The Response of Iron Metabolism to the Microbial Flora: Studies on Germfree Mice (33687)

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Ferrokinetic alterations which accompany infection have been the subject of considerable investigation (1, 2). Anemia is a relatively frequent consequence of infection. Ferrokinetic abnormalities, characterized by hypoferremia, shortened plasma from clearance, a slight increase in the plasma iron transport rate and a moderate decrease in red blood cell radioiron utilization have been described in bacterial infections in animals and man, however, the role which normal bacteriologic flora plays in iron metabolism has not been explored. Availability of germfree mice has recently made it possible to examine the effect of the germfree state on ferrokinetics.

In the present study the iron content of the tissues and the distribution of an intravenously administered dose of radioiron was determined in germfree and conventionalized mice.

Methods and Materials. Germfree ICR female mice, 12 weeks of age, were shipped from the Charles River Breeding Farms to the Walter Reed Army Institute of Research in a flexible film germfree tank. On arrival animals were maintained under germfree conditions. Illumination was provided on an automatic day–night cycle from 6 a.m. to 6 p.m. An autoclaved semisynthetic rice flour casein base diet (L-356)[1] and sterile canned water were supplied *ad libitum.*

Microbiologic assays for protozoa, fungi, bacterial flora, and certain pleropneumonia-like organisms were performed immediately on receipt of animals and weekly thereafter as described by Levenson and co-workers (3). No attempt was made to detect viruses or rickettsia.

After 2 weeks of acclimitization, the mice were randomized into two groups. One group was transferred to a second germfree isolator and were conventionalized: a portion of the cecal contents, aseptically removed from an open colony (specific pathogen free) mouse was suspended in 50 ml of sterile saline. Two-ml amounts were placed in water, on bedding, and in other areas of the germfree isolator. A second 3-week period was allowed to ensure establishment of the contaminating organism. No morbidity or mortality resulted from the contamination. The handling of the germfree animals is presented in detail in a previous publication (3).

Plasma iron clearance rates, 18 hr RBC radioiron incorporation and tissue distribution of radioiron was determined by previously described methods (4) in groups of germfree or conventionalized mice following the intravenous administration of a sterile solution of 0.2 μCi of ^{59}Fe/0.2 μg of ^{56}Fe sulfate.

At the termination of each experiment, the mice were anesthetized with ether and sacrificed. The liver, spleen, and an aliquot of blood were removed. Subsequently, the liver and spleen were weighed and the iron content of these organs and an aliquot of plasma was determined by atomic absorption spectrometry utilizing a modification of the technique of Sprague and Slavin (5).

Results and Comment. There was no significant difference between the total body weights of the germfree and conventionalized mice, however, both the liver and spleen weights were significantly increased in the conventionalized mice when compared to the germfree (Table I). This difference may be actual, or due to the increased mass of the cecum and its contents in the germfree animal which might artificially increase the total

[1] Iron content, 170.2 g/100 lb.

TABLE I. Hematocrit, Organ Weights, and Tissue Iron Content.[a]

	Germfree (no. of mice)	Conventionalized (no. of mice)	Significance[c]
Body wt. (g)	31.0 ± 2.9 (40)[b]	31.7 ± 1.7 (40)[b]	NS
Liver wt. (mg)	0.855± 0.149(34)	1.274± 0.240(32)	$p < 0.05$
Spleen wt. (mg)	79.4 ± 17.3 (32)	98.9 ± 17.1 (32)	$p < 0.05$
Hematocrit (vol %)	45.1 ± 0.3 (32)	42.4 ± 3.6 (32)	NS
Plasma iron[d] (μg/ml)	4.36 ± 0.76 (32)	4.46 ± 0.52 (32)	NS
Plasma iron[e] (μg/ml)	3.40 ± 0.92 (6)	3.22 ± 0.77 (6)	NS
Total iron binding capacity	5.00 ± 0.33 (6)	5.04 ± 0.47 (6)	NS
Liver iron[d] (μg/g of fresh liver)	249.3 ± 70.6 (32)	308.3 ± 60.4 (32)	$p < 0.05$
Spleen iron[d] (μg/g of fresh spleen)	941.1 ±417.3 (32)	1364.4 ±435.8 (32)	$p < 0.05$

