Relationship Between *in Vitro* Disintegration Time and *in Vivo* Release of Vitamins from a Triple-Dose Spaced-Release Preparation

By A. B. MORRISON, C. B. PERUSSE, and J. A. CAMPBELL

Studies were conducted on the relationship between *in vitro* disintegration time and *in vivo* release of riboflavin, thiamine, and niacinamide in a multivitamin product consisting of three component spheres which disintegrated at different times. *In vitro* disintegration times were determined using apparatus and fluids described in U.S.P. XVI. None of the three vitamins was fully available in the intact product, as measured by the rates of urinary excretion of riboflavin, thiamine, and N′-methylnicotinamide after dosing human subjects. Spheres which showed incomplete availability as measured by urinary excretion of riboflavin were also relatively unavailable when excretion of thiamine or N′-methylnicotinamide was used as the criterion of adequacy. No evidence was obtained that the delayed disintegration of some of the spheres produced sustained release properties *in vivo*. One of the spheres was recovered essentially intact from the feces of two subjects. It was concluded that measurement of riboflavin excretion provides a valid indication of physiological availability of vitamin preparations and that limits for *in vitro* disintegration time based on riboflavin excretion are also applicable to other vitamins.

EARLIER work from this laboratory (1, 2) demonstrated that sugar coated tablets must disintegrate within 60 minutes (30 minutes in simulated gastric juice and 30 minutes in simulated intestinal juice) in order to show full availability of riboflavin. In additional studies (3), it was concluded that enteric coated riboflavin preparations should withstand the action of simulated gastric juice for 60 minutes and then disintegrate within 30 minutes in simulated intestinal juice, in order to show full availability of riboflavin. Little information is available on the relationship between *in vitro* disintegration time and physiological availability for other vitamins. The appearance on the market of a new "spaced-release" vitamin preparation offered an opportunity to examine the validity of proposed limits for disintegration time, using other vitamins in addition to riboflavin. The present report deals with the results of these studies.

METHODS

The product was purchased on the retail market in the United States and consisted of three "spaced-release" spheres contained within a gelatin capsule. Each sphere contained 3.33 mg. of thiamine hydrochloride, 1.67 mg. of riboflavin, and 16.7 mg. of niacinamide; one-third of the total dose of each vitamin in the product. One sphere was designed to release its ingredients immediately, whereas the second and third spheres were designed to release the vitamins contained therein approximately 3 and 6 hours later, respectively. The three spheres differed from each other, therefore, only *in vitro* disintegration time.

Physiological availability values for riboflavin, thiamine, and niacinamide were determined with the intact preparation and each of the three spheres. The procedure used to determine physiological availability was the same as that described previously (4, 5). Riboflavin, thiamine, and N′-methylnicotinamide levels were determined in the urine of 5 to 7 normal male subjects before dosing and after administration of standard or test preparations. The amounts obtained in the urine after dosing were corrected by subtracting the appropriate blank values determined on the urine of the same subjects. The blanks were determined over several days, to reduce the effect of daily variation. The subjects received doses of 5 mg. of riboflavin or 50 mg. of niacinamide in rapidly disintegrating standard tablets, as well as in the intact product and its component spheres. Because each sphere contained only one-third of the total dose in the product, it was necessary to give three spheres of each type in this portion of the studies. To provide a valid comparison with the rates of excretion of riboflavin and N′-methylnicotinamide after giving the intact preparation, the subjects also received the standard doses of riboflavin and niacinamide in divided dose form, as described previously (4, 6).

In the thiamine studies, the dose was only one of each sphere, and the resultant urinary excretion of thiamine was compared with that found after giving 3.33 mg. of the vitamin in solution. The dose was intentionally kept low because of the fact that large doses of thiamine are not absorbed efficiently (5, 7). The availability of thiamine in the intact product was determined by comparing urinary excretion of thiamine after giving 10 mg. of the vitamin in divided doses with that found after giving the product. It was necessary to use the divided dose standard for this purpose because of previous findings (5) that administration of 10 mg. thiamine in divided dose form markedly increased urinary excretion of the vitamin over that found when the same total dose was given at one time.

Received September 6, 1961, from the Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada.
Accepted for publication October 16, 1961.

Riboflavin and thiamine in the product and in urine were determined by the U.S.P. XVI fluorometric procedure (8) and by the thiochrome method of Mickelsen, *et al.* (9), respectively. Niacin in the product was determined by the U.S.P. XVI procedure (8), whereas urinary N′-methylnicotinamide was determined by the fluorometric procedure of Pelletier and Campbell (10). Curves of net rate of excretion were plotted on semilogarithmic paper, as suggested by Swintosky, *et al.* (11).

