# Disappearance of Exogenous Erythropoietin (ESF) from the Blood of Germfree Mice[1] (36100)

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We have previously reported (1, 2) that an augmented response to erythropoietin (ESF) occurs in germfree mice. Several possible mechanisms for this enhanced response were proposed: (a) increased numbers of ESF-committed stem cells are present or that an increased sensitivity of these elements to ESF exists in the germfree animal, (b) the internal environment of the axenic animal favors an augmented activity of the ESF; this could conceivably occur if the level of a circulating erythropoiesis inhibitory factor (3) was reduced in the germfree animal, or (c) a diminution in the rate of destruction of the ESF characterizes the germfree animal. In the latter connection, the liver has been suggested as a site of inactivation of the ESF (4, 5). The fact that the liver is less developed in the germfree than in the conventional animal (6, 7) might be interpreted as favoring this possibility. This paper presents evidence supporting a slower rate of disappearance of exogenous ESF in the plasma of the germfree mouse.

*Materials and Methods.* Adult male germfree mice (26–30 g) of the Hauschka–Mirand/ICR Swiss strain were obtained from the Charles River Breeding Laboratories (Boston, MA). In order to maintain the axenic environment, they were kept in flexible plastic isolators of the Trexler type. Conventional mice of the same strain were raised under ordinary laboratory conditions. However, these were also placed in Trexler isolators to insure that the effects observed in the germfree mice were not due to confinement in the isolators. For the germfree animals, samples of food, water and stools were cultured daily to check the axenic environment of the isolators. Routine microbiological tests were conducted for bacteria, fungi, PPLO and other parasites. At the end of the experiments, the blood, fecal contents, lung, liver and spleen from the germfree mice were cultured as a final check on the axenic state.

Four groups of mice were established: (a) ESF-injected conventional mice, (b) ESF-injected germfree mice, (c) ESF-injected X-irradiated conventional mice and (d) ESF-injected X-irradiated germfree mice. 900R constituted the X-irradiation dose. The source of X-irradiation was a Maxitron 250 KVP operated at 250 kV and 30 mA with a filtration of 1.0 mm Al and 0.25 mm Cu. Axenic germfree mice were placed in a circular plastic restrainer (capacity 10 mice) within the isolator. The restrainer was then passed through a long plastic sleeve attached to the isolator in order to isolate the mice at a distance of 50 cm from the radiation source. The ESF used was Step 1 sheep plasma ESF obtained from the Connaught Laboratory, Toronto (Lot No. 127-1). Each mg of the preparation contained 0.80 IRP units of ESF. This material dissolved in saline was passed through a millipore filter (HA-0.45$\mu$) to insure sterility before use. Each mouse in the 4 groups received an iv injection of 5 IRP units of ESF dissolved in 0.5 ml sterile saline. In the 2 irradiated groups, the ESF was administered 3 days after exposure to the X-irradiation. The mice were killed at vary-

[1] This work was supported in part by U.S. Public Health Service grants CA-07745-07 and HE-03357-14 and the John A. Hartford Foundation, Inc.

