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A STUDY OF COMPARATIVE BLOOD ABSORPTION OF VITAMIN B_1 FROM DIFFERENT B-COMPLEX PREPARATIONS

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ABSTRACT

In order to judge the therapeutic efficacy of thiamine (vitamin B_1 in 9-complex preparations, three commercial syrup preparations were analysed for thiamine content and their blood absorption in rabbits was compared with that of thiamine as a single entity. A separate experiment was performed to find the effect of sugar concentration on the blood absorption of thiamine. The difference in the quantitative values of vitamin Ht absorbed in the single and compound preparations under the same conditions has been assumed as one of the criteria to ascertain the therapeutic efficiency of both the forms.

Introduction

Vitamins are usually supplied as manufactured dosage forms, both as single entities and also as multicomponent preparations. Among all the varieties the oral multivitamin and B-complex liquid preparations particularly exhibit stability problems due to chemical degradation and interaction of mixed substances (Hashmi, 1973). Interactions between thiamine and riboflavin, thiamine and pantothenic acid, cyanocobalamin and ascorbic acid were found to occur in all types of dosages forms (DeRitter, 1982). The therapeutic ef[f]icacy of thiamine (vitamin B_1) in liquid preparations is of doubtful nature, since thiamine may easily hydrolyse into thiazole and pyrimidine moieties in aqueous solution (Gisvold, 1977; Reynolds, 1989). In B-complex preparations thiamine interacts with riboflavin, pantothenic acid and cyanocobalamin (Macek, 1960; Ahmed, 1972). Accord[i]ng to Lhoest *et al.* (1958) the interaction of thiamine with pantothenic acid is largely a pH problem and adjustment to pH 4 to ensure thiamine stability results in the rapid decomposition of pantothenate. Interaction of thiamine with vitamin A has also been noted (Uprety *et al*, 1961).

The simultaneous administration of vitamin preparations is based upon two principles, fast the combinations must offer a definite therapeutic advantage over either of its component taken alone and secondly, this advantage must not be outweighed by an additional hazard. In oral liquid B-complex preparations the therapeutic efficacy of thiamine is influenced by its hydrolytic degradation depending upon the nature and composition of the formulation. Another important factor of thiamine instability in complex mixtures is its oxidation to thiochrome (Dale and Booth, 1976). Although manufacturers claim that over dosages are added to

compensate for the problem of stability - yet to satisfy each component of B-complex in a product, which has a quite different stability characteristics, is difficult. These factors might be responsible for the low blood absorption of vitamin B_1 in case of B-complex preparations when compared to that of the single B_1 preparation.

The aim of this work is, therefore, to determine whether vitamin B_1 is therapeutically more efficacious in liquid B-complex preparations or in preparations where B_l is present alone. The necessary information has been obtained by comparing the absorption of vitamin Bt in rabbits following the oral administration of some commercial liquid B-complex preparations as well as simple preparation of B_1 respectively, both having equal quantities of thiamine. The difference in the values of vitamin B_1 absorbed has been assumed as one of the criteria to ascertain the therapeutic efficacy of both the forms.

Materials and Methods

Thiamine hydrochloride (vitamin B_1) was obtained from Merck and was found to be chromatographically pure. The various B-complex syrups obtained from the market and used in this investigation ar*e* giv*en* in Table 1. All solvents and reagents used were of analytical grade or of the purest form available from Merck/B.D.H.

Animals used

Several groups of healthy rabbits each consisting of five in number were maintained on a normal diet of gram and water only. All the five rabbits in each group were designated as A, B, C, D and E having a weight of approximately 1.8, 2.0, 2.1, 2.2 and 2.4 kg respectively. Each group of rabbits was disposed of after thrice withdrawal of blood in oxalated tubes. The blood was used for analysis immediately after collection.

The minimum daily requirement of thiamine for an adult person is 1 mg (Marcus and Coulston, 1985; Bender, 1988). Taking this as standard an average daily requirement of thiamine for the rabbits was calculated assuming the average weight of rabbit as 2 Kg. Thus 1/2 mg of thiamine hydrochloride (which is in excess of the daily requirement, i.e. 2/70 Kg) of U.S.P. standard reference was dissolved in 5 ml of distilled water and transferred to a syringe attached to a rubber catheter. The catheter was then introduced into the rabbit's mouth and the solution was injected by means of the syringe. No food was supplied for at least four hours before and after the administration of B_1 solution. Three samples of blood, each 3 ml, were withdrawn by pricking the ear vein of the rabbit and collected in oxalated tubes. In this manner five different rabbits with a weight variation of 1.8-2.4 Kg was treated and Bi assayed on consecutive days.

