I. Introduction

To maintain a healthy state for a long life, many healthy people in developed countries prefer to continually take vitamins. In past, there have been many reports on the bioavailability of individual vitamins at a large dose and single administration. These are however unsuitable for understanding the efficacy and bioavailabilities of individual vitamins in view of recent real situation of general vitamin intakes. Usually, several vitamins are together taken as a multivitamin-preparation continuously over a long period.

When considering single vitamin intake for a long period, it is useful to know whether the baseline of blood vitamin levels changes, and whether a dose response appears. In addition, it may be important to know the problem of individuals with intakes of vitamin preparations with regard to the assessment of the nutritional status of vitamins. This also includes the danger of toxicity regarding the fat soluble vitamins A, D and E. However, there have been no reports, to our knowledge, on the bioavailabilities of the usual daily dose of vitamin preparations over a long period. From the above view, Takeda Chemical Industries who sponsor this symposium, promoted an investigation on the bioavailabilities of vitamins with long term intake of a multivitamin preparation for 44 weeks. Some of results of this study will be here.

II. Experiments:

The multivitamin preparation used listed in Table 1. The content of each vitamin was not high compared with the RDA values. This paper will show vitamins C, B6, nicotinamide, vitamins A and E. A randomized, placebo-controlled study on the bioavailabilities of vitamins with long-term intake of a multivitamin preparation for 44 weeks was carried out. Two dose levels and a placebo were examined to understand the dose responses. The pre-administration and post-administration study were also performed.

Table 1.

<table>
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<tr>
<th>Multivitamin Tablet Composition</th>
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<tr>
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<tr>
<td>Vitamin A (retinyl palmitate)</td>
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<td>Vitamin D2 (ergocalciferol)</td>
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<td>Vitamin E (d-α-tocopheryl acetate)</td>
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<td>Thiamin tetrahydrofurfuryl disulfide (TTFD)</td>
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<tr>
<td>Vitamin B2 (riboflavin)</td>
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<td>Vitamin B6 (pyridoxine hydrochloride)</td>
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<tr>
<td>Nicotinamide</td>
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<tr>
<td>Vitamin C (ascorbic acid)</td>
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<td>Calcium pantothenate</td>
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<td>Vitamin B12 (cyanocobalamine)</td>
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III. Results and Discussion

To understand difference in bioavailability of a vitamin, the same dose was taken either as a single vitamin preparation or together with other vitamins as a multivitamin preparation, a study was undertaken on vitamin C as
an example[1]. No differences were observed in the bioavailability between two forms of vitamin C. The ongoing study was then performed using only the multivitamin preparation.

1. Vitamin C[2]: Fig 1 shows the vitamin C changes in plasma and urine during administration. There were marked increases in the plasma concentration and urinary excretion at 4 weeks, followed by further gradual increases during the experimental period at both doses. These levels were significantly and effectively higher than in the placebo group. Dose-dependent differences in plasma concentrations and urinary excretion among the three groups were statistically significant by linear regression analysis. In this examination, no withdrawal effect was observed. Body saturation of vitamin C has been proposed at plasma level of 1.4 mg/100 ml. From the results of this study, body saturation may be reached with a daily dose of 500 mg in addition to dietary intake.

In the preliminary study [3] (data not shown), the time course of blood vitamin level was examined after single administration and daily administration for one week. This result shows that the profile of plasma vitamin C changes was observed in a similar pattern after administration of a single dose and the 7th day of administration in daily intake. However, the baseline level of plasma vitamin C was increased on the 7th day, as compared with the initial level. This was confirmed again in this study. Urinary excretion increased on the 7th day, reflecting the plasma baseline levels. The above finding indicates that the body must be saturated with vitamin C after repeated intake of the vitamin.

Seasonal nutrition survey of the dietary intake of vitamins A, B1, B2, nicotinamide and C was performed in this study. No significant changes in dietary intake of these vitamins were observed throughout the experimental period.

