Human Availability Studies of Five Vitamins in Sustained Release Form

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THE most desirable method of obtaining water-soluble vitamins for human metabolic functions is by continuous intake in our foods in the manner intended by nature. In patients with known or suspected inadequate vitamin intakes or deficiencies brought about by other mechanisms,[1,2] the dietary intake of vitamins needs to be increased by supplementation. Since the metabolism and excretion of some of the water-soluble vitamins is very rapid, a number of clinicians routinely give known or suspected vitamin-deficient patients daily multiple dosages of the deficient vitamin.[3]

In an attempt to simulate multiple daily dosing of vitamins with a single dosage form of the mixture, a sustained release capsule was designed. The design of these capsules and the analytical procedure for testing them are described by Souder and Ellenbogen.[4] The *in vitro* release pattern for all the vitamins contained in the sustained release product was 10 to 35 per cent at the end of a half hour, 40 to 65 per cent at the end of two hours, 60 to 80 per cent at the end of four and a half hours, and not less than 80 per cent at the end of seven hours.

The sustained release capsules were tested physiologically against capsules containing the same amounts of vitamin in an uncoated form. The test was performed on healthy male medical students. The study was designed closely after that used originally by Melnick et al.,[5] who showed that in normal well nourished human subjects the urinary excretion of water-soluble vitamins or their derivatives is directly proportional to the quantity consumed. Melnick and his coworkers established that a linear dose-response relationship existed between urinary excretion and the dosage level of the most completely available forms of ascorbic acid, thiamine, riboflavin, and niacinamide.

The experiment was designed so that five of the ten volunteer subjects (group A) took the immediate release vitamin capsule while the other five (group B) took the sustained release capsule. Ten days after the capsules had been taken (fourteen days after the start of the experiment) patients in the two groups were crossed over so that those in group A took the sustained release capsule while those in group B took the immediate release capsule.

All volunteer subjects were maintained on a basal diet similar to that used by Melnick et al.[5] for three days prior to the administration of the test capsules and for the thirty-six hours of urine collection following dosage with the vitamin capsules. Meals were prepared at the hospital and the test subjects ate their meals in a group. No snacks or other vitamin preparations were allowed during the experimental periods nor were the subjects permitted to smoke.

The following schedule was followed in running the test: All ten volunteer subjects were placed on the basal diet for three days. At noon on the fourth day, five men received a single capsule of sustained release vitamins (group B), and the remaining five received a

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single capsule of the immediate release vitamins (group A).

From noon of the third day, after urine was voided and discarded, to noon of the fourth day a twenty-four hour basal urine sample was collected. From noon of the fourth day, when the vitamin capsules were taken immediately before the noon meal, urine samples were collected every three hours for twelve hours and then every twelve hours for thirty-six hours. All urine samples were collected over enough hydrochloric acid to give a final concentration of approximately 0.1 normal; 100 ml. aliquots were taken from each sample period and immediately frozen.

During the interval between the end of the urine collections (thirty-six hours after the administration of the vitamins) and the start of the second basal diet period (four days before the second administration of vitamins), the volunteer subjects were allowed to return to their usual diets.

Two weeks after the start of the first experiment the "crossover" experiment was conducted in exactly the same manner as the first experiment, except that the patients in group A, who had been given capsules of immediate release vitamins, were now given the sustained release vitamins, and those in group B, who had been given capsules of sustained release vitamins, were now given the immediate release vitamins. This procedure permitted each subject to serve as his own control, while the crossover procedure helped to balance the effects of dietary or laboratory procedures for each of the two experimental periods.

ANALYTICAL PROCEDURES

The urine samples were analyzed for thiamine, riboflavin, ascorbic acid, niacin, and pyridoxine.

