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Thiamin and Pyrimidine Studies on Older Subjects.*†

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Most of the studies on human vitamin requirements have been conducted on young adults or children. Very little is known regarding the vitamin requirements with advancing age. The favorable reports of the clinical application of vitamins, particularly thiamin, in geriatrics raise the question as to why such deficiencies exist. Is it reduced intake, inability to utilize, or an increased need? As a first step toward understanding this problem, a study of daily urinary excretion of thiamin in reasonably healthy older subjects was undertaken.

In this study, 4 men and 4 women were used as experimental subjects. In order to determine the state of health of the individuals, a physician‡ examined each one before and after the experimental period. All members of the group were ambulatory and in the seventh or eighth decade of life.

It was impossible to control the type or amount of food eaten by the subjects, but complete dietary records were kept during the period of the experiment. The thiamin content of the daily diets was calculated, using the tables of Munsell[1] and Booher.[2]

The experimental period of approximately 3 weeks was divided into 3 parts: A preliminary period on the usual diet, a second period during which the diet was supplemented with 2 mg of thiamin daily, and a final period during which the supplement was 4 mg of thiamin daily. The thiamin was given orally in the form of one milligram tablets at mealtime. Complete 24-hour urine samples were collected daily and analyzed for their thiamin and pyrimidine content by the fermentation method of Schultz, Atkin, and Frey.[3]

Experimental Results and Discussion. The data concerning the experiment are summarized in Tables I and II.

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‡ We wish to acknowledge the coöperation of Dr. E. M. Holden, Amherst, Mass.

1 Munsell, H. E., *Milbank Memorial Fund Quart.*, 1940, **18**, 311.

2 Booher, L. E., and Hartzler, E. R., *U. S. D. A. Bull.*, No. 707, 1939.

3 Schultz, A. S., Atkin, L., and Frey, C. N., *J. Biol. Chem.*, 1940, **136**, 713.

TABLE I.
Thiamin and Pyrimidine Studies.
Average Daily Values for Thiamin Intake and Thiamin and Pyrimidine Excretion.

♀ Age	Wt Ht	Days of exper.	Thiamin intake Suppl. Gamma	Thiamin intake Diet Gamma	Thiamin excretion Gamma	Thiamin excretion % of intake	Pyrimidine* excretion Gamma	Thiamin retention† Gamma	Thiamin retention† % of intake
A.P.	130 lb	1-6	0	802	82	10.2	214	505	62.9
75 yr	5′ 4″	7-12	2000	859	246	8.6	343	2270	78.6
		13-16	2000	658	542	20.3	542	1810	68.1
		17-23	4000	829	509	10.5	463	3857	79.8
L.M.	150 lb	1-7	0	749	76	11.3	137	536	71.5
75 yr	5′ 1″	8,9	2000	842	138	4.8	232	2472	86.9
		10-13	2000	738	386	14.1	246	2106	76.9
		14-16	4000	1111	655	12.8	569	3887	76.0
A.W.	165 lb	1-8	0	620	81	13.1	220	319	51.5
68 yr	5′ 7″	9-13	2000	424	370	15.2	449	1605	66.2
		15,16	2000	630	445	16.9	561	1624	61.7
		17-26	4000	573	1110	24.2	757	3708	81.1
M.M.	121 lb	1-6	0	798	96	12.0	209	493	61.8
66 yr	5′ 3″	8-11	2000	835	234	8.2	305	2296	80.9
		12-15	2000	695	465	17.2	296	1934	71.8
		16-21	4000	794	603	12.5	397	3794	79.1

*Expressed in thiamin equivalents.
†Thiamin retention = thiamin intake — thiamin and pyrimidine excretion.

TABLE II.
Thiamin and Pyrimidine Studies.
Average Daily Values for Thiamin Intake and Thiamin and Pyrimidine Excretion.

♂ Age	Wt Ht	Days of exper.	Thiamin intake Suppl. Gamma	Thiamin intake Diet Gamma	Thiamin excretion Gamma	Thiamin excretion % of intake	Pyrimidine* excretion Gamma	Thiamin retention† Gamma	Thiamin retention† % of intake
F.K.	162 lb	1-8	0	986	169	17.2	219	598	60.6
70 yr	5′ 10″	10-15	2000	1003	246	8.1	356	2401	80.0
		16-19	2000	995	478	13.3	749	1768	59.0
		22-25	4000	1069	1120	22.0	551	3398	67.0
F.S.	148 lb	1-7	0	1020	261	25.5	219	540	52.9
74 yr	5′ 6″	8,9	2000	842	138	4.8	232	2472	86.9
		10-13	2000	738	386	14.1	246	2106	76.9
		14-16	4000	1111	655	12.8	569	3887	76.0
F.M.	152 lb	1-7	0	1224	136	11.1	324	764	62.3
73 yr	5′ 10″	8-10	2000	892	337	11.6	321	2234	77.3
		11-13	2000	892	366	12.6	488	2038	71.3
		14-16	4000	1268	740	14.0	650	3878	73.5
F.W.	155 lb	1-8	0	839	99	11.8	229	511	61.0
70 yr	5′ 8″	9-13	2000	681	377	14.7	386	1918	71.5
		15,16	2000	636	629	23.9	437	1570	59.2
		17-25	4000	841	1409	29.1	710	2722	56.4

*Expressed in thiamin equivalents.
†Thiamin retention = thiamin intake — thiamin and pyrimidine excretion.

It may be seen that the thiamin content of the average daily diet for this group ranges from 620 μg to 1224 μg. This range corresponds very well with the values given by Melnick, Field, and Robinson[4] for the dietary intake of normal subjects from 25 to 35 years of age.

