volume, every tenth section was used. From these studies no evidence emerged that the hormone caused hypertrophy of the cortex or changes in the cortical cells.

In conclusion, injecting gonadotrophin under the skin of fetal rats resulted in an increase in the size and number of interstitial cells of Leydig. While it seems likely that these cells secreted more androgen than normally, positive evidence of this was not obtained. The injections failed to cause any significant changes in the ovary and the adrenal.

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Urinary Excretion of Thiamine as a Characteristic of the Individual.*

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The urinary excretion of thiamine has been used as a measure of the intake and of the body stores of this vitamin. That there is some relation between these functions is shown by the fact that an increase in the intake produces an increase in the excretion. It is usually assumed that individuals on the same intake will excrete approximately the same amount of thiamine. There are very few reports in the literature which provide detailed data for individuals who have been maintained for any length of time on a constant vitamin intake. Consequently the individual variation, and hence the reliability of the estimation of intake from individual excretion, is practically unknown.

In the course of investigations on the response of normal young men to various levels of thiamine intake,[1] a marked variation between the urinary thiamine excretion of different individuals was observed. The inter- and intra-individual variations in the urinary thiamine excretions are considerable at intake levels from 0.6 to 2.0 mg per day.[2] In spite of the great intra-individual variation, there was an indication that over a period of time, the thiamine excretion level was highly characteristic of the individual.

Experimental. Ten different menus were used consecutively in this work. They all contained the same amount of thiamine (0.50 ± 0.07 mg per day) and were reasonably constant and "normal" in composition of carbohydrate, fat and protein. We found that in man large increases in the dietary content of carbohydrates, fats or proteins have no appreciable influence on the dietary excretion of thiamine.[3] In order to determine whether the marked variations in the daily thiamine excretion were due to variations in the dietary content of foodstuffs other than the above, we fed our subjects the same diet on 3 successive days. Most of the items in these diets, including the meat, were canned or dehydrated. Where this was not the case, large quantities of the food items were secured at the start of the experiment to insure uniformity in the diet. All food was served in weighed portions. An exact duplicate of all foods served during the day was saved for thiamine analysis.

* Work done in part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of Minnesota. Important assistance was also provided by the National Dairy Council operating in behalf of the American Dairy Association, Chicago, Ill., the Sugar Research Foundation, New York, N.Y., and the Nutrition Foundation, New York, N.Y., Merck and Co., Inc., Rahway, N.J., and Abbott Laboratories, North Chicago, Ill., provided a generous supply of vitamins. Most of the food materials were supplied by the Subsistence Branch, Office of the Quartermaster General, U. S. Army, and the J. R. Simplot Dehydrating Co., Caldwell, Idaho.

1 Keys, A., Henschel, A., Taylor, H. L., Mickelsen, O., and Brozek, J., *Am. J. Physiol.*, 1945, **144**, 5.

2 Mickelsen, O., Caster, W. O., and Keys, A., in press.

3 Mickelsen, O., Caster, W. O., and Keys, A., unpublished data.

TABLE I.

Thiamine excretion of normal young men maintained on the same diet for 3 days. The first 6 men received 1.0 mg thiamine per day while the others received 2.0 mg. All values for thiamine are expressed as μg of thiamine per day.

Subject	Urinary excretion				Stools
	Dec. 26	Dec. 27	Dec. 28	Mean	
A	118	75	79	91	540
Pa	36	21	39	32	510
E	60	48	53	54	800
M	85	83	86	85	550
S	55	46	49	50	720
R	62	52	60	58	666
Mean	69	54	61	62	631
D	276	294	359	310	1,090
C	82	87	88	86	490
B	240	141	158	180	1,240
W	128	193	109	143	960
Pe	114	128	98	113	1,840
H	148	122	138	136	—
Mean	165	161	158	161	1,124

The subjects in these experiments were 12 normal young men who had already been on the dietary regime for about 30 days. This length of time is required to secure a state of equilibrium in the urinary thiamine excretion.[2] Six of the men were given thiamine pills to raise their total daily intake to 1.0 mg while the other 6 were likewise increased to 2.0 mg. Placebos were given to make certain that all men received the same number of pills. The physical activity and mode of life for some time previous to and during this period were maintained reasonably constant. On each of the 3 days of this experiment, 24-hour urine samples were collected in bottles containing glacial acetic acid and toluol as preservatives. Stool samples, delineated by means of carmine, were also collected. The thiamine content of the urine and stools (total thiamine) was determined by our modification of the thiochrome technic.[4] Pyramin (the pyrimidine-like component of thiamine excreted in the urine) was determined by a modification of the yeast fermentation method for the estimation of thiamine.[5]

Results. The urinary and fecal excretions of thiamine are given in Table I. There is a considerable range in the urinary thiamine excretions of the men on each of the levels of intake. For the group on 1.0 mg of thiamine per day, the mean values range from 32 to 91 μg while for those on 2.0 mg they range from 86 to 310 μg. This variation in daily urinary thiamine excretion is not correlated with the fecal thiamine content. Najjar and Holt[6] suggested that the bacterial synthesis of thiamine in the lower gastro-intestinal tract could contribute to the body's requirement as shown by the absorption of thiamine from a retention enema. Using quantities of thiamine comparable to those normally excreted in the stools, Alexander and Landwehr[7] were unable to find any indications of thiamine absorption after a retention enema was given. Even if the thiamine synthesized by the bacteria in the lower gastro-intestinal tract is absorbed, there is no relation in our cases between the total thiamine content of the stools and the urinary excretion levels.