Thiamine was determined by oxidation to thiochrome which was measured fluorometrically.[6] Riboflavin was also determined fluorometrically.[6]

Ascorbic acid was determined colorimetrically by the method of Roe and Kuether.[7]

Niacin was ascertained by determining the concentration of the two major metabolites of this vitamin, N′-methyl-3-carboxylamide-6-pyridone and N′-methyl nicotinamide. The pyridone was determined by an ultraviolet procedure utilizing a combination of the methods of Rosen, Perlzweig and Leder[8] and Price.[9] The N′-methyl nicotinamide was determined fluorometrically utilizing the condensation reaction of Huff and Perlzweig.[10]

Pyridoxine was determined by analyzing the urine for 4-pyridoxic acid, which is reported to represent approximately 96 per cent of the urinary excretion product from this vitamin.[11] This metabolite was determined by the fluorometric procedure of Huff and Perlzweig as modified by Sarett.[12]

VITAMIN CAPSULES

The two vitamin formulations, the sustained release capsule (multivitamin "Spansule" capsule*) and the immediate release vitamin capsule (powdered or crystalline vitamins) contained the following vitamins in each capsule: ascorbic acid, 167 mg.; thiamine (as mononitrate salt, U.S.P.), 6.9 mg.; riboflavin (as 5′-phosphate sodium salt), 6.8 mg.; niacinamide, U.S.P., 68.8 mg.; pyridoxine (as hydrochloride salt, U.S.P.), 6.6 mg.; pantothenic acid (as dl-panthenol), 3.38 mg.; cyanocobalamin (crystalline, U.S.P.), 13.9 μg.; vitamin A (as palmitate ester), 16,500 U.S.P. units; and vitamin D, 1,500 U.S.P. units.

RESULTS

The average urinary excretion of each vitamin for the whole group of ten subjects on the immediate release preparation and on the sustained release preparation, and evaluations of the significance of the differences in urinary excretion of vitamins from the two formulations studied, are presented in Table 1.

From these values the availability of the sustained release preparation can be calculated on the basis of 100 per cent availability for the immediate release vitamin sample. The for-

* Fortespan,® Smith Kline & French Laboratories.

TABLE

Average Urinary Vitamin Excretion Values for Ten
(mg. of vitamin

Vitamin	Preparation (release)	Pre-Vitamin (control 24 hours)	Post-Vitamin (hours after	
			0–3	3–6
Riboflavin	Immediate	1.39	1.70	1.27
	Sustained	1.22	0.57	1.41
		Difference*	−1.13±0.23	0.14±0.15
		P†	‡	N.S.
Pyridoxine	Immediate	2.07	0.52	0.65
	Sustained	1.79	0.29	0.71
		Difference*	−0.23±0.07	0.06±0.11
		P†	‡	N.S.
Thiamine	Immediate	0.38	0.26	0.35
	Sustained	0.31	0.14	0.55
		Difference*	−0.12±0.03	0.20±0.08
		P†	‡	N.S.
Niacin	Immediate	21.7	12.2	13.7
	Sustained	20.1	5.5	12.8
		Difference*	−6.7±1.56	−0.9±0.84
		P†	‡	N.S.
Ascorbic acid	Immediate	19.1	12.5	17.1
	Sustained	17.8	6.9	17.3
		Difference*	−5.6±3.3	0.2 ±4.5
		P†	N.S.	N.S.

NOTE: N.S. $P > 0.05$ nonsignificant.

* Average quantitative difference in vitamin excreted during period indicated and standard error of the mean difference; negative signs used when vitamin excretion from immediate release form is greater than from sustained release form of vitamins administered.

mula used for these calculations over a specified time period is as follows:

$$\frac{\text{mg. excreted from sustained release} - \text{mg. excreted from control}}{\text{mg. excreted from immediate release} - \text{mg. excreted from control}} \times 100 = \text{per cent availability.}$$

The cumulative results of these calculations for each vitamin at the end of twelve, twenty-four- and thirty-six-hour periods are presented in Table II.

The logarithms of the urinary excretions (minus the calculated control value for each period) are presented as curves for the first twelve hours (Figs. 1 through 5). Control values for each three-hour period were calculated to be one-eighth the amount of vitamins excreted in the twenty-four-hour urine specimen collected before the test.

COMMENTS

Urinary excretion results are interpreted to be a reflection of the level of absorption of the vitamins. The results of Melnick et al.,[5] in which urinary vitamin levels increased linearly in the well fed healthy person as the intake of the vitamins increased, helps to substantiate this conclusion.

