RETICULOCYTES
CONTROL
600 r
C
10
24
2
4
7
8
14
28
HOURS
DAYS

Figure 7. Effect of 800 r X irradiation on the thrombocyte values of 37-day chicks.

Figure 8. Effect of 600 r X irradiation on the thrombocyte values of 23-day chicks.

FIG. 7

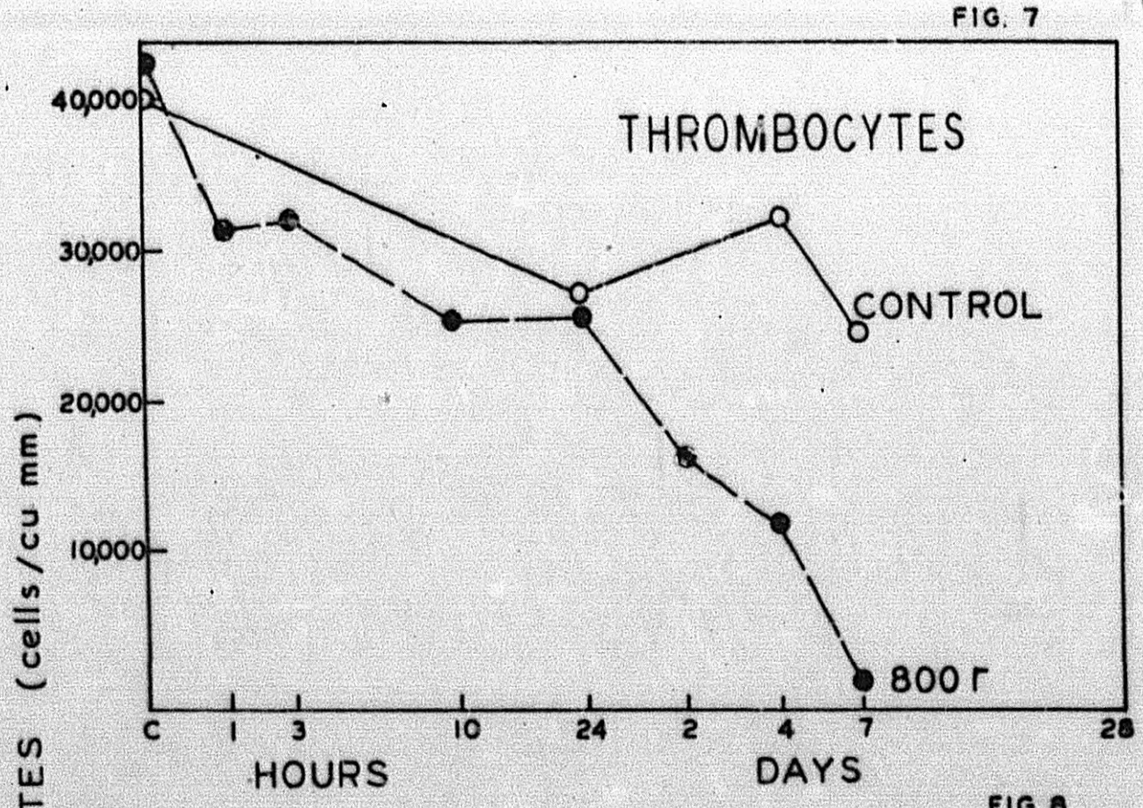

THROMBOCYTES
CONTROL
800 r
40,000
30,000
20,000
10,000
C
1
3
10
24
2
4
7
28
HOURS
DAYS

FIG. 8

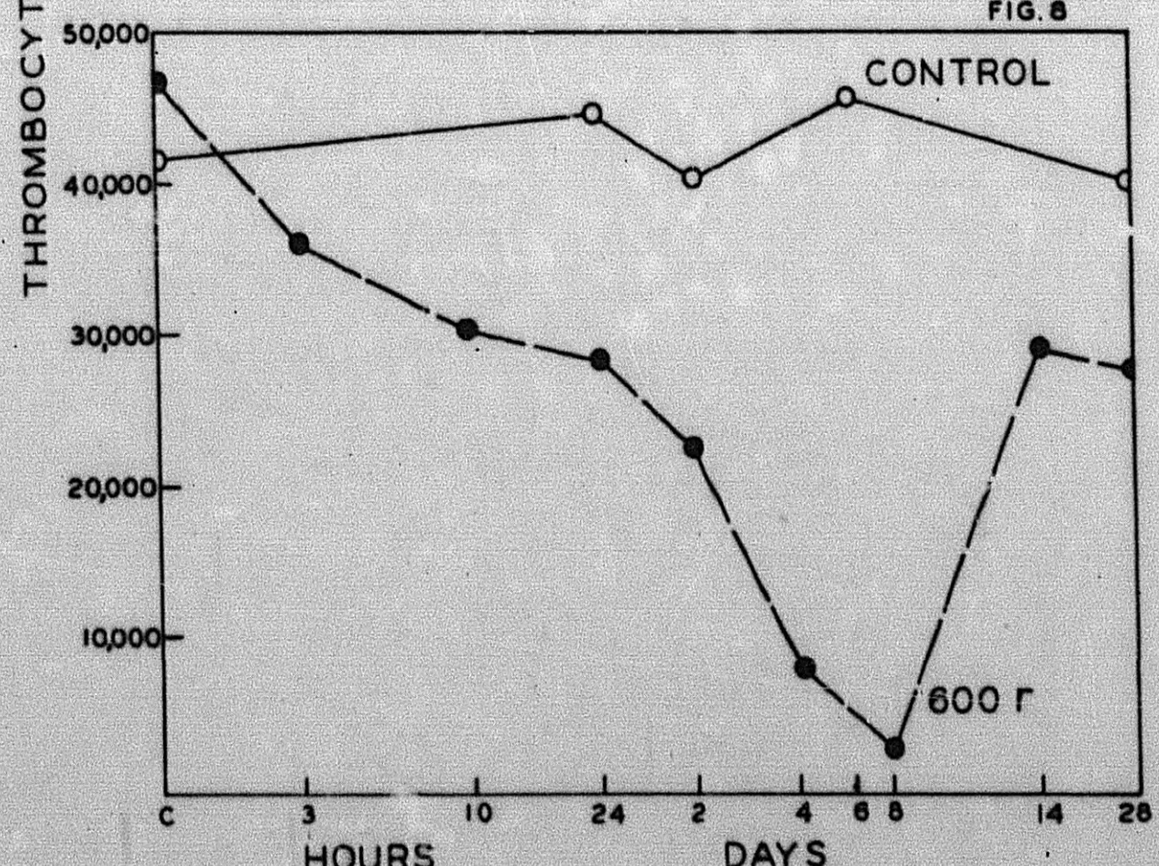

THROMBOCYTES (cells/cu mm)
CONTROL
600 r
50,000
40,000
30,000
20,000
10,000
C
3
10
24
2
4
6
8
14
28
HOURS
DAYS

Figure 9. Effect of 800 r X irradiation on the total leucocyte values of 37-day chicks.

Figure 10. Effect of 600 r X irradiation on the total leucocyte values of 23-day chicks.

FIG. 9

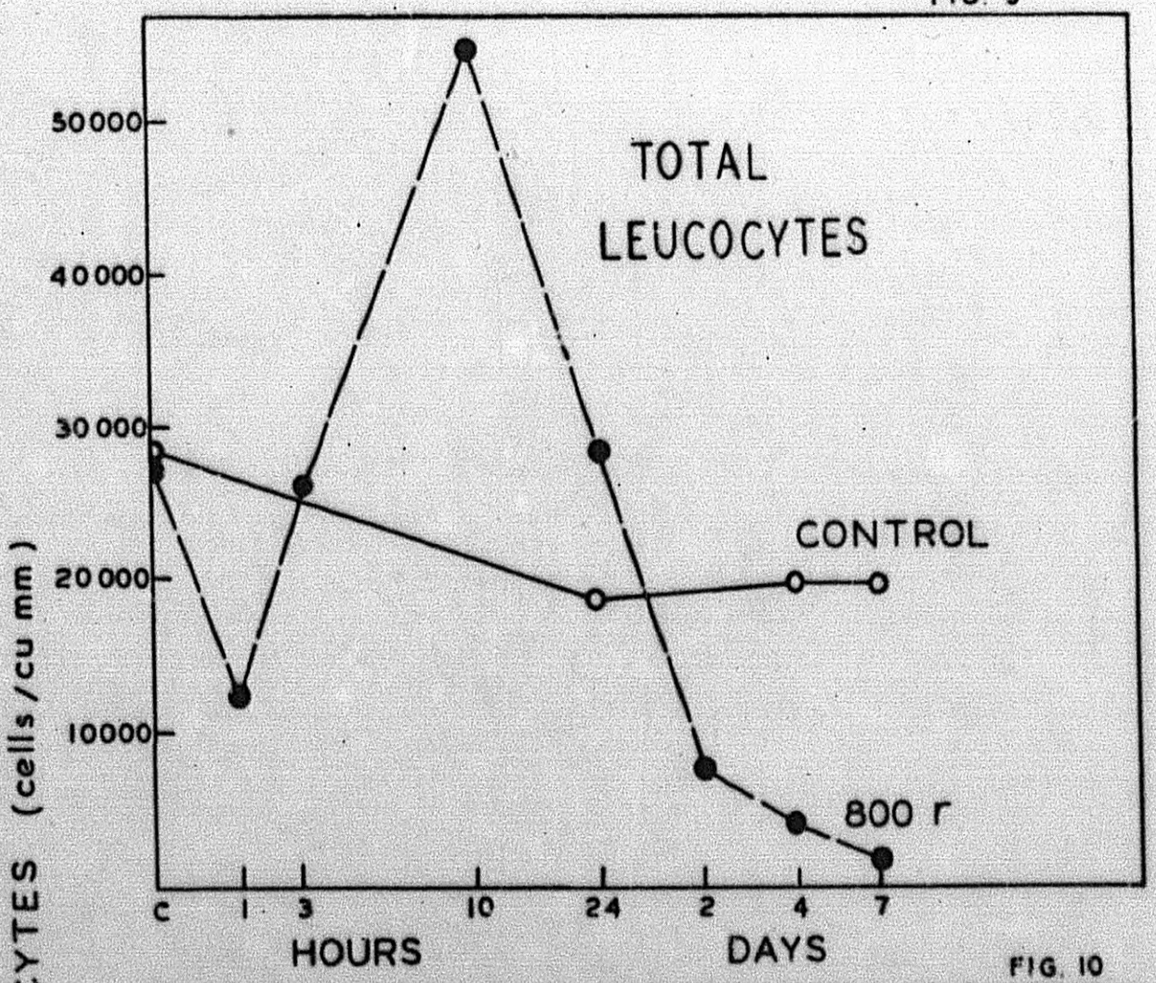

TOTAL
LEUCOCYTES
50000
40000
30000
20000
10000
CONTROL
800 r
TOTAL LEUCOCYTES (cells/cu mm)
C
1
3
10
24
2
4
7
HOURS
DAYS

FIG. 10

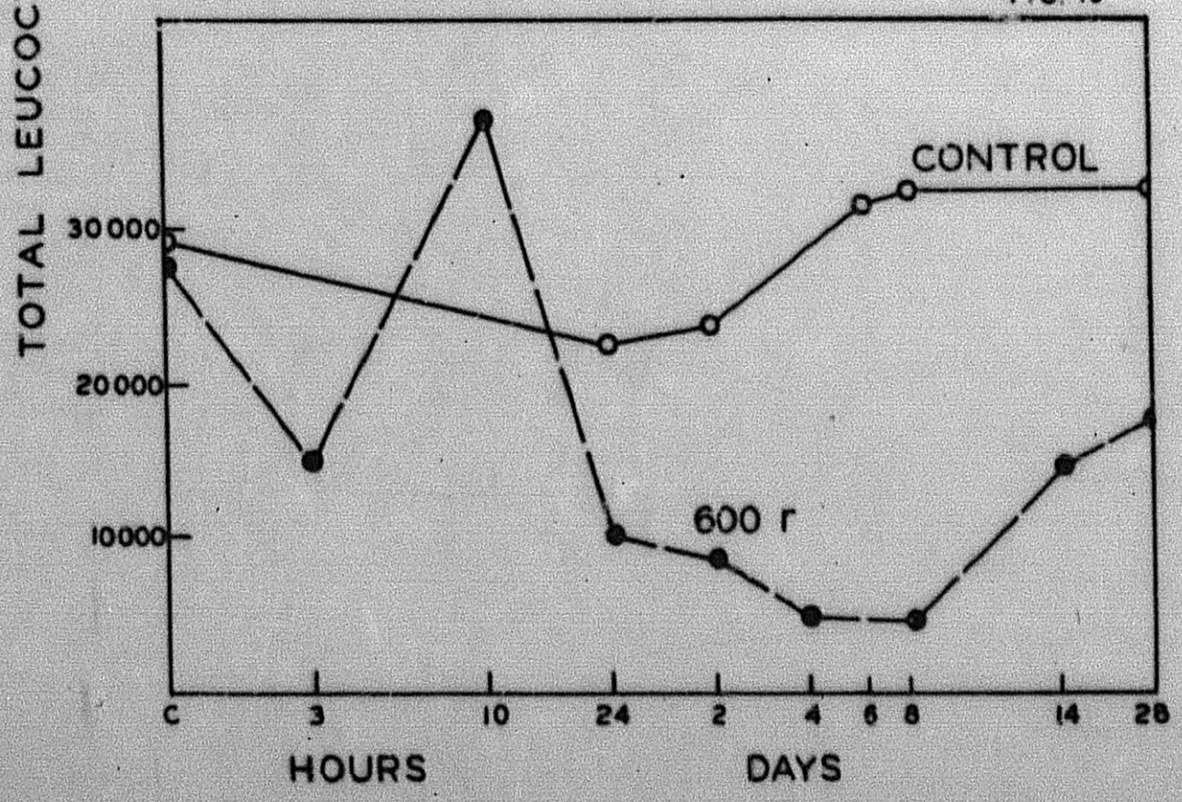

CONTROL
30000
20000
10000
600 r
TOTAL LEUCOCYTES
C
3
10
24
2
4
6
8
14
28
HOURS
DAYS

Figure 11. Effect of 800 r X irradiation on the heterophil values of 37-day chicks.

