Evidence Against Transferrin as a Binder of Either Vitamin B₁₂ or Folic Acid

By Elizabeth Jacob and Victor Herbert

Human transferrin is not a carrier protein for either folic acid or vitamin B₁₂.

Several reports raised the possibility that transferrin might be a carrier protein for both vitamin B₁₂ and for folic acid. In 1972, Carmel and Herbert¹ reported that B₁₂-binding protein from leukocyte sonicates electrophoresed at pH 8.6 at the same mobility as transferrin, and in 1973, Samuels et al.² reported that some radioactive B₁₂ bound to a material which moved with the speed of transferrin in disk acrylamide electrophoresis of serum and of a 95% pure preparation of transferrin. A further suspicion of relation between a vitamin B₁₂-binding protein and transferrin is raised by the reports³⁴ that unsaturated vitamin B₁₂-binding capacity is elevated with iron deficiency.

In 1972, Markkanen et al.⁵ reported that transferrin was a carrier protein for folic acid activity, using horizontal starch gel electrophoresis of human serum, and in 1973, Waxman and Schreiber reported that a folic acid-binding protein moved with the speed of transferrin in their system.⁶

Studies were carried out to evaluate directly whether transferrin in fact was a carrier protein for either vitamin B₁₂ or folic acid.

MATERIALS AND METHODS

⁵⁷Co-labeled vitamin B₁₂, of specific activity 15 μCi/μg, was purchased from Amersham/Searle, North Chicago, Ill. Pteroylglutamic acid (folic acid-3',5',9-H³), of specific activity 68 μCi/μg, was purchased from the same company. The purity of the vitamin B₁₂ was checked by coated charcoal separation,⁷ and the purity of the folic acid was checked by thin-layer chromatography.⁸

Pure (as determined by immunoimmuno-electrophoresis) human transferrin was purchased from Schwarz/Mann, Orangeburg, N.Y. Three milligrams of the pure human transferrin were dissolved in 1.0 ml of NaCl for the B₁₂-binding experiment and in 1.0 ml of phosphate buffer 0.1 M, pH 7.4, for the folate-binding experiment. To duplicate 100-μl aliquots of transferrin in NaCl, 500 μg of CoB₁₂ were added, and to duplicate 200-μl aliquots of transferrin in phosphate buffer, 0.27 ng of HPGA was added. After incubation for 30 min at room temperature, unbound B₁₂ and folic acid were removed with coated charcoal by standard methods.⁹

In order to determine whether uncoated charcoal, which is known to remove TCII from serum,¹⁰ will also remove unsaturated transferrin from serum, 2.0 ml of pooled normal human serum were mixed with 300 ng of uncoated charcoal, incubated at 37°C for 30 min, with several
mixings, and then centrifuged. The unsaturated iron-binding capacity of the supernatant was
determined.  

RESULTS

The pure human transferrin bound essentially no vitamin B₁₂ or folic acid. (Actual binding was 0.05 ± 0.05 pg B₁₂ bound per 0.3 mg transferrin, and 0.0027 ± 0.0027 ng ³HPGA bound per 0.6 mg transferrin. The standard deviations are for three separate experiments in duplicate.

Uncoated charcoal did not statistically significantly adsorb any unsaturated iron-binding capacity of normal human serum.

DISCUSSION

In view of the finding that pure human transferrin binds neither vitamin B₁₂ nor folic acid, it would appear that prior reports reflect the existence of material other than transferrin which, in the systems previously used, moves with the speed of transferrin. Furthermore, uncoated charcoal, in a quantity known to remove TCII from serum, does not remove endogenous unsaturated transferrin from serum. The possibility that charcoal could remove vitamin from a weak bond to transferrin is not excluded by these data.

REFERENCES

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