Urinary excretion studies are usually presented as "percentage availability" according to the method of calculation shown in Table II. Our excretion data are also presented as differences in vitamins excreted after ingestion of the two test forms (Table I) which are compared by an analysis appropriate to our cross-over design. The differences in vitamin excretions in Table I are based on the assumption that baseline (control) excretion values are equal for the two groups during any time period. The twenty-four-hour control periods before the administration of vitamins revealed no significant differences between the two test groups for any of the five vitamins tested.

I
Human Subjects Obtained in Crossover Study
per collection period)

Levels (mg.) administration)		Total Urinary Excretion (mg.) (hours after administration)		
6–9	9–12	0–12	0–24	0–36
0.61	0.29	3.87	5.31	6.36
0.84	0.41	3.23	4.56	5.68
0.23±0.09	0.12±0.04	−0.64±0.27	−0.75±0.36	−0.68±0.49
§	§	§	N.S.	N.S.
0.56	0.27	2.00	3.09	4.24
0.62	0.55	2.17	3.51	4.54
0.06±0.09	0.28±0.04	0.16±0.19	0.41±0.25	0.30±0.28
N.S.	‡	N.S.	N.S.	N.S.
0.17	0.08	0.86	1.15	1.41
0.34	0.16	1.19	1.51	1.80
0.17±0.05	0.08±0.02	0.33±0.10	0.36±0.13	0.39±0.43
‡	‡	§	§	N.S.
10.6	5.7	42.2	64.3	79.7
9.5	7.3	35.1	58.6	74.0
−1.1±0.99	1.6±0.57	−7.1±1.5	−5.8±1.4	−5.7±2.2
N.S.	§	‡	‡	§
11.9	4.8	46.3	72.4	95.8
16.7	9.0	49.9	67.2	91.2
4.8±3.8	4.2±3.1	3.6±14.2	−5.2±14.2	−4.6±18.0
N.S.	N.S.	N.S.	N.S.	N.S.

† "P" calculated as two-sided test of significance.
‡ P ≤ 0.01 highly significant.
§ P ≤ 0.05 significant.

In Table II are presented the twenty-four-hour vitamin availabilities which have been calculated directly from excretion data. Availability calculations for twelve- and thirty-six-hour collections are included for interest only and are based respectively on estimated control values calculated as a half and one and a half times the twenty-four-hour control urinary excretions. Statistical studies of the twenty-four-hour availabilities reveal that of the five vitamins studied only thiamine availability from the sustained release capsules differed from the immediate release form ($P < 0.01$).

The availability data (Table II) reveal that all the vitamins studied are equally or more completely available from the sustained release capsule than from the immediate release. Riboflavin, niacin, and pyridoxine availabilities show some increase between twelve and twenty-four hours which would seem to be a reflection of further sustained release

TABLE II
Percentage Availability* (Comparison of Sustained Release to Control) of Vitamins in Sustained Release Capsule as Measured by Urinary Excretion Studies

Vitamin	Availability (%) for Time Urine is Collected		
	12 hr.†	24 hr.	36 hr.‡
Riboflavin	82.6	85.2	90.0
Pyridoxine	132.3	168.6	163.4
Thiamine	153.7	155.8	158.9
Niacin	80.2	90.4	93.0
Ascorbic acid	111.7	92.7	96.0

* $\dfrac{\text{mg. excreted from sustained release} - \text{mg. excreted from control}}{\text{mg. excreted from immediate release} - \text{mg. excreted from control}} \times 100.$

† Excretion for twelve-hour control period calculated as one-half previtamin control twenty-four-hour excretion level.

‡ Excretion for thirty-six-hour control period calculated as 1.5 × previtamin control twenty-four-hour excretion level.