Assay of thiamine in blood samples

The method involves extraction of thiamine hydrochloride from blood by digesting it with dilute HCI, centrifugation, extraction and then adsorption on activated base exchange zeolite column from where it is eluted with KCI solution and oxidized to thiochrome with alkaline potassium ferricyanide solution. It is similar to that described for thiamine assay in the U.S.P. (1990). The details are as follows:

To 3 ml of oxalated blood 3 ml of 1 N HCI was added, the solution mixed and then 3 ml of 20% trichloroacetic acid was added. This solution was centrifuged for ten minutes at 800 r.p.m. The supernatent liquid was transferred to a 25 ml beaker, 10 ml of 5% sodium acetate solution was added to it and the pH adjusted to 4.5. This solution was then applied to the activated base exchange zeolite column. The column adsorbs thiamine only while the other ingredients pass away. Adsorption is quantitative in the pH range 3-6. The column was washed with hot distilled water and eluted with 10 ml of KCI solution. This 10 ml sample solution was placed into two reaction vessels each having 5 ml, one blank and the other sample. To the sample vessel 35 ml of alkaline $K_3Fe(CN)s$ solution was added while to the blank same quantity of distilled water was added. Then 3.5 ml of 15% NaOH was added to each vessel and finally 10 ml of isobutanol was added and vigorously shaken for three minutes.

After discarding the aqueous layer a small amount of anhydrous granular sodium sulfate was added and shaken well. When the sulfate particles settled down, the isobutanol layer was decanted into 1 cm cuvette for fluorimetric measurement. A great difficulty with this method results from quenching effects due to interfering substances which are largely eliminated by the adsorption process. In order to compare the blood absorption of vitamip B_1 in B-complex preparation with that of simple B_1 preparation, it was necessary to ascertain whether B-complex mixture which is orally administered to the rabbit contains exactly the same quantity of vitamin B_1 that is apparently present in it. Therefore, prior to the oral administration, all the commercial B-complex syrups were quantitatively estimated for their vitamin B_1 content by thin layer chromatography as described below:

Calculation

Amount of thiamine = $\frac{U - UB}{S - Sb}$ x µg of thiamine in standard x $\frac{100}{S}$

= µg of thiamine / 100 ml of blood.

Where U = Reading for sample.
UB = Reading for blank.
S = Reading for standard.
SB = Reading for standard blank.

Assay of thiamine in B-complex syrups

The method adopted in the present work is "ascending one dimensional thin layer chromatography" (Bolliger and Konig, 1969). Six spots of standard thiamine hydrochloride solution were made on the silica gel G thin layer plate with the help of micropipette adding 0.001, 0.002, 0.004, 0.06, 0.008, 0.01 ml aliquots respectively. The spots were made 2 cm apart from each other and 1.5 an above the lower edge of the plate. The concentrations of thiamine in these spots were 0.001, 0.002, 0.004, 0.006, 0.008, 0.01 pg respectively. The clear sample solution was also spotted on the plate in the same manner. The plate was dried for two minutes in air and then developed with the solvent system pyridine-acetic acid-n-propanol- water (5:5:60:90, v/v) and reddest for three minutes in a drier at 50°C. The dried plate was then sprayed evenly with alkaline potassium ferricyanide solution with the help of a micro-jet sprayer. The snow white spots of thiamine (R_f 0.34 ± 0.01) were visible against a pale white background. Thiochrome was then eluted in isobutanol and measured Buorimetrically using a standard curve (Fig. 1).

Calculation

Amount of thiamine = $\frac{S \times V \times 100 \times D \times C}{1000}$ = mg/100 ml of sample solution.

Where S = $\frac{\text{volume of diluted sample solution}}{\text{volume of spotted solution}}$
V = volume of isobutanol used to extract thiochrome.
D = initial dilution of assay sample.
C = concentration in pg obtained from the standard curve.

Results and Discussion

Data obtained from the analysis of different commercial preparations (Table 1) show that most of the manufactures use extra amount of vitamins to overcome the problem of instability and to increase the shelf-life of their products This extra amount is normally in the range of 10-15% (Table 1).

