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Interaction of Transferrin with Iron-Binding Sites on Rat Intestinal Epithelial Cell Plasma Membranes[1] (38417)

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Recent experiments suggest that the plasma membrane of intestinal epithelial cells contains iron receptor sites which are involved in iron absorption. Levine *et al.* (1) first demonstrated that both serum and pure transferrin enhance the release of ^{59}Fe from labeled isolated intestinal epithelial cells. These authors also demonstrated that transferrin binds to isolated epithelial cells and their data suggested that the amount of iron-free transferrin available at binding sites on the membrane may regulate the transfer of iron from the epithelial cell to plasma.

Yoshino and Manis (2) have isolated an iron-binding substance from the particulate fraction of rat intestine and have suggested that this substance may be a metabolic precursor for the transported iron pool as well as for other iron fractions in the mucosa. The observations of Yoshino and Manis implicate the plasma membrane in iron transport since the plasma membrane sediments with the cellular particulate fraction (3).

The observations of Levine *et al.* (1) and Yoshino and Manis (2) prompted us to examine iron binding by the plasma membrane fraction from intestinal epithelial cells. The experiments reported below describe the effect of both serum and transferrin on the release of ^{59}Fe from intestinal epithelial cell plasma membranes labeled *in vivo*.

Materials and Methods. Fasted, adult, male Sprague–Dawley rats were given 50 μg ^{59}Fe ($^{59}FeCl_3$, International Chemical and Nuclear Corp., Irvine, CA[4]) by gastric intubation. Forty-five minutes later, the rats were decapitated, and a 15-cm segment of proximal small intestine was removed from each animal. The segments were rinsed in cold 0.85% NaCl, after which the crude plasma membrane fraction was isolated by the method of Coleman *et al.* (3). The radioactivity in the membrane fraction and the remaining supernatant was determined in a gamma-well counter (Nuclear Chicago, Model 4233).

The labeled membrane fractions were suspended in 10-ml solutions that contained 0.85% NaCl, 0.85% NaCl and 4 ml female rat plasma, or 0.85% NaCl and 40 mg transferrin (Sigma Chemical Co., St. Louis, MO). The solutions were incubated for 1 hr at 33°, after which the membrane pellet and supernatant were separated by centrifugation at 5000*g* for 15 min. The radioactivity in the two fractions was determined in the gamma-well counter.

Following incubation with the labeled membranes, aliquots of each medium were chromatographed individually on a 1.5 × 30-cm column packed with Sephadex G-150 (Pharmacia Fine Chemicals, Piscataway, NJ). The gel had been equilibrated with 25 m*M* KH_2PO_4, pH 7.4, and the same buffer was used to elute the sample. Radioactivity in the collected fractions was determined in the gamma-well counter. To determine the elution volume of transferrin, a 5-mg sample of purified transferrin was dissolved in 3 ml distilled water and chromato-

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graphed as described above. The 280-nm absorbance of the eluted fractions was monitored with an LKB Uvicord II.

Results. Forty-five minutes after the oral administration of ^{59}Fe, 53.4 ± 10.8% of the total radioactivity in the mucosal homogenate was associated with the plasma membrane fraction. Moreover, as shown in Table I, both plasma and purified transferrin had a marked effect on ^{59}Fe release from the labeled plasma membrane fractions. Following incubation, 23% of the ^{59}Fe in the membrane preparations was released to the control medium, whereas 52% was released to the medium that contained rat plasma and 39% was released to the medium that contained transferrin. The greater removal of membrane-bound ^{59}Fe by rat plasma (approximately 10 mg transferrin) may be due to the presence of ferroxidase in the plasma.

When the incubation media were chromatographed on Sephadex G-150, ^{59}Fe from each of the solutions eluted in a single peak which corresponded with the elution volume of transferrin (Fig. 1). The recovery of ^{59}Fe from the chromatographed samples was as follows: plasma medium, 102 ± 4%; transferrin medium, 74 ± 5%; control medium, 71 ± 9%. We suspect that the ^{59}Fe recovered from the control medium was bound to endogenous transferrin since Levine *et al.* (1) demonstrated that transferrin binds to epithelial cells.

Discussion. Based on results obtained with isolated intestinal epithelial cells, Levine *et al.* (1) visualized an iron transport system in which transferrin binds to the intestinal cell membrane and enhances the release of ingested iron. The experiments described in this paper were conducted at the molecular level and the results indicate that a large fraction of ingested iron is bound to receptor sites on the plasma membrane of intestinal epithelial cells. Furthermore, in the presence of a source of transferrin, a significant portion of membrane-bound iron is released. These results both extend and substantiate the hypothesis of Levine *et al.* (1) and the results suggest that the plasma membrane of intestinal epithelial cells may function in regulating iron absorption.

TABLE I. Effect of Rat Plasma and Transferrin on ^{59}Fe Release from Labeled Plasma Membranes.[a]

Medium	Percent ^{59}Fe released from membrane fractions
NaCl	23.0 ± 11.7
Rat plasma	51.5 ± 7.1[b]
Transferrin	39.4 ± 2.1[b]

[a] Values are mean ± SD of five assays.
[b] $p < 0.01$ compared with control value.

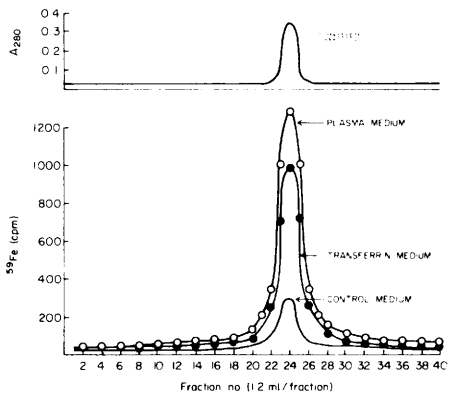

FIG. 1. Typical elution pattern from Sephadex G-150 of transferrin and the plasma membrane incubation media.

Metal-binding substances on the plasma membrane of intestinal epithelial cells may function in regulating the absorption of a variety of metals. Recent investigations in this laboratory indicate that the plasma membrane of intestinal epithelial cells contains receptor sites which bind both copper and zinc and subsequently transfer these metals to albumin (4, 5). Thus, the intestinal epithelial cell plasma membrane may be a regulatory site for the absorption of essential metals from the intestinal lumen.

Summary. Following oral administration of ^{59}Fe to rats, over 50% of the isotope in the intestinal mucosa was bound to the plasma membrane of the epithelial cells. Labeled plasma membranes incubated in a medium that contained either plasma or transferrin released a significantly greater quantity of ^{59}Fe than labeled membranes incubated in a control medium. These results suggest that transferrin interacts with iron-receptor sites on the epithelial cell plasma membrane.

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