

11. Reed, L. J., Muench, H., *Am. J. Hyg.*, 1938, v27, 493.
12. Hammon, W. McD., Yohn, D. S., Pavia, R. A., Sather, G. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1959, v100, 425.
13. Habel, K., Hornebrook, J. W., Gregg, N. C., Silberberg, R. J., Takemoto, K. K., *Virology*, 1958, v5, 7.
14. Sweet, B. H., Hilleman, M. R., *Proc. 2nd Inter. Conf. on Live Poliovirus Vaccines*, Washington, D.C., 6-10 June 1960.

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Hematologic Response of Monkeys to X-irradiation.* (26008)

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Previous reports from this institution have described pathology(1) and antibody response(2) in monkeys following total body x-irradiation. As part of the over-all project, the effect of irradiation on the peripheral blood picture was also studied. Most investigations into the effect of total body x-irradiation on cellular elements of the blood have been conducted in subprimate species. In the relatively few studies(3-5) concerned with monkeys, varying dose levels and schedules have been employed. This report describes hematologic response of monkeys following a single total-body exposure to 450 r.

Materials and methods. A total of 37 young adult monkeys (1½-3 years old, *Macaca mulatta*) received total body irradiation with 450 r by a previously described method (1). Group II (18 monkeys) was irradiated 6 months after Group I (19 monkeys). Seven control monkeys were handled in an identical manner, but received no irradiation. Blood counts done from ear lobes were obtained 4-5 times over a 2-week base-line period, and then at 2, 4, 10 and 12 days after irradiation; subsequent counts were then made twice weekly for 2 months and weekly up to 6 months. Hemoglobin content was determined with a Leitz-Rouy Photometer. Differential counts were made of 200 cells stained with Wright's stain.

Results. The effect of irradiation in the 37 monkeys was first reflected by the leukocytes, which were depressed significantly at 2 days, markedly at 4 days, and maximally between

10-17 days (Fig. 1). At the latter intervals, mean leukocyte count was only 5% of base-line level. Beginning recovery of leukocytes, noted at 19 days, was followed by a marked leukocytosis in 17 of 37 monkeys between 4 and 7 weeks post-irradiation. Recovery occurred at variable rates in the remaining animals, the low mean leukocyte count at 60 days reflecting the fact that half the animals had not attained base-line levels. By 3 months mean leukocyte count had returned to pre-irradiation level.

The effect of irradiation on erythrocytes (Fig. 1) was noted later and to a less marked degree than the effect on leukocytes. Erythrocytes were not significantly decreased at 2 and 4 days, and lowest levels were not reached until 17-19 days post-irradiation, at which time mean count was about half pre-irradiation value. Beginning recovery at about 4 weeks was followed by a gradual increase to base-line level at about 3½ months. In general, depression and recovery of hemoglobin paralleled that for erythrocytes. A gradual decline in hemoglobin concentration was noted in control, non-irradiated monkeys, but all other elements remained stable during the observation period.

The effect of irradiation on differential leukocyte count in 18 monkeys (Group II) is presented in Table I, with data on erythrocytes included for comparison purposes. Lymphocytes showed early reflection of irradiation with a rapid decline noted by the second day and a mean level of only 9% of base-line at 12 days. Gradual recovery began about the 20th day, with return to pre-

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TABLE I. Mean Erythrocyte and Leukocyte Counts in 18 Monkeys (Group II) after a Single Dose of 450 r.

