Physiologic Availability of Riboflavin and Thiamine in "Chewable" Vitamin Products

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VITAMIN preparations which can be readily chewed or dissolved in the mouth are of value to adults or children who have difficulty in swallowing tablets or capsules. Formulation of such products is made difficult, however, by the bitter and objectionable taste of the B vitamins, particularly riboflavin. Various procedures, such as coating the vitamins with lipid or other material, or combining riboflavin with an ion exchange resin,[1] have been used to produce relatively tasteless multivitamin preparations. Since it has been shown[2-4] that certain coating processes which retard disintegration or solution of a product in the gastrointestinal tract may thereby reduce the availability of its active component(s), it was considered necessary to obtain information on the availability to the body of the vitamins in chewable products. In the present studies, the *in vivo* availability of riboflavin and thiamine was determined in commercially available preparations and in individual coated vitamins used in the manufacture of such preparations. The availability of riboflavin in a riboflavin-ion exchange resin complex, and in a product containing the resinate, was also determined. Both human subjects and rats were used in the investigations.

METHODS

Commercial Products Studied

Five chewable multivitamin preparations (products A through E) and two coated products used in the manufacture of such preparations (products F and G) were tested for availability of riboflavin. The availability of thiamine was also determined in each of these products, with the exception of product E. Riboflavin availability was determined in two different lots of products B and F. The second lot of product F was prepared by what the manufacturer termed as an improved procedure. A riboflavin-ion exchange resin complex (product H) was also studied, along with a multivitamin product which contained the resinate (product I). Products A through E and I were obtained on the retail market in Canada or the United States, whereas products F, G and H were obtained from the manufacturer. The various preparations were analyzed for thiamine and/or riboflavin content prior to administration.

Physiologic Availability

The procedure used to determine physiologic availability was the same as that described previously.[2-5] In brief, riboflavin and thiamine levels were determined in the urine of five or six normal male subjects, before and after the administration of the standard or test preparations. The amounts of the vitamins found in the urine after the dose was given were corrected by subtracting the appropriate blank values determined on the urine of each subject prior to dosing. Physiologic availability was defined as

$$\frac{\text{percentage excretion after test preparation}}{\text{percentage excretion after standard preparation}} \times 100$$

The standard dose of riboflavin was 5 mg., given in a tablet which disintegrated rapidly. Standard doses of thiamine were given in solution. Because the percentage excretion of thiamine is markedly influenced by the size of the dose,[6,7] the excretion values for the standard dose were read from a dose-response curve previously determined with the same subjects.[6] The subjects received from 3 to 7.5 mg. of riboflavin, and from 1.8 to 3.0 mg. of thiamine in the various preparations.

Rat Studies

The basal riboflavin-deficient diet used in the two

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TABLE I
Physiologic Availability of Riboflavin in Chewable Vitamin Preparations

Product	Riboflavin Available (%)
A	95 ± 6.4*
B	
Lot 1	91 ± 5.6
Lot 2, trial 1	76 ± 10.5
Lot 2, trial 2	70 ± 7.6
C	125 ± 6.9
D	101 ± 12.4
E	30 ± 5.5
F	
Lot 1, trial 1	69 ± 6.1
Lot 1, trial 2	80 ± 8.2
Lot 2	92 ± 12.9
G	
Trial 1	57 ± 10.5
Trial 2	61 ± 6.9
H	57 ± 6.2
I	60 ± 11.6

* Standard error of the mean.

rat studies contained the following: sucrose, 62 per cent; vitamin-free casein, 18 per cent; corn oil, 10 per cent; non-nutritive cellulose, 5 per cent; U.S.P. intravenous mineral mixture, 4 per cent; vitamin mixture without riboflavin, 1 per cent. In both experiments, groups of eight male weanling Wistar rats of the Food and Drugs colony received the basal diet alone or supplemented with 50, 100 or 200 μg. of pure riboflavin per 100 gm. of diet. Other comparable groups received similar diets in which the riboflavin was supplied by products F and G, the two coated products used in preparing chewable multivitamin preparations (experiment 1), or by product H, the riboflavin resinate (experiment 2). The animals were housed individually in screen bottom cages kept in an air-conditioned room maintained at 74° to 76°F., and received the various diets *ad libitum*. In order to minimize vitamin losses, the diets were kept in a freezer at minus 20°F. During the three week experimental period, the animals were individually weighed at weekly intervals, and records were kept of the amounts of food consumed by each rat.

