plained by differences in the maximal formation and the stability of an intermediate complex, EAg.p.C'1,4,2.

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Effect of X-Irradiation and Hypophysectomy on Globule Leucocytes In Rat Intestine.* (22196)

JOHN F. KENT, BURTON L. BAKER, EDWARD C. PLISKE, AND JAMES G. VAN DYKE.
Department of Anatomy and Atomic Energy Commission Biological Effects of Irradiation Laboratory, University of Michigan, Ann Arbor.

Globule leucocytes are distributed widely in urinary(1), respiratory(2) and digestive mucous membranes (Fig. 1) of the rat. They are distinguished by presence in their cytoplasm of abundant, coarse, metachromatic acidophilic inclusions and by the fact that they occur only in the epithelium and tunica propria. Cytological evidence indicates that they arise by direct transformation from lymphocytes(3). Like lymphocytes, they are reduced in number by treatment with corticotropin or cortisone(2). Because of these observations and the well-known radiosensitivity of lymphocytes, this study was made of the response of globule leucocytes to total body x-irradiation. Observations are reported also on effect of hypophysectomy.

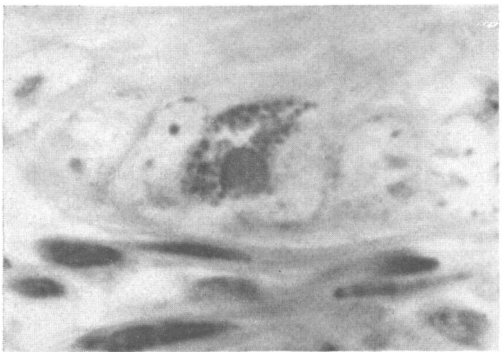

FIG. 1. Globule leucocyte in epithelium of duodenal crypt of hypophysectomized rat. ×1450.

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Procedure. Female Sprague-Dawley rats weighing 150-190 g were used. During early experiments, control rats were pair fed against irradiated animals. Since it was observed that reduction in food intake was without effect, the animals were fed *ad libitum* in the final experiments. Diet consisted of Purina Laboratory Chow supplemented twice weekly with greens, oranges, and a vitamin concentrate. Temperature of animal quarters was maintained at 75 ± 3°F. All irradiated animals (except as noted below with relation to shielding experiments) were exposed to whole body irradiation while held in flat cylindrical holder constructed of ¼-inch sheet Lucite and radially divided into 9 equal compartments. Radiation was administered by Keleket Theron X-ray therapy machine operated at 200 KV with ½ mm Cu and 1 mm Al filtration. Distance from center line of x-ray tube to table on which animal container rested was 110 cm. Dose rate was indicated by Victoreen rate meter and was held constant at about 11 r/min by varying x-ray tube cur-

TABLE I. Effect of X-Irradiation on Globule Leucocytes in the Ileum.

Days after irradiation	No. rats	X-ray dose (r)	Mean No. globule leucocytes/section	P values*
0	20	—	251.3 ±104.6†	
½	2	650	84.0 ± 15.3	<.001
¾	1	"	22.5	
1	4	"	19.2 ± 8.0	<.001
2	2	125	92.0 ± 13.5	"
2	3	650	7.8 ± 1.3	"
4	5	"	2.6 ± 1.2	"
5	2	"	2.5 ± 1.0	"
6	2	"	5.3 ± 2.3	"
7	5	"	6.5 ± 4.5	"
14	4	"	13.8 ± 1.2	"
21	2	"	21.5 ± 11.0	"
30	2	"	71.2 ± 6.5	"
35	2	"	201.5 ± 32.5	NS
43	2	"	220.4 ± 21.6	"
53	2	"	257.8 ± 8.8	"

NS = Not significant ($P > .05$).

* Counts compared with those for the rats which received no irradiation.

