

## Changes in Cell and Plasma Volumes Produced by Total Body X Radiation. (17866)

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There have been many reports on alteration in cellular and chemical composition of the blood following total body irradiation. The interpretation of such data, notably those recording only slight changes, is not possible unless the total plasma and cell volumes are known. A systematic study of the blood volume following irradiation has thus far, to our knowledge, not been made, although reference is made to some plasma volume determinations in X-rayed animals by Muntz and associates(1).

**Procedures.** The red cell volume was determined by the introduction of  $^{32}\text{P}$ -labeled homologous erythrocytes as described by Hevesy(2), and modified by Nieset *et al.* (3). The plasma volume was determined by the Evans blue (T-1824) technic and in some instances by a modification of the technic of Fine and Seligman(4) utilizing plasma tagged with  $^{131}\text{I}$ . The optical density of Evans blue solution was determined in a Beckman spectrophotometer using a Lowry cell(5) as described elsewhere(6).

The hematocrits were made with the capillary technic of Parpart and Ballentine(7).

The factors of irradiation were as follows: **Mice:** 250 kv;  $12\frac{1}{2}$  ma; tsd 90 cm; 1.5 mm Cu; inherent filtration 3 mm Al. Output 22.3 r per minute. **Rabbits:** 250 kv; 15 ma; tsd

53.5 cm; nofiltration other than above inherent filtration. Rate 91 r per minute. After 500 r exposure the rabbit was turned over.

The plasma and cell volume determinations were made in the same animal concurrently. Preliminary experiments have indicated that it was immaterial whether one or the other material was injected first. All rabbits were females. They were injected in one ear vein and sampled from the opposite ear vein. All mice were males of the Rf stock. They were injected into the tail vein and sampled from either the jugular or the heart blood.

**Results.** Fig. 1 is a graphic record of the sequence of cell, plasma and total blood volume changes in female rabbits following total body irradiation with 1,000 r. The upper part of the figure gives values calculated on the basis of injected  $^{32}\text{P}$ -tagged erythrocytes; the lower part gives values based on the conventional Evans blue technic. The ex-

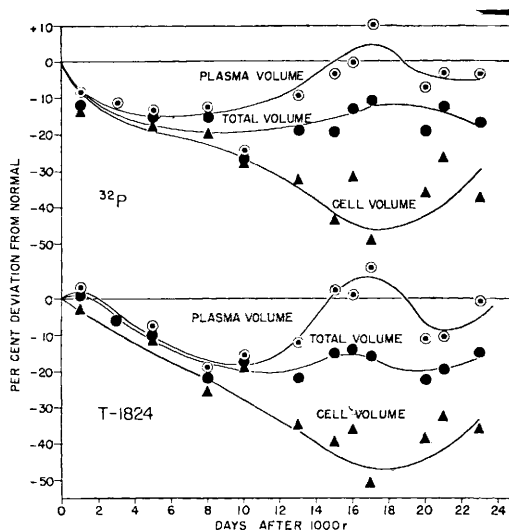


FIG. 1.

Cell, plasma, and total-blood volume of rabbits exposed to 1,000 r calculated on basis of pre-irradiation weights and expressed in percent deviation from normal.

1. Muntz, J. A., Barron, E. S. G., and Prosser, C. L., *Arch. Biochem.*, 1949, v23, 434.

2. Hevesy, G., and Zerahn, K., *Act. Physiol. Scand.*, 1942, v4, 376.

3. Nieset, R. T., Porter, B., Trautman, W. V., Jr., Bell, R. M., Parson, W., Lyons, C., and Mayerson, H. S., *Am. J. Physiol.*, 1948, v155, 226.

4. Fine, J., and Seligman, A. M., *J. Clin. Invest.*, 1943, v22, 285.

5. Lowry, O. H., and Bessey, O. A., *J. Biol. Chem.*, 1946, v163, 633.

6. Sobel, H., and Furth, J., *Endocrinology*, 1948, v42, 436.

7. Parpart, A. K., and Ballentine, R., *Science*, 1943, v98, 545.

TABLE I.  
Cell, Plasma, and Total-Blood Volumes in X-Rayed Rabbits.