Within each group, there is a 2 to 3 fold variation in the thiamine excretion in spite of the constancy of thiamine intake. There is some overlapping of the urinary thiamine

[4] Mickelsen, O., Condiff, II., and Keys, A., *J. Biol. Chem.*, 1945, **160**, 361.

[5] Schultz, A. S., Atkin, L., and Frey, C. N., *J. Ind. and Eng. Chem., Anal. Ed.*, 1942, **14**, 35.

[6] Najjar, V. A., and Holt, L. E., Jr., *J. Am. Med. Assn.*, 1943, **123**, 683.

[7] Alexander, B., and Landwehr, G., *Science*, 1943, **101**, 229.

TABLE II.

Daily pyramin excretion of normal young men maintained on the same diet for 3 days. The first 6 men received 1.0 mg of thiamine per day while the others received 2.0 mg. All values for pyramin are expressed as μg of 2-methyl-4-amino-5-ethoxymethyl pyrimidine.

Subject	Urinary excretion				Stools
	July 23	July 24	July 25	Mean	
A	187	169	157	171	383
Pa	162	170	142	158	208
E	148	178	178	168	37
M	178	198	179	185	532
S	188	173	146	169	61
R	159	169	150	159	94
Mean	170	176	159	168	219
D	211	264	248	241	42
C	231	270	245	249	66
B	261	204	235	233	63
W	211	202	253	222	47
Pe	254	209	272	245	35
H	248	247	243	246	30
Mean	236	233	249	239	47

excretions in the 2 groups. The lowest mean value of the 2 mg group is equal to or less than the 2 highest figures for the 1 mg group. This indicates that it may be difficult in certain cases to distinguish from a single thiamine excretion value whether a person is receiving 1 or 2 mg of the vitamin.

In view of these findings, it was deemed advisable to repeat the above experiment. This was done after the men were maintained on their respective thiamine intakes for an additional 7 months. The conditions of the first experiment were duplicated again for a 3-day period.

The results of the thiamine excretion were essentially the same as on the first occasion of the experiment. This time pyramin was also determined. The pyramin content of the urines is given in Table II. There is considerably less variation in the urinary excretion of pyramin than is the case for thiamine. Furthermore, there is no overlapping in the excretion levels of the 2 groups. This is discussed at greater length elsewhere.[2] The fecal excretion of pyramin is on an average less than one-third that of thiamine when the 2 are reduced to molecular equivalents. Here again, there was no positive correlation between the fecal and urinary excretion of pyramin. Actually there was an inverse relation in the 2 groups, the group receiving 1 mg of thiamine per day showed a higher average pyramin excretion than the group receiving 2 mg.

The above experiment was followed by a period of 14 days on an essentially zero thiamine intake after which all 12 subjects were on routine diets for a period of one month. At this time the controlled thiamine regime was instituted again except that the levels of intake were reversed for the groups. Those who had been on 1.0 mg of thiamine now received 2.0 mg and vice versa. All other factors of diet and supplementation were the same as before. Twenty-four-hour urine samples were collected for thiamine analysis after the men had been on their respective new levels of intake for about 2½ months.

The gross ranking of the urinary thiamine excretions on the 3 above occasions was made with the results shown in Table III. The correlation coefficients for the gross ranking of the men has been calculated according to the method described by Snedecor.[8] Under these conditions the values above 0.81 are significant. In all of our cases, the rank correlations of the urinary thiamine excretions are significant. This indicates that the urinary excretion of this vitamin is intimately related

[8] Snedecor, G. W., *Statistical Methods*, 1946, Iowa State College Press, Ames, p. 164.

TABLE III.

Gross ranking of urinary thiamine excretion of normal young men on 2 levels of thiamine intake. The first 6 men were on 1.0 mg of thiamine from Nov., 1944, through July, 1945, and on 2.0 mg in Nov. and Dec., 1945. The other 6 were on 2.0 and 1.0 mg at these respective times. The thiamine excretions are in μg per day and are the averages of 3-day periods. The figures in parenthesis are the gross ranks of the men. The correlation coefficients are for the gross rankings of adjacent columns.

Subject	Dec., 1944		July, 1945		Dec., 1945
A	91 (1)		83 (1)		411 (1)
M	85 (2)		63 (2)		356 (2)
R	58 (3)		51 (3)		303 (3)
E	54 (4)		47 (4.5)		292 (4)
S	50 (5)		47 (4.5)		255 (5)
Pa	32 (6)		28 (6)		212 (6)
Mean	62		53		305
Rank correlation coefficient		0.99		0.99	
D	310 (1)		316 (1)		88 (2)
B	180 (2)		245 (2)		92 (1)
W	143 (3)		171 (3)		56 (3)
H	136 (4)		154 (4)		36 (5)
Pe	113 (5)		134 (5)		45 (4)
C	86 (6)		89 (6)		26 (6)
Mean	161		185		57
Rank correlation coefficient		1.00		0.89	

to some unknown characteristic of the individual.