Days after irradi.	Erythrocytes		Total leukocytes		Neutrophiles		Lymphocytes		Eosinophiles		Basophiles	Monocytes
	$\times 10^6$	S.D.*	$\times 10^3$	S.D.	$\times 10^3$	S.D.	$\times 10^3$	S.D.	$\times 10^3$	S.D.	$\times 10^3$	$\times 10^3$
0	5.2	.7	14.8	5.2	6.5	4.1	7.0	1.8	.8	.5	.06	.48
2	4.9	.8	5.5	2.6	4.4	2.6	1.0	.5	.1	.2	.02	.03
4	4.7	.8	4.4	2.2	3.5	2.1	.8	.3	.1	.1	.01	.02
10	4.1	.8	1.0	.5	.3	.2	.7	.4	0	—	.00	.00
12	3.7	.9	.9	.6	.2	.2	.7	.6	0	—	.00	.00
17	3.2	1.1	1.4	.9	.5	.8	.8	.7	0	—	.01	.00
19	3.2	1.0	3.8	3.6	1.9	2.3	1.7	1.7	0	—	.01	.05
24	3.6	1.0	14.4	9.6	10.0	8.1	4.3	1.9	.1	.1	.00	.03
26	3.6	.9	17.7	12.0	12.9	10.0	4.7	2.4	.1	.1	.04	.10
31	4.1	1.0	16.6	7.5	12.5	7.2	3.1	1.2	.8	1.4	.08	.16
33	4.2	.6	15.7	6.8	10.2	6.5	3.9	1.4	1.4	2.3	.14	.05
38	4.4	.6	22.4	8.7	13.1	5.8	3.7	1.4	5.3	7.6	.11	.24
40	4.4	.7	26.3	8.3	13.4	7.1	4.6	2.0	7.8	6.9	.32	.20
45	4.7	.8	20.4	7.4	11.0	4.7	4.0	1.9	5.1	4.3	.09	.21
47	4.7	.6	17.2	6.3	10.2	4.3	3.1	1.2	3.7	4.1	.03	.15
52	4.9	.6	14.7	5.6	8.6	4.0	3.8	1.7	2.1	2.0	.06	.15
54	4.6	.6	11.0	2.5	5.1	2.1	3.8	1.2	2.1	1.5	.00	.03
60	5.1	.7	12.6	4.5	7.1	5.4	4.2	2.3	1.1	1.0	.04	.15
65	4.9	.5	11.8	2.6	5.6	3.0	4.7	2.0	1.3	.9	.00	.22
75	5.1	.5	13.8	9.1	5.7	4.6	6.4	3.2	1.5	1.1	.04	.16

* Stand. dev.

irradiation levels at about 2½ months. Neutrophiles were affected less promptly but more severely with the greatest depression to 3% of base-line level noted at 12 days. Recovery of neutrophiles paralleled the pattern described above in reference to total leukocytes (Fig. 1). Ten of 18 monkeys exhibited an increase in neutrophiles to at least twice base-line level between 24 and 47 days post-irradiation. Eosinophiles were essentially absent from the 4th to 26th post-irradiation days. Beginning recovery, noted at 31 days, was followed by definite eosinophilia in 12 of 18 monkeys. In monkeys not showing eosinophilia counts rose erratically to base-line level at about 2 months. Monocytes and basophiles were depressed to lowest levels from 10-17 days post-irradiation. Rate of recovery was variable and pre-irradiation values were reached at approximately 2 months.

Fig. 1 and Table I present data on both survivors and non-survivors since no differences were observed. Eleven of 37 monkeys died during the course of the study and all showed typical signs of radiation sickness. Three exhibited definite signs of recovery, hematologically, before death. One had a marked leukocytosis (45,000) just prior to

death at 31 days post-irradiation, and in one monkey, total leukocyte count remained at very low levels until death at 96 days. This monkey was the only one in which hematologic findings were not similar to those observed in survivors.

