an error would be small since antibody responses in these animals set an upper limit of 0.7 to 0.8 day for the half-life of macroglobulins. On the other hand, later antibody was eliminated at a rate approximating published values for the biologic half-life of mouse antiserum in mice. Apparently, equilibration did not appear to be an important factor in determining the rate of elimination of γ-globulin type antibody.

Differences in biological half-lives of early and late antibodies provide unmistakable evidence of some relationship between structural and biological properties of these molecules. In view of the fact that the specificity of immunologic phenomena is sensitive to small structural alterations of the reactants, it seems likely that the immunologic activities of early and late HI antibodies would differ. Indeed, in the rabbit, early and late hemolysins for sheep red cells appear markedly dissimilar in their hemolytic properties(12).

Summary. Hemagglutination - inhibition (HI) antibodies appeared in the serum of mice 3 days after injection of influenza virus vaccine. The initial antibody was sensitive to 2-mercaptoethanol, sedimented as an 18s macroglobulin, and had a biologic half-life of 0.5 days. Antisera obtained approximately 2 weeks after vaccination contained antibody that resisted treatment with mercaptoethanol, sedimented as 7s γ-globulin, and had a half-life of 2 days.

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Radiation Dose-Response Characteristics of Leucocyte Recovery in the Mouse. (28561)

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Early recovery of cell counts in peripheral blood has long been associated with protective and therapeutic treatment effects in irradiated animals(1,2) but few studies have employed these recovery times quantitatively. Although the survival end point is most generally used, in irradiated mice this is, at best, sensitive over a range of only about 250 rads. In practice, virulent infections such as *Pseudomonas* are frequently encountered(3) and they introduce major variations in survival, the cause of which may go undetected in laboratories which do not make routine bacteriological examinations. On the other hand, granulocyte and lymphocyte recovery times may be dose-related over a wider range and, importantly, at sublethal doses where variations introduced by infections do not interfere. Since there is much evidence of a causal relationship between granulocyte recovery and ability to survive(4,5) it is possible that, under certain circumstances, time of granulocyte recovery to a given level can be substituted for survival in studies of protective and therapeutic agents. While these remarks emphasize the problems arising from infection, clearly there are numerous other occasions in

which hematological end points can be used profitably.

Quantitative estimates of granulocyte and lymphocyte recovery times have been used in this laboratory(6,7) but their dose-response characteristics have not hitherto been described. In the present work the conditions under which dose increments result in significant response increments will be specified and a dose-response curve will be given which permits estimation of the dosage corresponding to a given level of response.

Methods. The mice used were (BALB/c X DBA/2)F_1 females, caged individually, and irradiated with Co^{60} at 270 or 510 rads per minute. The LD_{50} (28 days) for experimental groups which were essentially free of *Pseudomonas* infection was 820 (800-840) rads. Values in parenthesis represent 95% fiducial limits. Leucocyte counts were made on tail blood and differential counts on 100 cells. All estimates are based on log count.

The general method of estimating recovery time has been described(7). In the present experiments the end points used are day of recovery to 1500 granulocytes or lymphocytes per cu mm. For each radiation dose counts were made repeatedly on the same individuals throughout the recovery period. The observations include data from 12 experiments done at single doses and 3 experiments in which 2 or 3 doses were used. At 330 and 770 rads single experiments were done while pooled results from 2 to 4 experiments are given for the other doses. The number of mice per dose level ranged from 9 to 63, averaging 27.

Results. Granulocyte and lymphocyte counts were made during the course of recovery in mice exposed to radiation in doses of 275 rads to 770 rads (expected mortality at 770 rads, 30%). Estimated times of recovery to 1500 cells per cu mm are shown in Fig. 1. The fiducial limits in most cases reflect variations between mice within an experiment and not between different experiments. Straight lines were fitted to the data relating log recovery time to dose by the method of least squares, weighting inversely to the estimated variance.