Figure 12. Effect of 600 r X irradiation on the heterophil values of 23-day chicks.

FIG. 11

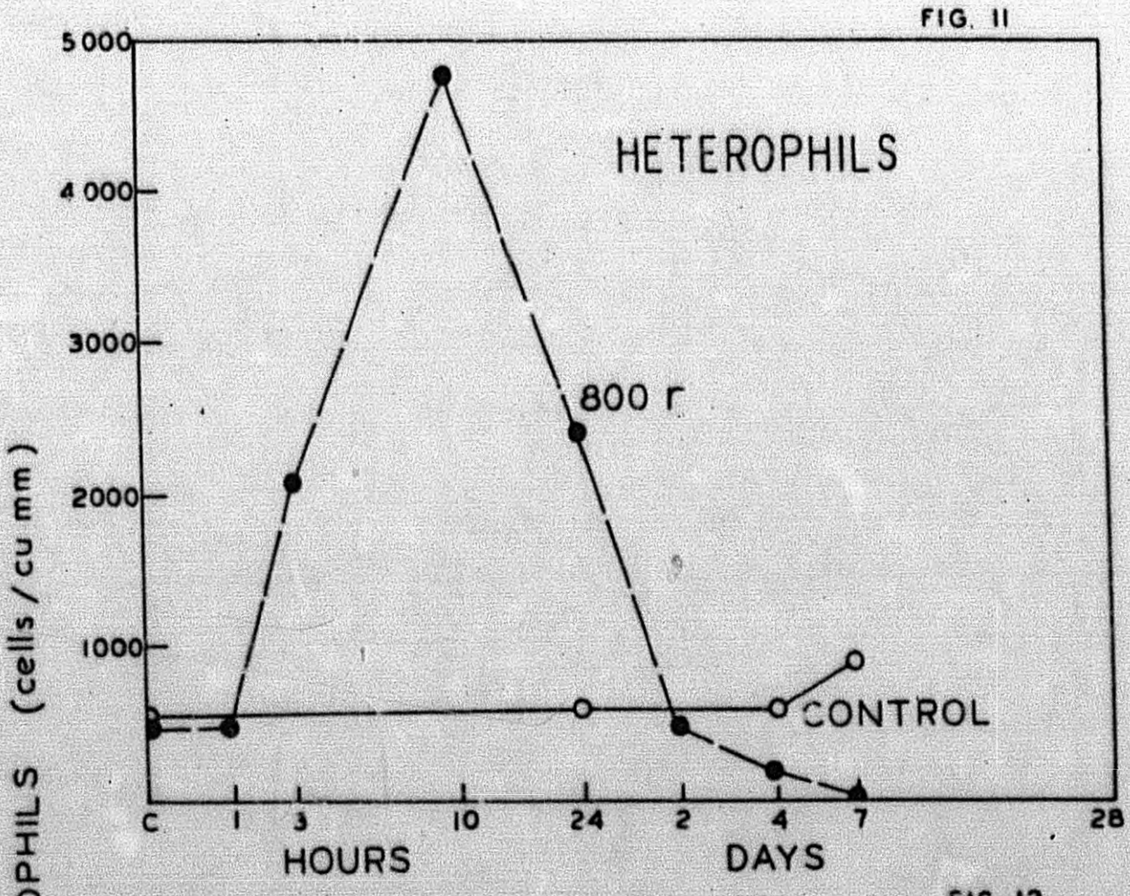

HETEROPHILS
800 r
CONTROL
5000
4000
3000
2000
1000
HETEROPHILS (cells / cu mm)
C
1
3
10
24
2
4
7
28
HOURS
DAYS

FIG. 12

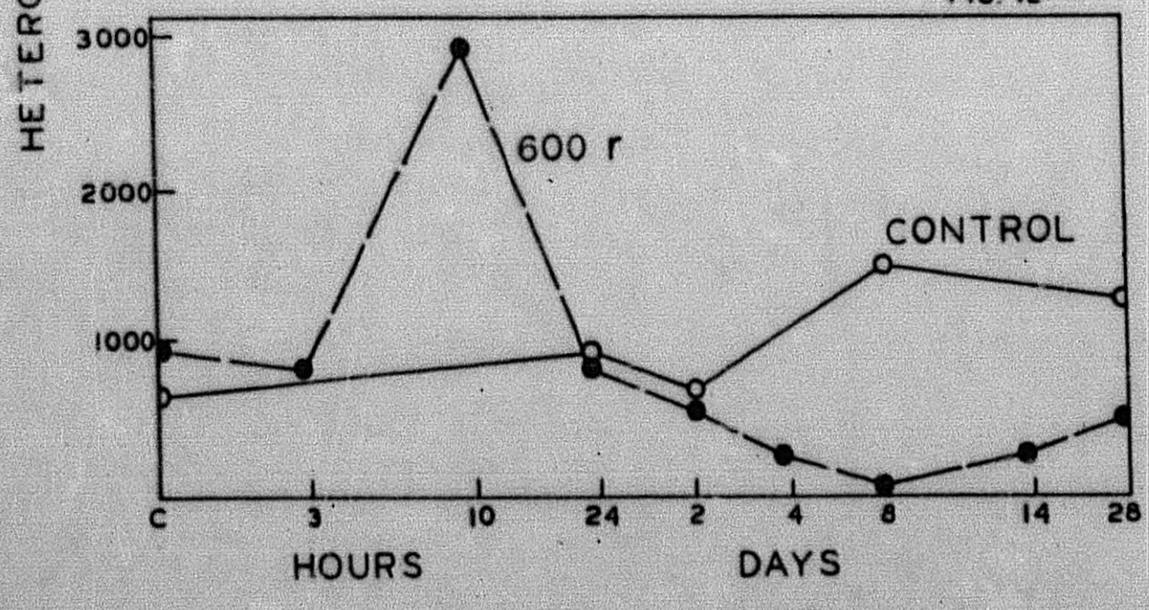

600 r
CONTROL
3000
2000
1000
C
3
10
24
2
4
8
14
28
HOURS
DAYS

Figure 13. Effect of 800 r X irradiation on the lymphocyte values of 37-day chicks.

Figure 14. Effect of 600 r X irradiation on the lymphocyte values of 23-day chicks.

FIG. 13

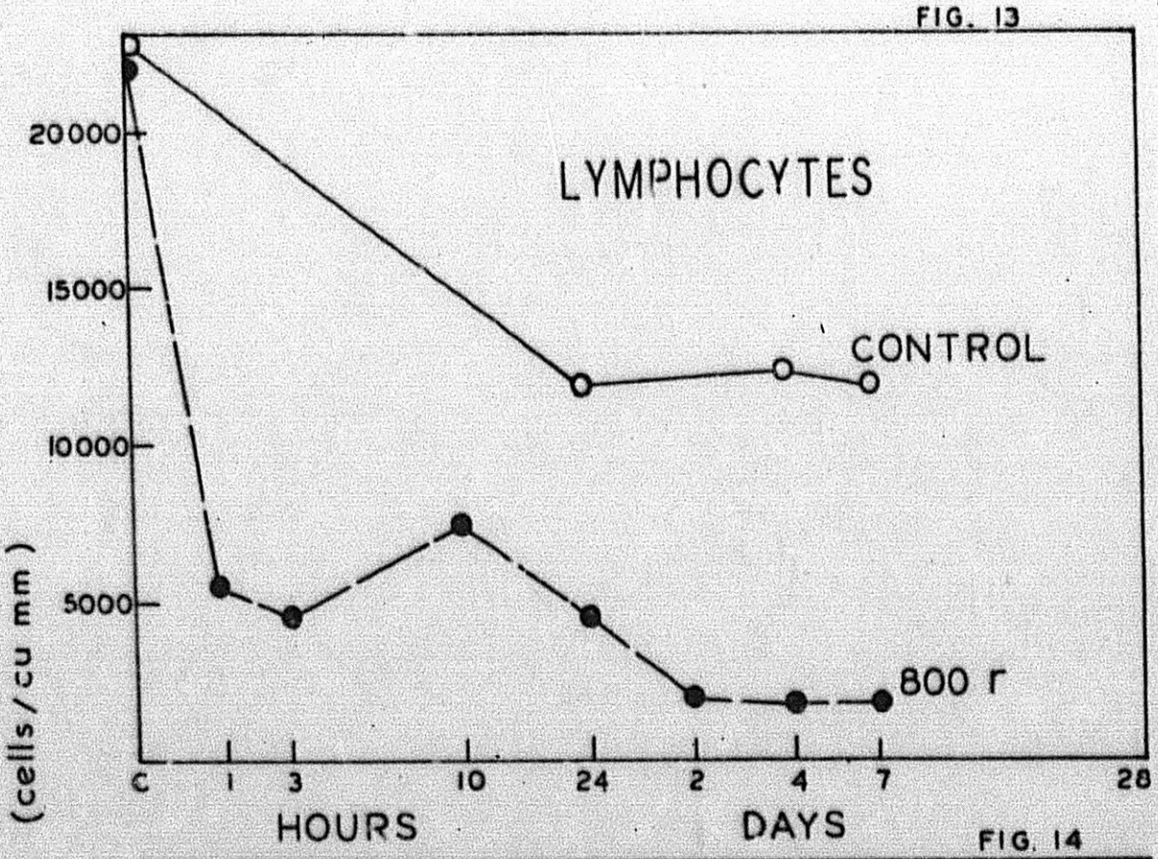
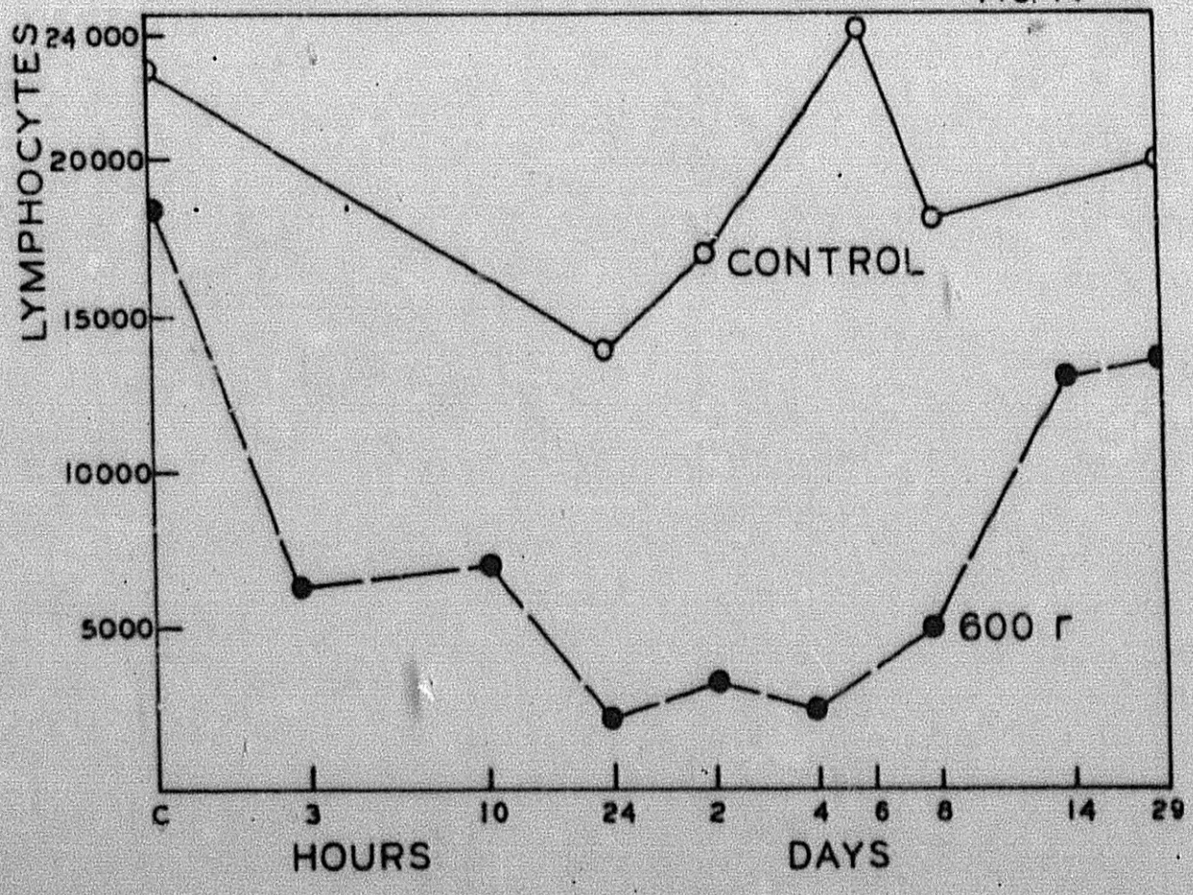

LYMPHOCYTES
20000
15000
10000
5000
(cells/cu mm)
CONTROL
800 r
C
1
3
10
24
2
4
7
28
HOURS
DAYS

FIG. 14


LYMPHOCYTES
24 000
20000
15000
10000
5000
CONTROL
600 r
C
3
10
24
2
4
6
8
14
29
HOURS
DAYS

in dry smears (see below). The continued depression in the number of heterophils at 4 weeks, however, points to the extensive damage actually suffered by this series of cells.