In vitro disintegration times for the three spheres were determined using the apparatus and fluids described in U.S.P. XVI (8). Disks were used in both the gastric and intestinal juice phases of the test. The spheres were immersed in simulated gastric fluid for 30 or 60 minutes and the remainder of the time in simulated intestinal fluid. Disintegration times reported were means of two separate tests on six spheres each.

Sphere R was given to two subjects and the feces were collected for a period of 48 hours after dosing. Undissolved spheres were recovered from the feces and analyzed for thiamine, riboflavin, niacinamide, folic acid, and ascorbic acid. With the exception of folic acid, which was analyzed by the procedure of Pelletier and Campbell (12), procedures given in U.S.P. XVI (8) were used for these analyses.

RESULTS

The *in vitro* disintegration times of the various spheres are summarized in Table I. Sphere O, designed to release immediately the vitamins contained therein, had the shortest disintegration time, whereas spheres Y and R, which were designed to release their vitamins approximately 3 and 6 hours after ingestion, had disintegration times which were much longer. The length of time in simulated gastric juice did not significantly affect the time required for disintegration in simulated intestinal juice.

Mean physiological availability values are shown in Table I and curves of excretion rate are given in Figs. 1-3. The percentage urinary excretion values for the standard single doses of riboflavin, thiamine, and niacinamide were 58, 14, and 19%, respectively. None of the vitamins was fully available in the intact product. Thiamine and niacinamide were fully available in sphere O, but riboflavin showed somewhat reduced availability in this component. Riboflavin and niacinamide showed similar low availability values in sphere Y, whereas thiamine, although not fully available, gave a higher value. All three vitamins showed low availability in sphere R.

Values found for thiamine with sphere Y showed considerable intersubject variation due, in part, to the fact that one subject was consistently unable to obtain any thiamine from this component. This subject was also unable to obtain any thiamine from sphere R.

The curves of excretion rates (Figs. 1–3) showed that sustained urinary excretion of riboflavin, thiamine, and N′-methylnicotinamide was obtained by giving the vitamins in divided doses. The intact product, however, showed no evidence of sustained release properties, as measured by urinary excretion of the vitamins tested. As shown in Fig. 1, the maximum rate of excretion of riboflavin after administration of the standard was higher than that found with the intact product or its component spheres. Spheres O and R showed maximum riboflavin excretion 2 hours after dosing, whereas the peak of excretion did not occur until 6 hours after ingestion of sphere Y.

The rate of excretion of N′-methylnicotinamide after ingestion of sphere O was very similar to that obtained with the standard (Fig. 2). Spheres Y and R, however, gave very low excretion curves. As with riboflavin, sphere Y gave peak excretion of N′-methylnicotinamide 2–4 hours later than did the other spheres.

As shown in Fig. 3, the rate of excretion of thiamine after dosing with sphere O was similar to that obtained with the standard. The curves for spheres Y and R were irregular and indicated low availability of the products.

The results of the fecal recovery studies are shown in Table II. Both subjects who consumed pellets of sphere R for this study recovered them from the feces. The amounts of niacinamide and folic acid obtained in spheres which passed through the gastrointestinal tract exceeded those claimed on the label. As well, 90% of the riboflavin, 84% of the thiamine, and 78% of the amount of ascorbic acid claimed on the label were found in spheres which were recovered from the feces.

DISCUSSION

The data again (13) emphasize the fact that processes which interfere with the disintegration or solution of a vitamin product in the gastrointestinal tract may have a marked effect on availability of vitamins. If the spheres are considered simply as plain coated tablets with varying disintegration times, they provide further evidence for the validity of a 1-hour limit for the *in vitro* disintegration time of vitamin preparations. Riboflavin tended to be somewhat less available in the various spheres than were the other two vitamins perhaps, in part, because of reduced solubility. Nevertheless, the results indi-

Table I.—Physiological Availability of Three Vitamins in a Spaced-Release Multivitamin Preparation and Its Component Spheres

Preparation	*In Vitro* Disintegration Time, min.			Availability, % ±S.E.		
	Gastric Juice	Intestinal Juice	Total	Riboflavin	Thiamine	Niacinamide
Intact product	..	...	...	38 ± 6.0	43 ± 5.9	64 ± 10.0
Sphere O	25	...	...	77 ± 5.1	98 ± 13.7	122 ± 14.7
Sphere Y	30	57	87	20 ± 1.9	73 ± 21.6	27 ± 6.4
	60	54	114	...	...	...
Sphere R	30	138	168	13 ± 4.5	28 ± 5.0	29 ± 7.8
	60	134	194	...	...	...