For granulocyte recovery the slope of the

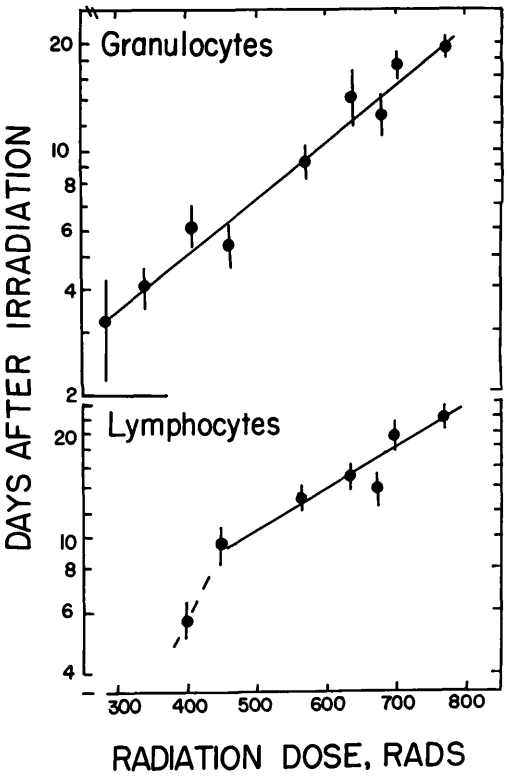

FIG. 1. Time of recovery to 1500 cells per cu mm (with 95% fiducial limits) for granulocytes and lymphocytes in mice exposed to 275-770 rads Co^{60} γ radiation.

line is 0.153, corresponding to a change in recovery time of 42.2 (39.2-45.3)% per 100 rad dose increment. Within the range of dosage covered, this line permits estimation of the dose corresponding to a given level of response. The index of inherent precision (8), λ, is estimated to be 122 rads. Thus, for any experimental configuration giving the same standard deviation of log recovery time, here 0.186, the dose to which a group of n mice has been exposed can be estimated with a probability of 0.95 that the error of the estimate will not exceed $(1.96)\ (122)/\sqrt{n}$.

At doses below 400 rads lymphocyte counts did not fall low enough for satisfactory estimates of recovery to 1500 cells per cu mm to be made. At 330 rads the minimum lymphocyte count, which occurred on day 4, was 2400 (1900-3040) compared to 1370 (1110-1670) for granulocytes. The least squares line relating log recovery time to dose would extrapolate to 7 days for 330 rads and to 8

days rather than the observed 6 days for 400 rads. It appears that the log time for lymphocyte recovery to 1500 cells is linear with dose only to about 450 rads. Between 450 and 770 rads the slope was found to be 0.117, corresponding to a change in recovery time of 30.2 (25.9-34.6)% per 100 rads. The value for λ over this dose range is estimated to be 76 rads.

The present estimates make it possible to use recovery times and percent mortality interchangeably. Increasing the radiation dose from 620 to 770 rads, for example, increased the 1500-cell recovery time for granulocytes from 11 days to 20 days and mortality, in experimental groups essentially free of *Pseudomonas* infection, from 3% to 30%. For the same dose interval the 1500-cell recovery time for lymphocytes was increased from 15 days to 22 days.

Discussion. The estimated values of λ for the 1500-cell recovery times of granulocytes and lymphocytes are approximately equal to the values of λ recently given for counts made 3 and 4 days after irradiation(9). The usable dose range, however, is about 400 rads less for granulocyte recovery time than for early count, being limited at one end by the small amount of initial depression and at the other by inability to survive. Under favorable conditions we have been able to extend the range to somewhat higher doses with streptomycin treatment(6). The usable dose range for lymphocyte recovery to 1500 cells is 450 to 770 rads compared to 0 to 600 rads for the early counts.

Although peripheral blood granulocyte and lymphocyte recovery time to 1500 cells per cu mm and cell count 3 or 4 days after irradiation are both responsive to dose within reasonable limits, these metameters are not both necessarily responsive to experimental variables other than dose. Both early count and recovery time respond to treatments which alter the effective dose, while only recovery time responds to treatments acting specifically on recovery processes. For example, colchicine given to mice prior to irradiation affects granulocyte recovery time and not the early count(10) whereas cysteine has been reported to affect both, as if it were reducing the dose(11). Taken together, the 2 metameters can thus be used in discriminating between the 2 types of treatment effect.

Summary. Dose-response characteristics of recovery times of granulocyte and lymphocyte counts to 1500 cells per cu mm in peripheral blood have been described for irradiated mice. The dose corresponding to a given time of recovery could be estimated with reasonable accuracy for granulocytes over the dose range 275-770 rads and for lymphocytes over the range 450-770 rads. For granulocytes recovery time increased 42% and for lymphocytes 30% per 100 rad dose increment. Together with earlier studies of 3- and 4-day counts, dose-response characteristics of recovery times are useful in distinguishing between dose-reducing effects and effects relating specifically to recovery processes.

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