# The Exchange of Iron with Interstitial Fluid.* (29046)

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The plasma $Fe^{59}$ disappearance data during the first 24-48 hours of the experiment, *i.e.*, prior to any possible return of radioiron from newly formed red cells, indicates the probable existence of 3 mutually interchanging compartments of the "preerythropoietic phase" of iron metabolism. These have been previously designated(1,2) as a plasma pool, an extravascular labile compartment and a marrow iron transit pool which transfers iron directly into the erythron maturation pool (the "erythropoietic phase"). This paper presents evidence identifying the extravascular labile iron pool as the interstitial fluid compartment.

*Materials and methods.* Subjects: The experimental subjects were patients hospitalized for various disorders; healthy mongrel dogs of approximately 30 kg weight were used in the animal studies.

Plasma $Fe^{59}$ studies. This procedure has been described(3,4,5). In one patient $Fe^{59}$ bound to autologous ascitic fluid having a large excess of iron binding capacity was injected intraperitoneally. Plasma and/or body fluid samples were obtained at suitable intervals and radioactivity was determined by counting in a well-type scintillation counter.

Determination of lymph $Fe^{59}$ activity: 1. Dogs: Under light nembutal anesthesia the thoracic duct was catheterized(6), protein bound $Fe^{59}$ was injected intravenously immediately after catheterization, and lymph and blood serially sampled thereafter. Lymph flow was 0.25 to 0.5 ml per minute; the midpoint of each collection interval was used as the sample time.

2. Patients: The thoracic duct, catheterized under local anesthesia(7,8), produced a lymph flow rate of 0.5 to 1 ml per minute at the time of the $Fe^{59}$ studies which were performed one to 3 days after surgery.

Plasma, lymph and body fluid iron content and iron binding capacity were determined by the methods of Ramsey(9) or Schade(10), and Ventura(11) or Schade(10).

Electrophoresis and Radioactive Strip Scanning: Paper electrophoresis was performed on samples of plasma, lymph and transudates, and $Fe^{59}$ radioactivity distribution determined by an automatic strip scanner.** Arcylamide gel electrophoresis was performed according to the method of Ornstein and Davis(12).

*Results.* I. Observations on pleural, ascitic and edema fluids: Observed values for iron concentration and iron binding capacity in specimens of pleural, ascitic and edema fluids are given in Table I. All samples of effusion fluids and 7 out of 9 samples of edema fluid contained significant iron concentrations and total iron binding capacities when compared with the corresponding sera. Paper electrophoresis and acrylamide gel electrophoresis of all fluids tested exhibited a band with the mobility of siderophilin and with the radioactive iron localized to this band.

The interchange of radioiron between plasma and transudates was studied in several patients with ascites, pleural effusion and generalized anasarca. Following the intravenous injections of protein bound $Fe^{59}$, transfer of radioiron to these fluids was observed (Fig. 1). The intraperitoneal injection of ascitic fluid bound $Fe^{59}$ in a patient with ascites,

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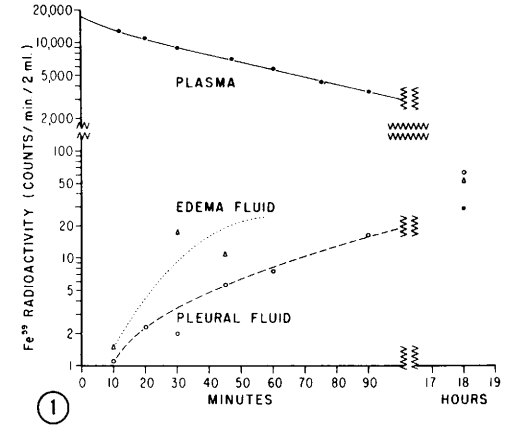
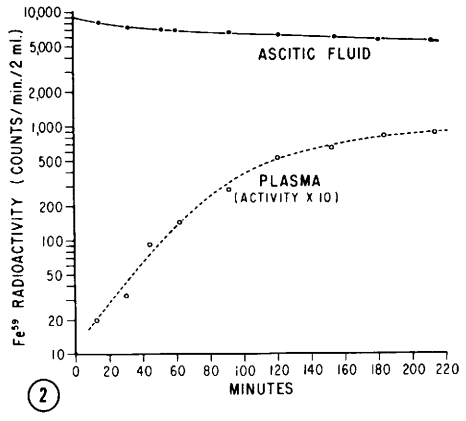
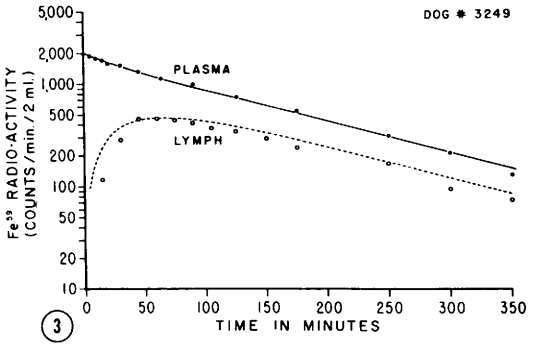
TABLE I. Fe and TIBC (in $\mu g$ per 100 ml) in Plasma and Body Fluids.

