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# Interrelationships of Iron and Cobalt Absorption: Mucosal Distribution of Cobalt During Absorption (38234)

RICHARD G. SHEEHAN
(Introduced by J. P. Sanford)
*Veterans Administration Hospital, Dallas, Texas 75216 and Department of Medicine, University of Texas Southwestern Medical School, Dallas, Texas 75235*

The mechanisms by which the upper small intestinal mucosal cell regulates iron absorption rates remain undefined. One thesis has proposed mucosal cell production of soluble cytoplasmic carrier substances which promote the transport of iron from the luminal to the basal membrane of the cell. The quantity or concentration of these "carriers" provides the controlled amount of iron to the basal membrane for transfer to the carcass. A number of such transport molecules have been suggested including high molecular weight, nonferritin binding proteins (1, 2) and transferrin-like binding proteins (3, 4). These studies have relied upon the identification of such substances in the normal animal, and the demonstration of a greater proportion of iron bound to the "carrier" during the process of enhanced iron absorption by the iron depleted animal.

Any theory of mucosal cell control of the rate of iron absorption must be consistent with the observation that iron absorption also is enhanced in the presence of increased rates of erythropoiesis regardless of the level of the iron stores (5, 6). We recently reported studies of mucosal supernatant fractions of iron during the period of rapid absorption. A transferrin-like iron binding substance was identified in the supernatant fraction of iron deficient animals, but no such compound was seen in animals with phenylhydrazine-induced hemolytic anemia. However, similarly increased rates of iron absorption were seen in both the iron deficient and the hemolyzing animals, when compared to the normal animals (7). We thus concluded that in these 2 different circumstances of increased iron absorption, no correlation could be made between the presence of iron binding molecules and the rates of absorption (7).

Several studies have provided evidence that the iron absorption mechanism is shared wholly or in part by 2 other cations, cobalt and manganese, in a competitive manner (8–14). Cobalt absorption is also enhanced in the iron deficient human (15). Thus, if soluble cytoplasmic "carrier" molecules do play a significant role in the absorption of these three cations, it might be expected that during the process of cobalt (or manganese) absorption, patterns of binding to these molecules, similar to that seen during iron absorption, would be observed. The present studies were designed to identify the form(s) in which cobalt appears in the soluble supernatant fraction of mucosal cells during the rapid phase of cobalt absorption in normal, iron deficient and phenylhydrazine treated animals, and to correlate these observations with rates of cobalt absorption.

*Methods.* Adult female Sprague–Dawley rats, weighing 180–250 g, were used in these studies. Normal animals (N) were maintained on a standard rat chow. Production of iron deficiency (FeD) and phenylhydrazine-induced hemolytic anemia (PHZ) has been previously described (7).

Carcass absorption of cobalt, as well as chromatographic separation of the mucosal supernatant fraction, were investigated utiliz-

ing the *in vivo* closed duodenal loop method previously described for iron absorption (7).

A freshly prepared aqueous solution of cobaltous chloride was placed in the closed loops. The total volume of 0.5 ml contained 0.72 $\mu$moles of elemental cobalt (an equimolar equivalent of the 40 $\mu$g dose of elemental iron previously used) and 50 $\mu$Ci of carrier free [$^{60}$Co]-cobaltous chloride (New England Nuclear Corp.). Loops were removed after 10 or 60 min of absorption time. Radioactivity of the animals was determined on a constant rotating table immediately after the instillation of the test dose of cobalt and again after removal of the loops. Carcass absorption was calculated as the ratio of net counts per minute in the animal after removal of the loop to the net counts per minute with the loop intact.

The separated loop was opened lengthwise and rinsed vigorously in cold isotonic saline. The mucosa was then scraped with a glass slide and homogenized in 5 ml of 0.01 *M* Tris–HCl buffer, pH 7.0. The homogenate was centrifuged at 6000*g* for 15 min and the radioactivity of an aliquot of the supernatant fraction was counted.