[a] Mean ± SD.
[b] Number of determinations (mice).
[c] Determined by Student's t test; NS, $p > 0.05$.
[d] Determined by atomic absorption spectrometry.
[e] Determined by chemical method.

body weight of the germfree mouse (6). There were no significant differences in the hematocrit values, but a tendency toward wider variability in the conventionalized animal. The plasma iron levels, performed either by chemical or atomic absorption methods, were essentially the same in the germfree and the conventionalized mice. Plasma iron values obtained by the chemical method, however, were approximately 1 μg/ml less than those obtained by atomic absorption. Plasma iron binding capacity was similar in both groups of mice. The splenic and hepatic concentrations of iron were higher in the conventionalized than in the germfree mice. Correction for total spleen and total liver iron content on the basis of total spleen and liver weight, respectively, would augment these differences (Table I). These findings are in agreement with those of Reddy and associates in rabbits (6) but at variance with the findings in rats (7). This is apparently indicative of species and strain variability.

The decreased hepatic and splenic iron content of the germfree animals may be secondary to the underdeveloped reticuloendothelial system characteristic of this animal (8). The reticuloendothelial system phagocytizes nonviable erythrocytes, removes iron from heme, recycles iron, and serves as an iron reservoir. Diminished mass or functional immaturity of the reticuloendothelial system could exert a pronounced effect on iron metabolism. Since there is evidence to suggest that the particulate removal function of the reticuloendothelial system is substantially intact in the germfree animal (9) the altered iron metabolism would presumably be secondary to ineffective storage or recycling of iron. A possible direct microbial effect on iron metabolism, however, cannot be excluded.

The results of tissue distribution of intravenously administered radioiron are presented in Table II. The livers of the germfree mice apparently picked up radioiron at a more rapid rate. However, by 18 hr there were no significant differences between the germfree or conventionalized mouse liver (Table I). A larger percentage of the administered dose of radioiron was found in the spleen of the conventionalized mouse at all time intervals studied (Table II). Clearance of the radioiron from the plasma was markedly slower in the germfree mouse (Table I). Determination of the mean plasma iron clearance rates from mean values of eight determinations at 30, 60, and 90 min resulted in a mean $T\ ½$ value of 147 min for germfree mice as opposed to 62 min for conventionalized mice. There was no difference in the erythrocyte radioiron incorporation at 18 hr.

These results indicate that microbial contamination plays an important role in ferro-

TABLE II. Tissue Distribution of Radioiron.[a]

	Time following iv ^{59}Fe administration			
(mice/group):	30 min 8	60 min 8	90 min 8	18 hr 12
Liver (%)				
Germfree	4.8 ± 1.6	4.9 ± 1.8	5.2 ± 1.3	12.5 ± 3.0
Conventionalized	6.1 ± 1.8	7.5 ± 1.7	8.7 ± 1.4	13.9 ± 1.9
Spleen (%)				
Germfree	1.3 ± 0.6	1.3 ± 0.7	2.5 ± 1.1	2.3 ± 1.2
Conventionalized	2.0 ± 1.0	4.3 ± 2.5	3.8 ± 2.2	5.8 ± 2.1
Plasma (%)				
Germfree	84.0 ± 16.0	76.9 ± 14.3	65.7 ± 12.9	—
Conventionalized	70.4 ± 18.2	50.7 ± 11.0	35.6 ± 4.0	—
Red blood cell (%)				
Germfree	—	—	—	27.2 ± 5.6
Conventionalized	—	—	—	33.1 ± 6.9

[a] Values are mean ± SD.

kinetics and that the alterations in ferrokinetics attendant to infection can be similated in germfree animals following conventionalization with normal nonpathogenic bacteriologic flora. These changes may be due to the microbial flora per se or secondary to the effect of the flora on the reticuloendothelial system and subsequent alteration in ferrokinetics.

Summary. The tissue iron levels and tissue distribution of an intravenous dose of radioiron were studied in germfree and conventionalized mice. The germfree animals demonstrated similar plasma iron levels, diminished plasma radioiron clearance rate, diminished iron concentration in the liver and spleen, a slower rate of accumulation of radioiron in the liver and spleen, and no difference in the percentage of radioiron found in the red blood cell at 18 hr when compared to conventionalized mice.

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