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Received October 16, 1957. P.S.E.B.M., 1958, v97.

Physiological Aspects of Aging. I. Efficiency of Absorption and Phosphorylation of Radiothiamine.* (23664)

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Mills and associates(1,2) have reported that the thiamine requirement, in terms of dietary concentration, is approximately twice as great for old rats as for growing rats. Thiamine was found to be unique among the B vitamins in this respect. Friedemann *et al.* (3) observed that oral doses of more than 5 mg were poorly absorbed by humans, whereas many times this quantity of other water-soluble vitamins is efficiently absorbed, and Kirk and Chieffi(4) estimated that 50% to 75% of a dose of 5 mg of thiamine hydrochloride apparently was unabsorbed by either young or old subjects. In the present study the absorption of radioactive thiamine by rats 1 month to 2 years old, and by young rats in deficient, normal and supernormal states of thiamine nutriture was studied. As an index of the influence of aging on the conversion of thiamine to its phosphoric acid esters, and on the efficiency of the general phosphorylation mechanism, the radioactive compounds in the liver were partitioned into phosphorylated and unesterified forms which were estimated separately.

Methods. Albino rats of the Sprague-Dawley strain were maintained on a stock diet with a supplement of 2500 I.U. Vit. A per month. Individuals of various ages were fasted for 24 hours and placed in a urinofecal separator(5). An oral dose of 120 μg of radiothiamine (thiazole-2-C^{14}) with a specific activity of 6 μc/mg was administered by stomach tube under light ether anesthesia. Urine and feces were collected for a 48-hour period; the animals then were sacrificed and the livers were removed and stored at –18°C. The feces were dried for 12 hours at 105°C, ground finely, and a 1 g sample was digested for 16 hours under toluene at 37°C, using 20 mg papain and 20 mg clarase in 25 ml 0.5% acetate buffer at pH 4.5. The hydrolysates were filtered through No. 1 Whatman paper and washed several times with distilled water. Duplicate 200 λ aliquots were plated for counting in a Tracerlab Q-gas windowless gas flow counter connected to a Nuclear scaler. The efficiency of the extraction procedure was determined by plating a fine suspension of the undigested residue and of the original ground samples, prepared by agitating vigorously for 5 minutes in a Waring blendor. For a series of samples a recovery of at least 95% of the total fecal radioactivity was obtained in the filtrate. Hydrolysates of feces from 2 treated animals were chromatographed on filter paper as described below and radioautographs were prepared. Only one spot, at R_f .66, corresponding to that of thiamine, was detected, indicating that this was the sole radioactive compound present in significant amounts after hydrolysis. Separation of thiamine and its phosphoric acid esters in liver was achieved

* This investigation was supported in part by a research grant from the National Institutes of Health, U. S. Public Health Service. The technical assistance of Carol Lowe is gratefully acknowledged.

by a modification of the procedure of Westenbrink and Steyn-Parvé(6) for the release of diphosphothiamine (DPT) from protein and of Siliprandi and Siliprandi(7) for its chromatographic separation from thiamine and other phosphate esters. It has been shown (6) that DPT may be released from animal tissues without hydrolysis of the phosphate ester by placing the minced pulp in a water bath for 5 minutes in .01N HCl, whereas in 1N HCl DPT is converted to monophosphothiamine (MPT). In the present study 200 λ aliquots of the supernatant obtained from this procedure were placed on strips of pretreated Whatman No. 1 filter paper(8) which were developed in the ascending direction(7). DPT and thiamine, which were detectable under ultraviolet light after spraying the dried strips with thiochrome reagent, exhibited R_f values of .21 and .66, respectively. Heating DPT in 1N HCl gave rise to a thiochrome-reactive compound at R_f .37, corresponding to that of MPT(7). The concentrations of triphosphothiamine (TPT) and MPT, reportedly present in liver(9,10), were too small for detection on sample strips by the thiochrome reaction or by scanning. The sample strips were cut into 2 segments so as to separate thiamine from its esters, as indicated by the development of standard strips with thiochrome reagent. The segments were eluted with distilled water and 200 λ aliquots were plated. Evidence that the intestinal wall performs a regulatory function in thiamine absorption(3) and the suggestion that an impairment of thiamine absorption may occur in rats deficient in this vitamin(11), prompted a study of the absorption of radiothiamine in 7-week-old rats which had received inadequate, "normal" and supernormal amounts of thiamine in the diet. Three groups of 6 weanling rats were fed a synthetic thiamine-deficient basal diet. One group received no supplementary thiamine, a second received 2 μg per g and a third 50 μg per g of diet. The absorption tests were made, as described previously, when the deficient animals had begun to lose weight. In order to prevent wide disparities in body weight between groups, the weights of the rats fed the supplemented diets were controlled by regulating their food supply so that all animals were of comparable size when the test was administered. Following the dose, all rats were given 4 g of the basal diet per day for the 2-day collection period.