2.3 Morphological changes: (a) Lymphocytes: In addition to the rapid reduction in number, as described above, many lymphocytes showed varying degrees of degeneration. An hour after irradiation some 10 per cent of these cells showed damage ranging from clumping of nuclear chromatin to complete disappearance of the nuclear outline and a scattering of chromatin clumps throughout the cytoplasm. The pattern of damage remained essentially the same at intervals through 2 days, but the number of degenerating forms decreased as the lymphocytes themselves were progressively reduced in number, and no degenerating mononuclear cells occurred at 4 days.

At 2 days there were few small but many medium-sized lymphocytes and many transitional forms (monocytes?). At 4 to 14 days there was an even greater prominence of cells which were slightly smaller than the medium-sized lymphocytes and which had the nuclei of normal lymphocytes together with deeply basophilic-staining cytoplasm. By 8 and 14 days the number of small lymphocytes was again increasing, and by 27 days the lymphocytic picture of the treated animals differed from that of the controls only in the persistent absolute reduction in the number of lymphocytes and in the continued presence of many lymphocytes with deeply basophilic-staining cytoplasm.

Only two macrophages were found, both at 24 hours.

(b) Thrombocytes: Differentiation of degenerating thrombocytes from degenerating small lymphocytes is often difficult if not impossible, for a similar nuclear damage takes place in both types. The maximum destruction, however, seemed to occur later for the thrombocytes (3, 10 and 24

hours) than for the lymphocytes (1 hour). Thrombocytes with enlarged, vacuolated cytoplasm were prominent during the first day, less so at 2 and 4 days, and largely absent thereafter. At 14 and 28 days the thrombocytes appeared somewhat more shrunken than in control smears.

(c) Heterophils: No actual degeneration of heterophils was seen at any of the intervals studied through 7 days after 800 r and through 27 days after 600 r. A larger proportion of mature heterophils, however, was seen from 3 to 24 hours after irradiation, and a smaller proportion thereafter through 7 or 8 days. Seven days after 800 r there were only a few late heterophils and no intermediate forms, but a fairly large increment of lymphocytic cells with a rather deply basophilic-staining cytoplasm, possibly "blast" forms, was present. The 8-day specimen of the 600 r series had, in addition to many of these young forms, myelocytes in various stages of maturation as well as normal late heterophils. At 14 and 27 days the picture was essentially normal, though the number of heterophils was still reduced and the percentage of immature granular forms was still high.

Some heterophils showed another kind of change between 3 and 24 hours, as the rod-shaped, cytoplasmic bodies became thicker, more distinct, and more conspicuous.

The eosinophils had disappeared by 24 hours, and returned along with the other granulocytes.

(d) Erythrocytes: The degeneration undergone by lymphocytes during the first day after irradiation was largely paralleled among the erythrocytes, which showed simply clumping of nuclear chromatin, or pyknosis, or karyorhexis and dissolution. After 24 hours, when these variations were already minimal, such changes were no longer seen.

Basophilic and polychromatophilic erythroblasts were present from 1 to 8 days, particularly after 600 r, and polychromatophilic and eosinophilic erythroblasts occurred with bi-lobed nuclei or with two or three distinct nuclei. At 14 and 27 days after 600 r this increase in polychromatophilic erythroblasts had been largely reduced to the level of the controls.

3. Organs

3.1 Bone Marrow: The bone marrow was damaged at all doses from 25 r to 1000 r. The effects, however, vary with the size of the dose, but are otherwise similar in all treated animals. Gelatinous marrow was found, however, only after 800 r and 1000 r. At all doses of 100 r or more, and most strikingly at the two highest doses, there were at least two waves of depletion and regeneration, particularly among the erythrocytes.

(a) 800 r: The 3-week chicks underwent a considerable destruction of marrow after treatment with 800 r. Erythropoiesis was completely wiped out at 1 hour, and granulocytopoiesis at 14 hours (Figure 15b). A few late forms were still present at these intervals. Most of the large lymphocytes of the lymphatic nodules - and of the myeloid tissue survived, however, despite extensive damage to the smaller lymphocytes. Hemocytoblasts in the myeloid tissue of the marrow also survived between 2 and 8 hours (Figure 15b). Transient waves of regeneration were seen at 2-14 hours and 2-5 days in the erythropoietic series, and in the granulocyte series possibly at 5 hours and certainly from 1 to 4 days. These sporadic attempts at recovery failed, and the marrow at 9 days was entirely aplastic.

The damage was marked as early as the first intervals, from 45 minutes to 2 hours after treatment. Mitosis had ceased. The basophilic erythroblasts

Figure 15. Effect of 1000 r (A) and 300 r (B) on the bone marrow of 3-week chicks.

FIG. 15 A BB 1000 r BONE MARROW 3-Wk. Chicken

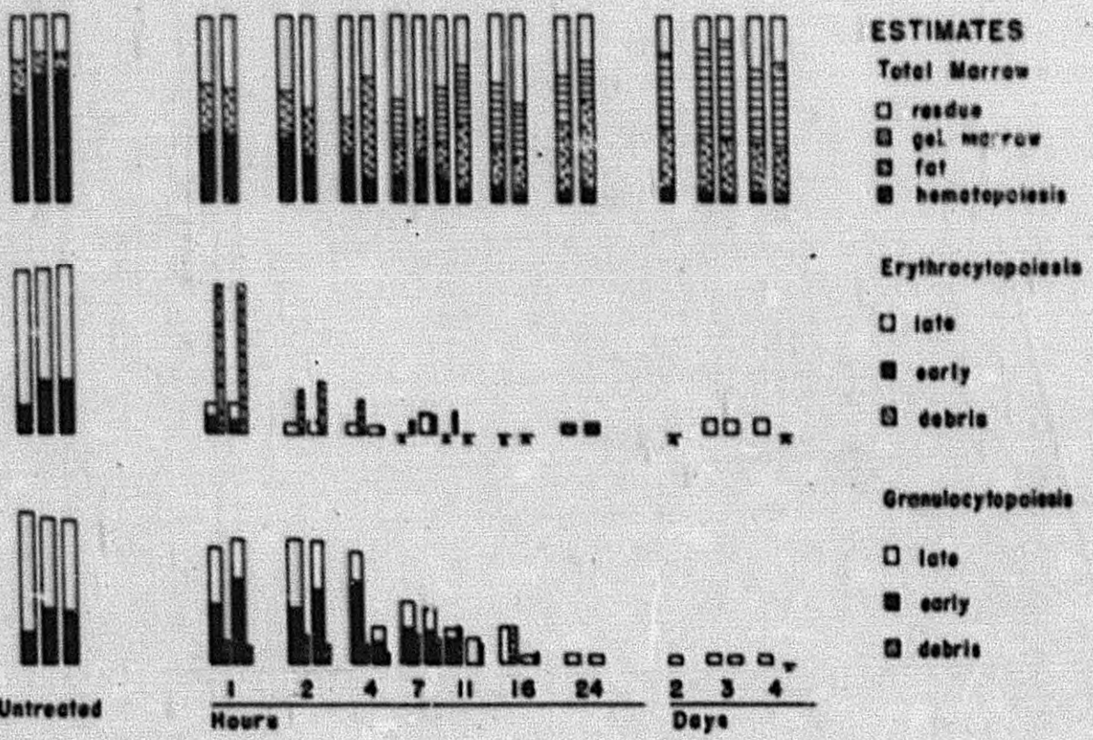
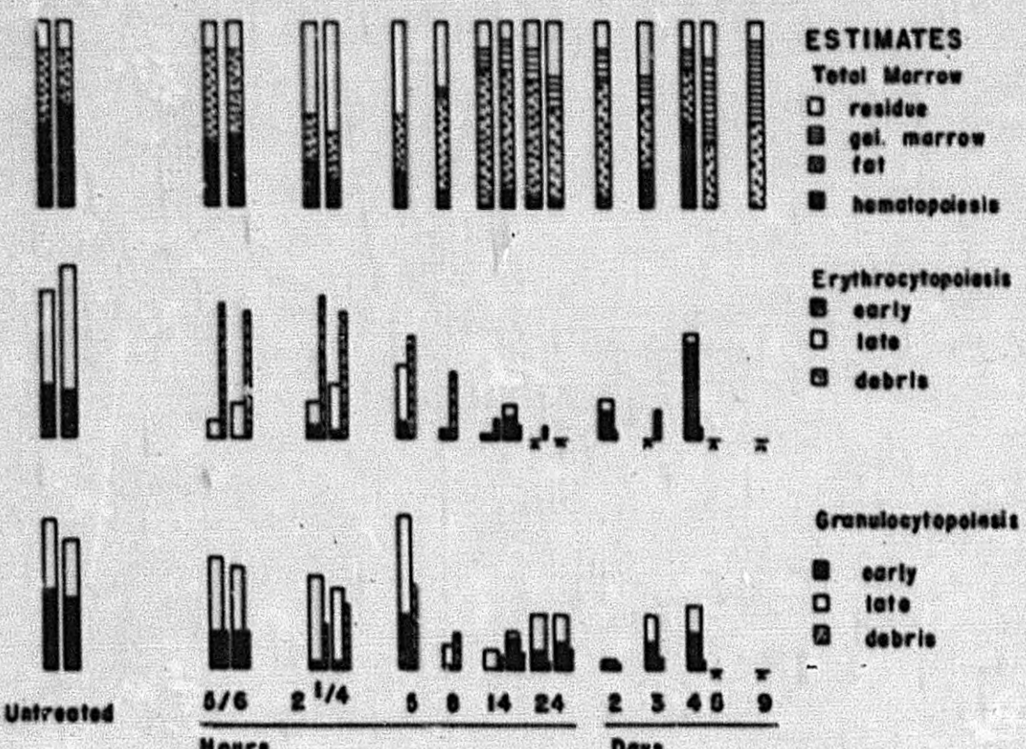


FIG. 15 B BB 800 r BONE MARROW 3-Wk. Chicken



were almost completely destroyed, and only the mature or nearly mature erythrocytes survived. The lymphatic follicles contained many dead small lymphocytes, and a few large lymphocytes constituted the only living free cells in the lymphatic tissue. A few granulocytes also showed degeneration.

At 5 hours a partial regeneration was apparent in the midst of the continued destruction. Mitotic activity was again present in the myelocytes, though not in the erythroblasts. Many hemocytoblasts survived, and more had formed from the reticulum, giving rise to myelocytes. Practically all the debris was now contained in macrophages of the reticulum.

From 8 to 14 hours, the marrow continued to be depleted of cells, granulocytes continued to degenerate, and adipose tissue was often present where there had been granulocytes before. In the sinuses, replacing erythrocytes, there were now some macrophages and a granular material, probably blood plasma. At 14 hours, the intravascular material had increased, and some gelatinous marrow was found extravascularly. The lymphatic tissue was composed principally of reticular cells, some polyblasts, and much intercellular substance. Debris from all types of cells had been almost entirely cleaned up.

Although myelopoiesis and lymphopoiesis were greater at 24 hours than earlier (Figure 15b), the gelatinous marrow had also increased, and the granulocytopoietic areas contained a number of plasma cells. This myelocytopoietic activity had subsided by 48 hours, at which time erythropoiesis was resumed and single layers of basophilic erythroblasts appeared. These erythroblasts, derived from the endothelium of the venous sinuses, were less in evidence at 3 days, but once more very numerous at 4 days.

At 5 and 9 days, the last stages studied, the marrow was aplastic and gelatinous, containing groups of plasma cells and macrophages, but hemopoiesis

had virtually ceased. The venous sinuses contained much plasma and only an occasional circulating red blood cell.

(b) 1000 r: After treatment with 1000 r (Figure 15a) the marrow in the 3-week chicks was even more severely damaged, and very little hemopoiesis was seen at 4 days. Erythropoiesis had practically ceased at 2 hours, and although there were a few basophilic erythroblasts at 24 hours, there was almost no evidence of mitosis among the erythroblasts. The decrease in granulocytopoiesis was more gradual, but by 16 hours the remaining myelocytes were mainly late forms and there was little subsequent mitosis.

At 1 and 2 hours, the erythroblasts were dead or degenerating. Considerable cellular debris was present, particularly intravascularly, but phagocytosis was already in progress. Myelocytopoiesis, on the other hand, was active, though a few of the early myelocytes had abnormal nuclei or were otherwise damaged. The sinuses contained monocytes and a few plasma cells, and there was a marked increase in the number of stem cells outside the sinuses. No mitoses were observed.

At 4 hours most of the erythroblasts were dead, fewer myelocytes were present, and the fat in the marrow increased. A badly damaged lymphatic nodule contained only a few lymphocytes, interspersed among many phagocytic cells. By 7 hours the marrow was further depleted, and, in one of the two specimens, somewhat gelatinous. The very few erythroblasts found at this interval were usually degenerated. Some new fat cells were beginning to develop from reticular cells.

At 11 to 16 hours all erythroblasts were dead, though the vessels still contained circulating erythrocytes. There was an increase in the number of reticular cells and phagocytosing macrophages, and in the amount of fatty

and gelatinous marrow. Myelocytes were numerous and generally normal at 11 hours, but at 16 hours late forms predominated, some of them beginning to disintegrate. No mitoses were observed.

