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1. Ackerman, G. A., Knouff, R. A., *Blood,* 1960, v15, 267.
2. Baserga, A., *Haematol.,* 1948, Suppl. 32.
3. Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R., Roberston, J. R., in Stohlman, F., Jr., *Kinetics of Cellular Proliferation,* Grune and Stratton, New York, 1959, 188.
4. Bond, V. P., Odartchenko, N., Cottier, H., Feinendegen, L. E., Cronkite, E. P., in *Erythropoietin and regulation of Erythropoiesis,* San Francisco, 1962, in press.
5. Cronkite, E. P., Bond, V. P., Fliedner, T. M., Paglia, D. A., Adamik, E. R., in, Johnson S. A., *Blood Platelets,* Little and Brown, Boston, 1961, 595.
6. Jones, O. P., *Anat. Rec.,* 1960, v138, 105.
7. Lindell, B., Zajicek, J., *Experientia* (Basel), 1957, v15, 27.
8. Odell, T. T., Jr., Tausche, F. G., Gude, W. D., *Am. J. Physiol.,* 1955, v180, 491.
9. Pease, D. C., *Blood,* 1956, v11, 501.
10. Simpson, S. M., *Int. J. Rad. Biol.,* 1959, v2, 181.
11. Yamada, E., *Acta Anat.,* 1957, v29, 267.
12. Zajicek, J., *Acta Physiol. Scand.,* 1957, v40, (Suppl. 138).
13. Zajicek, J., Zeuthen, E., *Exp. Cell Res.,* 1956, v11, 568.

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Radiation Dose-Response Characteristics of Leucocytes in the Mouse. (27739)

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Lymphocyte and granulocyte counts in peripheral blood are known to be sensitive indicators of exposure to ionizing radiation. Suter(1) and Lawrence *et al.*(2) have published extensive data for the rat, Jacobson *et al.*(3) for the rabbit and Cronkite and Brecher(4) for granulocyte counts in the dog. Total leucocyte counts in mice exposed to several dose levels have been published by Ting *et al.* in studies of relative biologic effectiveness of 200 KV and 23.5 MEV X-radiation(5), but recent studies of RBE(6,7) have not utilized these counts. Little information is available, especially in the mouse, concerning the reliability with which lymphocyte and granulocyte counts can be used to detect differences in dose. Satisfactory dose-response relationships within the first few days after exposure would provide additional dimensions for quantifying dose and treatment effects within the various biological systems. An early dependence of count on dose would also be useful in distinguishing between a dose-reducing effect of treatment and an effect which does not reduce initial damage but does specifically accelerate recovery processes. The present experiments deal with dose-response characteristics of lymphocyte counts, granulocyte counts, and granulocyte counts following mobilization by endotoxin, in tail blood of mice 1 to 4 days after irradiation.

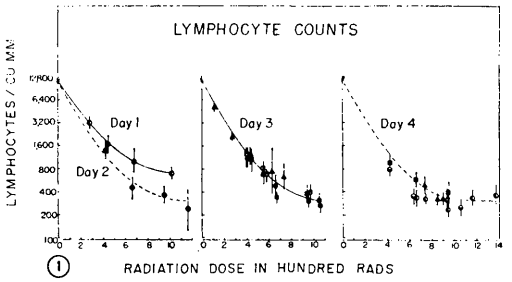
Methods. The mice used were (BALB/c $\times$ DBA/2) F_1 females 13 to 17 weeks old, caged individually, and irradiated in groups of 10 to 30 with a cobalt-60 source at rates of 270 or 510 rads per minute. In these experiments 780 rads was the LD_{50} (28 days) and 950 rads the LD_{95}. The endotoxin used was prepared by Dr. Edgar Ribi* from an aqueous ether extract of *Salmonella enteritidis*(8,9). Mice were injected intraperitoneally and counts made on tail blood 18 hours later. Values are given as geometric means with 95% confidence limits.