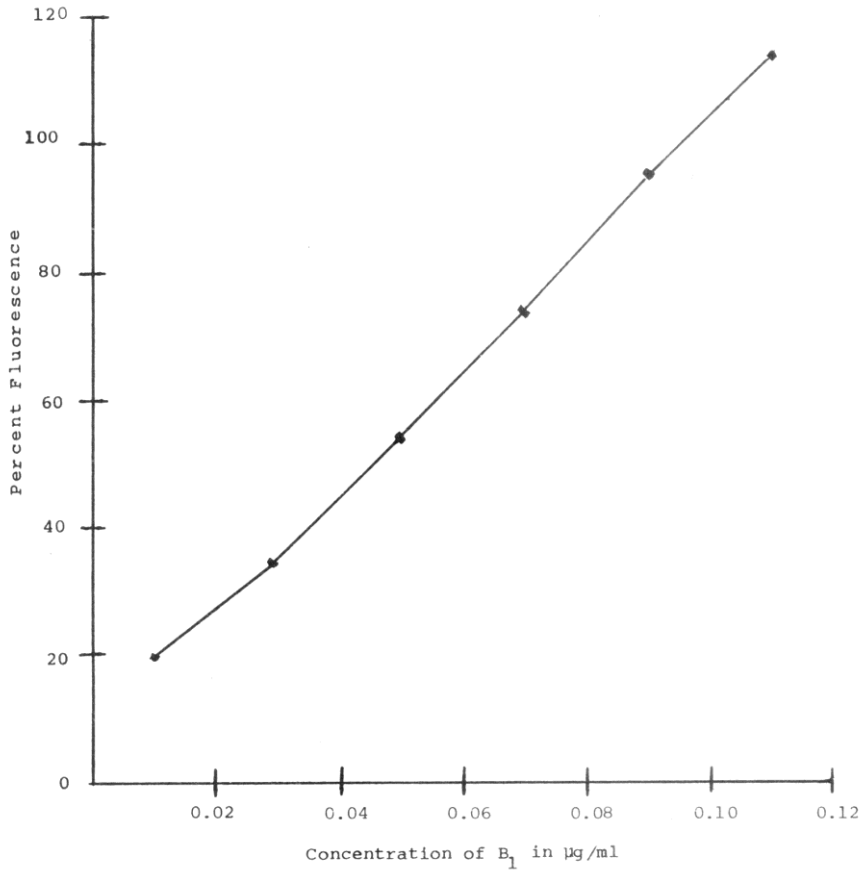
120
100
80
60
40
20
0
Percent Fluorescence
0.02
0.04
0.06
0.08
0.10
0.12
Concentration of B_1 in µg/ml

Table 1

B-Complex preparations used in present investigation.

Sample	Product	Labelled amount of B_1 mg/100 ml	Determined Amount of B_1 mg/100 ml	Labelled amount of other vitamins mg/100 ml
1.	Syrup	10	10.98	Vit-A 50000 units Riboflavin 12 mg Nicotinamide 100 mg Vit-C 300 mg Vit-D 5000 units
2.	Syrup	30	33.00	Vit-A 18 mg Vit-B_2 24 mg Vit-B_6 20 mg Vit-B_{12} 60 mcg Nicotinamide 200 mg Vit-C 1000 mg Vit-D 200 mcg
3.	Syrup	60	65.02	Vit-A 100000 units Niacinamide 400 mg Pantothenic acid 20 mg Vit-B_{12} 100 mcg Vit-D 10000 units Riboflavin 40 mg

Data for the determination of blood concentration of thiamine in case of liquid B-complex preparations (Table 2) show that different the B-complex preparations vary in their blood absorption of thiamine within a specific time limit. This time limit is the time at which peak concentrations of thiamine are obtained after the oral administration of simple vitamin B_1 preparation compared with that obtained for the B-complex preparation. Therefore, it appears that the duration of blood absorption might be longer in case of B-complex preparations and it may take more time to reach the same peak level. This timing may also be different within different B-complex preparations. However, not more than a rise of 0.2 to 03 pg of thiamine/100 ml of blood after 10 minutes of the time specified for peak concentration, i.e. after 1:20 hours, has been noticed.

Table 2

Thiamine content of rabbit's blood after oral administration of simple vitamin B_1 and B-complex preparations

Rabbit*	Concentration of thiamine (μg/ 100 ml)**				
	normal	with vitamin B_1	with B -complex syrups		
			1	2	3
A	9.4±0.53	24.2±0.74	16.06±0.6	18.40±0.5	21.2±0.57
B	9.9±0.49	24.8±0.69	16.30±0.63	19.09±0.6	21.5±0.60
C	10.2±0.52	25.1±0.70	17.20±0.59	19.09±0.61	21.5±0.56
D	10.6±0.51	2.51±0.68	17.50±0.57	19.30±0.59	21.8±0.61
E	11.0±0.49	26.8±0.72	13.10±0.62	20.60±0.60	22.1±0.62

* In each group five rabbits (A-E) were taken for normal B_1 value as well as for simple B_1 and syrups treatment. In all 25 animals were used. The average weights of rabbits were between 18-24 Kg.
**values indicate mean ± standard deviation (n = 3).

It is evident from the overall analysis that blood absorption of thiamine in liquid B-complex preparation is considerably lower (about one-third) compared with that of the single B_1 preparation. This lowering of blood absorption is highly significant in terms of the therapeutic efficacy of B_1 and may be attributed to interactions between different B-components as well as the sugar concentration in the syrups. In case of sample 1 where sugar percentage is high (74%), the absorption of B_1 is lowest indicating that sugar concentration may exert an effect on B_1 absorption. The variations in B_1 absorption among the three B-complex preparations may be due to different sugar levels as well as other formulation factors (Hoener and Benet, 1990) and measurement of blood levels provides a means of assessing absorption directly (DeRitter, 1982). Accord'ng to Beckett (1969) the problem of vitamin combinations and their usefulness should be approached in a natural manner with due consideration of the condition under which simultaneous administration of vitamins having different biological half-fives is justified.

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