amine, 48/80(14), and the binding of heparin by this substance(15) suggest that this granule constituent may exchange histamine for various other amines, thereby forming complexes with the latter substances. In any event, ingestion and subsequent detoxification of this material by fibroblasts would tend to reduce the stimuli for the inflammatory response. Under physiological circumstances, noxious amines arising as the result of metabolism and/or cell death could be dealt with in these manners and the production of inflammatory lesions avoided(16).

Summary. It has been observed that following mild trauma to the skin of mice, degranulation of mast cells occurs in the subcutaneous connective tissue and is followed by the appearance of metachromatic granules within the cytoplasm of surrounding fibroblasts. Investigation of this phenomenon by subcutaneous injection of isolated mast cell granules showed that fibroblasts began to ingest the granules within 15 minutes after injection. Granulation appeared to be maximal within one to two hours and digestion of granules was apparent within four hours. These observations on the fibroblastic uptake of mast cell granules were confirmed by using S^{35}-labeled mast cell granules for injection. It is suggested that this phenomenon represents one aspect of local detoxification in the tissues.

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Effect of Total Body X-Irradiation on the Parakeet.* (22446)

HANS G. SCHLUMBERGER AND ULRICH K. HENSCHKE.†
Departments of Pathology and Radiology, College of Medicine, Ohio State University, Columbus, O.

The biological effect of total body x-irradiation on vertebrates has been investigated chiefly in mammals; a few data have also been reported on fishes, amphibians, and reptiles(1). Although birds retain many characteristics of reptiles, they differ in being homeothermal and having a metabolic rate akin to that of mammals. Nevertheless, relatively few radiation studies have been conducted on birds, and most of these have employed the chicken(2-5). To provide a broader base for comparing the response of birds with other classes of vertebrates we have

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† Present address: Dept. of Radiotherapy, Memorial Center for Cancer and Allied Diseases, New York.

carried out this study on the shell parakeet, *Melopsittacus undulatus.*

Material and methods. The 336 parakeets used in these experiments were obtained from commercial dealers and the previous genetic history of the birds is unknown, except that no inbred strains were represented. Both sexes were used, but there was a slight excess of males; in age the birds ranged from 3 to 18 months. Before and after irradiation they were kept in roomy cages, usually 15 birds in a 1 x 2 x 3 foot cage. They were fed a mixture of equal parts millet and canary seed *ad libitum.* Once weekly water-soaked oats mixed with cod liver oil as well as carrot greens were provided. Water and oyster shell grit were always available. At the time of irradiation each bird was confined in a 4 x 4 x 10 cm compartment of a plexiglass box. The box measured 20 x 20 x 4 cm and contained 10 such compartments arranged in 2 parallel rows of 5 each. The outer walls and partitions were perforated by holes 5 mm in diameter and 10 mm apart. During irradiation, when 4 boxes were usually stacked one above the other, they were separated by a 1 cm space and ventilated with a gentle current of air from an electric fan; the room was air conditioned. The boxes rested on a turntable that rotated at about 1 rpm. The motion was transmitted by a lucite rod 5 cm in diameter that elevated the turntable 65 cm above the motor housing. To eliminate the influence of any dose difference in the vertical axis the order of stacking the boxes was systematically changed during irradiation. The containers were removed from the stack at different times depending on the dosage to be delivered to any group of 10 birds. To exclude the possibility that close confinement in the boxes during irradiation might affect the mortality, several experiments were carried out in which only 3-5 birds were kept in a commercial plastic bird cage as large as all 4 of the experimental cages combined. No difference in radiation response was noted. A constant potential Quadrocondex therapy machine operated at 250 KV and 15 ma served as the x-ray source. A filter of 0.5 mm copper and 1.0 mm aluminum was located 7 cm from the anode. An additional compensating filter of 3 copper sheets whose contours followed the isodose curves, was placed 12 cm from the anode. This filter reduced a dose variation of 8% within the field occupied by the boxes to 4%. The half value layer in the center of the field was 1.8 mm copper. The boxes were 1 meter from the anode; a Siemens integrating dosimeter was located at the level of the stack center, 2 meters from the anode and 40 cm from the axis of the x-ray beam. By previous calibration with a 100 r Victoreen chamber this dosimeter was used to measure accurately the total dose received by the birds at any time during the period of irradiation. The dose rate delivered at the boxes was approximately 22 r/min.