Results. A. Lymphocytes. Lymphocyte counts in mice irradiated 1 to 4 days previously are shown in Fig. 1. A curve of the form

$$Y = A + Be^{-kx}, \qquad (1)$$

where Y is count and x is dose in 100 rads, was fitted to the values observed on day 3,

* *Se 209.* We are indebted to Dr. Ribi for a generous supply of this preparation.



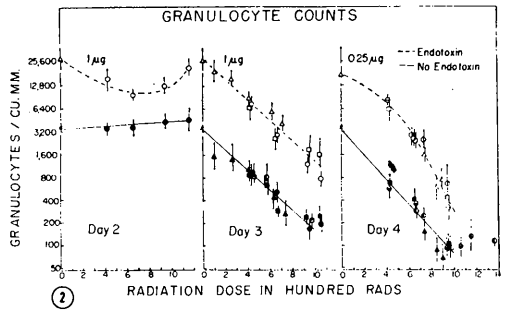


FIG. 1. Lymphocyte counts, with 95% confidence limits, in mice 1 to 4 days after exposure to radiation.

FIG. 2. Granulocyte counts, with 95% confidence limits, 2 to 4 days after exposure to radiation in mice given no endotoxin and in mice given a prior mobilizing inj. of *S. enteritidis* endotoxin.

with A = 300, B = 11,000 and k = 0.600. One day after irradiation the lymphocyte counts had not fallen to the 3-day level, but counts made 2 days and 4 days after irradiation fell approximately on the 3-day curve (broken lines). The slope of the curve relating $\log_{10}$ count to dose in 100 rads at any dose is given by

$$\frac{-k}{2.3\,[1 + (A/B)e^{kx}]} \qquad (2)$$

At 200 rads the slope is thus estimated to be –0.2392, corresponding to a change of 42% over an interval of 100 rads, while at 800 rads the slope is –0.0605, corresponding to a change of only 13% over an interval of 100 rads.

A dose-response curve such as that of Fig. 1 permits estimation of the dosage corresponding to a given level of biological response. The error in that estimate, λ, in units of dosage, is provided by the ratio of the standard deviation of the response metameter to the absolute value of the slope of the curve (10). Thus, if equation (1) is used to estimate the dose to which a group of n mice has been exposed, the probability that the error of the estimate will exceed $1.96\,\lambda/\sqrt{n}$ is 0.05.

Using the slope estimated from equation (2) and the pooled standard deviation of the log lymphocyte count for 0-950 rads on days 2, 3 and 4 (0.157 for 343 mice at 9 dose levels), λ is found to be approximately 66 rads for doses up to about 550 rads. In the lethal range the value of λ increases rapidly, however, so that it soon becomes impossible to distinguish between doses by means of lymphocyte counts.

B. Granulocytes, no endotoxin. Unlike lymphoctyes, granulocyte counts did not show a fall one or 2 days after irradiation (see also ref. 3 and 12). Mean counts in the irradiated mice did not differ significantly from controls [3670 (3270-4120) cells per cu mm] and, for a given dose, counts on day 1 did not differ significantly from counts on day 2. In both experiments, however, granulocyte counts were higher in the groups given 950 rads [pooled mean, 4330 (3700-5070)] than in those given 400 rads [3190 (2780-3660)].

Granulocyte counts 2, 3 or 4 days after irradiation in mice not given a prior injection of endotoxin are shown in the unbroken lines of Fig. 2.† Three and 4 days after irradiation these counts decreased linearly as the dose increased over the range 0 to 950 rads. On day 3 the slope of the line relating log count to dose was –0.1341, corresponding to a change of 27(25-28)% per 100 rads. On day 4 this slope was –0.1706, corresponding to a change of 32(31-34)% per 100 rads. The pooled standard deviation for day 3 (10 dose levels, 216 mice) was 0.194 and for day 4 (7 dose levels, 165 mice), 0.183. The values for λ are therefore estimated to be 145 rads for day 3 and 107 rads for day 4.