At 24 hours the marrow was greatly depleted, but a few erythroblasts appeared. Occasionally in mitosis, while the myelocytes continued to represent almost entirely the later stages. This erythropoietic activity was not apparent in the 48-hour animals, which showed a spreading of young fat cells, and a similar picture — fatty, gelatinous marrow depleted of hemopoietic cells — was found at 3 days. At 4 days, however, there was another partial, abortive regeneration of red blood cells in one of the specimens, which contained in a few sinuses a number of polychromatophil erythroblasts.

(c) 400 r: A dose of 400 r (Figure 16a) by 7 hours had brought about severe depletion of lymphocytes, myelocytes, and especially early erythroblasts, but recovery was rapid, and at 13 hours the marrow was practically normal. There was a slight secondary depletion of cells at 30 hours, but the normal pattern was once more restored by 48 hours, and variations at later intervals were slight.

Erythroblasts and lymphocytes were the first to show damage, as early as 1/2 hour after treatment. At 4 hours only some 20 to 25 per cent of these cells remained, and the myelocytes were also beginning to show definite signs of degeneration. At 7 hours all three cell types were at their lowest point, but the presence of many new hemocytoblasts suggested the beginning of a regenerative process which was much more marked at 13 hours.

At 18 hours, erythroblasts were present in somewhat greater than normal numbers, but both erythroblasts and early myelocytes were perceptibly reduced in number by 30 hours, only to return once more to normal at 48 hours.

Figure 16. Effect of 400 r (A) and 100 r (B) on the bone marrow of 3-week chicks.

FIG. 16 A BB 400r BONE MARROW 3-Wk. Chicken

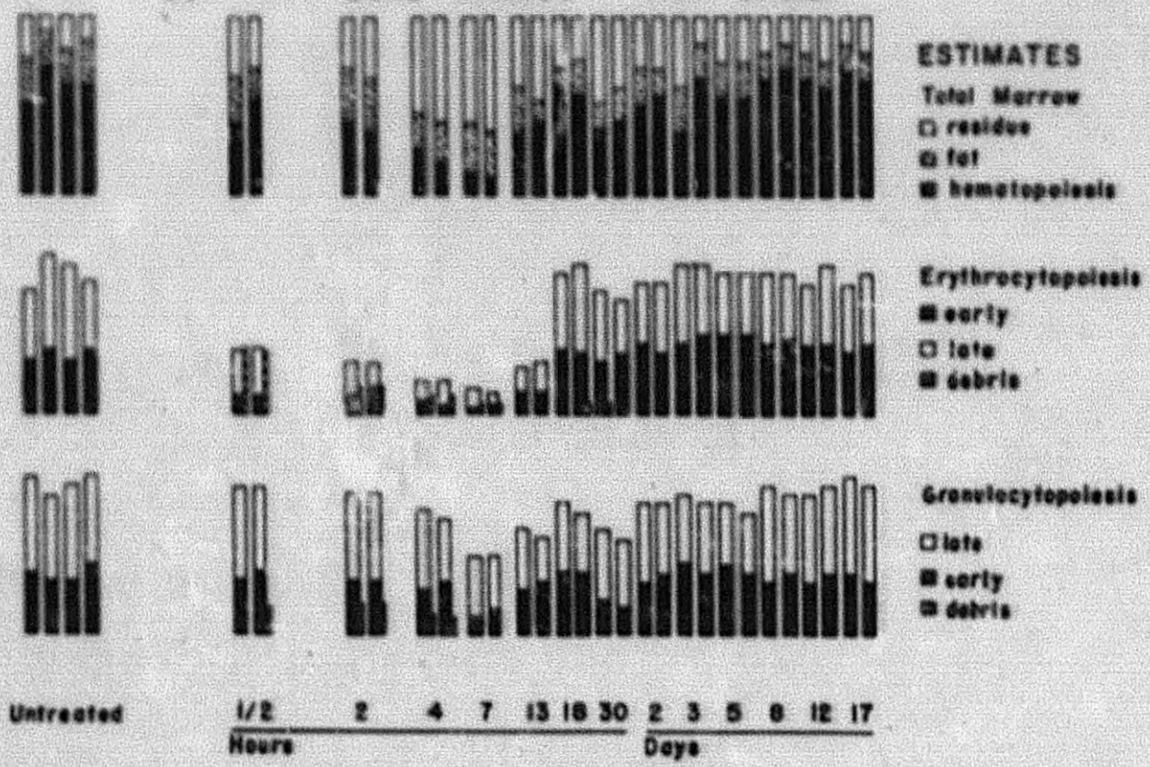
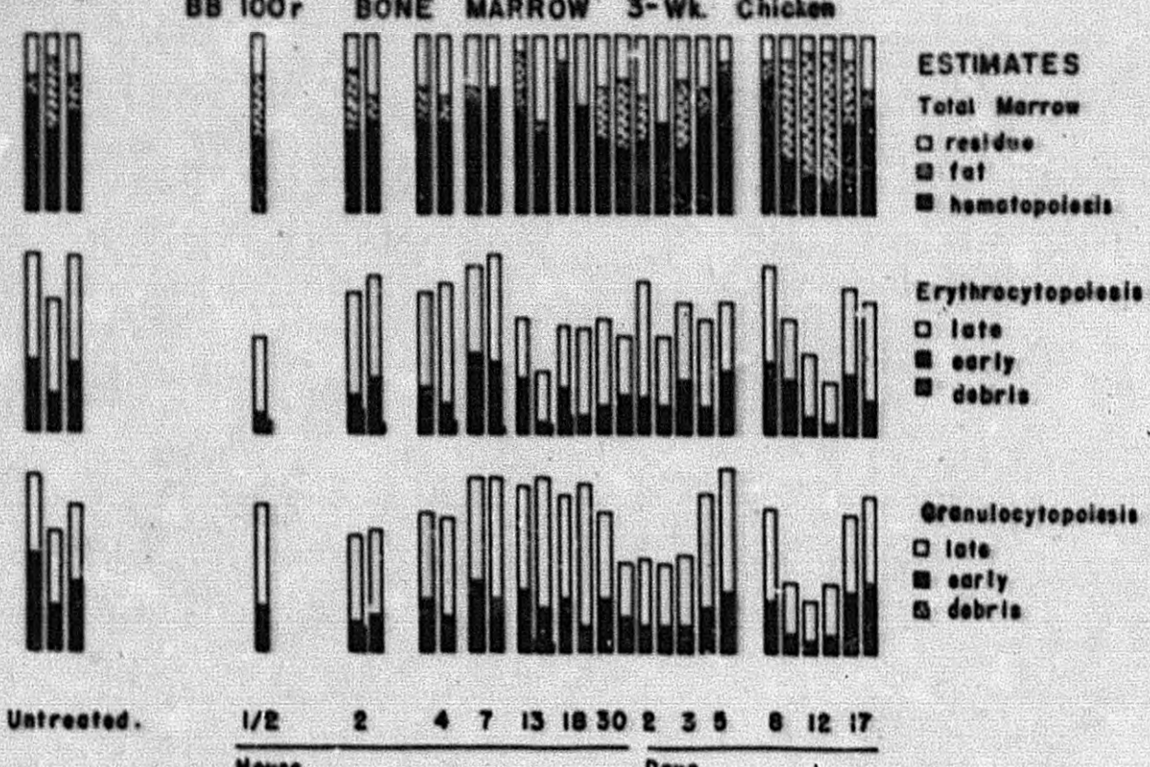


FIG. 16 B BB 100r BONE MARROW 3-Wk. Chicken



(d) 100 r: After a dose of 100 r (Figure 16b), there was an irregular reduction of hemopoiesis which extended as late as 12 days, but the damage was slight compared to that following 400 r. Mitotic activity had decreased markedly at 1/2 hour, but was normal again at 2 hours. A few dead young erythroblasts and myelocytes were present at intervals of 4 hours or less, but the marrow was not depleted. There seemed to be, however, two periods of de letion, one from 13 to 72 hours, and a second and more severe one at 8 to 12 days. It should be noted that sections of marrow from untreated animals were occasionally almost as fully depleted as those of the most heavily damaged specimens, presumably because hemopoietic areas are unevenly distributed in the marrow cavity of the chick, and because the region of marrow sampled at autopsy was not always uniform.

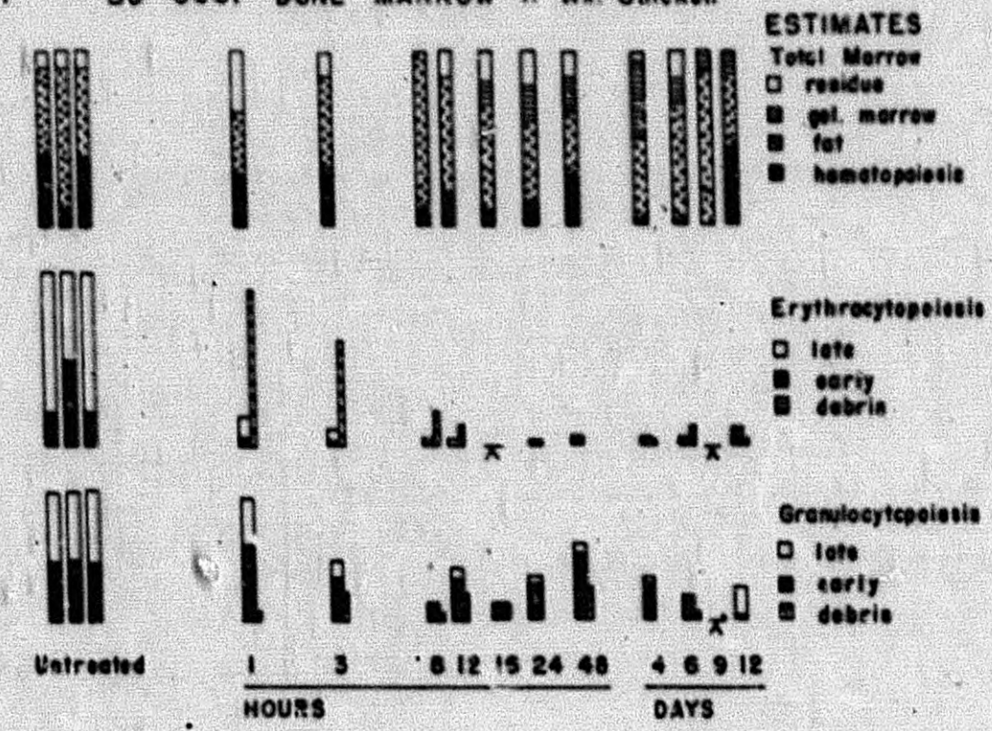
(e) 25 r, 6 r, 2 r: Lymphocytes in the marrow were damaged after 25 r, but no effects of treatment were observed at either of the lower doses.

(f) 11-week chicks: The extensive damage to 11-week chicks after 800 r (Figure 17) was comparable to that suffered by the younger animals. Erythropoiesis particularly was affected, being virtually absent from 3 to 16 hours, recovering very slightly at 24 and 48 hours, disappearing again at 9 days, and returning once more at 12 days. Myelopoiesis was considerably reduced from 3 hours on, but was not eliminated until 9 days, and at the last stage observed, 12 days, it was beginning again. The cells of the lymphatic nodules also underwent degeneration.

3.2 Spleen: In normal 3-week chickens, the white pulp of the spleen is not yet completely developed. There are very few active germinal centers, and the principal mass of the white pulp is made up of the very prominent sheathed arteries.

Figure 17. Effect of 800 r on the bone marrow of an 11-week chicken.

FIG. 17 BC 800r BONE MARROW 11 Wk. Chicken



(a) 250 r: In such children, a dose of 250 r resulted in complete destruction of all lymphocytes, though the hemocytoblasts survived somewhat longer than the small lymphocytes. Many new cells were transformed from the cells of the reticulum, but most of these cells or their progeny turned to plasma cells. However, the last stage (9 days) did suggest a possible substantial recovery.

Mitotic activity had ceased and lymphocytes were destroyed by 45 minutes, and at 2 hours practically no small lymphocytes remained. From about 2 hours to 9 days, furthermore, the entire spleen was smaller than the spleen of control animals. The small lymphocytes degenerated principally by fragmentation, and the large lymphocytes or hemocytoblasts by vacuolation of the cytoplasm and clumping of the nuclear chromatin. A few very large hemocytoblasts with vacuous nuclei were present at 45 minutes, increasing in number up to 14 hours (when very large reticular cells with vacuous nuclei were also found) but decreasing again between 1 and 3 days, when these latter nuclei became pyknotic and the cytoplasm vacuolated. From 5 hours on, and especially at 9 days, many hemocytoblasts and some medium lymphocytes turned into plasma cells. The extensive intercellular substance seen at 5 days was less in evidence at 9 days.

Recovery started before maximum destruction had occurred. Phagocytosis began at 45 minutes, by 2 hours practically all of the debris was in macrophages, and at 5 hours there was relatively little direct evidence of former damage. Most of the macrophages were inactive, many lymphocytes were developing from the reticular cells and sheath cells, and mitosis of medium and large lymphocytes was resumed, though often with lagging chromosomes. By 9 days, although numerous plasma cells were present, the centers of some follicles contained many normal medium-sized lymphocytes, possibly suggesting a future recovery.