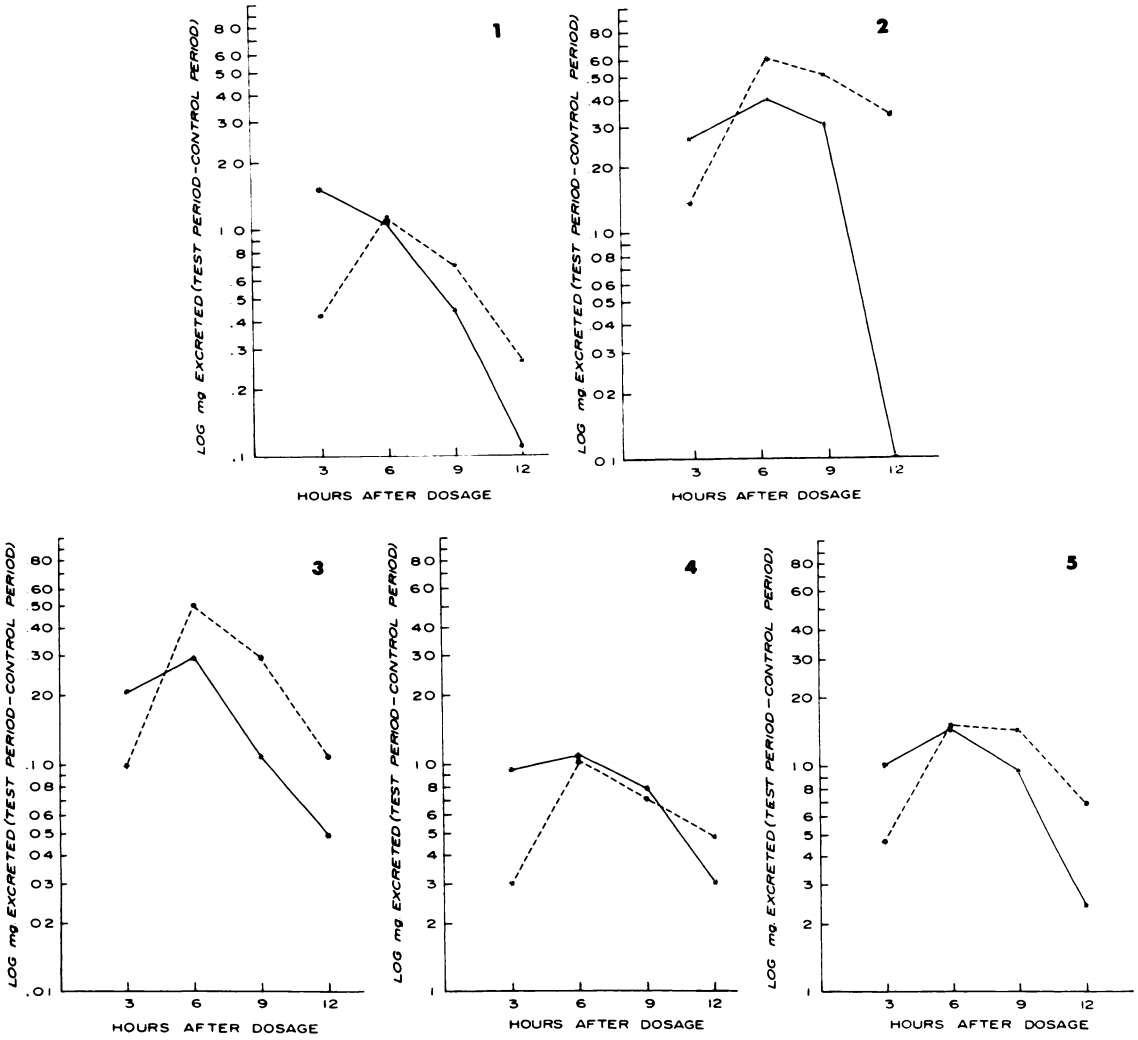

FIG. 1. Riboflavin. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

FIG. 2. Pyridoxine. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

FIG. 3. Thiamine. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

FIG. 4. Niacin. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

FIG. 5. Ascorbic acid. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

effect. The stabilization of values, except for riboflavin which continued to increase after twenty-four hours, indicates that this action is no longer operational after this time.

Data published by Friedemann et al.[13] concerning the absorption of orally administered thiamine to man offers a logical explanation for our finding that the absorption of thiamine in the sustained release form is significantly increased ($P<0.05$ as calculated in Table I and <0.01 as calculated in Table II)

over that obtained from a single dose of immediate release thiamine. These authors found that thiamine is poorly absorbed in man and that the maximum which could be taken orally without an increase in the fecal excretion of thiamine was slightly less than 2 mg. at each meal or single ingestion. Since, as these authors indicate, there seems to be a very efficient barrier mechanism for thiamine absorption in the intestinal tract, the administration of thiamine in the sustained release form may help overcome this barrier by presenting the vitamin for absorption more slowly and thereby permitting greater absorption than from a single dose of immediate release vitamins.

