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Receptors for vitamin B₁₂ related to ileal surface area and absorptive capacity

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SUMMARY

Isolated microvillous membranes from hamster ileum bind in vitro 1.4 × 10⁻¹² moles of intrinsic factor–vitamin B₁₂ complex while the maximum absorption from a single dose of vitamin B₁₂ in vivo is about 3 times as great (4 × 10⁻¹⁰ moles). The limited number of intrinsic factor–vitamin B₁₂ receptor sites (0.5 × 10¹¹ per hamster ileum) are distributed over approximately 7000 cm² of ileal surface area, corresponding to less than one receptor per microvillus. The restricted capacity for vitamin B₁₂ absorption in the intact hamster may be explained by the small number of receptor sites and the slow rate of release of vitamin B₁₂ from the receptor.

Gastric intrinsic factor binds vitamin B₁₂ in a macromolecular complex and promotes its absorption by the ileum ¹, ². Although small quantities are efficiently absorbed by this mechanism, the capacity of the intestine to absorb vitamin B₁₂ is extremely limited, and the process takes several hours. Man, for example, absorbs no more than 1–2 μg (10⁻⁹ moles) of any single oral dose ³, ⁴, and when radioactive vitamin B₁₂ is given 6–8 h must elapse before blood radioactivity is maximal ⁵. Previous studies from this laboratory ⁶, ⁷ showed that microvillous membranes isolated from absorptive cells of hamster ileum, but not jejunum, contain receptor sites specific for intrinsic factor-bound vitamin B₁₂. We have now estimated the number of intrinsic factor–vitamin B₁₂ complex receptor sites relative to ileal absorptive surface area and show that these membrane receptors maximally bind approximately the same quantity of intrinsic factor–vitamin B₁₂ complex that the hamster is capable of absorbing in vivo.

Golden hamsters weighing 150–250 g were given by gastric tube ⁶⁰Co-labeled

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cyanocobalamin (spec. act. 1.0 μCi per μg) bound to neutralized hamster gastric juice, which served as the source of intrinsic factor. Absorption of the test dose of intrinsic factor—vitamin B₁₂ complex was estimated, by determining fecal excretion of radioactivity for 7 days. When given 2.5, 5, 10, 20, 40 and 100 ng of intrinsic factor-bound cyanocobalamin, 4–6 hamsters absorbed an average of 1.6 ± 0.3 (S.E.), 2.4 ± 0.5, 4.1 ± 0.2, 5.0 ± 0.5, 5.5 ± 0.4 and 5.2 ± 0.7 ng of the vitamin, respectively. Thus the absorptive capacity of the hamster for vitamin B₁₂ was limited to 5.5 ng (4·10⁻¹² moles).

To quantitate binding of intrinsic factor—vitamin B₁₂ complex to the ileal absorptive surface in vitro, we isolated microvillous membranes from hamster ileum, i.e. from the distal half of the small intestine. Membranes were then incubated in 4 ml of Krebs–Ringer bicarbonate buffer, pH 7.0, with increasing quantities (0.2–17.2 ng) of Co-labeled cyanocobalamin (spec. act. 15 μCi per μg) bound to hamster intrinsic factor. The concentration of membranes was adjusted so that each incubation flask contained 0.8 mg of membrane protein as determined by the method of Lowry et al. After incubation for 30 min at room temperature, we measured the amount of radioactivity bound to the centrifuged, washed microvillous membrane pellet. The procedures used to isolate microvillous membranes, to prepare intrinsic factor—vitamin B₁₂ complex, and to measure binding of radioactivity have been described previously.

A Scatchard plot of the binding of intrinsic factor—vitamin B₁₂ complex to microvillous membranes was linear (Fig. 1). Thus, intrinsic factor—vitamin B₁₂ complex, over an 80-fold range of concentrations, appeared to bind to a uniform species of receptor with an affinity constant calculated to be 3.1·10⁻¹⁰ M⁻¹. Maximal membrane uptake of intrinsic factor—vitamin B₁₂ complex determined from the Scatchard plot was 3.9·10⁻¹⁰ moles per mg of microvillous membrane protein. Analysis of binding data by the Hill equation yielded a linear plot with a slope of 0.986 indicating that over a wide range of intrinsic factor—vitamin B₁₂ complex concentrations there was no cooperative binding and that each intrinsic factor—vitamin B₁₂ complex molecule attached to a separate receptor.