(b) 1000 r: Treatment with 1000 r caused the spleen to be depleted of cells and to be reduced in size. Small lymphocytes were killed and replaced sporadically, principally by transitions from reticular and sheath cells, or by large lymphocytes. Large lymphocytes degenerated, but regained their normal appearance. The fluctuations in the numbers of cells and extent of debris suggested a wave-like pattern with degeneration up to 7 hours, regeneration between 7 and 11 hours, and renewed degeneration thereafter.

Only small lymphocytes were affected at 1 hour, but at 2 hours medium-sized and large lymphocytes were being transformed into plasma cells, and healthy small lymphocytes began to reappear at 7 hours. Regeneration of the large lymphocytes was apparent at 11 hours. The lymphocytes formed during this period of recovery did not survive, however, and after 36 hours there were few small or medium-sized lymphocytes in the spleen. By 3 days the spleen has shrunk to less than one-fourth its former size.

At 16 hours, 3 days, and, to a lesser extent, at intervals between, the chromatin of the sheath cells had a clumped appearance similar to that seen in reticular cells of the rabbit and mouse.

(c) 400 r: Four hours after a dose of 400 r, over three-fourths of the small lymphocytes and many of the large and medium lymphocytes were dead or dying. Regeneration was evident in one specimen at 7 hours in the form of numerous young large lymphocytes, but there were two periods of secondary degeneration and regrowth before the organ returned to normal at 5 days. Except for the disappearance of plasma cells, which had not reappeared by 17 days, and a minor reduction in the number of sheath cells, there were no other changes. The erythroblasts and early myelocytes underwent a single and somewhat slower process of degeneration and subsequent regeneration.

Destruction of the small lymphocytes began at 1/2 hour, when mitotic activity in all cell types was reduced. At 2 hours, perhaps half the small lymphocytes were dead or damaged, and the medium lymphocytes followed at 4 hours. Meanwhile there had been an overgrowth of hemocytoblasts, however, and at 7 hours the total damage had been so fully repaired that only the presence of considerable debris gave indication of the earlier destruction.

At 13 hours the small lymphocytes were again reduced to about half the normal number, but by 30 hours recovery had been so extensive that an overgrowth of some 20 per cent was apparent. At 48 hours the damage was again seen, now affecting the medium-sized lymphocytes and adult granulocytes as well. The continued partial depletion of the spleen at 3 days suggested that recovery was occurring this time at a slower rate than before. At all subsequent intervals, however, the number of cells was normal or slightly above normal.

(d) 100 r: Damage after doses of 100 r was relatively slight, and recovery was complete after 18 hours. At 1/2 hour, but more clearly at 2 hours, there was an increased number of dead lymphocytes in the white pulp, principally in the active nodules and in the margins of the sheaths. Debris was still in excess of normal at 4 hours and 7 hours but not at subsequent intervals. At intervals from 2 to 18 hours the nuclear chromatin was often clumped in the large lymphocytes.

(e) 25 r, 6 r, 2 r: Lymphocytes were again affected at 25 r, but not at smaller doses.

(f) 11-week chicks: The effects of 300 r on the 11-week animals included the destruction of most of the small lymphocytes, and a marked decrease in the size of the spleen. This decrease was apparent only at 4 days,

however, whereas spleens of the 3-week chickens grew progressively smaller from 2 hours through the last available interval at 9 days. The presence of many more follicles in the older animals makes a close comparison difficult, but at 9 days the follicles of the 3-week specimen were full of plasma cells, while those of the 11-week specimen were normal. Both series are alike, however, in showing early destruction and then rapid repair of the small lymphocytes, approximately the same amount of extramedullary myelopoiesis, and at 9 days, a tremendous increase of plasma cells.

At 1 hour, the small lymphocytes around the sheathed arteries and those of the red pulp were nearly all dead, by 3 hours, those in the follicles were destroyed also, and maximum damage was apparent at 12 hours, by which time the hemocytoblasts were also showing extensive degeneration.

The reparative process began with phagocytosis as early as 1 hour after treatment, and included regeneration of the diffuse lymphatic tissue of the red pulp at 3 hours, when there were transitional forms from the reticulum as well as mitoses in the red pulp. At 12 and 16 hours, the edges of the sheathed arteries showed especially numerous transformations. Many of the lymphocytes of the diffuse lymphatic tissue had turned into plasma cells at 4 and 9 days, but these areas were once more normal by 12 days. Reconstitution of the follicles was somewhat slower, becoming evident at 12 hours but not significant before 48 hours; the follicles at 9 and 12 days were normal.

3.3 Thymus: The thymus of young chickens does not differ significantly in structure from the mammalian thymus as described in Bloom's paper[4], except for the presence of myo-epithelial cells in the medulla of the chickens. The pattern of damage from radiation treatment is outlined on the accompanying chart, where it is apparent that doses of 800 r resulted in the almost complete

destruction of small lymphocytes and a noticeable reduction in the number of large lymphocytes, whereas 400 r produced much more moderate effects. The reaction was almost as severe in the 11-week chickens as in the 3-week chickens, but regeneration was more rapid and more persistent. The sensitivity of the lymphocytes was similar in chickens and rabbits, but here there was some evidence of damage to epithelial cells as well; the process of recovery was roughly comparable to that observed in the rabbits, but more sporadic.

(a) 800 r: A dose of 800 r given to the 3-week chick resulted in destruction of almost all the lymphocytes, and of some of the myo-epithelial cells. The medium and large lymphocytes survived longer than the small lymphocytes; the reticular cells were apparently not affected. The cortex became progressively more and more depleted of lymphocytes, so that by 4 days it was impossible to distinguish between cortex and medulla.

At 45 minutes, many small lymphocytes had been destroyed both in the cortex and in the medulla. At 2 hours, the medulla contained only large and medium-sized lymphocytes, with plasma cells and transitional forms appearing by 5 hours, but many apparently healthy small lymphocytes were again present at 14 hours. The cortical region at 14 hours was reduced, and showed many active macrophages. Some of the epithelial cells were meanwhile also undergoing a change that was probably due to treatment rather than to the normal formation of Hassal's bodies. At 2 and 3 days, the cytoplasm of these cells was vacuolated, and their nuclear chromatin was often clumped.

At 4, 5, and 9 days there was considerable regrowth of lymphocytes in both cortex and medulla, but regeneration was never more than a third of normal. Whether the process of recovery was uninterrupted or wave-like

is difficult to determine. At 2 hours, there were many medium-sized lymphocytes in the medulla; at 5 hours, very few; at 14 hours, many; at 24 hours, almost none; and at 3 days and thereafter they seemed to remain constant. The small lymphocytes, however, appeared to be killed, removed, and replaced only once throughout the series, perhaps because they were more quickly affected by treatment.

It cannot be said from these preparations whether or not some of the transitional forms derived from epithelial cells.

(b) 1000 r: The damage resulting to the thymus from 1000 r was even more quickly felt than after 800 r, and the organ was almost completely depleted of lymphocytes and much reduced in size within 1 to 4 hours after treatment. Regeneration was not evident by 4 days, at which time the last surviving animals died.

Within an hour after receiving X radiation, all small lymphocytes in the medulla were dead, as well as a moderate number of those in the cortex. The destruction and phagocytosis of small lymphocytes continued until by 16 hours, the cortex and medulla were indistinguishable. Meanwhile more large lymphocytes were dying, or showing damage; medium-sized lymphocytes became fewer in number, though the survivors were not damaged.

At 24 hours the lymphocytes continued to die off rapidly, but new lymphocytes were possibly being transformed from reticular cells, which were abundant and prominent at this stage. The organ remained shrunken in size from 24 to 48 hours. The specimens available at 3 and 4 days showed a considerable, though irregular, regeneration of the lymphatic tissue.

(c) 400 r: Damage after 400 r was much less severe than after 800 r and regeneration was complete by 30 hours. Approximately two-thirds of the small lymphocytes had disappeared by 7 hours, and many of the large

and medium lymphocytes were dead or dying in both the cortex and the medulla. The reticular and epithelial cells were not visibly altered.

Recovery was already under way at 13 hours, and 5 hours later, the number of cells in the thymus once more approached the normal number. At 2 to 5 days, granulocyte formation, normally inconspicuous in the medulla, was prominent in that region. Except for a slight reduction in the number of cells at 5 days, and a slight overgrowth at 12 days, there were no further significant variations.

(d) 100 r: There was more than a normal amount of debris in the thymus at early intervals after a dose of 100 r, especially in one specimen at 7 hours. No important changes were apparent after 11 hours. No damage was observed at doses lower than 100 r.

(e) 11-week chicks: A dose of 800 r caused almost as much damage to these animals as to the 3-week chicks, though the recovery here was more rapid. Small lymphocytes were again very nearly eliminated, and other effects included early cessation of mitosis, the presence of hemocytoblasts with vacuous nuclei, death of epithelial cells, and edema of the surrounding and interlobular tissue.

The peak of destruction of small lymphocytes appeared in the cortex at 8 hours, and in the medulla at 1 to 3 hours. Phagocytosis and other reparative processes were prominent in the cortex after 12 hours, but began in the medulla immediately and were almost complete at 8 hours. Regeneration of lymphocytes was apparent after 12 hours in the medulla, and after 24 hours in the cortex, and by 2 days the whole thymus was nearly reconstituted.

Between 2 and 4 days there was a second degenerative period, but by 9 and 12 days the organ was again almost normal, suggesting once more the existence of waves of damage and repair.

3.4 Liver: Damage to the liver included the typical reduction (followed by restoration) of lymphatic areas, and, at doses of 800 r or more, degeneration of some hepatic cells. Kupffer cells were very active in disposing of circulating debris in the sinuses. The definite evidence of death and regeneration of epithelial cells is at variance with the observed effects of radiation upon the liver of rabbits, though changes have been recorded in rats and mice with internal emitters.

(a) 800 r: A dose of 800 r caused very early damage to lymphocytes, and, somewhat later, damage to liver cells. Repair seemed to be effected within 5 days.

Degeneration of free lymphocytes and of patches of lymphatic tissue was particularly noticeable at 45 minutes following irradiation. Healthy, free lymphocytes reappeared at 5 hours, but the lymphatic areas, which were replaced by reticular cells and granulocytes, were not distinguishable again as lymphatic patches until 4 days after treatment.

Small areas of damaged liver cells were increasingly apparent from 2 to 14 hours, after which these areas were filled with macrophages, reticular cells, and heterophils. There was active mitosis at 14 hours, and repair seemed to be almost complete by 5 days. The 9-day specimen contained areas of bacterial invasion, with degeneration of the liver cells involved, possibly suggesting that the radiation made the cells more susceptible to damage by bacteria.

(b) 1000 r: The damage after 1000 r was more striking. Many blood cells appeared in the sinuses, only to be quickly taken up by swollen Kupffer cells. The hepatic cells showed only minimal changes; swelling of the nuclei, and perhaps, at the earliest intervals, a clumping of chromatin.

One and 2 hours after treatment, the sinuses of the liver contained a greatly increased number of blood cells, many of the cells were damaged, and many were undergoing phagocytosis. Swollen or otherwise irregular macrophages, lymphocytes, hemocytoblasts, hepatic cells, and Kupffer cells were also found. At 4 hours, areas of acute focal necrosis were discernible. The active phagocytosis had decreased somewhat by 7 hours, the damage was being repaired, and most of the cells in the sinuses were normal erythrocytes. At 11 hours, debris persisted in the larger vessels, but had been completely phagocytosed from the sinuses. The Kupffer cells had contracted considerably and were no longer swollen. From 16 to 36 hours few circulating blood cells remained in the sinuses, and by 48 hours, the debris had been cleared away and the Kupffer cells were practically normal.

Heterophils were numerous in the sinuses at 3 and 4 days. At 4 days there were many lymphoid cells in the large blood vessels, the hepatic cells seemed to be filled with glycogen, and focal necroses, which could not be accounted for, appeared.

(c) 400 r: The smaller dose brought about no destruction of liver cells, but there was increased phagocytosis of debris by the Kupffer cells during the first few hours after treatment. The occasional islands of blood- and lymph-forming tissue followed the typical cycle of degeneration and recovery.

(d) 100 r: Occasional dead lymphocytes were present at 2 hours, and there was a little debris in the Kupffer cells and in the sinuses in one specimen at 18 hours and one at 30 hours. The hepatic cells were not significantly damaged.

(e) 11-week chicks: A dose of 800 r to these animals caused a marked increase in the activity of the Kupffer cells, and the usual rapid

destruction of lymphatic tissue. The debris in the Kupffer cells was extensive at 1 hour, but had been largely disposed of by 12 hours, though many such cells continued to show debris as late as 12 days.

Scattered degeneration of hepatic cells was found especially at 3 and 8 hours, and some at 16 hours. Many necrotic areas, probably not caused by irradiation, were present in the 3-hour specimen and in the 16-hour specimen.

3.5 Glandular Stomach (Proventriculus): The glandular stomach of the chicken has a central lumen with the epithelium formed in villi. Opening upon this central lumen are smaller lumina with deep zymogenic glands. Damage to parietal cells appeared after 800 r and 1000 r, and the glandular epithelium was damaged by all doses of 400 r or more. Hemorrhage in the villi of the central lumen occurred in the 3-week chicks that had received 800 r or 1000 r. Effects of treatment were usually most severe within the first few hours, and recovery, though often irregular, was generally complete within several days.

(a) 800 r: The principal effects of 800 r on the 3-week chick were hemorrhage in the lamina propria; the destruction of epithelial cells in the glands of the central lumen, especially at the base of the villi; and death and nuclear change in the lymphatic tissue of the lamina propria and in the epithelium of the zymogenic glands.