† Stand. dev. $= \sqrt{\frac{\Sigma x^2 - \bar{x}^2}{n}}$.

rent within range of 20-25 ma. Output was calibrated by giving a timed exposure to Victoreen r-meter chamber inserted within animal chamber in a position receiving average radiation intensity. At autopsy, samples of ileum were fixed in Helly's fluid. Paraffin sections were cut at 5 μ and stained in phosphotungstic acid hematoxylin and basic fuchsin. Globule leucocytes were counted in 3 nonadjacent sections from each animal and these counts averaged. The number of animals sacrificed at various time intervals after irradiation and radiation doses employed are summarized in Table I. To determine whether x-irradiation acted directly or indirectly on globule leucocytes, additional animals were divided into 3 groups: a. those having a segment of intestine exteriorized and protected by lead shield during irradiation; b. those treated like group a, but with shield made of paraffin; c. those treated like group a, but without exposure to x-irradiation. Animals were anesthetized with subcutaneous injections of Nembutal (0.04 or more mg/g body weight), and a loop of small intestine was exteriorized through incision in the flank. In initial experiments, 4 rats were irradiated simultaneously while fastened to plywood square with their ventral sides up and shield toward center of board. They were arranged symmetrically with relation to the x-ray beam. For experiments at 600 r and 800 r, smaller shields were used to permit irradiating anesthetized animals in the regular Lucite holder instead of tying them down as in earlier experiments. The intestinal loop was anchored in depths of the shield by a loose stay suture passed around the intestine (through the mesentery) and around a wire hook at end of shield. The shield itself was fastened to skin of the animal by sutures through wire loops at each end of the shield. These groups were irradiated at 20 r/min by a Pikens X-ray machine operated at 235 KV. In all shielding experiments, one limb of the shielded segment of intestine was marked at the entrance to the shield with a mattress suture with knotted end pointing toward shielded loop. At autopsy, small samples of shielded and nonshielded intestine were taken approximately 1.5 cm on each side of this suture. The procedure for preparation and study of these tissues was the same as for those from nonshielded animals. Experiments on hypophysectomized animals were performed in 8th or 9th post-operative week. In all cases, completeness of operation was established by microscopic examination of serial sections of the pituitary capsule. All incompletely hypophysectomized animals were eliminated.

Observations. In non-hypophysectomized rats there was a statistically significant reduction in number of intestinal globule leucocytes within 12 hours after irradiation, with a 99% disappearance of cells by 4th and 5th days after irradiation with 650 r (Table I). Beginning about the 6th day after irradiation and continuing at a relatively slow rate until about the 30th day, globule leucocytes increased in number. During 4th week after irradiation, reappearance of cells accelerated and by 35 days their number was within the normal range. Since globule leucocytes arise within the tunica propria from small lymphocytes(3), which are radiosensitive, it is pertinent to note that rate of increase in absolute lymphocyte count in the circulation(4)

TABLE II. Effect of X-Irradiation and Shielding on Globule Leucocytes in the Ileum.

Exp.	Type of shield	Dose (r)	Days after irradiation	No. rats	Mean No. globule leucocytes/section	P values
I		0	1, 4, 7	6	170 ± 50 *	NS †
	Lead	0	1, 4, 7	6	215 ± 40	" †‡
II		650	2, 4, 7	13	5.6 ± 2.3	<.001†
	Paraffin	"	2, 4, 7	3	4.4 ± 4.3	NS ‡
	Lead	"	2, 4, 7	6	286.7 ± 44.4	" †
III		800	3	3	5.9 ± 3	<.001†
	Paraffin	"	3	1	9.7	
	Lead	"	3	2	188.2 ± 20.9	NS †
IV		"	4	11	1.6 ± 1.9	<.001†
	Paraffin	"	4	1	4	
	Lead	"	4	9	148.4 ± 42.8	" †
V		0	0	11	109.8 ± 63.4	" †
Hyp.		600	4	8	10.9 ± 9.4	NS §
	Lead	"	4	6	100.6 ± 85.7	" ‖

* Stand. dev. $= \sqrt{\frac{\Sigma x^2 - \bar{x}^2}{n}}$. † Compared with non-hypophysectomized, non-irradiated rats (Table I). ‡ Compared with non-shielded animals of same experiment. § Compared with hypophysectomized, non-irradiated rats (Exp. V). ‖ Compared with non-hypophysectomized rats 4 days after irradiation with 650 r (Table I).

Hyp. = Hypophysectomized. NS = Not significant ($P > .05$).

roughly parallels recovery of globule leucocytes. The extended period of slow recovery, therefore, may be interpreted as being due to scarcity of parent cells from which new globule leucocytes may be formed.