A known volume (usually 0.5 ml) supernatant fraction was placed on a freshly prepared Sephadex G-200 (Pharmacia Fine Chemicals, Inc.) 60 × 0.9 cm column. The sample was eluted with 0.01 *M* Tris–HCl buffer, pH 7.0, containing 0.15 *M* NaCl and 2.4 ml fractions were collected. The radioactivity of each fraction from one bed volume was counted. Values for each fraction were expressed as a percentage of the total radioactivity applied. Results were analyzed for statistical significance by the Student *t* test (16).

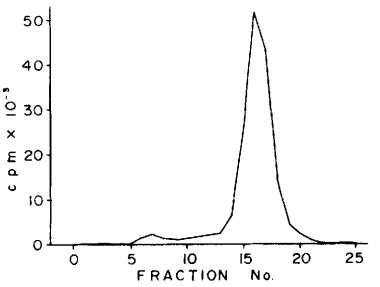


FIG. 1. Pattern of cobalt distribution in the normal animal. Typical elution pattern of radioactivity from the 6000*g* supernatant fraction of mucosal cells after 10 min absorption period. Void volume, fraction 7. Bed volume, fraction 16.

*Results.* Table I expresses the values for carcass absorption at 10 and 60 min for the 3 groups of animals. The FeD animals demonstrated a statistically significant increase in cobalt transfer at both 10 and 60 min compared to the *N* animals. In addition, the PHZ animals also had increased cobalt transfer at both times, and these values became statistically significant at the 60 min absorption period.

Figure 1 is a typical elution pattern of the radioactivity of the 6000*g* supernatant fraction of the mucosal preparation from a normal animal atfer a 10 min absorption period. Essentially all of the radioactivity appeared in a single peak eluting at the total bed volume. In all instances, greater that 95% of the applied radioactivity was

TABLE I. Mucosal Transfer and Concentration of Radiolabelled Cobalt.

| Group[a] | Absorption time (min) | % Carcass[b] absorption | *P* Value[c] | $\mu$gm Co per g supernatant protein | *P* Value |
|---|---|---|---|---|---|
| N (5)[d] | 10 | 2.4 ± 0.3 | — | 58.0 ± 11.6 | — |
| FeD (4) | 10 | 13.8 ± 1.5 | <.001 | 262.2 ± 46.9 | <.005 |
| PHZ (4) | 10 | 3.4 ± 0.6 | N.S. | 68.6 ± 14.0 | N.S. |
| N (4) | 60 | 6.1 ± 0.97 | — | 74.9 ± 13.1 | — |
| FeD (4) | 60 | 43.7 ± 2.7 | <.001 | 246.8 ± 25.1 | <.005 |
| PHZ (4) | 60 | 15.7 ± 2.3 | <.05 | 92.8 ± 23.0 | N.S. |

[a] *N* = Normal; FeD = Iron Deficient; PHZ = Phenylhydrazine-Induced Hemolysis.
[b] Mean ± 1 SEM.
[c] Compared to normal group at same absorption time. N.S. = not significant.
[d] Figures in parentheses indicate number of animals.

eluted. The qualitative patterns from all 3 groups of animals at both absorption periods were identical to Fig. 1. The only differences between the three groups was the concentration of radiolabeled cobalt in the supernatant fraction of the FeD animals (Table I). The elution pattern and proportion of radioactivity recovered was the same when an aqueous solution of cobaltous chloride was placed on the column. The small amount of radioactivity (<1%) appearing at the void volume was seen in all patterns and also was seen when the free cobaltous chloride was applied to the column.

*Discussion.* The close interrelationship of iron, cobalt and manganese absorption has been elucidated (8–15). All three metals appear to share, at least in part, a common absorptive pathway demonstrating competitive inhibition kinetics. In addition, the uptake and transfer of these metals is enhanced in iron deficient animals and cobalt absorption is impaired in FeD mice with a hereditary defect in iron absorption (17). The results reported here again confirm the increase in cobalt absorption in iron deficiency and further demonstrate that enhancement also occurs when hemolysis and resultant increased erythropoiesis exist in the iron replete animal. These effects mirror those of iron absorption (7).