TABLE I. Survival after Total Body X-Irradiation.

Dose (roentgens)	No. of birds	No. dead in 30 days	Avg time of death (days)	Birds surviving 30 days (%)
500	12	1	13.0	92.5
1250	33	3	12.3	90.9
1500	88	24	12.9	72.7
1750	47	21	10.3	55.3
2000	77	54	10.5	30.0
2250	18	13	13.3	27.7
2500	30	24	12.6	20.0
2750	20	20	13.8	0.0
3000	24	22	11.5	8.3

Results. The parakeets proved to be surprisingly resistant to total body x-irradiation. The doses employed ranged from 500 to 3000 r; even at the highest dose an occasional bird survived (Table I). Approximately 55% of birds live for 30 days after exposure to 1750 r; at 2000 r the survival is only 30%. The LD 50/30 was calculated to be 1800 $\pm$ 75 r. Mortality, regardless of the dose, was highest during the second week after irradiation. Birds that did not die in the first 3 weeks usually survived indefinitely; many are still under observation 1 to 2 years after irradiation.

Radiation Sickness. During the first 5 days after irradiation the birds showed little change in activity or behavior. At the end of this period, however, those that had received a large dose of radiation became quiet, often sitting on their perch with eyes closed and feathers ruffled. They stopped eating and as

TABLE II. Effect of 2000 r Total Body Irradiation on Peripheral Blood Count.

Sex	Days past radiation	RBC count* Before radiation	RBC count* After radiation	% RBC surviving	WBC count Before radiation	WBC count After radiation	% WBC surviving
♀	2	2.95	2.32	78	24,000	14,800	62.0
♂	2	4.12	2.97	72	26,000	8,880	34.1
♀	4	3.33	2.24	67	26,660	9,760	36.6
♂	4	4.44	2.49	56	29,320	9,320	31.8
♀	6	3.01	2.49	82	12,220	1,540	12.6
♂	6	4.18	2.05	48	18,660	5,320	28.5
♂	8	3.99	2.42	60	17,540	1,320	7.5
♂	8	3.87	2.37	61	9,100	1,330	14.5
♀	11	4.31	3.26	75	29,100	1,100	3.7
♀	11	4.12	2.28	55	46,660	880	1.8
♀	13	4.37	2.75	62	13,320	100	.7
♀	13	4.36	2.36	54	30,440	3,540	11.6
♀	15	4.05	2.26	55	42,220	9,100	21.5
♂	15	4.79	2.54	53	27,760	220	.8
♀	19	4.80	1.75	36	38,880	3,100	7.9
♂	19	3.42	1.09	31	40,220	2,880	7.1
♀	22	4.23	2.14	50	25,320	2,376	9.3
♂	22	4.00	3.17	79	28,880	2,132	7.4
♀	26	3.71	2.18	57	24,440	5,540	22.6
♀	26	3.81	2.63	68	28,880	4,220	14.6

* Expressed in millions.

they became weaker would leave the perch to rest on the bottom of the cage. The excrement, which in birds is a mixture of intestinal and renal wastes, often became watery, but never was streaked with blood. Shortly before death the birds showed definite evidence of emaciation. The birds used to obtain the data in Table I were not disturbed following irradiation. To determine the sequence of events in the peripheral blood and marrow an additional 25 birds were irradiated with 2000 r 24 hours after red and white cell counts had been made. Every 2-3 days thereafter 2 birds were exsanguinated and blood studies carried out (Table II). Despite the great variation in the total counts of the various birds and the difficulty of the counting technic(6) because of the nucleated erythrocytes, it is clear that within 48 hours after irradiation the number of leucocytes in the peripheral blood had dropped precipitously and after a week these cells had almost disappeared. However, within 2 weeks after irradiation their number began to increase. The erythrocytes showed similar though much less marked changes in number (Table II). During this period the blood uric acid level of 9 birds 2 to 22 days after irradiation averaged 6.4 mg % in a range of 5.3 to 8.0 mg %. This is within normal limits for the parakeet.