RESULTS

Human Studies

Data on percentage excretion of the standard dose of riboflavin were presented previously[3,6] as were those for blank excretion values.[8]

TABLE II
Physiologic Availability of Thiamine in Chewable Vitamin Preparations

Product	Thiamine Available (%)
A	110
B	
Lot 1	116
Lot 2	104
C	88
D	98
F	85
G	94

The mean values for physiologic availability of riboflavin in the various preparations are shown in Table I. Products A, C and D were fully available. Lot 1 of product B appeared to be fully available but two subsequent trials with a second lot of this product showed mean availability values of 76 and 70 per cent. Product E showed a low availability value of 30 per cent. Product G, the coated material used in the manufacture of products B and E, was also not completely available to the body, as evidenced by physiologic availability values of 57 and 61 per cent in two trials. Lot 1 of product F also showed incomplete availability in two trials. However, the riboflavin was found to be fully available in a second lot of this product, produced by an improved procedure. Product H, the riboflavin resinate, showed a low availability of only 57 per cent, almost identical with that of product I which contained the resinate.

Data on mean rate of excretion of riboflavin after ingestion of products B, E, F, G and H were compared with those obtained after the standard dose (Fig. 1). As suggested by Swintosky et al.,[9] a semi-log plot was used. Since the percentage of riboflavin excreted after ingestion of a dose remains constant over a wide range of intakes,[6] all excretion values were converted to the basis of a 5 mg. dose, to be directly comparable with the standard dose. Peak excretion values tended to be directly related to physiologic availability values. Product E, which showed the lowest availability, also showed the lowest peak excretion value of 2.29 μg. per minute. The peak of

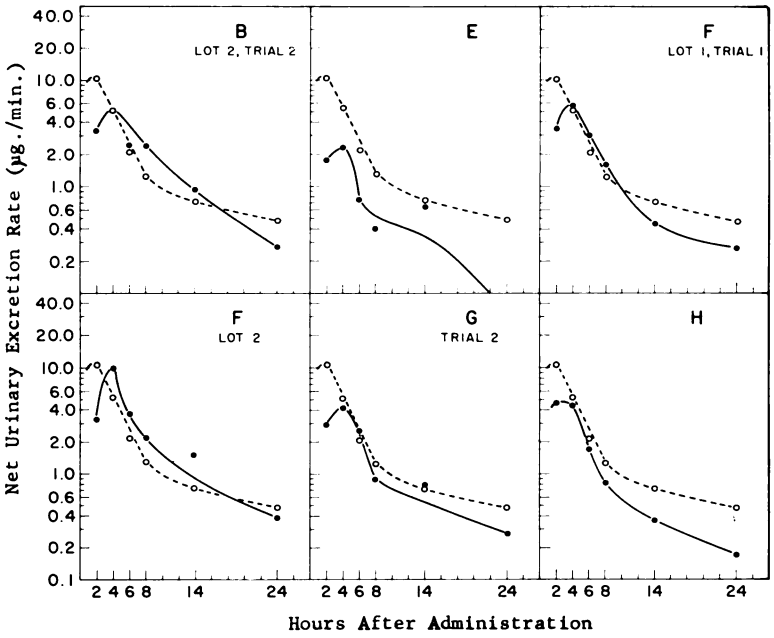

FIG. 1. Urinary excretion curves for riboflavin given in a standard tablet (O—O—O) compared with curves for riboflavin given in various "chewable" products (●——●).

riboflavin excretion occurred later with the coated products than with the standard preparation. With lot 2 of product F, which was fully available, the peak excretion value was similar to that obtained with the standard preparation. None of the products showed any sustained release of riboflavin.

Data on the availability of thiamine in the various products are summarized in Table II. Because the percentage excretion values after ingestion of the standard preparation were read from an average curve for all subjects, it was not possible to calculate standard errors for the physiologic availability values. All the products were fully available, as measured by urinary excretion.

Rat Studies

The rat studies are summarized in Table III. In both experiments, the weight gains of the animals were directly related to the riboflavin content of the diet. In experiment 1, the animals which received the basal diet supplemented with various levels of riboflavin supplied by products F and G grew at comparable rates to those fed similar diets containing pure riboflavin. In the second experiment, weight gains of the rats given the basal diet supplemented with pure riboflavin were similar to those obtained in animals given the basal diet supplemented with the riboflavin resinate. These results indicated that the riboflavin resinate was fully available for growth in the rat.