Discussion. Results of the present study are much like those obtained by other workers(3-5), but minor differences, possibly dose or dose-rate dependent are evident. The pattern of the late eosinophilia seen in our monkeys after 450 r (23 r per minute) was similar to that described by Eldred(3) in monkeys

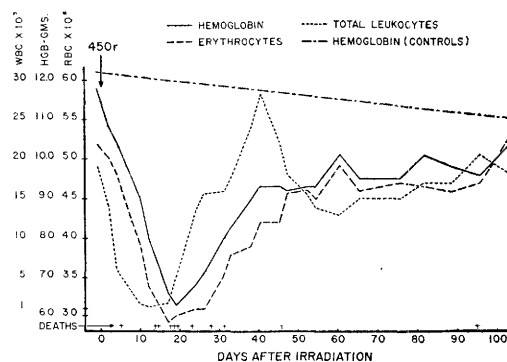


FIG. 1. Mean leukocyte, erythrocyte, and hemoglobin values in 37 monkeys after a single dose of 450 r.

given 550-600 r (13.7 r per minute) in a single session. The depression of all cellular elements was more severe in our series than in that of Riopelle, Ades and Morgan(4) who irradiated 4 monkeys with 350 r (17 r per minute). Our results show certain marked similarities to those of Haigh and Paterson (5) who irradiated 50 monkeys with 260 r, 28 with 500-550 r, and 11 with 600 r (all at 3 r per minute). The effect of 450 r on erythrocytes, eosinophiles and lymphocytes in our monkeys was much like that observed after their intermediate dose, 500-550 r. The response of neutrophils was also similar except that the secondary elevation appeared to begin earlier in our study. Haigh and Paterson(5) observed peak neutrophil counts on the 26th and 41st days after 500-550 r, and attributed this phenomenon to "a consistent and exact duration of inhibition of precursor cells, related to dose." An almost identical picture was observed in our monkeys in that peak neutrophil and total leukocyte counts were seen consistently on the 26th and 40th days after 450 r (Table I). The secondary

elevation of monocytes to supernormal levels observed by Haigh and Paterson(5) was not noted in our series.

Summary. Total body x-irradiation of monkeys with 450 r resulted in significant depression of leukocytes as early as second day with maximal effect noted between 10-17 days. Erythrocytes were affected at a later interval with maximum depression at 17-19 days post-irradiation. Recovery of leukocytes and erythrocytes to normal levels was observed between 3-3½ months.

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1. Schlumberger, H. G., Vazques, J. J., *Am. J. Path.*, 1954, v30, 1013.
2. Saslaw, S., Carlisle, H. N., *J. Lab. Clin. Med.*, 1959, v53, 896.
3. Eldred, E., *Blood*, 1959, v14, 187.
4. Riopelle, A. J., Ades, H. W., Morgan, T. E., *Rad. Res.*, 1957, v7, 581.
5. Haigh, M. V., Paterson, E., *Brit. J. Radiol.*, 1956, v29, 148.

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Electrophoretic Fractionation of B₁₂-Binders in Gastric Juice from Patients with Pernicious Anemia and from Controls.* (26009)

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The intrinsic factor (I.F.) of gastric juice has not been chemically isolated. There is some evidence that it may be associated with B₁₂-binding(1-4), although several B₁₂-binders without I.F. activity have been isolated (5-8), as have substances with high I.F. activity and a remarkably low B₁₂-binding capacity(9,10). However, whether or not I.F. actually binds Vit. B₁₂, it seems to be intimately associated with a B₁₂-binder in gastric juice(4), which may accordingly be used to trace I.F. The present study compares B₁₂-binders in gastric juice from pernicious anemia patients with those from controls.

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Methods. *Human gastric juice* was collected by a previously described method, with *in vivo* neutralization of the acid juice to prevent peptic autodigestion(11). Precautions were taken to avoid extragastric contaminants. The fasting secretion as well as the histamine, carbamylcholine chloride, and insulin-stimulated secretions were investigated. Within 3 hours after collection, B₁₂Co⁶⁰ was added to the juice. In some cases total B₁₂-binding capacity of gastric juice was estimated by a dialysis technic(12). In these cases, amount of Vit. B₁₂ added for electrophoresis is expressed as percent of total binding capacity (Tables I, II). Following concentration and ultrafiltration through collodion membrane for about 24 hours at +4°C,