Pathologic findings. In birds dying after irradiation there were usually few gross lesions. Hemorrhages in skin and intestinal tract, so often noted in a number of mammals(7) were not encountered. Frequently one or both lungs showed hemorrhagic areas of consolidation. Histologically these were usually associated with a more or less luxuriant growth of a fungus, identified on culture as *Aspergillus*. These were not postmortem growths for they were found in birds examined immediately after death. This fungus is a frequent cause of pulmonary disease in a variety of birds(8). Occasionally widespread bacterial colonization was found in many organs, in the lungs this was associated with yellow miliary lesions. The latter were not true abscesses because leukocytes were absent, but tissue necrosis and fibrin deposits surrounded dense masses of bacteria which on culture proved to be *Staphylococcus aureus*.

The most striking changes were found in the hematopoietic tissue. In the normal adult parakeet the thymus and bursa of Fabricius have involuted; lymph nodes are absent, as in

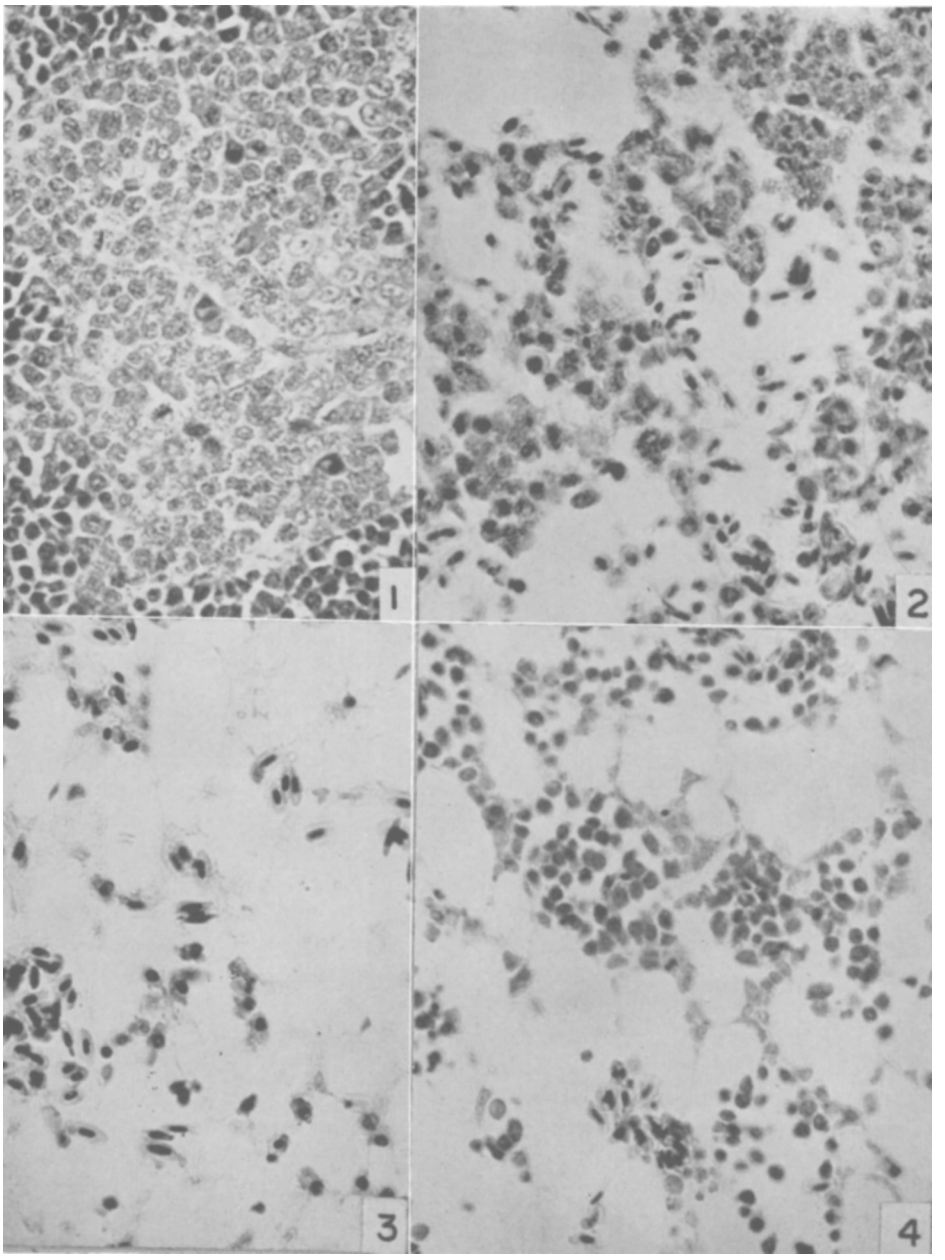

FIG. 1. Femoral marrow of a normal parakeet. Pale gray cells are masses of granulocytes; dark cells are immature elements of erythroid series filling vascular sinuses. Giemsa stain. Mag. × 425.

FIG. 2. Femoral marrow 48 hr after exposure of bird to 2000 r. Note almost complete loss of erythropoietic cells from sinuses and great depletion of granulocytes from interstitial tissue. Giemsa stain. Mag. × 425. Bird 35-1.

FIG. 3. Femoral marrow 8 days after exposure of bird to 2000 r. A few mature nucleated erythrocytes are present in partially collapsed sinuses; interstitial tissue is empty except for an occasional reticulum cell and "primitive lymphocyte." Giemsa stain. Mag. × 425. Bird 35-7.