The death or degeneration of epithelial cells at the bottom of the villi was marked as early as 45 minutes after treatment, and continued up to approximately 5 hours, when the necrosis extended toward the tip of the villus. At 14 hours, the interrupted mitotic activity was resumed, and mucus was being secreted, although occasional epithelial cells contained intranuclear vacuoles and others were dead.

A second degenerative period apparently occurred between 48 and 72 hours, during which time many epithelial cells were flattened, others had vacuoles in the cytoplasm, and still others were large and vacuous. The 4- and 5-day stages showed progressive recovery, so that by 9 days the epithelium was almost normal.

Many lymphocytes in the lymphatic tissue of the lamina propria were destroyed at early intervals, with the damage appearing particularly at 2 hours, though at no time was the reduction of lymphatic tissue as extensive as in the spleen or bone marrow. Large hemocytoblasts with vacuous nuclei were present, resembling those seen in the rabbit. There was some phagocytosis at 2 hours, but most of the debris was not phagocytosed until about 5 hours. At 8 hours there was an extensive hemorrhage in the lamina propria and the villi were greatly extended, but at 14 and 24 hours there was no evidence of extravasation. At 48 hours there was an even more severe hemorrhage, extending through the epithelium as well as the connective tissue. The hemorrhage was greatly decreased by 4 days, and the lymphatic tissue, having shown recovery at 14 and 24 hours, was now very actively forming lymphocytes. By 5 and 9 days the lamina propria was normal except for a slight increase in plasma cells.

The zymogen cells showed no mitosis at 45 minutes, and many seemed to have degenerated by 2 hours. A second degenerative period was apparently reached at 48 hours, when more zymogen cells died, while others contained vacuolated cytoplasm, swollen nuclei, discharged granules, or basophilic granules. Edema was extensive in the lamina propria of the zymogen or parietal glands at this time, extending between the muscle layers also. During this degenerative period, mitotic activity was increasing, so that at 5 and 9 days the zymogen cells were again normal.

The epithelium of the zymogen glands other than zymogen cells showed degenerative changes similar to those found in the epithelium of the central lumen.

(b) 1000 r: The area around the central lumen was again found to be the most sensitive portion of the stomach after a dose of 1000 r. Here debris of lymphoid cells accumulated in the lamina propria and some of the epithelial cells at the base of the glands degenerated, particularly at 2 hours. Debris was somewhat less extensive at 4 hours, and phagocytosis was active at 7 hours, although some specimens at intervals after 16 hours showed considerable hemorrhage in the lamina propria.

At 1 and 2 hours the debris was extensive, and dead or damaged epithelial cells appeared at the base of the glands as also along the ducts. Repair was well established by 4 hours and was progressive though irregular at subsequent intervals. In one specimen at 7 hours, however, the lymphatic tissue was composed principally of stroma, and there were enormous hemocytoblasts undergoing degeneration beneath the stratified squamous epithelium. Similarly, in one aniral at 11 hours a great infiltration of eosinophils accompanied other signs of incomplete recovery.

A great deal of hemorrhage occured in the lamina propria in one of the two specimens at each interval from 16 hours on. In these animals varying amounts of the epithelium around the central lumen had been destroyed, and other epithelial cells were swollen and vacuolated. The other specimens at these intervals were relatively normal, though some residue of damage usually remained.

(c) 400 r: Except for the suppression of mitotic activity at 2 and 4 hours, and the appearance of an unusual number of vacuoles in the

epithelium, there were no changes in the stomach after a dose of 400 r. The parietal glands were not damaged.

(d) 100 r: No irradiation effects were observed.

(e) 11-week chicks: Generally the first effects of 800 r were the same in both the older and younger chickens. These effects were partial destruction of the lymphatic tissue, early degeneration of the epithelium in the bottom of the villi, and the interruption of mitosis. The hemorrhages described above, however, were not found in the 11-week animals, nor was degeneration of zymogen cells so severe.

Destruction of small lymphocytes was complete by 3 hours, by which time phagocytosis was also at a maximum, and a definite reconstitution of the follicles was begun at 8 hours and completed at 48 hours. A striking characteristic of the 12-day stage was the number of myelocytes and mature granulocytes at the periphery of many follicles. At earlier stages, too, in the epithelium, there were cells with nuclei morphologically identical to small lymphocytes, having a few coarse, red granules in the cytoplasm.

Degeneration of the epithelial cells in the villi of the central lumen was apparent at 45 minutes, reached a maximum at 3 hours, and was still prominent at 8, 12, and even 16 hours. Mitoses of epithelial cells could again be seen by 24 hours, and the whole epithelium was normal at 4 days. Occasional dead zymogen cells were seen at intervals up to 48 hours but by 4 days the zymogen cells were again normal.

3.6 Intestine: After doses of 400 r or more, the intestinal epithelium showed damage proportionate to the size of the dose. The effects were comparable to those found in the rat, and much greater than those in the rabbit. Destruction was regularly most severe within the first few hours

after irradiation, and recovery was rapid. The usual pattern of damage and repair was observed in the lymphatic tissue.

(a) 800 r: In the 3-week chickens, the maximum damage was evident at the earliest interval, 45 minutes, when nearly all the lymphatic tissue and a few epithelial cells were destroyed. The lymphatic tissue showed more regeneration than in either the spleen or lymph node. The debris had been cleared away at 14 hours, by macrophages which were present at injury, and by those formed from reticular cells during the reparative process.

At 45 minutes, the epithelial cells at the tips of the villi were edematous, and many at the base of the villi were destroyed, with the cytoplasm vacuolated and the nuclei darkened. The small lymphocytes of the connective tissue were almost all destroyed also, while in the reticular cells the nuclei were more heavily outlined than usual, and contained chromatin in larger clumps. Plasma cells and large and medium-sized lymphocytes were not affected. In the lower part of the small intestine there was an increase in the number of goblet cells.

At subsequent intervals, a gradual repair was brought about. Macrophages had already engulfed some of the lymphocytic and epithelial debris at 2 hours, and transitional forms from reticular cells and large lymphocytes were present. In the connective tissue at the base of the villi there was an infiltration of eosinophils which continued through the 5-hour stage. By 8 hours, the very small number of lymphocytes was the only remaining abnormality in the epithelium, but at 14 hours lymphocytes and many transitional forms were again seen. The debris had been cleared away, the macrophages were empty, and the only remaining trace of damage was in the large number of goblet cells. After 72 hours, these too were normal. At 4 and 5 days experimental and control animals were indistinguishable.

(b) 1000 r: A dose of 1000 r caused destruction that increased through the first 4 hours, before repair was evident. The damage to the duodenum was greatest at the base of the crypts of Lieberkühn. Many of the epithelial cells degenerated, as well as large aggregates of leucocytes from the lamina propria. The cecum also contained debris at the base of the crypts, but in smaller quantity.

The damage was evident in the duodenum 1 hour after treatment; debris at the base of the crypts, swollen epithelial cells, and dead eosinophilic leucocytes. At 2 and 4 hours, this damage was progressively intensified as mitosis ceased and ameboid plasma cells and degenerating heterophils appeared. At 7 and 11 hours, the debris was being removed, and at 16 hours the epithelium was normal, with only a few degenerating heterophils imbedded in it. At 1-4 days, the recovered epithelium showed a fairly active mitosis, and the lamina propria contained plasma cells and other cells which appeared to be intermediate forms between hemocytoblasts and plasma cells.

(c) 400 r: After 400 r the damage to the epithelium was severe at 2 hours, but progressively less severe at subsequent intervals up to 18 hours; increasing numbers of mitotic figures at 30 and 48 hours indicated that regrowth was taking place.

(d) 100 r: No damage was observed at this dose.

(e) 11-week chicks: A dose of 800 r was less destructive to these animals than to the younger chicks, but the same kinds of damage were found: the epithelium at the base of the villi was destroyed, mitosis was inhibited, and some of the small lymphocytes died. The most severe damage was apparent at 1 hour. At 3 hours the debris was being phagocytosed and mitosis resumed. The reparative processes steadily increased until the normal condition of the intestine was restored at 2 days after irradiation.

All the small and medium-sized lymphocytes within nodules were destroyed, while those at the villi tips and within the villi remained undamaged. The replacement of lymphocytes was complete by 16 hours. Plasma cells, chrome cells, lymphocytes containing Russell's bodies, and cells beyond the bases of the villi were not affected.

3.7 Kidney: The formation of nephrons is still in progess in the 3-week chick, and the new tubules and developing glomeruli, characterized by hyperchromatic nuclei and numerous mitotic figures, can be seen at the periphery of the lobules. Radiation caused cell destruction in these areas, and in the epithelium of the ureters and renal pelvis, in the 3-week chicks, but not in the 11-week chicks.

(a) 800 r: A dose of 800 r resulted in the destruction of many tubular and glomerular cells in the metanephrogenic zone of the 3-week chick, beginning at 45 minutes after irradiation and reaching a maximum at 8 hours. A large percentage of the cells composing new convoluted tubules and glomeruli were damaged, containing heavy dark granules or resembling empty spheres surrounded by dark membranes. The organ appeared normal 14 hours after irradiation.

Following irradiation the epithelium of the ureter and of the renal pelvis was found damaged at 2 and 5 hours, but was repaired after several days; sections were not available for the intervals in between. Lymphocytes found in the kidney were destroyed at 45 minutes, and began to reappear by 5 hours.

(b) 1000 r: The maximum damage seemed to be reached 2 days after a dose of 1000 r, and though regeneration was well under way by 11 hours, complete recovery had not been effected by the last available interval, 4 days. At 36 hours and again at 3 and 4 days, some tubules were somewhat

injured; the lumen held coagulated material, some of the cells were markedly vacuolated, and the nuclei stained deeply. The coagulated material was even more extensive in the lumen at 3 and 4 days.

(c) 400 r: Similar but milder changes were observed after 400 r. The nephrogenic tissue showed a slight depletion of cells during the first 8 hours, and returned to normal by 2 days. The blood-and lymph-forming elements followed the customary pattern of damage and regeneration.

(d) 100 r: No effects of irradiation were observed.

(e) 11-week chicks: Injury to the kidney in the older animals was confined to the lymphocytes, with greatest damage apparent at 1 hour. Complete repair was evident by 8 hours after irradiation.

3.8 Adrenal: Chromaffin cells occur throughout the adrenals of the chicken, and are not confined to the central part as is the case in the adrenals of most mammals. Similarly, the cells analogous to the cortical cells of the mammalian adrenal are not restricted to the outside. In the chicken the terms "cortical" and "medullary" are thus descriptive of cell types, rather than of location. The adrenal was damaged in the 3-week animal, but not in the older animal.

(a) 800 r: In the 3-week chicks a dose of 800 r caused a prolonged degeneration of chromaffin cells, and an apparent late change in the "cortical" cells. The chromaffin cells were reduced in number, contained damaged nuclei, and showed a smaller number of chrome granules within individual cells. These changes, greatest in the region of the capsule, were evident as early as 2 hours, were well sustained during the first day, and were not fully removed until 9 days. The "cortical" cells, at 3, 5, and 9 days, usually contained very prominent nucleoli.

(b) 1000 r: A similar but much less severe and less prolonged damage to chromaffin cells occurred after 1000 r irradiation. The number of cells and the amount of chrome in individual cells varied greatly, but not in any consistent fashion.

(c) 400 r: One specimen at 2 hours contained a small area of damaged and dead chromaffin cells, as well as very occasional dead chromaffin cells elsewhere in the section.

(d) 11-week chicks: No damage was observed.

3.9 Pancreas: Doses of 800 r caused damage to acinar cells, and in the younger chick, to the epithelium of the duct. The lymphatic tissue was affected at lower doses also. The organ showed full recovery several days after irradiation.

(a) 800 r: The principal effect of 800 r upon the pancreas of the 3-week chick was upon the acinar cells, which were often found vacuolated or dead, particularly after 3 days. The epithelium of the duct contained dead epithelial nuclei at 1, 2, and 3 days, when dead embryonic duct cells were also seen. The usual degenerative changes were seen in the lymphatic tissue.

(b) 400 r: There was no effect after 400 r except for the typical pattern of damage and regeneration to the islands of blood- and lymph-forming tissue.

(c) 11-week chicks: Acinar cells were damaged, from 3 to 24 hours following irradiation, by which time whole acini were destroyed. The usual lymphatic changes were apparent. The pancreas was again normal by 4 days, although one specimen, at 12 days, showed on area of degeneration.

3.10 Heart: The heart, like other organs in the chicken, is the site of extramedullary myelopoiesis and also contains areas of lymphocytopoiesis.

Both these functions were inhibited by irradiation.

(a) 800 r: In the 3-week chicks at 45 minutes after 800 r X irradiation, hemopoiesis was reduced, and lymphocytes, heterophil leucocytes, myelocytes, and developing erythrocytes were being destroyed. The damage was marked at 8 hours, with debris filling the macrophages, but the heart was normal after 3 or 4 days. No degeneration was seen in the muscle of the heart.

(b) 1000 r: A number of the specimens from 11 hours to 4 days after exposure to 1000 r showed edema in the pericardium; a few dead cardiac cells were found at 16 and 24 hours following irradiation.

(c) 400 r; 100 r: With doses of 400 r or 100 r only the occasional areas of blood- and lymph-forming tissue were affected. In the ventricle of one specimen, 13 hours after 100 r, there was a very large inflammatory area extending to the pericardium; in this area most of the muscle cells had disappeared, and considerable irregularity was observed among the other cells. The inflammation was probably not caused by irradiation.