To calculate the total number of receptors on the ileal surface we measured ileal maltase activity. Since all of the maltase activity present in the intestine is confined to the microvillous membranes of the epithelium, one can determine the total amount of microvillous membrane protein in a segment of intestine by measuring the total maltase in that segment together with the maltase activity of microvillous membranes from the segment. In 6 hamsters total ileal maltase activity measured by the method of Dahlqvist was 16.6 ± 1.3 (S.E.) units per ileum while microvillous membranes isolated from hamster ileum contained 4.8 ± 0.5 (S.E.) maltase units per mg membrane protein. Each hamster ileum therefore contained an average of 3.6 mg of microvillous membrane protein. As indicated above, maximal binding of intrinsic factor—vitamin B₁₂ complex by ileal surface receptors was 3.9·10⁻¹⁰ moles per mg of membrane protein. Thus all of the receptors present on hamster ileum bind a total of 1.4·10⁻¹⁰ moles (8.5·10¹⁰ molecules) of intrinsic factor—vitamin B₁₂ complex. This value is about 3-fold the maximum amount of intrinsic factor—vitamin B₁₂ complex absorbed by the hamster as measured by in vivo absorption tests (4·10⁻¹² moles).
Fig. 1. Scatchard plot of binding of intrinsic factor–vitamin B₁₂ complex to hamster microvillus membranes. Vₚ nanomoles of intrinsic factor–vitamin B₁₂ complex bound per mg of microvillus membrane protein. C nanomoles of unbound intrinsic factor–vitamin B₁₂ complex per 1.

Fig. 2. Pressure–area isotherm of microvillus membrane lipid. Total lipids were extracted from microvillus membranes⁹ dissolved in hexane, and spread on water at 22 °C in a 14 cm x 50 cm Langmuir trough. Lipids were compressed at a rate of 6 cm per min, and the surface pressure and surface area were continuously recorded. A liquid-condensed film began to increase surface pressure when compressed to 6150 cm⁻² per mg lipid (point A). The film collapsed (point C) at a pressure of 49 dynes/cm and an area of 3210 cm² per mg.

To relate the total number of receptor sites to the absorptive surface area of hamster ileum, we quantitatively extracted lipids from isolated microvillus membranes by methods previously described in detail.¹⁹ Membrane lipid was then spread as a monolayer on a Langmuir trough which continuously recorded the surface pressure exerted by the molecules in dynes/cm as a function of surface area occupied by the molecules.¹⁹,²⁰ In Fig. 2 surface pressure is plotted against the surface area occupied by the monolayer of membrane lipids. Depending upon the extent to which lipid is actually compressed in microvillus membranes, one mg of microvillus membrane lipid would occupy 3210 cm² as a highly compressed monolayer or 6150 cm² if minimally compressed. At 20 dynes/cm² the surface area is 6400 cm², a value we selected as a crude but reasonable estimate for lipid molecules packed in a membrane. The lipid:protein ratio of ileal microvillus membranes was 0.85 ± 0.17 (S.E.). Thus hamster ileum contained an average of 3.1 mg of
microvillous membrane lipid which occupied 14,000 cm² as a monolayer. Assuming that this lipid exists in microvillous membranes as a bilayer compressed to about 20 dynes/cm, we calculated the total absorptive surface area of the ileum to be 7000 cm².

Since hamster ileal microvillous membranes maximally bind 8.5-10¹⁴ intrinsic factor--vitamin B₁₂ complex molecules and since each intrinsic factor--vitamin B₁₂ complex molecule appears to bind to a separate receptor site, hamster ileum with an absorptive surface area of 7000 cm² contains 8.5-10¹⁴ receptor sites or one receptor per 8-10⁷ Å² of ileal absorptive surface. For comparison, the surface area of a single microvillus is approximately 3·10⁵ Å² (refs 21–23), and it is therefore unlikely that, on the average, there could be more than one intrinsic factor--vitamin B₁₂ complex receptor per ileal microvillus.

Thus the total number of receptor sites on the absorptive surface of hamster ileum is sufficiently small to account for the restricted capacity of the hamster to absorb intrinsic factor--vitamin B₁₂ complex. Since the available sites are capable of binding approximately 1/3 that quantity of vitamin maximally absorbed from a single oral dose, each membrane receptor probably transports only a small number of vitamin B₁₂ molecules during intestinal absorption. This relation between number of receptors and number of molecules transported suggests that each intrinsic factor--vitamin B₁₂ complex molecule is slowly processed by its receptor. Prolonged interaction between intrinsic factor--vitamin B₁₂ complex and the membrane receptor would be consistent with the extremely slow rate of absorption of vitamin B₁₂ observed in vivo.

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REFERENCES

1 Castle, W.B. (1968) in Handbook of Physiology, Alimentary Canal (Code, C.F., ed.), p.1529, Williams and Wilkins, Baltimore, Md
2 Glass, G.B.J. (1968) Physiol. Rev. 43, 529