As the radiation dose was increased beyond

† One of a group of 15 mice exposed to 500 r had the exceptionally high granulocyte count of 2236 on day 3 compared to a mean of 712 (598-848) for the remaining 14, and was excluded. Counts from all other animals were included in the analysis.

950 rads granulocyte counts showed no further decrease. Three days after exposure to 1060 rads the mean count was 222. Four days after 1060, 1160, and 1380 rads mean granulocyte counts were 98, 136, and 113 respectively.

C. Granulocyte counts after endotoxin mobilization. Although the LD_{50} of the *S. enteritidis* endotoxin preparation for normal 21-day-old mice was about 200 μg per mouse (11), when mice exposed to 950 rads were given an injection of 1 μg on the third afternoon 7 of 10 died within 24 hours. There were no deaths within this time among the 950 rad mice given no endotoxin, the mice given endotoxin after exposure to lower radiation doses, and the mice injected on the afternoon of the first or second day after irradiation (for counts on days 2 and 3) even though exposed to doses as high as 1160 rads. The endotoxin dose was reduced to 0.25 μg, but this did not eliminate the deaths within 24 hours in the mice given endotoxin on the third afternoon after exposure to 950 rads (32% of 38 mice) or 1060-1380 rads (69% of 64 mice compared to 26% of 35 given no endotoxin). This obviously deleterious effect of endotoxin must be kept in mind when mobilized counts made 4 days after exposure to 950 rads or more are under consideration. It should also be noted that granulocyte counts 18 hours after injection of 0.25 μg of the endotoxin [17,600 (15,700-19,700)] were somewhat lower than when 1 μg was given [22,900 (19,000-27,600)].

Granulocyte counts 18 hours after endotoxin injection are represented by the broken lines of Fig. 2. When an injection of 1 μg per mouse was made on the afternoon of the first day (*i.e.*, 30 hours) after irradiation, granulocyte counts were elevated as shown in the first panel. This elevation was less in irradiated than in control mice, however, and less in mice given 670 rads than in those given 1160 rads. When endotoxin given on the afternoon of the second day was followed by counts on the third day, the counts decreased with increasing dose, as shown in the second panel, at about the same rate as when no endotoxin was given. For the dose range 0 to 950 rads the slope relating log count to dose was –0.1354, corresponding to a change of 27(25-29)% per 100 rads. The pooled standard deviation for these counts (8 dose levels, 165 mice) was 0.181 and the value for λ, therefore, 134 rads.

When an injection of 0.25 μg of endotoxin was given on the third afternoon and counts taken on the fourth morning, count and dose were related by a curve of the following form:

$$Y = Ae^{-(k_1x + k_2x^2)} \qquad (3)$$

where Y is count, x is dose in 100 rads, A = 17,600, $k_1 = 0.059$ and $k_2 = 0.035$. The slope of the curve relating count to dose at any dose is given by

$$-\frac{1}{2.3}(k_1 + 2k_2x). \qquad (4)$$

At doses of 200, 600 and 800 rads the slopes are estimated to be –0.0872, –0.2100 and –0.2714, corresponding to changes over an interval of 100 rads of 18%, 38% and 46% respectively. The pooled standard deviation of log count for all dose levels (7 levels, 132 mice) was 0.204. Values of λ for 200, 600 and 800 rads are therefore estimated to be 230, 95 and 74 rads respectively.

Discussion. Early workers have been aware that the lymphocyte counts after irradiation fall rapidly, the extent of the fall reflecting dose only at the lower dose levels. Data are incomplete, however, and are not readily usable for estimations of damage or protection in terms of dose or equivalent dose. Our results indicate that differences of the order of 50 rads (*i.e.*, $1.96\ \lambda / \sqrt{10}$) can be detected with probability 0.95 by lymphocyte counts 1 to 4 days after irradiation, using mice in groups of 10 and limiting the dose effect to the sublethal range.