This phenomenon did not occur on these 3 occasions only but was apparent throughout the entire course of the 8 months' experiment. The intra-class correlation was +.97 for the group receiving 1 mg of thiamine per day and +.85 for the group on 2 mg. Both of these values are significant.[2] The intra-class correlation is a measure of the consistency with which the various individuals in a group maintain their relative positions within the group throughout the entire experiment. This holds true whether the level of intake is increased or decreased. Those individuals who excrete large amounts of thiamine do so at various levels of intake. Additional evidence of this has been obtained from another experiment in which the thiamine intake was 5.0 mg. Three of the men in one of the above groups were subjects for this experiment. The gross ranking of the urinary thiamine excretion was the same as for the lower levels of intake.

There is no apparent explanation for the relationship between an individual and his level of thiamine excretion. A number of factors might be suggested. Fecal excretion as a measure of bacterial synthesis has been ruled out.[7] Under this category, we should also mention possible variations in the absorption of this vitamin. It is highly unlikely that there is any great variation in the absorption of thiamine in these cases. Physical activity does not account for the differences. In all experiments, a large share of the daily activity of these men was regulated by walks on a motor-driven treadmill. In the evenings and on Sundays, the physical activity was unrestricted. The most active men on off-hours are randomly distributed throughout the 2 groups. In separate experiments to determine the effect of severe physical exertion, there was no change in the thiamine excretion.[3] This has also been observed by Wang and Yudkin.[9] Differences in kidney function might be another possible explanation. No evidence is available on this point, but if this were the only factor then, over even a short period of time, the thiamine level in the blood of the "low excreters" should increase to such a point that they would gradually approach the "high excreters."

[9] Wang, Y. L., and Yudkin, J., *Biochem. J.*, 1940, **34**, 343.

There was a positive but non-significant correlation between the B.M.R. and the urinary thiamine excretion. The weights of these men ranged from 137 to 168 lbs. Here also, there is no correlation between weight and thiamine excretion. Variations probably exist in the way that these individuals metabolize thiamine. We know that only about 30% of the thiamine intake at these 2 levels can be accounted for as the sum of the urinary thiamine and pyramin excretions.[2] Breakdown products of thiamine other than pyramin probably vary inversely as the thiamine excretion.

No accurate prediction of the magnitude of one subject's excretion at a higher or lower level can be made from data of any one control period. For instance, in the 2 different groups D and A had approximately the same level of excretion on an intake of 1.0 mg per day whereas there was a marked difference in the excretion levels when the intake was 2.0 mg (313 versus 411). The overall excretion of the group on 2.0 mg thiamine in December 1945 was considerably higher than that of the other group when it was on this intake. The excretion of the subjects on 1.0 mg was approximately the same for both groups.

There are a few references to the variations of individuals on the same level of thiamine intake.[9,10] In these reports the great variation in the thiamine excretion is apparent but the data do not permit any more detailed analysis. In both of these reports, there is a strong suggestion that the thiamine excretions of their subjects are characteristic of the individuals. De Jong states that "The mean of the daily excretions of a weekly period is, for the same individual, more or less constant."[10, p 127]

Summary. 1. The thiamine and pyramin excretions by 2 groups of normal young men maintained on 1 and 2 mg of thiamine per day have been determined on 2 different occasions when the same diet was served on 3 successive days. Each of these periods occurred only after the men had been stabilized at their respective intakes.

2. The thiamine excretion on a constant diet shows a 2 to 3 fold variation for the men within each group and an overlapping of the 2 groups. The pyramin excretion is much more constant and shows no tendency for the values of the 2 groups to overlap.

3. The excretion of thiamine is characteristic of the individual as shown by the rank correlations of the excretion values. This was true over the entire 8 months of the dietary regime and was also true when the above thiamine intakes were reversed. There is no such correlation for the pyramin excretions.

4. The explanation of the thiamine excretion as a characteristic of the individual cannot be found in the fecal excretion of thiamine, the physical activity of the subjects, in the B.M.R. nor in their body weights.

[10] de Jong, S., *Arch. néerl. de physiol.*, 1940, **25**, 57.

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Persistence of Elevated Values for the Thymol Turbidity Test Following Infectious Hepatitis.*

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The thymol turbidity test introduced by Maclagan,[1] because of its simplicity and the ease with which results can be reproduced, is finding wide application in the study of patients with liver disease.[2,3] It is extremely

* The Bureau of Medicine and Surgery of the U. S. Navy does not necessarily endorse the ideas set forth in this paper.

[1] Maclagan, N. F., *Nature*, 1944, **154**, 670.

[2] Recant, L., Chargaff, E., and Hanger, F. M.,