FIG. 4. Femoral marrow 13 days after exposure of bird to 2000 r. Region was selected to show an area of regeneration. Sinuses are beginning to fill with primitive cells identified as hemocytoblasts and later erythrocyte precursors. Granulocytes are appearing in interstitial tissue accompanied by "primitive lymphocytes." Giemsa stain. Mag. × 425. Bird 35-12.

most birds. The bone marrow of all parakeets dying 7-14 days after irradiation showed an almost complete absence of blood forming elements. The spleen was almost wholly depleted of lymphocytes. The sequence of changes was followed in the birds killed at intervals of 2-3 days after exposure to 2000 r.

In the normal parakeet marrow the erythropoietic cells occupy large blood sinusoids, granulopoiesis takes place in the interstitial tissue (Fig. 1). Within 48 hours of irradiation there was a striking depletion of immature elements (Fig. 2) and after 4 days the marrow was almost wholly aplastic (Fig. 3). However, small nests of cells resembling lymphocytes could still be found sparsely scattered through the marrow during the first week after irradiation. These cells resemble those identified by Jordan(9) as "lymphocytes" and which he believes enlarge and become hemocytoblasts that then differentiate into the various cell types. By the end of the second week these centers of regeneration are increasing in size and the primitive lymphocyte-like cells are assuming the appearance of precursors of granulocytes and erythrocytes (Fig. 4). During the third week after exposure the marrow becomes solidly filled with hematopoietic cells.

The changes in the spleen parallel those in the marrow and are characterized by a rapid loss of lymphocytes. By the end of the second week lymphocytes reappear accompanied by extensive areas of erythropoiesis. The precursors of these cells have not been identified with certainty although the reticulo-endothelium appears to play an important role.