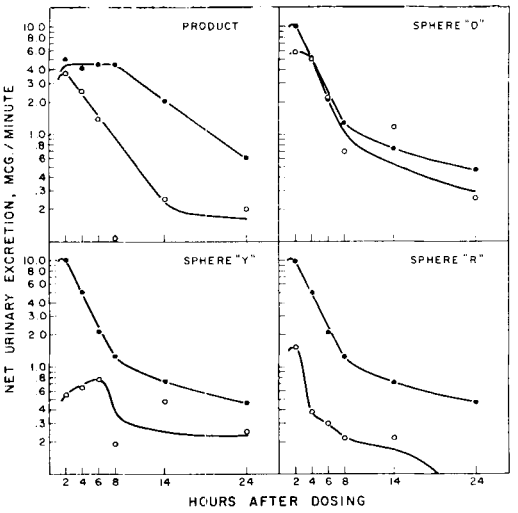

Fig. 1.—Urinary excretion curves for riboflavin in product or its component spheres O—O—O compared with curves for riboflavin standard ●—●—●.

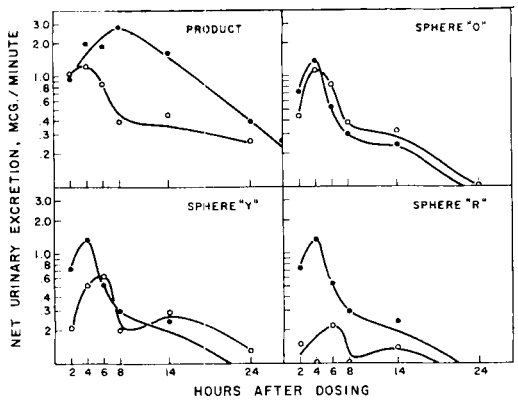

Fig. 2.—Urinary excretion curves for thiamine in product or its component spheres O—O—O compared with curves for thiamine standard ●—●—●.

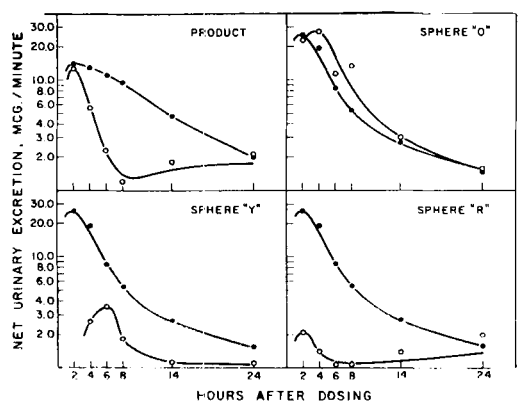

Fig. 3.—Urinary excretion curves for N′-methylnicotinamide for product or its component spheres O—O—O compared with those for niacinamide standard ●—●—●.

Table II—Vitamin Content of Sphere R Recovered from Feces

Vitamin	Amount Recovered, % of Label Claim
Riboflavin	90
Thiamine	84
Niacinamide	140
Folic acid	118
Ascorbic acid	78

cated that spheres which showed incomplete availability as measured by urinary excretion of riboflavin were also unavailable when excretion of thiamine or N′-methylnicotinamide was used as the criterion of adequacy. This finding would appear to allay the fears of Endicott and Kirchmeyer (14) that vitamins more soluble than riboflavin might yield an entirely different relationship between *in vitro* disintegration time and physiological availability.

The time limit given in U.S.P. XVI (8) for plain coated decavitamin tablets is 30 minutes in simulated gastric juice plus 2 hours in simulated intestinal juice. Sphere R would not pass such a test, but sphere Y would. The latter sphere, however, showed inadequate availability of all three vitamins tested. It is obvious that riboflavin, thiamine, and niacinamide in coated tablets which pass the U.S.P. test may not be fully available to the body.

Because of the resistant coatings used, it might appear more appropriate to consider spheres Y and R as enteric coated preparations. In a previous communication (3) it was suggested that enteric coated products containing riboflavin should withstand simulated gastric juice for at least 60 minutes and should then disintegrate within 30 minutes in simulated intestinal juice, to ensure full availability *in vivo*. The *in vitro* disintegration times for spheres Y and R exceeded this time limit, and both spheres showed incomplete availability of all three vitamins tested. These results suggest that the proposed time limit is also applicable to enteric coated preparations containing other vitamins.