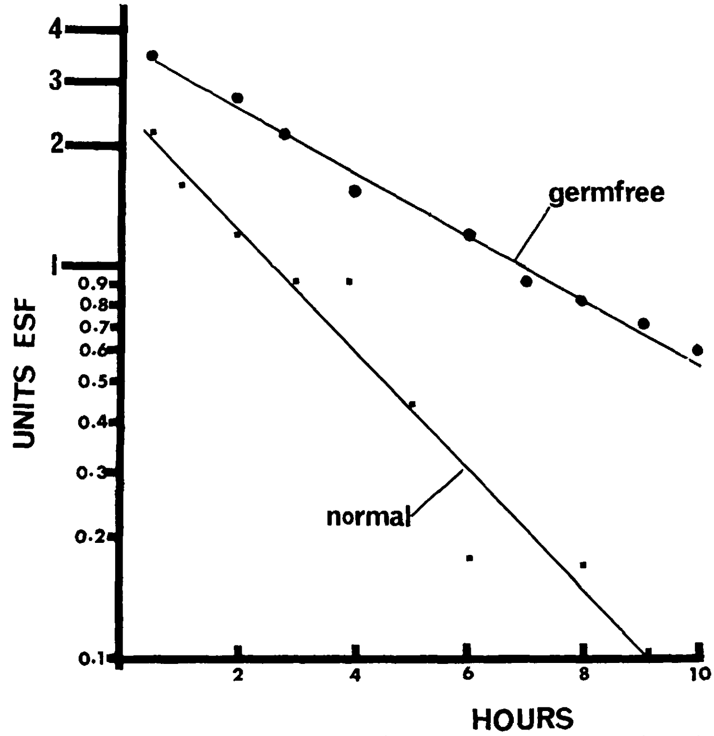


FIG. 1. Plasma disappearance of exogenous ESF in normal and germfree mice. Lines plotted by method of least-squares.

ing times, beginning with 20 min after injection of the ESF. At these intervals, the plasmas of the differential groups of mice were collected and pooled. Thirty to 40 mice served as plasma donors at each of the time intervals following ESF administration. A total of approximately 300–400 mice were employed in this capacity for each of the 4 groups.

Determinations of ESF levels in the pooled plasmas were made utilizing adult (25–30 g) mice (Hauschka-Mirand/ICR Swiss strain) that had been rendered plethoric by ip injections on 2 successive days of 1 ml homologous packed red cells. For each plasma tested, 10 assay mice each received 0.5 ml of the sample ip on 3 successive days. On the 4th day, each mouse was given an iv injection of 1 $\mu$Ci $^{59}FeCl_3$ in 0.2 ml saline. Twenty-four hours later, the mice were bled from the dorsal aorta and the percent incorporation of $^{59}Fe$ into the circulating red cells was estimated (8). All radioiron incorporation values were converted into IRP units of ESF by reference to the standard curve for the International Reference Preparation of ESF (9). Each assay included saline and ESF standard-injected control groups.

*Results.* Figure 1 indicates that the rate of disappearance of the ESF from the plasma of germfree mice was considerably slower than for that of conventional mice. Thus, although no remaining ESF was observed in the conventional mice at about 9 hr post-injection, considerable activity was present in the germfree animals at this time. In addition, significantly greater quantities of ESF ($p<.05$) were noted in the plasmas of the latter mice at all time intervals shown.

X-irradiation resulted in a highly significant delay in the rate of disappearance of the iv administered ESF in both the conventional and germfree mice (Fig. 2). Thus, significant amounts of the ESF were present in the plasmas of the irradiated non-axenic mice even at 21 hr after ESF injection (Fig. 2). In contrast, the ESF was no longer evident in the non-irradiated conventional mice at 9 hr post-injection (Fig. 1). A similar delay in the

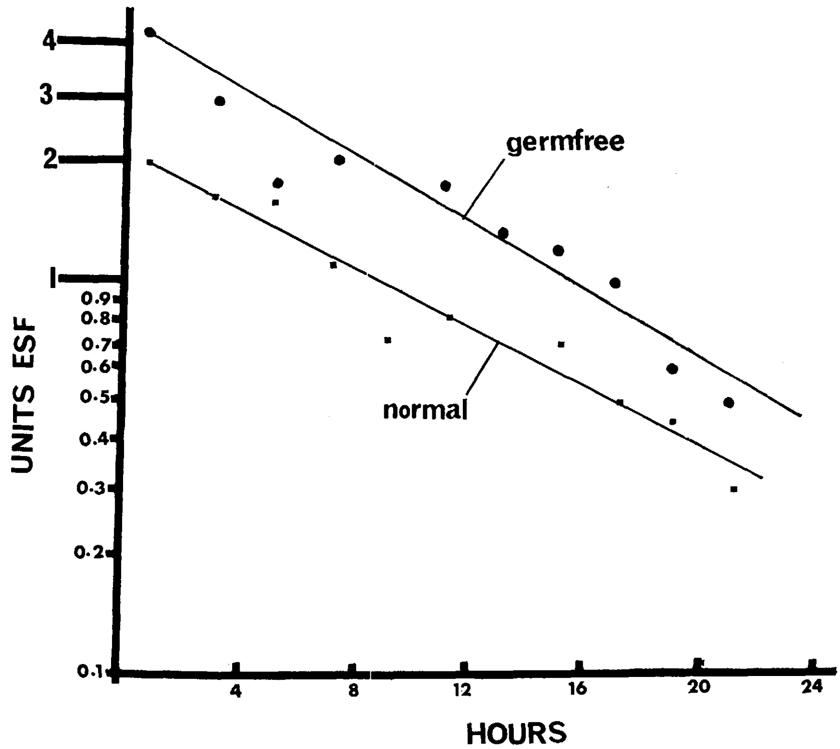


FIG. 2. Plasma disappearance of exogenous ESF in X-irradiated normal and germfree mice. Lines plotted by method of least-squares.