COMMENTS

The results of the present studies showed that wide variation existed in the availability of riboflavin in chewable vitamin preparations. Of nine products studied, six were partially unavailable to the body. Of these, three contained the same coated preparation (products B, E and G). It should be noted that the manufacturers of the coated preparations have now modified their processes to produce products with full availability of riboflavin. A riboflavin resinate and a multivitamin preparation which contained the resinate were also found to be only partially available *in vivo*, although Brudney[1] reported that the riboflavin was released from the resinate *in vitro*. Contrary to published

TABLE III

Weight Gains of Male Weanling Rats Fed Diets Containing Various Levels of Riboflavin Supplied by the Pure Vitamin, Coated Products F and G or Resin Product I

Riboflavin Added to Basal Diet (μg./100 gm.)	Riboflavin Supplied by				
	Experiment 1			Experiment 2	
	Standard Preparation	Product F	Product G	Standard Preparation	Product H
0	7 ± 1.4*	...	...	7 ± 1.5	...
50	21 ± 2.5	20 ± 1.7	20 ± 2.0	15 ± 1.3	15 ± 2.1
100	38 ± 3.2	38 ± 3.9	38 ± 3.8	31 ± 2.4	28 ± 4.0
200	68 ± 2.0	58 ± 3.1	59 ± 3.3	57 ± 2.2	58 ± 3.3

* Standard error of the mean.

claims,[10] assumptions as to the rate of release of a drug from a resin complex *in vivo* cannot be made from such *in vitro* data.[1] The lack of sustained urinary excretion of riboflavin from the resinate, in part, may be due to the fact that riboflavin is absorbed efficiently only high in the intestinal tract.[11,12] These results provide further evidence that some resinates are unavailable to the body, as was found previously for resinates containing creatinine, acetylsalicylic acid and amphetamine.[13] Considerable variation was found between products containing the same coated material. This variability is probably related, in part, to interlot variation in the thickness of the coating used.

In contrast to riboflavin, thiamine was fully available in all the products studied. This difference between the two vitamins may be related to the thickness with which riboflavin was coated to reduce its bitter taste, which is more objectionable than that of thiamine. The much greater solubility of thiamine than of riboflavin may also partially explain the difference in availability of the two vitamins in coated products.

Although the results of the human studies indicated that the riboflavin in coated products F and G and in resin product H was only partially available, the rat studies demonstrated full availability of the vitamin in these products. It would appear that the availability of riboflavin in vitamin preparations designed for human use should be determined in man, since results obtained with experimental animals may prove misleading. It might be argued, of course, that the animal studies actually reflected the true availability of the product more accurately than did the urinary excretion measurements. In answer to this, it should be pointed out that a constant relationship between riboflavin intake and excretion in the urine of normal subjects has been demonstrated in several laboratories.[2,14,15] Urinary excretion of riboflavin, therefore, must accurately reflect intake, and consequently may be used as a valid indicator of the amount of riboflavin available to the body from a given dose. It would appear likely that differences in the physiology of the rat's gastrointestinal tract, as compared to that of human subjects, may explain the differences in the availability of riboflavin for the two species. The fact that the riboflavin resin was fully available in the rat, but only partially available in man, raises questions as to the validity of the statement[10] that release of the active principle from an ion exchange resin is controlled by "the normal laws governing velocity of chemical reactions and is unaffected by enzyme action, peristalsis or other physiologic processes."

It may be concluded that any process which interferes with the rate of disintegration or solution of a vitamin product in the gastrointestinal tract may result in reduced physiologic availability of riboflavin. Products such as B, E, F, G, H and I, which were not completely available *in vivo*, in effect do not meet label claims, and could not be expected to

produce blood riboflavin levels comparable to those found with a standard preparation. It is obvious that such products would be unlikely to yield the desired clinical effects.

SUMMARY

The physiologic availability of riboflavin and thiamine was determined in a number of "chewable" vitamin preparations. Of nine products studied, six showed incomplete availability of riboflavin, as measured by urinary excretion of the vitamin by human subjects. Of these, four were coated products or preparations containing them, while the other two were a riboflavin resin and a product containing riboflavin resin. Thiamine was fully available in all products studied. Two coated riboflavin products and a riboflavin resin found only partially available in man were fully available for growth in the rat. It was concluded that (1) any process used to coat or otherwise modify the rate of release of a vitamin for human use should be tested in man to ensure that the availability of the vitamin is not affected; (2) any process which interferes with the disintegration or solution of a vitamin product in the gastrointestinal tract may have a marked effect on the physiologic availability of riboflavin.

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