| | Plasma | | Ascitic fluid | | Pleural fluid | | Edema fluid | |
|---|---|---|---|---|---|---|---|---|
| Patient | Fe | TIBC | Fe | TIBC | Fe | TIBC | Fe | TIBC |
| E. P. | 35 | 177 | | | | | 45 | |
| M. A. | 83 | 217 | | | | | 3 | 7 |
| A. A. | 60 | 244 | | | 26 | 70 | 16 | 30 |
| We. | 60 | 188 | | | | | 50 | 103 |
| T. S. | 38 | 240 | 85 | | | | 45* | 45* |
| | | | | | | | 15† | 15† |
| S. B. | 120 | 183 | | | | | 13 | 33 |
| E. H. | 37 | 245 | | | | | 8 | 8 |
| Hy. | 44 | | | | | | 0 | |
| A. H. | 40 | 352 | | | 30 | 139 | | |
| C. F. | 40 | 262 | | | 28 | 133 | | |
| R. L. | 98 | 397 | | | 40 | 313 | | |
| E. L. | 53 | 356 | 30 | 147 | | | | |
| A. C. | 73 | 343 | 38 | 180 | | | | |
| T. L. | 65 | 399 | 98 | | | | | |
| J. M. | 124 | | 84 | 159 | | | | |

* Left leg. † Right leg.



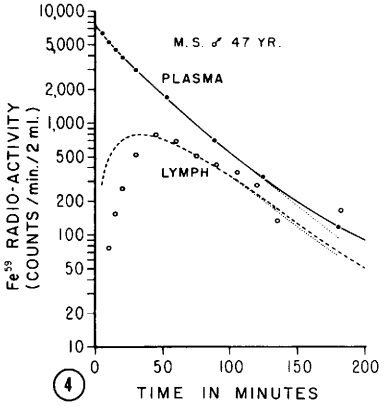




FIG. 1. Subject A.A. (heart failure): Experimental plasma $Fe^{59}$ disappearance and pleural fluid and edema fluid $Fe^{59}$ appearance data after an intravenous injection of $Fe^{59}$ tagged autologous plasma. -●——●- Experimental plasma $Fe^{59}$ data. -○--○- Experimental pleural fluid $Fe^{59}$ data. --△----△-- Experimental edema fluid $Fe^{59}$ data.

FIG. 2. Subject A.C. (Kimmelstiel-Wilson Disease): Radioactivity in ascitic fluid and in plasma after intraperitoneal injection of $Fe^{59}$ bound to autologous ascitic fluid. -●——●- Experimental ascitic fluid $Fe^{59}$ data. -○--○- Experimental plasma $Fe^{59}$ data (multiplied by 10).

FIG. 3. Dog #3249 (normal): Radioactivity in plasma and thoracic duct lymph after an

intravenous injection of $Fe^{59}$ tagged autologous plasma. ● Experimental plasma $Fe^{59}$ data. ○ Experimental lymph $Fe^{59}$ data. ——— Approximation of the experimental plasma radioactivity data by a sum of two exponential functions (*i.e.*, theoretical values based on an initial two-pool system)(1,2): $q_{pl} = 200e^{-0.028t} + 1750e^{-0.00714t}$. – – – – Theoretical radioactivity in the second (extravascular labile) pool, based upon the approximation of plasma radioactivity by two exponential functions, and multiplied by an appropriate constant (1, 2): $q_{evlp} = 1000\ (e^{-0.00714t} - e^{-0.028t})$.

FIG. 4. Subject M.S. (mild iron deficiency anemia): Radioactivity in plasma and thoracic duct lymph after intravenous injection of $Fe^{59}$-beta-1-globulin. ● Experimental plasma $Fe^{59}$ data. ○ Experimental lymph $Fe^{59}$ data. ——— Approximation of the experimental plasma radioactivity data by a sum of three exponential functions (*i.e.*, theoretical values based on an initial three-pool system of iron metabolism)(1, 2): $q_{pl} = 3000e^{-0.041t} + 4050e^{-0.022t} + 46e^{-0.00077t}$. – – – – Theoretical radioactivity in the second (extravascular labile) pool, based upon the above approximation of experimental plasma radioactivity by three exponential functions, and multiplied by an appropriate constant (1, 2): $q_{evlp} = 65\ (-52.406e^{-0.041t} + 52.204e^{-0.022t} + 0.202e^{-0.00077t})$. • • • • Theoretical plasma and extravascular labile pool data based on an initial two-pool system (1, 2). Values preceding the dotted line (during the first 100 to 150 min) cannot be distinguished from those based on an initial three-pool system.

was followed by the reverse transfer of radioiron to plasma (Fig. 2).