TABLE I. Excretion of C^{14} following a 120 μg Oral Dose of Radiothiamine by Young Rats Maintained on Three Different Levels of Thiamine. 6 rats/series.

Group	Body wt (g)	Thiamine pretreatment (μg/g diet)	48-hours C^{14} excretion (% of dose) in Feces*	Urine*
1	85.5 ± 1.4	None	4.3 ± .5	14.7 ± .6
2	85.5 ± 2.3	2	5.2 ± 3.3	11.9 ± 2.8
3	91.5 ± 3.8	50	8.8 ± 2.8	28.3 ± 1.2

* Mean and stand. error.

Results. Absorption of the 120 μg dose of labelled thiamine was found to be about 95% efficient in young rats and to be unaffected by the previous level of thiamine intake (Table I). However, individual animals exhibited considerably poorer absorption, the highest fecal counts being 18.9% and 22.8% of the dose, while in other cases absorption was essentially quantitative. The increased urinary excretion in group 3 probably is a reflection of greater tissue saturation resulting from the high thiamine intake.

Since it has been found(12,13) that a small quantity of radioactivity is excreted in the feces of the rat after injection of thiazole-2-C^{14} thiamine or S^{35}-thiamine, the data for fecal C^{14} excretion may slightly over-estimate the unabsorbed portion by the amount of the endogenous contribution. As a check, 6 young rats, previously fed a stock ration, were given 120 μg of radiothiamine intraperitoneally and the feces were collected for 48 hours. Radioassay indicated that 0.3-1.4% of the injected activity was excreted in the feces, amounts in close agreement with those reported previously(12,13). The slightly higher value for fecal activity obtained for group 3 may be partially attributable to an increased endogenous contribution to the feces coincident with the increased urinary excretion.

The fecal excretion of C^{14} by rats of different age groups following the 120 μg oral dose of radiothiamine is summarized in Table

TABLE II. Fecal Excretion of C^{14} by Rats of Different Ages 48 Hours after a 120 μg Oral Dose of Radiothiamine.

Age (mo)	No. rats	Body wt (g)*	48-hr C^{14} excretion in feces (% of dose)*
1- 2	23	94 ± 6	4.8 ± 2.8
5- 8	12	391 ± 14	6.8 ± 1.8
14-16	5	435 ± 14	6.0 ± 5.1
19-20	6	436 ± 11	12.9 ± 3.2
22-24	11	400 ± 28	23.4 ± 3.6

* Mean and stand. error.

II. No agewise effect is evident until the animals reached 19-20 months, when a reduction in absorption is indicated, and a further decrease is apparent in the 22-24-month-old group. In the rats up to 16 months old, absorption in several cases was essentially quantitative and in only 8 out of 40 observations was it less than 90%. In the two oldest age groups (19-24 months) 13 out of 17 animals absorbed less than 90% of the dose, the minimum values being 55.7% and 57.2%.

The *results* of the assays for C^{14}-thiamine and its esters in the liver are given in Table III. Significantly more radiothiamine ($P = <.01$), expressed either as total disintegrations per minute or as % of absorbed radiothiamine, was found in the livers of the early mature rats than in those of the growing animals. However, this difference becomes insignificant when adjustment is made by covariance analysis for the disparity in body weight between the 2 groups. Among the adult animals, significantly less activity ($P = <.05$) was found in both the esterified and unesterified fractions of the older group (22-24 months) when compared with the younger (5-8 months), after adjustment for differences in body weight and radiothiamine absorbed. A shift in the ratio of the vitamin to its esters appears to accompany maturity, since 57% of the total activity was found in the esterified form in the livers of the growing animals and only 22% and 18% in the early mature and old rats, respectively.