| Volume—% body wt        |                 |                |                     |           |              |                         |                  |         |            |
|-------------------------|-----------------|----------------|---------------------|-----------|--------------|-------------------------|------------------|---------|------------|
| Days<br>after<br>irrad. | No. in<br>group | Cell by<br>32P | Plasma by<br>T-1824 | Total     |              |                         | Body wt          |         | Hematocrit |
|                         |                 |                |                     | by<br>32P | by<br>T-1824 | Combined<br>(corrected) | Before<br>irrad. | At test |            |
| 0                       | 8               | 1.96           | 4.06                | 4.87      | 6.80         | 5.72                    | —                | 3430    | 40.2       |
| 1                       | 2               | 1.67           | 4.18                | 4.28      | 6.84         | 5.54                    | 3516             | 3308    | 39.1       |
| 3                       | 2               | 1.74           | 3.81                | 4.32      | 6.40         | 5.27                    | 3393             | 3140    | 40.6       |
| 5                       | 2               | 1.61           | 3.74                | 4.14      | 6.15         | 5.07                    | 3675             | 3437    | 39.3       |
| 8                       | 2               | 1.57           | 3.29                | 4.13      | 5.31         | 4.59                    | 3822             | 3572    | 38.2       |
| 10                      | 2               | 1.41           | 3.43                | 3.59      | 5.65         | 4.59                    | 3742             | 3459    | 39.6       |
| 13                      | 2               | 1.32           | 3.57                | 3.95      | 5.34         | 4.62                    | 3738             | 3484    | 33.3       |
| 15                      | 2               | 1.11           | 4.15                | 3.91      | 5.81         | 4.95                    | 3055             | 2774    | 28.4       |
| 16                      | 2               | 1.35           | 4.09                | 4.24      | 5.85         | 5.13                    | 3675             | 3596    | 32.3       |
| 17                      | 2               | 1.00           | 4.40                | 4.36      | 5.74         | 5.07                    | 3742             | 3325    | 21.4       |
| 20                      | 2               | 1.25           | 3.61                | 3.95      | 5.29         | 4.59                    | 3648             | 3162    | 31.3       |
| 21                      | 2               | 1.45           | 3.64                | 4.27      | 5.50         | 4.83                    | 3737             | 3750    | 33.9       |
| 23                      | 2               | 1.23           | 4.04                | 4.05      | 5.79         | 4.97                    | 3393             | 3024    | 30.3       |

The values are expressed as percent body weight immediately before irradiation. The combined corrected total-blood volume is the sum of the cell volume determined by  $^{32}\text{P}$ -labeled erythrocytes and the plasma volume by T-1824 divided by 1.08. The latter is the correction factor for Evans blue withdrawn from blood 5-6 minutes after injection—as indicated by the presumably more correct  $^{131}\text{I}$ -plasma technique(11).

perimental data are shown in Table I. The graphs are based on calculations using the body weight immediately before irradiation, since the animals lose weight during the first few days after irradiation apparently without losing blood. There is an apparent rise of blood volume if expressed in percent of body weight at time of determination.

Fig. 1 and Table I indicate a marked cell volume drop beginning soon after irradiation and reaching a depth at about the 17th day. The plasma volume drops more slowly and

reaches a depth at about the 10th day, after which it rises. Concomitant with the latter there begins an elevation of the total-blood volume which has not reached the normal in 23 days.

It remains to determine how much of the red cell volume drop is due to a dropping out of aged cells while the marrow is depleted and how much to red cell injury. Erythrocytes are believed to be relatively resistant to X-rays but it is possible that irradiation hastens their death. The plasma volume drop

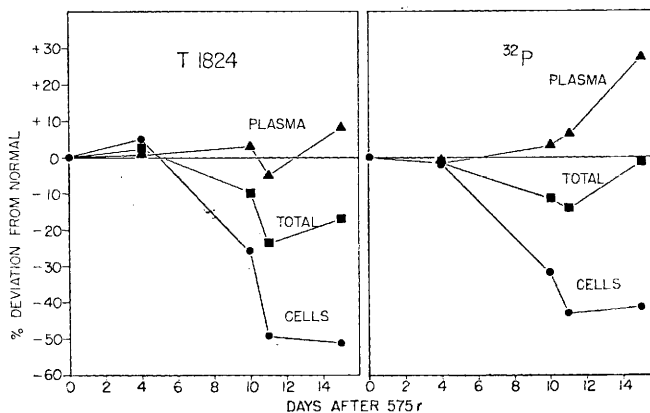


FIG. 2.