2. Vitamin B6[4]: A similar pattern to vitamin C was observed for vitamin B6. Plasma levels increased during the first 4 to 12 weeks of intake which were followed by a plateau level during further administration, while urinary excretion of 4-pyridoxic acid, a metabolite of vitamin B6, slightly increased throughout the administration periods. Since the requirement of vitamin B6 depends on protein intake, it was assumed to be about 2 mg/day for a Japanese adult[5]. The doses in this study corresponded to 3.4 times (in group of 9 mg/day) and 6.7 times (in group of 18 mg/day) higher than the level of the vitamin B6 requirement. The body pool of vitamin B6 is thought to saturate as the plasma vitamin B6 level reaches a steady state. Dose-dependent differences
were also statistically significant in plasma concentrations and urinary excretion among the three groups. From the results of this study, the body pool of vitamin B6 may be increased by increasing doses because of the finding of only a slight increase in urinary excretion of vitamin B6 with continuous intake of the vitamin. In addition, the ordinary doses of vitamin B6 as shown in group of 9 mg/day, will be recommended because general intake of vitamin B6 from the diet, did not seem to satisfy RDA in several reports [6] and there may be many common drugs usually used including contraceptives which contribute to an abnormality of vitamin B6 metabolism.

3. Nicotinamide[4]: Nicotinamide in whole blood shows a different pattern from vitamins C and B6. No significant changes occurred in the whole blood level throughout the administration period at both doses. The urinary excretion of N1'-methyl nicotinamide, a metabolite of nicotinamide, increased after administration and the value slightly increased throughout the administration period and fell rapidly to the baseline level one week after cessation. A dose response was clearly found in urinary excretion while it was not seen in the whole blood levels. As previously shown in our preliminary study [7], the metabolic turnover rate of nicotinamide was found to be enhanced by repeated intake. The urinary excretion indicates enhancement of nicotinamide metabolism rate during continuous administration. In this study, nicotinamide for metabolism comes from intake of the vitamin itself and metabolically from tryptophan in ingested foods. From the nutritional survey, we can calculate the nicotinamide levels from ingested tryptophan. A correlation between total nicotinamide from the preparation and from tryptophan, and excretion levels of N1'-methyl nicotinamide was then studied. A linear correlation bound to 0 point was observed. From this finding based on excretion of nicotinamide, the levels of ingested nicotinamide used in this experiment shows similar bioavailability at both doses.

4. Vitamin A: Vitamin A is known to cause toxicity such as hypervitaminosis A, in which the plasma retinyl ester level is reported to increase [8]. In RDA research, the vitamin A requirement is known to depend on liver storage of the vitamin [9]. In this study, the plasma retinol level showed no changes with daily intake of 4,000 IU other than dietary intakes. In addition, no peaks corresponding to retinyl ester in HPLC patterns were observed. Therefore, no danger of toxicity was observed during the experimental period indicating that the nutritional status of vitamin A will be enhanced, since liver storage of retinyl ester will probably increase to a non-dangerous level.

5. Vitamin E: Differing from the retinol pattern, plasma alpha-tocopherol
levels increased with daily intake of 20 mg, but not the 10 mg of dl-alpha-tocopheryl acetate, the dose of which was accompanied by slight lowering of the gamma tocopherol level, while no difference was observed between the placebo group and the 10 mg administration group. If alpha-tocopherol levels become sufficient, plasma gamma-tocopherol is known to decrease accompanied with an increase in the plasma alpha-tocopherol level (10). This indicates that in daily ingestion of 20 mg dl-alpha-tocopheryl acetate the vitamin E status in the body will improve without adverse effects.

6. Side effects and toxicity: Standard clinical screening tests were designed to examine hepatic function, renal sufficiency, the neuromuscular system and hematologic normality. On the basis of this result, it must be concluded that no signs of toxicity were demonstrated in this study.

IV. Conclusions:
Recently, from the prospective survey of human nutrition and diseases in the elderly, it has been emphasized that prevention of development of aging diseases including ischemic heart diseases and cancer might be closely related to enhancement of the nutritional status of these vitamins A, C and E [12]. A significant enhancement of the nutritional status of vitamins C, B6 and E was assessed in a 44 weeks administration study using a daily dose of 250-500 mg vitamin C, 9-18 mg vitamin B6 and 20 mg vitamin E. Plasma retinol levels at daily doses of 2,000 and 4,000 IU in addition to dietary intake during the experimental periods did not change and stayed the recommended adequate level. This may assist the goal of enhancement of vitamin A status. The metabolic turnover of nicotinamide was accelerated with a daily doses of 75-150 mg. Neither side effects nor toxicity were documented in all the volunteers including the placebo group on the basis of the finding of the questionnaire and the clinical screening test.

Fig 3. Changes in plasma alpha- and gamma-tocopherol levels after administration.
adult males. Vitamin (Japanese) 64, 37-43.