A number of studies have demonstrated that during the process of iron absorption, iron can be shown to be bound to various soluble intracellular molecules (1–4, 7). The major, and in our previous studies, the only soluble high molecular weight compound which bound iron during the rapid phase of iron absorption was ferritin. These findings have been recently confirmed (18). By comparing the quantitative relationships of ferritin-binding of newly absorbed iron in three physiologic states (*N*, FeD and PHZ), no correlation was found between ferritin binding and iron absorption rates (7). An iron binding molecule with similar, if not identical characteristics to transferrin, has also been reported (3, 4). We found a similar substance only in the FeD animals and thus, could not explain the increase in iron absorption in the PHZ animals on the basis of this binding molecule (7). Therefore, it was of interest to find, that despite the fact that cobalt absorption was increased in both FeD and PHZ animals, no binding of cobalt to soluble cytoplasmic components occurred in the mucosal cells of either of these groups of animals nor in the normal animals. The quantity of iron or cobalt uptake that is subsequently transferred appears to be the major rate limiting step and is increased in the FeD animal (12–14). Our studies would suggest that this is also true in the PHZ animals. Thus, we conclude that although carcass transfer of cobalt is enhanced in both FeD and PHZ animals, this increase in transport is not mediated by a soluble cytoplasmic carrier molecule. Therefore, either the binding of Fe to cytoplasmic molecules is not a factor in controlling iron absorption or a portion of the intracellular mechanisms for the control of iron and cobalt absorption are different.

*Summary.* The carcass transfer and subcellular distribution of cobalt during the period of rapid absorption were studied in normal rats and animals with iron deficiency or phenylhydrazine induced hemolytic anemia. Although carcass transfer of cobalt was increased in the latter 2 groups, no binding to soluble cytoplasmic molecules was found. Since iron and cobalt appear to share a common absorptive mechanism, these findings support the proposition that soluble cytoplasmic molecules do not play a major role in the control of absorption of these cations.

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1. Pearson, W. N., and Reich, M. B., J. Nutr. 99, 137 (1969).

2. Worwood, M., and Jacobs, A., Brit. J. Hematol. 22, 265 (1972).

3. Huebers, H., Huebers, E., Forth, W., *et al.* Life Sci. 10, 1141 (1971).

4. Pollack, S., Campana, T., and Arcario, A., J. Lab. Clin. Med. 80, 322 (1972).

5. Erlandson, M. E., Walden, B., Stern, G., *et al.* Blood 19, 359 (1962).

6. Bothwell, T. H., Pirzio-Biroli, G., and Finch, C. A., J. Lab. Clin. Med. 51, 24 (1958).

7. Sheehan, R. G., and Frenkel, E. P., J. Clin. Invest. **51**, 224 (1972).

8. Pollack, S., George, J. N., Reba, R. C., *et al.* J. Clin. Invest. **44**, 1470 (1965).

9. Schade, S. G., Felsher, B. F., Bernier, G. M., *et al.* J. Lab. Clin. Med. **75**, 435 (1970).

10. Schade, S. G., Felsher, B. F., Glader, B. E., *et al.* Proc. Soc. Exp. Biol. Med. **134**, 741 (1970).

11. Thomson, A. B. R., Shaver, C., Lee, D. J., *et al.* Amer. J. Physiol. **220**, 674 (1971).

12. Thomson, A. B. R., and Valberg, L. S., Amer. J. Physiol. **220**, 1080 (1971).

13. Thomson, A. B. R., Valberg, L. S., and Sinclair, D. G., J. Clin. Invest. **50**, 2384 (1971).

14. Thomson, A. B. R., and Valberg, L. S., Amer. J. Physiol. **223**, 1327 (1972).

15. Valberg, L. S., Ludwig, J., and Olatunbosun, D., Gastroentero. **56**, 241 (1969).

16. Mode, E. B. Elements of Statistics. Englewood Cliffs, N. J. Prentice–Hall (1961).

17. Toskes, P. P., Smith, G. W., and Conrad, M. E., Amer. J. Clin. Nutr. **26**, 435 (1973).

18. Yoshino, Y., and Manis, J., Amer. J. Physiol. **225**, 1276 (1973).

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