Cell, plasma, and total-blood volume of mice exposed to 575 r calculated on basis of preirradiation weights and expressed in percent deviation from normal.

TABLE II.  
 Cell, Plasma, and Total-Blood Volumes in X-Rayed Mice.

| Days<br>after<br>irrad. | No. in<br>group | Volume—% body wt |                     |           |              |                         | Body wt<br>at test | Hematocrit |
|-------------------------|-----------------|------------------|---------------------|-----------|--------------|-------------------------|--------------------|------------|
|                         |                 | Cell by<br>32P   | Plasma by<br>T-1824 | Total     |              |                         |                    |            |
|                         |                 |                  |                     | by<br>32P | by<br>T-1824 | Combined<br>(corrected) |                    |            |
| 0                       | 7               | 2.67             | 5.47                | 6.39      | 9.46         | 7.22                    | 29.9               | 42.0       |
| 4                       | 6               | 2.62             | 5.51                | 6.29      | 9.70         | 7.22                    | 26.6               | 41.9       |
| 10                      | 2               | 1.82             | 5.64                | 5.66      | 8.56         | 6.52                    | 27.9               | 31.2       |
| 11                      | 3               | 1.52             | 5.20                | 5.48      | 7.23         | 5.86                    | 27.6               | 27.9       |
| 15                      | 3               | 1.56             | 5.94                | 6.31      | 7.89         | 6.51                    | 24.8               | 24.6       |

The values are expressed as percent body weight immediately before irradiation. The combined total-blood volume is the sum of the cell volume determined by  $^{32}\text{P}$ -labeled erythrocytes and the plasma volume by T-1824 divided by 1.2. The latter is the correction factor for Evans blue withdrawn from the blood 5-6 minutes after injection as indicated by the presumably more correct  $^{131}\text{I}$ -technic.

and the subsequent rise likewise require further analysis. Changes similar to those found in rabbits were observed in mice which received 575 r over the entire body. (Fig. 2 and Table II). Reticulocytes are known to be depleted after irradiation during 1-2 weeks (8,9,10). Thus the drop during about the 2-8-day period may be a measure of the sum of the erythrocytes dying because of aging and of irradiation damage.

*Observations on the rate of disappearance of material used for blood volume determinations.* In preliminary experiments the rate of disappearance of the substances used for blood volume determinations was assayed in both mice and rabbits at various intervals after irradiation. The blood-volume values are based on samplings at 5 to 6 minutes with all materials used. Such samplings made with tagged erythrocytes and plasma probably give more correct values while the Evans blue drop from 1- to 2-minute peak is due to removal of the dye from the circulation by Evans blue-affin cells. We believe that the peak Evans blue value following its introduction would more closely express the cor-

rect plasma volume but, since this point is difficult to determine, the conventional Evans blue technic was adhered to. Data to be described indicate that the Evans blue value divided by 1.2 in mice and 1.08 in rabbits equals the  $^{131}\text{I}$  plasma value(11).

The 30-minute rate of disappearance of the injected material was assayed in a large number of animals. The results were highly variable but the sum of the data suggests that often there is a greater disappearance of these materials in the irradiated than in the normal mice and rabbits. These data will be fully reported in the final paper, and will be correlated with drop of red cell counts following irradiation and calculations on the aging rate of the erythrocytes and the influence of irradiation on their survival time.

*Summary and conclusion.* Following irradiation of rabbits by 1,000 r of X-rays there is a large drop in erythrocyte volume as measured by  $^{32}\text{P}$ -tagged cells beginning with the 3rd day and reaching a depth at about the 17th day after irradiation, followed by a slow recovery. There is at first a slight concomitant plasma-volume drop followed by a rise on about the 10th day that elevates the lowered total volume but does not bring it up to normal in 23 days.

Similar changes occur in mice that have been exposed to 575 r.

Mary Knoohuizen and Maryann Huddleston assisted in the work.

8. Andrews, G. A., unpublished data.

9. Hagen, C. W., Jr., Jacobson, L. O., Murray, R., and Lear, P., Atomic Energy Commission Declassified Report No. MDDC-999.

10. Jacobson, L. O., Marks, E. K., Simmons, E. L., Hagen, C. W., and Zirkle, R. E., Atomic Energy Commission Declassified Report No. MDDC-1174.

11. Wish, L., Storey, R. H., and Furth, J., unpublished data.