The explanation for the increased availability of pyridoxine from the sustained release form of vitamins is not readily apparent since pyridoxine is reported to be easily absorbable.[14] Whether the increased availability of pyridoxine (only approaching significance in both tables) is as real as that of thiamine seems to be, or is a reflection of alteration of the percentage of 4-pyridoxic acid formed and excreted in the urine, cannot be judged from these data.

The results of average urinary excretion levels of the vitamins reported in Table I clearly show that larger amounts of the vitamins were excreted into the urine by the subjects on the immediate release vitamin capsule than by those on the sustained release vitamin capsule during the first three hours of urine collection. Conversely, during the nine- to twelve-hour collection period larger amounts of vitamins were excreted by those receiving the sustained release capsules than by those receiving the immediate release vitamin capsules.

The urinary excretions of the five vitamins tested are plotted as semilogarithmic curves, as suggested by Swintosky et al.[15] (Figs. 1 through 5) and are based on the three-hour collection periods from the time the capsules were taken to the end of the first twelve hours thereafter. Similar patterns of urinary excretion were exhibited by all five of the vitamins studied (riboflavin, pyridoxine, thiamine, niacin, and ascorbic acid).

The urinary excretion data are presented with a statistical analysis for differences in excretions of vitamins for each three-hour period from zero to twelve hours and for total excretion of zero to twelve, zero to twenty-four and zero to thirty-six hours (Table I). These data show clearly that significantly more riboflavin, pyridoxine, thiamine, and niacin, but not ascorbic acid, were excreted during the first three hours by those who took the immediate release capsules than by those who took the sustained release capsules. Conversely, during the last three-hour period (nine to twelve hours) significantly more riboflavin, pyridoxine, thiamine, and niacin, but not ascorbic acid, were excreted by those receiving the sustained release capsules than by those receiving the immediate release vitamin capsules. These results indicate a significant sustained release effect for four of the five vitamins measured and at least equal availability of ascorbic acid from the two vitamin forms (immediate release and sustained release). Although niacin was calculated to be "statistically" more available from the immediate release form of the vitamin than from the sustained release form (as calculated from Table I but not from Table II), this effect was due to the low degree of variability of patient to patient results and is of little practical importance since at the end of twenty-four hours the average urinary excretion levels of niacin from the immediate and sustained release vitamin capsules were 64.3 and 58.6 mg., respectively.

Extremely high standard deviations of urinary levels of ascorbic acid made evaluation of any sustained release effects of this vitamin impossible. Four of the ten subjects failed to show any appreciable increase in urinary excretion levels of ascorbic acid on either the immediate or sustained release forms of the vitamin. Subsequent studies in this laboratory have shown that saturation of the test subjects with ascorbic acid was probably not attained on the diet used. In order to use urinary excretion as an adequate index of ascorbic acid absorption, preliminary tests should be used to show that experimental subjects are saturated with the vitamin. In view of our re-

sults with ascorbic acid, special attention should be paid to the degree of saturation of test subjects on any vitamin assayed for availability by the method used here.

Careful control of experimental variations have made statistical analyses of these data possible; however, in a relatively small population study, such as this, data should be judged more by their practical or medical significance than by the results of statistical tests. The over-all results of these studies reveal that sustained release of vitamins is possible and that the availability of vitamins from the sustained release form are comparable, and in the case of thiamine, superior to that of the immediate release form.

SUMMARY

The availability and pattern of absorption of five vitamins, as measured by urinary excretion (thiamine, riboflavin, ascorbic acid, niacin, and pyridoxine) from immediate and sustained release forms, were studied in a crossover experiment with ten healthy human subjects.

Based on a twenty-four-hour urine collection the availabilities of sustained release vitamins were calculated as the following percentages of those from the immediate release formulation: riboflavin, 85.2; pyridoxine, 168.6; thiamine, 155.8; niacin, 90.4; and ascorbic acid, 92.7.

All vitamins except ascorbic acid showed significant sustained release effects when administered in a sustained release formulation and compared with equal amounts of vitamins administered in an immediate release form. Ascorbic acid studies suffered from a lack of adequate saturation of the test subjects with the basal diet used.

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