It is also well known that granulocyte counts may be elevated for a day or two after irradiation, but at varying times thereafter fall according to the dose received. Our data indicate that in mice differences of approximately 100 rads can be detected by granulocyte counts 3 and 4 days after irradiation, using groups of 10 and limiting the dose effect to the range from 0 to a dose 98% lethal (in our experiments, 950 rads).

It was thought that mobilization with bacterial endotoxin might bring out dose effects not otherwise apparent in peripheral blood granulocyte counts. Earlier experiments showed ratios of mobilized to non-mobilized counts to be the same 4-11 days after exposure to 0-50% lethal radiation as in non-irradiated mice(13) but we had not studied endotoxin mobilization nearer the time of exposure or at dose levels in the 100% lethal range. The advantage of this procedure for detecting dose differences was found to be doubtful, however. On day 3, the earliest day on which granulocyte counts decreased with increasing dose, the error of the dose estimate, λ, was essentially the same as for mice given no endotoxin. We were again impressed with the constancy of the ratio of mobilized to non-mobilized granulocyte counts. On day 4 the error of the estimate was smaller in the lethal dose range, but the acutely toxic effect of the endotoxin when given at that time to lethally irradiated mice severely limited its usefulness.

It is clearly possible to achieve an advance in recovery without diminishing the immediate radiation damage, namely by postirradiation treatment with endotoxin(14,15). Assuming the dose-reducing effect to apply to the system under consideration (*i.e.*, granulocytes or lymphocytes), the type of data presented here should help to differentiate between changes in immediate damage and changes limited to recovery. Thus, if a given treatment should accelerate recovery without reducing the initial cell damage, granulocyte counts on days 3 and 4 and lymphocyte counts on days 1-4 would be expected to be unaltered by the treatment provided the recovery processes have not already contributed to the counts. Indeed, such an argument was used against direct protection of myelopoietic cells by glutathione(16). Early differences may have been missed, however, because total rather than differential counts were used and the radiation doses were probably too high for changes to be registered by the lymphocyte counts.

Questions pertinent to the present considerations arise from several studies currently under investigation in our laboratory. For instance, pretreatment with colchicine and related substances causes an advance in hemopoietic recovery and increases survival in irradiated mice(17) and one needs to know whether the effect is protective against immediate cell injury or is limited to the initiation of recovery. In studies of the effect of age upon radiation damage to hemopoiesis(18) one question to be answered is whether the earlier recovery of granulopoiesis in mice 10 weeks old compared to 5 weeks old, for example, is the result of a smaller amount of immediate damage or is due to a superior ability to recover. It is hoped that application of closely controlled studies of the type presented here will help to clarify some aspects of these problems.

Summary. 1. In mice, changes in dose in the sublethal range could be detected by means of lymphocyte counts 2 to 4 days after irradiation with an estimated accuracy of 66 rads, but as the dose was increased it soon became impossible to distinguish between doses by means of lymphocyte counts. 2. Changes in dose in the range 0 to LD_{95} could be detected by granulocyte counts 3 days after irradiation with an estimated accuracy of 145 rads and 4 days after irradiation, 107 rads. 3. Prior injection of endotoxin did not improve the accuracy of dose discrimination 3 days after irradiation. Four days after irradiation accuracy was increased in the higher dose range but usefulness of the procedure was limited by the acutely toxic effect of the endotoxin on lethally irradiated mice.