rate of ESF disappearance was also apparent in the irradiated-germfree mice (Fig. 2). In addition, whereas the half-life for the injected ESF in the non-irradiated conventional mice was significantly shorter ($p<.05$) than that observed in the irradiated germfree mice (2.2 hr vs. 5.7 hr),[2] this difference did not occur for the 2 irradiated groups (9.5 hr vs. 9.0 hr).[2] Although a longer half-life for the ESF was observable in both groups of irradiated mice, the absence of a significant difference between the 2 irradiated groups is traceable to a relatively greater increase in ESF half-life in the irradiated conventional than in the irradiated germfree animals.

It should be stressed that no increase in endogenous ESF levels was noted in either the normal or germfree mice at 3–4 days after irradiation which corresponds to the time the present experiments were conducted. This is in conformance with recent studies by McDonald *et al.* (10) showing that significant quantities of ESF do not appear in mice until 8 days after exposure to similar intensities of X-irradiation. Thus, the disappearance lines in the irradiated mice (Fig. 2) relate only to the removal of the exogenous ESF administered to these 2 groups of mice.

*Discussion.* It seems evident that a lower rate of disappearance of exogenous ESF occurs in the plasma of the germfree than in the conventional mouse. This most likely forms the basis for the previously reported greater effectiveness of the ESF in the axenic as compared to the non-axenic mouse (1, 2). In this regard, it has been suggested that the liver is a site of inactivation of the ESF (4, 5). The observation that the liver is less developed, both on a morphological and functional basis, in germfree than in conventional mice (6, 7) suggests the possibility that a diminished rate of destruction of the ESF may occur in the axenic animal. Differences in the ability of the kidneys of these 2 types of animals to eliminate exogenous ESF seems

[2] Half-life determined from the regression lines and errors of estimate.

unlikely in view of the finding that excretion rates for this hormone are uniformly very low (11).

The results also indicate that X-irradiation delays the plasma disappearance of ESF in the normal and germfree mice. This delay may arise, as proposed by Stohlman and Brecher (12), from a diminished rate of inactivation or utilization of the ESF by the blood-forming tissues of the X-irradiated animals. Evidence for this contention is also provided in experiments by McDonald *et al.* (10) indicating that mice given homologous bone marrow transplants did not exhibit the characteristic increase in ESF levels evident at 10 days after X-irradiation. On the other hand, Naets and Wittek (13) were unable to detect any effect of the marrow hypoplasia induced by X-irradiation on ESF levels in mice subjected to hypoxia.

A final question that remains to be answered relates to the differences in early disappearance rates of ESF in the germfree and conventional groups of mice. Extrapolation of the disappearance curves in both figures to zero would make it seem that the germfree animals had 50–100% more ESF than the conventional mice. Obviously this is not the case since both groups were similar in starting weight and each received the same quantity of ESF. In the present studies, the determinations were initiated at 20 min after administration of the ESF. It has been reported that the disappearance curves for exogenously administered ESF is biphasic in nature with the initial phase occurring within 30–45 min after injection (11, 14). The marked early changes in the levels of ESF may reflect significant differences in the disappearance patterns in the 2 groups in even this rapid phase. This could conceivably involve differences in distribution and clearance rates of the hormone in the germfree and conventional mice.

*Summary.* A slower rate of disappearance of exogenous ESF from the plasma occurs in germfree mice as compared to normal mice. Higher plasma levels of ESF are maintained in X-irradiated than in non-irradiated mice. Possible mechanisms for these findings include a reduced ability of the liver to destroy ESF in the germfree animal and a decreased capacity of the blood-forming organs in the irradiated mouse to utilize the ESF.

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Received June 7, 1971. P.S.E.B.M., 1972, Vol. 139.