If decavitamin preparations are enteric coated, the time limit given in U.S.P. XVI (8) is 5 hours (1 hour in simulated gastric juice plus 4 hours in simulated intestinal juice). Sphere R, which would have passed the U.S.P. XVI test, was recovered essentially intact from the feces of human subjects. These results indicate that the present U.S.P. time limit for enteric coated decavitamin preparations is too long, and that products which pass the test may not be available to the body.

The results of the fecal recovery studies indicated that tablets with an *in vitro* disintegration time similar to that of sphere R would exhibit very low availability not only of riboflavin, thiamine, and niacinamide, but also of folic acid and ascorbic acid. These findings suggest that the relationship between *in vitro* disintegration time and physiological availability for folic acid and ascorbic acid is similar to that obtained with riboflavin, thiamine, and niacinamide.

No evidence was obtained that the intact preparation possessed sustained-release properties, nor that the total dose was released at three separate times. Sphere R, which had the longest *in vitro* disintegration time, was recovered almost intact from the

feces of two subjects. It is obvious that the vitamins in this component of the product were largely wasted and that the sphere could not exert desired effects *in vivo*. The results obtained do not support claims that the product provides improved nutritional benefits with less loss of vitamins, and are in agreement with similar findings obtained previously with other sustained release vitamin preparations (4, 5). Furthermore, the data again (4) emphasize that it is most difficult to formulate vitamin preparations in true sustained-release form.

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Kinetics of Copper Catalyzed Oxidation of 2,3-Dimercapto-1-propanol by Molecular Oxygen

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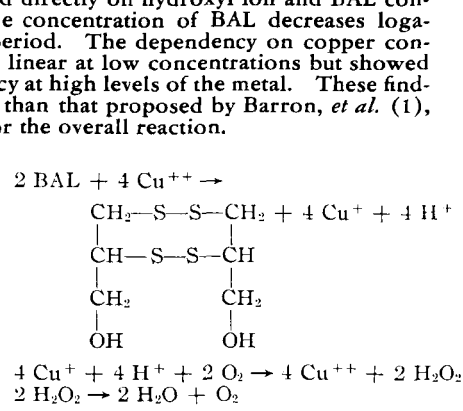
The rate of disappearance of the sulfhydryl groups of 2,3-dimercapto-1-propanol (BAL) in aqueous solutions in the presence of molecular oxygen and catalytic quantities of copper has been shown to depend directly on hydroxyl ion and BAL concentrations and on oxygen pressure. The concentration of BAL decreases logarithmically following a short induction period. The dependency on copper concentration was found to be approximately linear at low concentrations but showed an apparent limiting zero-order dependency at high levels of the metal. These findings suggest that some mechanism other than that proposed by Barron, *et al.* (1), must be responsible for the overall reaction.

BARRON, *et al.* (1), studying the oxidation of (BAL) by dissolved molecular oxygen in aqueous solutions in the presence of copper, manometrically, have reported that BAL and and similar dithiols apparently do not undergo oxidation by free oxygen in the absence of certain metal ions and that any observed oxidation in the apparent absence of these catalysts is due to residual trace quantities of metals (probably copper). They suggest a mechanism involving three stoichiometric reactions



Every dithol molecule oxidized, according to this mechanism, involves reaction with copper. Present findings based on iodometric determination of residual sulfhydryl groups appear to be in partial conflict with these suggestions. A possible alternate route is presented.

EXPERIMENTAL

Reagents.—Reagent grade mono- and dipotassium phosphate; 0.1 *N* iodine solution; 0.1 *N* thiosulfate solution; oxygen U.S.P.; catalase (Worthington); water redistilled from all glass apparatus; 2,3-dimercapto-1-propanol containing not more than 0.93% 1,2,3-trimercaptopropane.

Received September 11, 1961, from the School of Pharmacy, University of Minnesota, Minneapolis 14.
Accepted for publication September 28, 1961.
Based in part on a thesis submitted by E. G. Rippie to the Graduate School, University of Wisconsin, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
This investigation was supported in part by a grant from the Research Committe of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.
The authors wish to acknowledge the help given to us by Dr Albert A. Kondritzer, Chief of the Physiological Chemistry Branch, Army Chemical Center, Md., in carrying out these studies.
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