(d) 11-week chicks: Myelopoiesis and lymphopoiesis were affected in the usual pattern, but no further damage was seen.

3.11 Nervous System: Some of the neurilemma cells of the somatic nerves were destroyed by all doses from 100 r to 1000 r, and particularly by the larger doses. Some were destroyed also in the visceral nervous system, but no autonomic ganglion cells were destroyed. The damage was most prominent from 8 to 30 hours after treatment, but was observed as late as 5 days. Neurones in the central nervous system were not examined, but those in the peripheral system were not visibly affected.

(a) 800 r: Degeneration and death of the Schwann cells in the ganglia and peripheral nerves, and especially in the nerves adjacent to the adrenal, had occurred in the 3-week chick 45 minutes after treatment with

800 r. This condition reached a maximum at 14 hours and decreased thereafter through 5 days. The debris was not seen to be phagocytozed, and its disappearance could not be accounted for.

(b) 1000 r: The dead sheath cells and satellite cells were present in varying numbers at all intervals from 1 to 48 hours following irradiation, but at 3 and 4 days little debris remained.

(c) 400 r; 100 r: A small percentage of the sheath cells was damaged at intervals from 2 to 30 hours.

(d) 11-week chicks: A much less extensive destruction of Schwann cells was found in the older animals, principally at the intervals between 8 and 16 hours. The ganglion cells were not affected.

3.12 Lung: The scattered areas of lymphatic tissue in the lung were regularly damaged, but further effects of radiation were limited to a suggestion of epithelial damage in the younger chicks which received high doses.

(a) 800 r: In the 3-week chick a dose of 800 r X radiation caused, in addition to the destruction of lymphatic cells, an increase in the activity of goblet cells through 14 hours. Lymphocytes were destroyed within 2 hours; phagocytosis continued from 2 to 14 hours, with the identity of the phagocytes difficult to determine. Dead cells, probably lymphocytes but possibly epithelial cells, were found also among the respiratory epithelial cells of the bronchioles. Edema appeared among the control as well as the experimental animals.

(b) 1000 r: Dead cells were numerous in the vessels during the first day after treatment with 1000 r. At 2 and 4 hours there were a few swollen epithelial nuclei in the air ducts.

(c) 400 r: There was no change at 400 r except in the occasional islands of blood- and lymph-forming tissue.

(d) 100 r: In one specimen, at 1/2 hour and one at 4 hours, there were a few dead lymphocytes and many plasma cells in the lymph nodules of the lung.

(e) 11-week chicks: Only the usual damage to lymphatic tissue was suffered by the older animals.

3.13 Testis: Primitive germ cells were destroyed by all doses of 100 r or more, in numbers varying with the size of the dose received. The 11-week chick was more resistant to treatment, and showed less damage after 800 r than the 3-week animal (which even normally had developed only a few primitive germ cells) showed after 100 r irradiation. The sustentacular and interstitial cells were not injured in any of these series.

(a) 800 r: Primitive germ cells were virtually eliminated in the younger chicks after a dose of 800 r. The damage was apparent at 45 minutes, after which time there was beginning repair, ending in the replacement of much of the gland by connective tissue at 9 days.

At the earliest intervals there was degeneration of many cells of all types — germinal epithelial, Sertoli, and interstitial — accompanied by active phagocytosis. The partial regeneration took place at 5 through 8 hours, as mitosis increased and a good deal of the debris was being cleared up. But at 14 hours, mitosis was again diminished, and more degenerate forms were present.

No specimens were available in this series between 14 hours and 5 days, by which time the tubules contained no germinal cells, the tubules themselves were small, and interstitial tissue was abundant. At 9 days, part of the organ resembled the 5-day stage, and part had been replaced by connective tissue - fibroblasts, a few lymphocytes, and macrophages. The central portion of the testis seemed to be more severely affected than was the peripheral part.

(b) 1000 r: The primitive germ cells were destroyed within the first 2 days, and there was no subsequent mitosis. The tubules gradually contracted to a columnar epithelium around a central lumen, while the connective tissue, including the interstitial cells, became relatively more prominent.

At 1 and 2 hours, many of the primitive germ cells showed a marked degeneration, and mitosis had ceased. Lymphocytes and eosinophils were also being destroyed. By 7 and 11 hours, still fewer primitive germ cells remained, and the amount of tissue in the tubules was decidedly reduced. The 16-hour specimens were hemorrhagic and otherwise badly damaged, but so was one of the control testes in this series, though to a somewhat lesser degree; the condition of these animals thus cannot be attributed entirely to the treatment. At subsequent intervals the testis was composed mainly of connective tissue. The tubules were decreased in size, and the supporting cells, instead of forming cords, became a columnar epithelium around a central lumen. By 48 hours, nearly all the primitive germ cells had been destroyed, and no new ones were apparently formed, as neither mitotic figures nor resting forms of primitive cells were present at 4 days. In general, the supporting cells appeared to be undamaged. With the great decrease in the size of the tubules, the interstitial cells made up an increasingly large proportion of the entire organ.

(c) 400 r: The spermatogonia disappeared almost completely during the first few hours with a dose of 400 r, and did not reappear until 8 or 10 days after treatment. All mitotic activity had ceased and most of the primitive germ cells were dead at 2 hours. The new cells formed at 8 and 12 days were smaller than those in the untreated specimens. At 17 days they were present in approximately normal numbers, but they were still not normal in size. Sustentacular cells and interstitial cells were not affected.

(d) 11-week chicks: By contrast with the almost complete destruction of primitive germ cells in the 3-week chicks after 800 r, there was little effect on the testis of the 11-week animals after this dose. The death of spermatogenic cells was increased from 3 to 16 hours, but the only evidence of damage thereafter was a reduced spermatogenesis in some specimens.

3.14 Bone: Doses of 800 r or 1000 r caused severance of the cartilage from spongy bone, and within the extent of the experiment, effectively stopped the growth of bone. The bones recovered rapidly from the moderate damage following 400 r, and showed no effects after 100 r.

(a) 800 r: Increasing signs of damage to the bone after a dose of 800 r were climaxed at 9 days by a complete cessation of bone growth. From 14 to 48 hours, there was a progressive loss of osteoblasts and a slight increase of osteoclasts around most of the metaphyseal spicules, as well as in the provisional zone of calcification. At 4 days, however, osteoblastic activity was resumed throughout the metaphysis, probably, as in the marrow, representing attempted regeneration. But even at 4 days severance of spongiosa from the epiphyseal cartilage was beginning, and at 5 and 9 days this severance was more extensive, osteoblasts were absent, and bone growth had stopped.

(b) 1000 r: The damage caused by 1000 r was similar to that caused by 800 r, and the beginning of repair was even less clearly indicated. The disappearance of osteoblasts, as well as the spread of osteoclasts around the spongy bone, developed steadily from 1 to 16 hours, whereas the normal osteoclastic activity in the zone of eroding cartilage decrease, especially at 16 hours. There were occasional dead osteocytes, and a number of empty lacunae were seen from 7 hours to 4 days after irradiation. By 4 days the cartilage and spongiosa had been extensively severed, some of the spongy bone also had been resorbed, and the bone had stopped growing.

The only observed attempt at regeneration, found in one specimen at 24 hours, was marked by mitoses of spindle or mesenchymal cells in the metaphysis, transitions from these cells to osteoblasts, and the presence of osteoblasts around some of the metaphyseal spicules. Even in this animal, however, osteoclasts were still numerous at the end of the shaft.

(c) 400 r: A dose of 400 r was sufficient to cause a progressive reduction of osteoblasts from around the metaphyseal spicules of bone, from 2 to 18 hours, but insufficient to cause severance or cessation of bone growth. At 30 hours and thereafter there was again active osteoblastic and osteoclastic activity.

(d) 100 r: No effects were observed.

(e) 11-week chicks: No sections of long bone were available.

4. Correlation of Blood and Histological Studies

Within the first week after exposure, the essential pattern of the massive effects of irradiation upon the peripheral blood was found to be the same for 23- and 37-day-old chicks with doses of 600 r and 800 r, respectively. These findings can be grouped together for correlation with the findings for the hemopoietic organs in 3-week chickens receiving 800 r. All of the animals treated with 800 r succumbed or were sacrificed within 11 days. Thereafter hematological data are available only for the specimens treated with 600 r. Four animals survived for 28 days after receiving 600 r, but no histological material is available for animals treated with this dose. However, certain general correlations may be made between this series and the 400 r series which was followed histologically.

4.1 Hemoglobin and red blood cells: The hemoglobin and red blood cell counts rose significantly during the first 10 hours following irradiation. This rise could not be explained by mobilisation of young erythrocytes from

the marrow, since the reticulocyte count dropped at the same time. It would seem, rather, to be due to a change in blood volume and a resultant concentration of cells. Between 24 and 48 hours after exposure, the hemoglobin level and red blood cell counts dropped to the pre-exposure level, which also suggests a readjustment in blood volume since it was normal to 48 hours.

4.2 Animals exposed to 800 r: In these animals there was a steady fall in the hemoglobin and erythrocyte values to a level moderately below normal before death on the 9th day, and anemia, per se, was not sufficiently severe to be a cause of death. The essential factor in the development of the progressive anemia in these animals lay in the damage sustained by the bone marrow, and which was reflected by the drop in the reticulocytes in the peripheral blood. After a significant rise at 24 hours, the reticulocyte count also dropped during the second day, and remained at the same level to the fourth day, showing only a slight rise before death on the seventh day. It may be inferred that young red cells were mobilized from the marrow at 24 hours, but that the delivery of young forms was insufficient and not long enough sustained to maintain a normal number of red cells in the blood. Since the histological findings in the marrow showed extensive destruction of erythroblasts as early as 1 hour after treatment, with only transient waves of regeneration during the first 8 hours and again between 2-5 days, the reason for the failure of the continued delivery of young erythrocytes to the blood is apparent. Blood loss evidenced by hemorrhage into the lamina propria of the stomach of animals exposed to 800 r was probably a factor in the anemia, although this finding was not observed after 400 r and cannot be relied upon as a significant feature causing the fall in the red blood cell and hemoglobin count of the chicks exposed to 600 r. The removal of damaged circulating erythrocytes by phagocytes was suggested by the finding of engorgement of the

Kupffer nuclear cells of the liver by debris; however, since this debris consisted of masses of the various blood cells it was not possible to estimate the importance of this factor especially since there was no histological evidence that the circulating leucocytes were harmed.

4.3 Animals exposed to 600 r: In these chickens the recovery from anemia followed rapidly upon the regeneration of the erythroid elements of the bone marrow and spleen.

A rise in the red blood cell count occurred between the 14th and 28th day, and this was preceded by a rapid rise in the reticulocyte count between the 8th and 14th day. It is noteworthy that the reticulocyte rise did not occur until after the 8th day, and that erythroblasts in the marrow showed recovery 18 hours after X irradiation and appeared to be normal 24 hours after the exposure to 400 r.

4.4 Leucocytes: In the chicks exposed to 800 r the total leucocyte count dropped 50 per cent within the first hour, rose to 50,000 almost twice the pre-exposure level at 10 hours, then fell again, reaching 5,000 at 48 hours, and a low point of 2,000 at the time of death on the seventh day. The initial drop during the first hour after exposure was due to the precipitous fall in the number of lymphocytes, at which time there was no parallel drop in the number of granulocytes. Throughout the first day, the granulocytes were undamaged in the peripheral blood and continued to increase in number, due to the mobilization of mature granulocytes from the hemopoietic centers into the blood, but their numbers fell thereafter throughout the remaining seven days of life. At the time of death no granulocytes were present in the blood. This drop in the granulocyte count was not explained by destruction of the granulocytes in the blood, which showed no degenerative changes, but was due to the destruction of myelocytes and their precursors in the

marrow, which showed extreme damage as early as 5 hours. Granulopoiesis ceased after 48 hours and failed to recover. Though the hemacytoblasts survived longer than the myelocytes, a few myelocytes were formed at 8 and 24 hours in chicks exposed to 600 r.

The early effects were similar to those produced by 800 r, while the late effects were those of recovery. During the first four days, the curve of the total leucocyte count showed the same initial drop and subsequent rise above the pre-exposure level at 10 hours as after 800 r. This was followed by a progressive fall until the fourth day; then after the eighth day, the curve rose to reach a level of approximately 50 per cent of normal by the 28th day. The granulocyte curve mirrored that of the total leucocyte count, dropping to the lowest level at 8 days, then rising to 30 per cent of normal at 28 days. The lymphocyte curve was identical after 600 and 800 r only during the first 24 hours, showing recovery at 48 hours. Later there was a precipitous fall within the first 3 hours, and a more gradual drop to the lowest level at 24 hours, after which there was a slight rise at 48 hours, fairly well sustained at 4 days, and a significant rise at 8 days, with a continued rise to a pre-exposure level at 28 days.

Since histological studies were not available for a series of chickens exposed to a dose level of 600 r but only for those of 400 and 800 r, a direct correlation between the blood and histological findings after 600 r is not possible. However, an interpolation of the histological changes observed after the higher and lower doses may permit the following deductions to explain the blood cell changes.

