Hydroxocobalamin. II. Absorption from the Site of Injection and Uptake by the Liver and Calf Muscle in Man

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RESULTS OF STUDIES reported in Part I of this paper gave evidence that hydroxocobalamin is excreted in the urine more slowly and builds up consistently higher and more prolonged vitamin B₁₂ levels in the blood as compared to cyanocobalamin.

We therefore made a comparative study of the absorption of labelled hydroxocobalamin and cyanocobalamin from the site of injection and the uptake of each by the liver and calf muscle to determine the applicability of hydroxocobalamin as a long-acting B₁₂ in man. A report of our findings follows.

MATERIALS AND METHODS

Six individuals with normal B₁₂ blood serum levels and no evidence of circulatory, renal or hepatic failure received intramuscular injections of radioactive hydroxocobalamin and cyanocobalamin in doses of from 50 to 1000 µg. of each, each dose containing 2.5 to 5.0 µc. of Co₅⁷- or Co₅⁸-labeled material. In all 6 subjects the hydroxocobalamin and cyanocobalamin injections were spaced 2 weeks apart and given alternately in the left and right gluteal areas.

The measurement of the disappearance of radioactivity from the site of injection, the hepatic uptake of radioactivity, and the deposition of the radioactivity in the calf muscle were determined with the use of a scintillation detector with a NaI-thallium crystal and by the technic described by Glass, Boyd and Gellin. For the present study, a scaler connected to a gamma ray spectrometer was used. The counts were taken at the optimum setting of the energy spectra of Co₅⁷ and Co₅⁸, with a background count of only 10–12 cpm. The background was deducted and the counts were corrected for physical decay of radioactivity.

The sites of injection were counted immediately after injection, then 30 minutes, 1, 2, 4, 6 and 24 hours later, followed by daily counts for a total of 2 weeks. For 4 weeks in all, daily counts were also taken from the left mid-calf to determine the uptake of radioactivity by the muscle. For the second series of counts over the mid-calf when cyanocobalamin was injected into the same patient 2 weeks after administration of hydroxocobalamin, or vice versa, the baseline of radioactivity was determined over this area. This was done by averaging the counts for 3 consecutive days prior to the second injection, and by calculating the new increment of radioactivity from this baseline.

For 4 weeks the 3 cutaneous projections of the liver (anterior, anterolateral and lateral) were measured daily for radioactivity. The counts obtained were then averaged and corrected for background and physical decay. When cyanocobalamin was injected into the same patient 2 weeks after administration of hydroxocobalamin, or vice versa, the baseline for new increment of hepatic radioactivity was determined, as was that over the mid-calf. For the duration of the study, each counting area (the 3 hepatic projections, the...
RESULTS

Absorption of Radioactive Hydroxocobalamin from the Site of Injection

Figure 1 shows the results obtained in 4 subjects who were injected alternately with 50–100 μg doses of hydroxocobalamin and cyanocobalamin. The semilogarithmic tracings in figure 1 show marked differences between the absorption of hydroxocobalamin and that of cyanocobalamin throughout the 2-week period of the study. By 24 hours only 0.7–2.0 per cent of the radioactivity from the labeled cyanocobalamin remained at the site of injection. After the same time interval, however, 6.0–12.0 per cent of the radioactivity emanating from the labeled hydroxocobalamin could still be traced at the injection site. Four days after injection of hydroxocobalamin, the counts at the site of injection were still 3–5 per cent of the amount injected as compared with 0.5–1.0 per cent after administration of a similar dose of cyanocobalamin. After 1 to 2 weeks, this difference was still from 2- to 6-fold, although at this point the radioactivity at the site of injection of hydroxocobalamin represented only 2–3 per cent of the dose injected.

Similar results were obtained in 2 other subjects (not shown in fig. 1) when the absorption of Co58-labeled hydroxocobalamin, injected i.m. at a dose of 1000 μg., was compared with that of an equivalent dose of Co68-labeled cyanocobalamin. The absorption of hydroxocobalamin from the site of injection again lagged far behind that of cyanocobalamin throughout the entire 2-week investigational period.

Measurements of the Uptake of Radioactivity by the Liver and Calf Muscle

The results are shown in figure 2. The hepatic deposition of radioactivity in
Radioactive hydroxocobalamin, given intramuscularly to 6 individuals,
HYDROXOCOBALAMIN II

proved to be absorbed more slowly than cyanocobalamin from the site of injection. Its accumulation in the liver and muscles, as judged by the results of surface counting after i.m. injection of 100 µg., was either equal to or higher than that of cyanocobalamin. The pattern of hepatic uptake of hydroxocobalamin was grossly similar to that previously reported by our group1–3 for cyanocobalamin and also found in the work of others.4–7

Although the results of our surface scanning suggest that more hydroxocobalamin than cyanocobalamin is retained in the tissues, more direct data on the storage of hydroxocobalamin in the tissues are required.

Moreover, the inferences drawn from this work will have to be supported by direct evidence obtained on patients with B₁₂ depletion before it can be definitely stated that hydroxocobalamin is a better form of vitamin B₁₂ than cyanocobalamin for the treatment of vitamin B₁₂ deficiency states. These investigations, now in progress, will permit a better evaluation of the possible place of hydroxocobalamin in the treatment of conditions due to vitamin B₁₂ deficiency.

Summary and Conclusions

Labeled hydroxocobalamin, administered intramuscularly in man, is absorbed more slowly from the site of injection and deposited in the liver and calf muscle to a degree equal to or higher than that of cyanocobalamin at an equivalent dose. These findings, together with those reported in Part I of this paper, suggest the applicability of hydroxocobalamin as a long-acting vitamin B₁₂ in man. These conclusions, however, require the support to be obtained from long-range observations on patients with vitamin B₁₂ depletion.

Summario in Interlingua

Hydroxocobalamina in forma marcate e administrate per via intramuscular al homine—in comparation con un dose equivalente de cyanocobalamina—es absorbite plus lentemente ab le sito del injection sed deponite in le hepate o le musculo sural a un grado equal o superior. Iste constatationes, insimul con illos reportate in le prime parte de iste communication, suggere le applicabilitate de hydroxocobalamin como un vitamina B₁₂ de deposito. Tamen, iste conclusiones require le supporto de observationes perdurative in patientes con depletion de vitamina B₁₂.

REFERENCES


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