Discussion. The above results indicate that thiamine is efficiently absorbed by rats up to an age of approximately 19-20 months, beyond which a substantial decrease in efficiency occurs. The single dose of labelled thiamine used may be regarded as about tenfold the minimum daily requirement for growth. Watanabe(14) has reported that young rats absorb doses of 120, 240 or 400 μg of unlabelled thiamine with about the same efficiency, indicating that there is no threshold value within this range.

The postulation that the ability of thiamine-deficient rats to absorb this vitamin is impaired because of lesions of the gut wall (11) is not supported by the results shown in Table II. Magyar and Gábor(15) have reported that the absorption of thiamine from an isolated loop of intestine is reduced by prior administration of large doses of thiamine or other B vitamins, and they suggested that this effect is due to an exhaustion of the phosphorylation mechanism. No impairment of radiothiamine absorption was encountered in the present study after continuous administration of about 50 times the necessary dietary concentration of thiamine. The lower recovery of labelled thiamine in the livers of the aged rats may be attributable to a reduction in protein binding or to a faster turnover rate.

The influence of pathology on the outcome of this study, particularly as it may have affected the absorption of thiamine, is impossible to assess. Although some of the rats in the oldest age group were slowly declining in

TABLE III. Liver C^{14} Distribution in Rats of Different Ages 48 Hours after a 120 μg Oral Dose of Radiothiamine.

Age group (mo)	No. rats	Body wt (g)	DPM* ($\times 10^{-3}$) in liver as		% of absorbed radiothiamine in liver as	
			Thiamine	Thiamine esters	Thiamine	Thiamine esters
1- 2	6	143 ± 4†	10.6 ± 2.5	13.8 ± 6.3	.67 ± .16	.87 ± .40
5- 8	10	408 ± 19	43.2 ± 7.6	12.2 ± 2.8	2.97 ± .51	.83 ± .18
22-24	8	342 ± 12	20.0 ± 1.4	4.4 ± .8	1.50 ± .12	.33 ± .06

* Disintegrations/min. † Mean and stand. error.

weight there was no apparent correlation between recent weight changes and the efficiency of absorption. A number of animals were discarded because of tumors and others because of a chronic respiratory disease which was the most prevalent health problem.

Summary. Absorption of an oral dose of 120 μg of C^{14}-labelled thiamine by the rat has been studied as a function of age and the state of thiamine nutrition. Approximately 95% was absorbed up to an age of 19-20 months, when the efficiency declined to about 75% at 22-24 months. Absorption by thiamine-deficient animals was not different from that of rats receiving normal or excess quantities of thiamine in the diet. The proportion of absorbed radiothiamine found in esterified and unesterified forms in the livers of rats 22-24 months old was less than that in rats 5-8 months old.

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Received October 18, 1957. P.S.E.B.M., 1958, v97.

Chlorpromazine Blockade of Water Imbibition by Frog Gastrocnemius. (23665)

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The imbibition of water by frog gastrocnemius muscle has been studied extensively by Halpern and Reuse(4) and by Courvoisier and Ducrot(1), in an attempt to determine the mechanism of action of various drugs, chiefly phenothiazine derivatives, in blocking water uptake by this tissue. These investigators were able to rule out antihistaminic and local anesthetic potency as actions capable of being correlated with water imbibition block. Van der Kloot(6) has measured rate of sodium extrusion from frog gastrocnemius in hyponatric media and has related this to cholinesterase activity, but has not correlated the phenomenon with water transport.

Since phenothiazine compounds possess other pharmacologic properties than those mentioned above, it has seemed of interest to us to examine selected structures of this type for correlation of these activities with water imbibition block. In the course of this work, a number of additional non-phenothiazine compounds were used for activity analogy in elucidation of the phenomenon.

Methods. The technic was essentially that of Halpern and Reuse(4), modified as follows: Gastrocnemius muscles were removed as rapidly as possible after pithing of the frogs, rinsed in distilled water, blotted and weighed. They were then assigned at random to beakers containing 45 ml of distilled water containing either no drug or the drug under examination. The drugs were used in a final concentration of 0.0016 M as employed by