The early drop in the total leucocyte (all white blood cells) count reflects the destruction of lymphocytes in the circulating blood and in the lymphatic nodules throughout the hemopoietic system. Lymphocytes were destroyed in the blood as well as in the lymphatic tissues and marrow. Degenera-

ting forms in the blood were found in large numbers during the first hours after exposure and in lesser numbers for the first 2 days. The rise in the total leucocytes at 10 hours was due to the mobilization of mature injured granulocytes into the blood. The subsequent fall in the total leucocytes was due to the failure of the marrow to supply young granulocytes, and to the similar failure of the lymphatic tissue to supply lymphocytes. The fall was a direct reflection of the damage sustained by these organs. The rise in the total leucocytes after the 8th day was due primarily to the recovery of the lymphatic structure which occurred as early as 2 days after exposure to 400 r. Within 30 hours after this dose the spleen and thymus which had been markedly reduced in size and showed extreme destruction of the lymphocytes, were seen to have increased in size and regeneration of lymphocytes in the nodules had begun; regeneration was complete at 5 days. It may be emphasized that small lymphocytes were more susceptible to damage than large lymphocytes although destruction of both occurred. Regeneration was shown by the appearance of large lymphocytes in the germinal centers of the nodules. In the chicks receiving 600 r, the slight rise in the blood lymphocytes at 2 days indicates that a similar early recovery occurred at this dose level as well although the blood lymphocyte level would not indicate full recovery of lymphopoiesis until the 8th to the 14th day.

Recovery of granulocytopoiesis began at 48 hours in the chicks exposed to 400 r, but is not reflected in the blood of chickens exposed to 600 r until the 14th day. Again without direct evidence by study of sections of the organs of the 600 r chicks, it requires interpolation regarding the observations of the regenerative pattern of granulopoiesis to explain the delay in the rise of the granulocyte count in the blood.

Granulocytopoiesis was practically absent in the bone marrow within 14 hours after exposure to 800 r, and did not recover during the 9-day period

of observation. After 400 r, depletion of early granulocytes was most severe within 7 hours after exposure and waves of regenerative effort had occurred at 13 and 18 hours. However, regeneration was not sustained until after 48 hours. Estimates of the proportion of early and late granulocytes in the marrow indicated that the early forms were slightly increased above normal until the 8th day.

It is reasonable to assume that recovery of granulocytopoiesis after 600 r would follow the same pattern as after 400 r and it is consistent with findings in other species exposed to near lethal doses, to say that although the qualitative pattern of recovery in the hematopoietic organs was the same as after lower dose levels, the quantitative restoration was delayed. Since the quantitative estimates of granulopoiesis in the marrow indicate that young granulocytes predominated until the 8th day after 400 r, it is possible that full restoration of the marrow did not occur until considerably later after 600 r. However, it is significant that there appears to be a delay in the delivery of young cells to the blood for a long time after the marrow shows substantial recovery, in contrast to the more rapid reflection of recovery of the lymphatic tissues by a rise in the blood lymphocyte count. Whether this delay presents a normal relationship between blood and marrow activity is not revealed by this study; whether there is a disturbance in the mechanism for release of cells from the marrow, and whether there is a delay in the maturation of young forms will require more detailed study.

4.5 Thrombocytes: The thrombocyte count began to drop within 3 hours after exposure, and continued to fall, totalling less than 1000 at the time of death. This curve parallels the drop in the granulocyte count and may reflect the destructive changes in the marrow, although any attempt to correlate the findings relative to blood and marrow thrombocytes is defeated

ly the great difficulty in distinguishing between erythroblasts and disintegrating thrombocytes in marrow sections, and between thrombocytes and damaged erythroblasts in blood smears.

5. Discussion

These series of X ray studies have demonstrated the value of the chicken as a biological test object for radiation effects. The separation of erythrocyte and granulocyte formation in the bone marrow, together with the presence of lymph nodules, made possible conclusive demonstration of the great susceptibility of erythroblasts to X radiation, and an evaluation in the same tissue of the relative sensitivity of erythroblasts, myelocytes, and lymphocytes. Moreover, the time sequence of damage and repair in the three principal cell lines could be individually followed not only because of the special separation but also because of the relative rapidity of hemopoietic and lymphopoietic processes in the chicken. The intervals of sacrifice were sufficiently closely spaced so that the dramatic sequence of changes occurring within a few hours could be followed. The demonstration of these relative sensitivities in chickens confirms the similar pattern found in mammals[4], where the mixture of these cell types in the marrow rendered their identification much more difficult, and obscured the origin of unidentifiable debris.

At doses in the median lethal range (400 - 800 r) both erythroblasts and lymphocytes were badly damaged. In the bone marrow, particularly, there was perhaps more severe damage to erythroblasts than to small lymphocytes. This may indicate a genuinely greater susceptibility of this cell type under these conditions. On the other hand, as the dose decreased, a point was reached (100 r) where possibly more small lymphocytes than erythroblasts were destroyed, and at still lower doses, lymphocytes, but not erythroblasts were hit[5]. From these results the conclusion that small lymphocytes are in some sense more susceptible than erythroblasts cannot be denied.

The capacity to recover and reproduce again must also be considered in a comprehensive account of relative sensitivity of cell types. Erythroblasts recovered more rapidly than lymphocytes at doses of 400 r or less, although recovery was completed in both. At 800 r or more, on the other hand, neither erythrocytopoiesis not granulocytopoiesis was permanently restored within the limits of the experiment, whereas the return of lymphocytopoiesis, although slight in the bone marrow, was considerable and sustained in the spleen and thymus. These differences may reflect in part differing sensitivity of the immediate precursors of these two cell lines, but probably not to a very great extent, for both lines have a common origin in hemocytoblasts and undifferentiated reticular cells.

The rapidity of damage and repair, probably greater in chickens (and certainly in young chickens) than in mammals, correlates to some extent with the meager lethal data available. With high doses at least, the chickens were much more likely to die within the first day. Only one of twelve chickens survived the first few hours after 1200 r, for example, whereas only a small percentage of the mammals exposed to X radiation die so early.

Waves of beginning regeneration and subsequent secondary degeneration were more clearly noted in the chickens than in any other species studied, again possibly because of the rapidity of such processes in birds, or perhaps because the sacrifice series was more complete. In the testis, for example, after the primary degeneration, a period of mitotic activity suggesting recovery was followed in turn by renewed degeneration. Similarly, sporadic attempts at regeneration of both red cells and granulocytes in the marrow, the formation of blood cells had ceased at 9 days. Had these waves been spread out over longer periods they might have been more difficult to follow without sacrificing many more animals.

The young chickens differed from the mammals similarly studied in their reaction to X ray in the damage apparent in the chicken kidney, adrenal, lung, and nervous tissue. When the greater resistance of the older chickens is considered, however, most of these differences appeared to be due to the relative maturity of the animals, and not to differences in species (mammals studied were all older than the young chicks). In general, there is a very close parallel between mammals and chickens in the cell types damaged, the degree of damage, the relative sensitivities, and the sequence and source of repair. One exception is the death of numerous neurilemma sheath cells in the 11-week as well as the 3-week chickens. Careful study of several mammalian series (4) has revealed no comparable damage. The general similarity between the effects produced in birds and mammals in all other respects suggests that this may be a case of our inability to detect microscopically in the mammals a change that very likely has occurred.

Certain interesting observations, more or less incidental to the description of changes occurring after X irradiation, should be emphasized. The potencies of several cell types was well illustrated in the abundant transformations of endothelial cells to erythroblasts in the bone marrow, reminiscent of similar transformations in the embryonic yolk sac, and in the transformation of sheath cells of the splenic white pulp into lymphocytes. The presence of large amounts of debris (primarily of erythroblasts) in the vessels, adrenal, and liver, and in the liver phagocytes, probably does not represent damage to circulating cells but rather reflects the intravascular location of the badly damaged erythrocytopoietic activity. This impression is strengthened by the virtual absence of such debris in mammals, where the formation of erythrocytes is presumably extravascular. Or one might say, the absence of debris of erythroblasts in the vessels and liver phagocytes

of mammals may be considered evidence for the extravascular origin of erythrocytes in mammals.

6. Summary

Three-week old chickens were X radiated with doses ranging from 2 r to 1200 r. Another group comprised of 11-week-old birds was given 800 r. Small groups of 3-week-old and 5-week-old chickens were studied for changes in the peripheral blood after treatment with 600 r and 800 r, respectively. The severe damage suffered by the younger chicks was progressive until death after exposure to 800 r or more, but full recovery was effected with all lower doses. The following effects were observed in the 3-week-old chickens, and except where stated otherwise, the effects on the 11-week chickens were similar but less severe.

1. The median lethal dose of 200 kv X rays for 3-week chickens appeared to lie between 400 r and 800 r.

2. A slight modification of Kyes' method for enumeration of fowl blood was applied successfully to chickens. Values for leucocytes in controls were always several times the corresponding mammalian values.

3. The hemoglobin and erythrocyte values were significantly depressed after 600 r and 800 r; the anemia was moderate but progressive until the times of death 8 days after exposure to 800 r. Recovery was rapid after 600 r and was proceeded by a reticulocytosis. Reduction of erythroblasts in the bone marrow was the essential factor in the production of anemia.

4. The granulocyte count showed an early fall, followed by a precipitous fall within 4 days after exposure to 600 and 800 r. Recovery was only moderate 4 weeks after 600 r and was delayed after the marrow appeared normal.

5. The lymphocyte count dropped immediately and sharply after 600 and 800 r, and approximated the normal level only 4 weeks after 600 r, later than recovery of the lymphatic tissue seemed apparent.

6. Thrombocytes and lymphocytes in the peripheral blood fell precipitously after 800 r and 600 r, recovering only moderately 4 weeks after 600 r.

7. Morphologic changes in circulating cells, as seen in dry smears, included early clumping of nuclear chromatin, pyknosis, and karyorhexis in lymphocytes, erythroblasts, and thrombocytes. The average maturity of granulocytes rose in the period from 3 to 24 hours after treatment, but thereafter decreased rapidly so that at 4 days only a few early forms were present.

8. Doses of 800 r or 1000 r caused severance of cartilage from spongy bone, and within the extent of the experiment effectively stopped the growth of bone. The bones recovered rapidly from the moderate damage following 400 r, and showed no effects after exposure to 100 r.

9. The bone marrow was damaged at all doses from 25 r to 1000 r, the effects varying in magnitude with the size of the dose, but being otherwise similar in all treated animals. The marrow was reduced to fat cells, gelatinous ground substance, and reticulum 9 days after 800 r. Recovery was rapid and complete 2 days after moderate damage with 400 r.

10. Following 800 r and 1000 r the spleen was greatly reduced in size by the complete and rapid loss of virtually all lymphocytes. Beginning regeneration was not yet substantially successful at 9 days. After 400 r, depletion was nearly as great, but recovery, first by transitions and later by mitosis, was complete at 5 days. Damage to small lymphocytes occurred at doses as low as 25 r but not at 6 r.

11. Doses of 800 r to the thymus resulted in the almost complete destruction of small lymphocytes and a noticeable reduction in the number of large lymphocytes, whereas 400 r produced much more moderate effects. Recovery was only moderate 9 days after 800 r but was complete 2 days after 400 r.

Small lymphocytes were damaged at 400 r but not at lower doses.

12. The liver showed striking phagocytosis of circulating debris after 400 r or more. Focal areas of degenerating liver cells after 800 r and 1000 r, with and without obvious bacterial invasion, suggested lowered resistance but no directly observable damage to liver cells.

13. The stomach showed moderate damage to epithelial cells, and sporadic hemorrhage in the lamina propria in the first few hours after 400 r or more. Recovery was complete in a week even with 1000 r.

14. After all doses of 400 r or more, the intestinal epithelium showed damage proportionate to the size of the dose. The effects were comparable to those found in the rat, and much greater than in the rabbit, as reported elsewhere. Destruction was regularly most severe within the first few hours, and recovery was rapid. The pattern of damage and repair observed in the lymphatic tissue resembled that in the spleen.

15. A number of epithelial cells in the metanephrogenic areas of the kidney, and in the ureter and renal pelvis, were destroyed in the 3-week chickens at doses from 400 r and upwards.

16. There was mild damage to chromaffin and cortical cells in the 3-week chickens after exposure of 800 r or more.

17. A few acinar cells of the pancreas were destroyed in the 3-week chickens at 800 r and 1000 r.

18. In the immature testis of 3-week chicks, primitive germ cells were destroyed by all doses of 100 r or more, in numbers varying with the dose. Damage to the relatively mature 11-week testis after 800 r resembled that in the 100 r 3-week chicks, but nearly complete 17 days after 400 r. No damage was seen at 25 r or lower doses.

19. Some of the neurilemma cells of the somatic and visceral nerves in

both 3-week and 11-week chickens were destroyed at doses from 100 r to 1000 r. Damage was greater with higher dose, and greater in the 3-week chicks than in the 11-week chickens. Neurones were not affected.

20. With the one exception of damage to neurilemma cells in the chickens, differences in reaction to X irradiation in chickens and mammals of comparable maturity are insignificant.

7. Literature Cited

1. Hagen, C. W., and R. E. Zirkle: Methods of Exposure of Animals to X rays and to Cyclotron Fast Neutrons. PPR, Vol. 22b.
2. Wetmore Psyche: A Direct Method of Determining the Erythrocyte, Leucocyte, and Thrombocyte Counts of Fowl Blood. Science 92:386 (1940).
3. Kyes, Preston: Normal Leucocyte Content of Birds' Blood. The Anatomical Record, No.2. 47:197-198 (1929).
4. Bloom, William, et al: Histopathology of Irradiation from External and Internal Sources. PPR, Vol. 22b.
5. Bloom, M. A., and W. Bloom: The Radiosensitivity of Erythroblasts. J. of Lab. and Clin. Med., No. 6. 32:654-660 (1947).

END