II. Observations on lymph: Values for concentration of iron, and iron binding capacity in thoracic duct lymph in dogs and human subjects are given in Table II. The concentrations in the central lymph and in the corresponding plasma were quite similar. Electrophoresis of lymph demonstrated a band with the same mobility as plasma siderophilin, and with the radioactivity localized to the same region.

Simultaneous observations of plasma and lymph $Fe^{59}$ tracer behavior, were performed on 8 dogs and 4 patients after intravenous injection of radioiron. Typical experimental findings in one dog and one patient are noted in Fig. 3 and 4. Lymph radioactivity increases and achieves a maximum at approximately 60 and 45 minutes, respectively. After about 2 to 3 hours the lymph radioactivity begins to parallel plasma $Fe^{59}$ disappearance when plotted on semilogarithmic paper.

*Discussion.* Interstitial fluid is of variable composition and is not a single anatomically delineated compartment(13,14). Its easiest accessible compartments occur in pathological states as effusions: ascitic, pleural and edema fluids. The presence of iron bound to siderophilin and the interchange of radioiron between these fluids and plasma has been demonstrated. No attempts at the quantitation of the kinetics of iron movement could be made in the studies. These fluids may not be in a steady state and represent a partial accumulation pool or reservoir of iron. The decreasing radioactivity in ascitic fluid (Fig. 2) may represent transport into the blood stream, delayed mixing with pockets of fluid and may be influenced by numerous other variables. At 18 hours more radioactivity was found per unit volume in pleural and in edema fluid than in plasma (Fig. 1); this again may indicate delayed mixing or stagnation rather than a dynamic state of such pathological samples of interstitial fluid.

TABLE II. Fe and TIBC (in μg per 100 ml) in Plasma and Thoracic Duct Lymph in Dogs and Humans.

| Subject | Plasma Iron | Lymph Iron | Plasma TIBC | Lymph TIBC |
|---|---|---|---|---|
| Dog #3161 | 177 | 186 | 347 | 345 |
| Dog #3234 | 250 | 245 | 324 | 336 |
| Dog #3249 | 280 | 300 | 441 | 430 |
| Dog #4369 | 52 | 46 | 306 | 314 |
| Dog #3637 | 51 | 45 | 363 | 231 |
| Human, J. B. | 128 | 75 | | 103 |
| Human, M. S. | 55 | 80 | 270 | 263 |

Because of these considerations, a study of lymph(1,2) as the only accessible normal subcompartment of the interstitial fluid(13,14) was undertaken. The role of lymph, and therefore of interstitial fluid, as a possible medium for transport of iron has been investigated previously(15-17).

The similarity of iron binding proteins and of iron concentrations in plasma, in lymph, and in other interstitial fluids has been shown. Rapid interchange of radioiron between plasma and lymph was directly demonstrated from studies of plasma and of thoracic duct lymph after intravenous injection of $Fe^{59}$. Turnover of iron in the central lymph sub-

compartment of interstitial fluid was estimated to be 0.75 mg per day¶ in 2 patients studied. The 0.75 mg of iron leaving the thoracic duct per day comprises only a fraction of the entire lymph turnover, which on the basis of lymph protein turnover studies, (14,18,19) is estimated to be 2 to 4 mg/day (*i.e.*, the plasma iron content(20)).

The theoretical tracer behavior in the extravascular labile compartment calculated on the basis of multiple pool analysis(1,2) gives a sufficiently good approximation of the experimental lymph data (Fig. 3, 4) to identify the lymph as a subcompartment of the extravascular labile pool. Using this type of multicompartment analysis, the turnover of iron in the extravascular labile pool in normal volunteers ranged from about 5 to 50 mg/day depending upon the model selected. Recent evidence indicating direct blood to marrow (21,22) or tissue(23,24) transport of iron would favor the lower values obtained in the analysis.

*Summary.* The extravascular labile iron pool of the preerythropoietic phase of iron metabolism has been demonstrated to be the interstitial fluid compartment. There is no direct means of measuring total iron turnover in interstitial fluid. From the evidence presented one may conclude that interstitial fluid iron turnover per day is at least as great as the size of the plasma iron pool but probably less than the plasma iron turnover itself.

¶ (Flow rate, ml/min) (min/day) (Fe conc. in mg/ml) = 0.75 × 1440 × 0.00075

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