On the diet alone, the average daily excretion of thiamin by the women ranged from 76 μg to 96 μg; for the men, from 99 μg to 261 μg. There was considerable variation from day to day in the amount of thiamin excreted by the same individual and between individuals on essentially the same level of thiamin intake. It seems improbable that a single measure of the thiamin excretion of an individual on any one day would be a reliable index of his usual excretion. The average dietary thiamin intake for the men was higher than that for the women, which accounts in part for the larger amounts excreted. For 2 of the men, the percentage of ingested thiamin excreted was significantly higher than that for the women. This observation confirms the experience of other workers.

In all cases, when a 2 mg supplement of thiamin was given, there was a marked increase in the amount of thiamin excreted. However, the response of the individual subjects was not uniform. Six out of 8 subjects excreted about one-half to one-third as much thiamin during the first few days of the "2 mg" period as they did during the latter part of this period. This reaction might indicate that there was a definite need of the body for more thiamin than it had been receiving; the larger amount being excreted after an equilibrium had been established. The rise in the latter part of the "2 mg" period manifested itself sharply instead of being a gradual increase. The time when the rise occurred varied with the individual, ranging from the third to the seventh day. Two cases, A. W. and F. M., showed only slightly less excretion during the earlier than during the latter part of the "2 mg" period. A break was made arbitrarily in this period for these 2 people for comparison with the other subjects.

It was expected that thiamin excretion would increase when the daily diet was supplemented with 4 mg of thiamin. Four subjects, F. M., A. P., A. W., and M. M., excreted the same or smaller amounts of thiamin on the first day of the "4 mg" period than they did on the preceding day (last day of the "2 mg" period). This may indicate that there is some lag in the excretion of the ingested thiamin, although it did not manifest itself in the other 4 cases in which excretion increased immediately. The percentage of ingested thia-

[4] Melnick, D., Field, H., Jr., and Robinson, W. D., *J. Nutr.*, 1939, **18**, 593.

min excreted was not much greater than on the previous level of intake and, in some cases, it was less.

The presence of 2-methyl-5-ethoxymethyl-6-aminopyrimidine, a fraction of the thiamin molecule, in the urine is of interest and may be of more significance than we know at present. The pyrimidine may be present in the food as such and results indicate that it is also a breakdown product in the metabolism of thiamin. With increase in the thiamin intake, the pyrimidine fraction increased considerably in the urine although less than the thiamin fraction. The amount of urinary pyrimidine was remarkably constant irrespective of sex, and was usually greater than the urinary thiamin on the lower levels of intake. If the normal pyrimidine excretion can be considered above 200 μg, one subject, L. M., falls appreciably below normal. It is interesting to note that this subject showed the lowest initial response to the 2 mg supplement of thiamin. Applying the interpretation of Pollack,[5] that low pyrimidine and thiamin values indicate a protracted insufficiency of vitamin B_1, one might assume that a deficiency had existed in this case. However, this subject experienced no beneficial subjective effects from the increased thiamin intake.

If it is assumed that both the urinary thiamin and pyrimidine may be derived from dietary thiamin, the sum of these 2 fractions subtracted from intake may be considered as the amount of thiamin retained or destroyed. When this "retention" figure is calculated, it appears to increase more nearly in proportion to intake than does the excretion (see table). However, a large portion of this thiamin may be destroyed in digestion and absorption. Preliminary work indicated that, at the levels of thiamin used in this study, the loss in the feces was negligible. It would seem impossible to determine by measuring the amount excreted in the urine how much of a given amount of thiamin ingested was acutally used or stored by the body tissues.

Clinical and Subjective Observations. The physical examination of the subjects before the experiment indicated that they were all in good health and showed no evidence of any thiamin deficiency. Reëxamination after the completion of the experiment showed no changes that could be measured clinically.

The subjects were asked to note any change in their general well-being during the experimental period. Two of the 8 subjects noticed no difference in how they felt. Of the remaining 6, 2 noted definite

[5] Pollack, H., Ellenberg, M., and Dolger, H., PROC. SOC. EXP. BIOL. AND MED., 1941, 47, 414.

improvement in chronic constipation, 4 felt less fatigued or were "peppier", 2 enjoyed improved appetites, and one noted an increased thirst.

Summary. 1. The thiamin and pyrimidine excretion in the urine of older subjects, after different levels of thiamin intake, was measured by the fermentation method of Schultz, Atkin and Frey. 2. There appears to be a sex difference in the excretion of thiamin which is not apparent in the excretion of pyrimidine. 3. The response of people in this age group to increased thiamin intake is similar to that of younger people so far as excretion is concerned. 4. "Retention" calculated as thiamin intake minus thiamin plus pyrimidine excreted, was more nearly proportional to intake than was the amount excreted. 5. Six out of the 8 subjects reported some type of subjective reaction to the increased thiamin intake. Physical examinations before and after the experimental period revealed no significant changes.

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Response of Intraocular Prostatic Implants in Rabbit to Gonadotropic Hormone Administration.*

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Recent work by Krichesky, *et al.*, has demonstrated the effect of testosterone propionate on prostatic implants in the eye of the male rabbit, and has confirmed earlier reports that maintenance of the transplanted accessory tissue is dependent on the normal functioning of the testes, since castration results in atrophy of the transplanted tissue.[1] In undertaking a similar experiment in which 2 different gonadotropic agents were administered, a consistent response was observed which, to the writers' knowledge, has heretofore been unreported.

Fifteen male rabbits from 4 to 6 months of age were completely

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† B. Rosenberg and B. Krichesky, Ph.D., from the Department of Zoology, University of California, Los Angeles; J. A. Benjamin, M.D., Portland, Oregon.

1 Krichesky, B., Benjamin, J. A., Belt, E., and Schwartz, M., *J. Urology*, 1941, **46**, 303.