

Effect of Ethylenediamine Tetra-acetate on Vitamin B₁₂ Absorption in the Rat

HERBERT¹ has reported that calcium is obligatory for absorption of vitamin B₁₂ by liver slices in the presence of intrinsic factor, since the addition of a calcium binder—the di-sodium salt of ethylene diamine tetra acetate (EDTA) decreased the intrinsic factor effect. Minard and Wagner² made similar observations with liver homogenates in which calcium increased vitamin B₁₂ uptake in the presence of intrinsic factor.

The *in vivo* work on this problem has not been so direct, nor is there agreement concerning the requirement for calcium for vitamin B₁₂ absorption from the gut of the living animal. Abels *et al.*³, in studies with gastrectomized rats, administered vitamin B₁₂ — ⁵⁸Co with neutralized rat gastric juice, and observed no impairment of intestinal absorption of vitamin B₁₂ when large amounts of ethylene diamine tetra acetate were added to the administered materials. Gräsbeck and Nyberg⁴ administered ethylene diamine tetra acetate to normal human subjects along with a test dose of radiovitamin B₁₂ and showed a definite decrease in vitamin B₁₂ absorption which was reversed by simultaneous administration of calcium lactate. Gräsbeck *et al.*⁵, have also implicated calcium in the failure of steatorrhea patients to absorb vitamin B₁₂.

In the course of studies on agents which would promote vitamin B₁₂ absorption, both the di- and tetra-sodium salts of ethylene diamine tetra acetate were tested for their effects on vitamin B₁₂ absorption in the rat. Intact rats received daily 1 μgm. of vitamin B₁₂ — ⁶⁰Co in 2.5 ml. of solution for three consecutive days. The test vitamin doses were contained in water, 2 per cent or 4 per cent solutions of di-sodium ethylene diamine tetra acetate, or 2 per cent or 4 per cent solutions of tetra-sodium ethylene diamine tetra acetate. Forty-eight hours after the final test dose the animals were killed and the radioactivity levels in their blood, livers, and kidneys were estimated by scintillation counting. The results for the kidneys as per cent of the radioactivity administered as vitamin B₁₂ — ⁶⁰Co per gram of tissue are presented in Table 1. Liver and blood values

caused an increase in the tissue radioactivity as compared with that of the control (water) group.

In no case was there a significant decrease in vitamin B₁₂ absorption due to concomitant administration of vitamin B₁₂ and ethylene diamine tetra acetate. A decrease had, however, been expected in the case of tetra-sodium ethylene diamine tetra acetate because the pH of this salt in solution (approximately pH 11) is unfavourable to the stability of the vitamin. The increase in vitamin absorption found with the di-sodium ethylene diamine tetra acetate (pH 5) may, on the other hand, be the result of a pH buffering or of a weak chelating effect which stabilizes the vitamin in the gut, and not due to the removal of some interfering substance by chelation with the ethylene diamine tetra acetate. Unfortunately, it is not clear which salt of ethylene diamine tetra acetate was used by Abels *et al.*³ or by Gräsbeck and Nyberg⁴, although Abels *et al.*, did point out that Herbert¹ had used the di-sodium salt in his *in vitro* experiments.

There is no evidence of a calcium requirement for vitamin B₁₂ absorption under the conditions of our experiment; indeed, it was noted that as the amount of di-sodium ethylene diamine tetra acetate administered was increased vitamin B₁₂ — ⁶⁰Co absorption was increased. The relatively large amount of the vitamin used in our experiments makes it possible that some secondary mechanism might be operative in the absorption process. These results in intact rats substantiate the findings of Abels *et al.*³, in gastrectomized animals.

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¹ Herbert, V., *J. Clin. Invest.*, **37**, 646 (1958).

² Minard, F. N., and Wagner, C. L., *Proc. Soc. Exp. Biol. Med.*, **98**, 684 (1958).

³ Abels, J., Woldring, M. G., Nieweg, H. O., Faber, J. G., and de Vries, J. A., *Nature*, **183**, 1395 (1959).

⁴ Gräsbeck, R., and Nyberg, W., *Scand. J. Clin. Lab. Invest.*, **10**, 448 (1958).

⁵ Gräsbeck, R., Kantero, I., and Siurala, M., *Lancet*, **i**, 234 (1959).

Table 1. PER CENT VITAMIN B₁₂-⁶⁰Co FOUND IN KIDNEYS 48 HOURS AFTER FINAL ORAL DOSE

| | Administered with vitamin B ₁₂ - ⁶⁰ Co | | | | | |
|--|--|------------------|------------------|------------------|------------------|------------------|
| | Tetra-sodium EDTA | | | Di-sodium EDTA | | |
| | 0 per cent | 2 per cent | 4 per cent | 0 per cent | 2 per cent | 4 per cent |
| Per cent of radio- active dose per gm. of kidney | 1.50 | 1.92 | 1.84 | 1.40 | 2.98 | 2.70 |
| | 3.07 | 1.58 | 1.64 | 1.34 | 2.97 | 2.72 |
| | 2.02 | 1.30 | 3.32 | 1.90 | 1.66 | 4.80 |
| | 1.62 | 1.43 | 1.76 | 2.08 | 2.05 | 3.13 |
| Average ± S.E. of Mean | 2.05 ± 0.357 | 1.56 ± 0.133 | 2.14 ± 0.396 | 1.68 ± 0.183 | 2.42 ± 0.333 | 3.34 ± 0.497 |
| p Value* vs. H ₂ O | — | 0.15 | 0.5 | — | 0.1 | 0.05 |

* One-sided *t* test.

showed differences of the same order between the control and experimental groups in all cases and are therefore not given.

Administration of the test dose of vitamin B₁₂ in either 2 per cent or 4 per cent solutions of tetra-sodium ethylene diamine tetra acetate caused no observable change in the tissue vitamin B₁₂ — ⁶⁰Co levels over that observed with the water solution. When the test dose was administered in di-sodium ethylene diamine tetra acetate, both 2 per cent and 4 per cent solutions

Heat Inactivation of Rinderpest-infected Bovine Tissues

INFORMATION on the survival of rinderpest virus in stored infected bovine tissues is pertinent to the problem of the export of meat from rinderpest-endemic areas. The literature contains numerous snippets of contradictory information¹. The confusion has arisen through the use of thermal death points which are meaningless and fail to express the response of a virus to heat. Accordingly, a fresh appraisal of the effect of heat on the infectivity of rinderpest-infected bovine tissues was made.

The experimental design was factorial such that the heat stabilities of three strains of rinderpest virus in the form of three types of infected bovine tissues were determined at four temperature-levels over the range 7–56°C. In addition, immediate and storage death at temperatures below the freezing-point were examined. The rinderpest strains were the virulent Kabete 'O', the attenuated Kabete caprinized and the attenuated Nakamura III lapinized. The two latter are widely used in equatorial Africa as live virus vaccines. The 'O' and the caprinized strains were titrated in cattle and the lapinized strain in rabbits.