Significant lesions were not observed in the crop, esophagus, gizzard, or intestine. The absence of ulceration in the crop and gizzard is particularly surprising because of the presence in these organs of seeds and sand respectively. The traumatic effect of these materials would be expected to produce extensive erosions if the mucosa had been damaged by radiation. In several instances, however, a pulmonary fungus infection, particularly of the right lung seemed to be an extension of a similar lesion in the crop. This suggests that the crop, into which fungi are frequently introduced with the seeds, is more susceptible to mycotic invasion after irradiation.

The liver and pancreas showed no evidence of radiation injury. The cytoplasm of the renal tubular epithelium was granular and the cells were swollen. These renal changes usually disappeared after the first week; however many of the birds that have survived for many months are now dying in renal failure. This would suggest that in some instances irradiation led to a progressive nephritis similar to that occasionally seen in patients treated with more than 2000 to 3000 r over the kidney region. These and other late manifestations of radiation injury in the parakeet are still under investigation.

In birds dying after irradiation with 1250 or more roentgen units, active spermatogenesis had ceased and in the ovaries some of the ova showed degenerative changes. However, because irradiation was administered without reference to the cyclic nature of spermatogenesis and oogenesis in these birds, no definitive conclusions can be drawn from these experiments.

The pituitary, thyroids, and adrenals were weighed on a Roller-Smith torsion balance. No consistent difference in the weight of the endocrines of the irradiated birds was found when compared with that of normal birds. Histological studies were likewise without evidence of radiation effect.

Discussion. The LD 50-30 of 1800 r for parakeets is 3 times as great as that obtained with the monkey in this laboratory using similar equipment and technic with a delivery rate of 23 r per minute(10,11). The dose is also 3 times greater than that reported for most mammals(1) and over twice that for the frog(12) and goldfish(13). Most studies on birds have been carried out with fowl chicks(4,5) and embryos(2). From data available it is difficult to determine the LD 50-30 for adult chickens when irradiated at a rate of 20 to 45 r per minute, but it appears to be approximately 600 to 800 r(3). The reason for the unusual resistance of the parakeet is not clear. The fact that in birds the circulating erythrocytes and thrombocytes are intact nucleated cells rather than the effete

erythrocytes and platelets found in mammals may be significant. The loss of leukocytes from the peripheral blood and marrow is approximately as rapid as that observed in mammals, indicating that the cellular mechanism for defense against infection is depressed. The relative resistance of the parakeet intestinal tract, however, may be an important factor in limiting access of pathogenic organisms to the blood stream and thus permit survival. Fluid and electrolyte loss through vomiting, diarrhea, and hemorrhage, often observed in mammals, is also not as great in the parakeet.

The observation of Stearner *et al.*(4) that chicks exposed to 1000 r at a rate of 43 r per minute died within 24 hours in acute renal failure is of interest in view of the cloudy swelling seen in the renal epithelium of the parakeet. Although in the parakeet these changes were not accompanied by an elevation of the blood uric acid level, there is evidence of progressive kidney damage followed by renal failure months after irradiation. Kidney damage following ionizing radiation has also been observed in mice(14).

Summary. The LD 50-30 for adult parakeets exposed to total body irradiation by x-rays at the rate of 23 r per minute is 1800 ± 75 r. The destruction and regeneration of the hematopoietic tissue is similar to that observed in mammals. The kidneys may be more susceptible and the intestinal tract less so than the same organs in mammals.

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Plasma Iron Studies in Normal Beagle Dogs.* (22447)

JOHN E. PARKINSON.† (Introduced by Thomas F. Dougherty.)

Department of Anatomy, University of Utah College of Medicine, Salt Lake City.

Plasma iron values ranging from 50 μg% to 292 μg%(1) and 71 μg% to 285 μg%(2) have been observed in mongrel dogs. Plasma iron values were therefore studied in a highly bred stock of dogs maintained under relatively standard environmental conditions. Studies included comparisons among animals, weekly fluctuations in individual animals, differences between 24-hour fasting and non-fasting states, sex differences, and correlations with weight and age. This study is part of a larger study on the effects of radioisotopes in beagle dogs.

Methods. The 36 animals are a part of a colony of about 350 beagle dogs. They are

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