The effect of radiation on globule leucocytes is primarily a direct one as shown by shielding experiments (Table II). Segments of intestine which were either unshielded or paraffin-shielded showed the same reduction in number of globule leucocytes as in irradiated animals not subjected to shielding procedure. In contrast, samples of small intestine which had been lead-shielded during irradiation showed no reduction at 650 r, the LD_{20} 30 days(5). At 800 r, the LD_{100} 30 days(5), only 40% reduction from normal occurred. X-irradiation may act directly on globule leucocytes themselves or indirectly through changes elicited in their tissue environment. The latter possibility must be considered in view of local disappearance of these cells in areas of tracheal inflammation.

It is probable that radiation-induced stress is responsible for partial disappearance of these cells in lead-shielded segments of intestine of non-hypophysectomized rats receiving 800 r. In the absence of the hypophysis, the comparable LD_{100} 30-day dosage for hypophysectomized rats (600 r) failed to induce a significant reduction in number of globule leucocytes in lead-shielded segments of the intestine (Table II, Exp. V). The shielding procedure was not involved, since in non-irradiated animals, neither shielded nor unshielded segments of intestine showed a statistically significant change in population of globule leucocytes. Neither was it likely to be due to radiations which penetrated the shield, since the theoretical protection afforded by shields was in the neighborhood of 99.5%. Finally, we have demonstrated that treatment with corticotropin or cortisone reduces the number of globule leucocytes(2).

Hypophysectomy, without irradiation, caused a marked reduction in number of globule leucocytes just as it decreases thymic and splenic weight from that of normal rats (6). After x-irradiation of hypophysectomized rat, the number of globule leucocytes was reduced to essentially the same level as in irradiated non-hypophysectomized rat (Table II). The percentage of reduction, however, was significantly less than that of

non-hypophysectomized animals. Hypophysectomy also caused a shift in position of a considerable portion of the globule leucocytes from the tunica propria (where the largest percentage occurs in non-hypophysectomized rat) to the crypt epithelium.

Summary. Within 4 days after whole body x-irradiation (650 r), globule leucocytes were reduced to 1-2% of their normal number in the intestine of the rat. Reestablishment of normal numbers of cells began toward end of the first week after irradiation and was completed by the 5th week. The effect of irradiation on globule leucocytes was chiefly a local one, being prevented by shielding the intestine with lead during irradiation. Only with the LD_{100} 30 days dosage (800 r) was there a significant reduction in number of globule leucocytes in lead-shielded intestinal segments. This appeared to be the indirect result of stress induced by irradiation, since the effect was prevented by hypophysectomy. Hypophysectomy alone reduced markedly the number of intestinal globule leucocytes.

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Strontium^{85} Metabolism in Man and Effect of Calcium on Strontium Excretion.* (22197)

HERTA SPENCER, MILTON BROTHERS,† EVA BERGER, H. E. HART, AND DANIEL LASZLO.
(Introduced by Louis Leiter.)
Division of Neoplastic Diseases, Montefiore Hospital, N. Y. City.

Sr^{90}, a β emitting isotope with a 25 year half life is one of the major hazards of nuclear fission because of its selective localization in the skeleton. Its entry into the human body and its pathways of elimination are, therefore, of great concern. Availability of a carrier-free γ emitting isotope of strontium, Sr^{85}, with a half life of 65 days, facilitated this investigation of the metabolism of radiostrontium in man(1). Data on metabolism of intravenously administered Sr^{85} are reported in this communication.‡

Methods. Carrier free Sr^{85}, 0.1-0.4 μc/kg body weight was injected intravenously in 4 human subjects. Blood samples and urine were obtained at 1, 4, 8 and 24 hour intervals initially. Subsequently, urine, stool and plasma were analyzed daily. Radioactivity was determined in 1-5 ml plasma and urine and on stool samples weighing up to 5 g. Significant counts on these samples were obtained up to 24 days. A Nuclear scintillation well counter, Model DS3 was used: through special lead shielding the background was reduced to 140-180 CPM.

Results. Fig. 1 shows plasma levels, urinary and fecal excretions of Sr^{85} of a 72 year

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† Trainee, National Cancer Institute.

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‡ Produced in Oak Ridge National Laboratory Cyclotron by the nuclear reaction of Rb^{85} (p,n) Sr^{85}. Radionucleides of Sr^{85} were isolated from the target material, Rb_2CO_3, and other impurities with no strontium carrier added. This separation was performed by H. T. Russell, Radioisotope Operations Division of Oak Ridge National Laboratory. We thank these laboratories for their cooperation.