1. Suter, G. M., *Response of Hematopoietic Systems to X-Rays.* MDDC-824, AEC, Oak Ridge, Tenn., 1947.

2. Lawrence, J. S., Dowdy, A. H., Valentine, W. N., *Radiol.,* 1948, v51, 400.

3. Jacobson, L. O., Marks, E. K., Lorenz, E., *ibid.,* 1949, v52, 371.

4. Cronkite, E. P., Brecher, G., *Ann. N. Y. Acad. Sci.,* 1955, v59, 815.

5. Ting, T. P., Johns, H. E., Jaques, L. B., *Blood,* 1952, v7, 826.

6. Storer, J. B., Harris, P. S., Furchner, J. E., Laugham, W. H., *Radiation Res.,* 1957, v6, 188.

7. Sinclair, W. V., *ibid.,* 1962, v16, 394.

8. Ribi, E., Milner, K. C., Perrine, T. D., *J. Immunol.,* 1959, v82, 75.

9. Ribi, E., Hoyer, B. H., Milner, K. D., Perrine,

T. D., Larson, C. L., Goode, G., *ibid.*, 1960, v84, 32.

10. Bliss, C. I., *The Statistics of Bioassay,* Academic Press, Inc., New York, 1952, p271.

11. Haskins, W. T., Landy, M., Milner, K. C., Ribi, E., *J. Exp. Med.,* 1961, v114, 665.

12. Gershon-Cohen, J., Hermel, M. B., *Am. J. Roentgenol.,* 1954, v71, 846.

13. Smith, W. W., Alderman, I. M., Cornfield, J., *Am. J. Physiol.,* 1961, v201, 396.

14. Smith, W. W., Alderman, I. M., Gillespie, R. E., *ibid.,* 1957, v191, 124.

15. ———, *ibid.,* 1958, v192, 549.

16. Cronkite, E. P., Brecher, G., Chapman, W. H., *The Military Surgeon,* 1951, v109, 295.

17. Smith, W. W., Alderman, I. M., *Radiation Res.,* 1962, v17, 7.

18. ———, *The Physiologist,* 1960, v3, 146.

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A Histochemical Comparison of Glycogen Synthesis from Glucose-1-Phosphate and Uridinediphosphoglucose in Uterine Sections.* (27740)

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In vitro, synthesis of glycogen-like polysaccharides is easily obtained by the combined action of amylo-1,4-1,6 transglucosidase with either a phosphorylase or uridinediphosphoglucose (UDPG)-glycogen transferase. The latter 2 enzymes synthesizing 1,4 linkages with glucose-1-phosphate (G-1-P) and UDPG as their respective substrates. Unlike UDPG-glycogen transferase the action of phosphorylase is readily reversible, so that this enzyme may be involved in both synthesis and breakdown of glycogen. However, there seems to be conclusive evidence that in most tissues the synthesis of glycogen proceeds largely through the action of UDPG-glycogen transferase and of the branching enzyme, while the function of phosphorylase in conjunction with that of a debranching enzyme (amylo-1,6-glucosidase) being chiefly involved in the breakdown of glycogen to G-1-P,(1).

Takeuchi and Kuriaki(2) and Takeuchi and Glenner(3) were able to demonstrate histochemically the presence of amylophosphorylase and UDPG-glycogen transferase by incubating tissue slices with G-1-P or UDPG respectively and staining the incubated tissues with iodine.

It has previously been demonstrated histochemically that in the uteri of rats and rabbits phosphorylase activity is confined to the myometrium and that the activity of the enzyme was at its highest following estrogen stimulation(4,5). This investigation, in addition to confirming and extending my previous findings, was designed to determine whether glycogen synthesis from UDPG can be demonstrated in uterine sections of the ovariectomized, ovariectomized-hormone treated rats and rabbits and how the activity of UDPG-glycogen transferase, if present, is altered by the ovarian hormones.

Materials and methods. Twenty-three adult virgin female rabbits were used in this study. All the animals were bilaterally ovariectomized. Three weeks following ovariectomy 5 rabbits were killed without further treatment. The remaining animals were divided into groups and received the following daily hormone† treatment: I. 10 μg of estrogen (6 animals); II. 2.5 mg progesterone (6 animals); III. 10 μg estrogen plus 2.5 mg progesterone (6 animals). In Groups I and II the hormones were injected subcutaneously on 7 consecutive days while in Group III estrogen was injected for 7 consecutive days followed by injection of progesterone for 7 consecutive

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