Observations on the Urinary Excretion of Thioctic Acid

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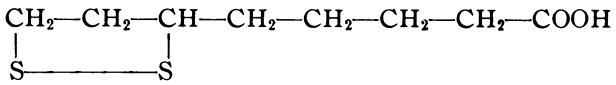
THIOCTIC ACID, 6,8-dithiooctanoic acid, is a biologically active substance which may occur in various molecular forms including the dithiol, the monosulfoxide and conjugates. Substances with the biological activity of thioctic acid occur in a large variety of plant and animal tissues.[1] Thioctic acid has a coenzymatic function in the oxidative decarboxylation of α-keto acids[2,3,4] and it was suggested that in photosynthesis a conjugate of thioctic acid is the substance in which the quantum absorbed by the plant pigments first appears as chemical bond energy.[5] It is an essential nutrient for certain micro-organisms and is active in very low concentrations.[6–8] Its structural formula is as follows:



Attempts to produce a deficiency in animals by dietary means or with antagonists have not been successful in our hands, probably because the intestinal microflora or the animal itself may have the ability to synthesize thioctic acid.[9] The latter is suggested by experiments in which the thioctic acid content of the developing chick embryo showed a continued increase from the first to the 21st day.[9] However, DeBusk and Williams[10] have recently reported a growth-promoting effect of DL-thioctic acid (also known as DL-α-lipoic acid) for chicks and rats. Beneficial effects of DL-thioctic acid in the management of hepatic coma in man,[11–13] and a reduction of the thioctic acid activity in the livers of rats having abnormal ketogenesis have been reported.[14]

This paper reports the results of a study made of the urinary excretion of thioctic acid, and some observations on a new form of thioctic acid found in the urine.

BIOASSAY

Thioctic acid activity was measured microbiologically by the pad plate assay using *Corynebacterium bovis*[15] except in certain instances when *Streptococcus faecalis* was used as indicated.[16] The activity of fresh urine was 10 to 180 times as much by the *C. bovis* assay as with the *S. faecalis* assay (Table I). The *S. faecalis* assay value of urine stored several days at 4° C increased to essentially the value obtained with *C. bovis* which had not changed. Thioctic acid and thioctic acid monoxide are equally active for these two organisms. It appears that in fresh urine thioctic acid activity is in an unidentified form that changes during storage to thioctic acid, as identified by bioautographs and solvent counter-current distribution.

TABLE I

The Growth Response of *Streptococcus faecalis* and *Corynebacterium bovis* to Human Urine

Sample (DL-thioctic acid orally)	μg equivalent DL-thioctic acid per ml	
	C. bovis	*S. faecalis*
Normal	0.32	0.005
0–1 hr after 100 mg	164	5.1
1–6 hr after 100 mg	54	0.33
0-24 hr after 10 mg	2	0.03

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THE EFFECT OF THE ADMINISTRATION OF DL-THIOCTIC ACID ON THE THIOCTIC ACID ACTIVITY OF THE URINE

The thioctic acid activity in the urine of several species (Table II) varied over a threefold range. When DL-thioctic acid was administered the thioctic acid activity in the urine was increased (Tables III and IV), yet only one biologically active form was found by paper chromatography and solvent countercurrent distribution. The per cent recovery based on bioactivity varied with the route of administration. For example, when 5 mg of DL-thioctic acid was administered to 250-g rats the amount recovered in the urine following intraperitoneal injection was 0.6 per cent;

TABLE II

The Thioctic Acid Activity of Urine from Several Species

Animal	Average Thioctic Acid Activity per ml (μg equivalent of DL-thioctic acid)
Man	0.20
Dog	0.44
Rat	0.21
Rabbit	0.30
Horse	0.60
Cow	0.52
Sheep	0.25

TABLE III

The Excretion of Thioctic Acid Activity in the Urine of Rats and Rabbits After Administration of DL-Thioctic Acid

No. of Animals	Dose (mg DL-thioctic acid)	μg equiv. DL-thioctic acid in urine per animal	Recovery,	Urine Collection Period
(250 g avg)			%	*hr*
1 rat	—	3.5 (avg)	—	0– 24
1	0.5 I.V.	29	5.9	
1	5 I.V.	294	5.9	
1	5 I.P.	29	0.6	
3	15 I.P.	51	0.29	
1	15 I.P. + 15 mg B_1 I.P.	42	0.28	
1	25 I.P.	40	0.16	
1	50 I.V.	1630 (died 30 hr)	3.2	
5	0.1 Subcut.	11	7.5	0– 18
5	1.0	110	10.0	
5	5.0	280	5.5	
(2 kilo avg)				
10 rabbits	—	110	—	
	100 I.V.	5040	5.0	24– 48
	—	80		48– 72
	—	300		72–144
	100 I.V.	8000	8.0	144–168
	—	560		168–192
2	—	1500		0– 24
	500 I.V.	10,000	1.7	24– 48
	—	400		48– 96
	500 oral	23,700	4.4	96–120
	—	2600		120–144
	—	1100		144–168
2	—	2000		0– 24
	500 oral	24,000	4.4	24– 48
	—	750		48– 96
	500 I.V.	20,000	3.8	96–120
	—	2900		120–144
	—	900		144–168

I.V.—intravenously
I.P.—intraperitoneally
Subcut.—subcutaneously
B_1—vitamin B_1

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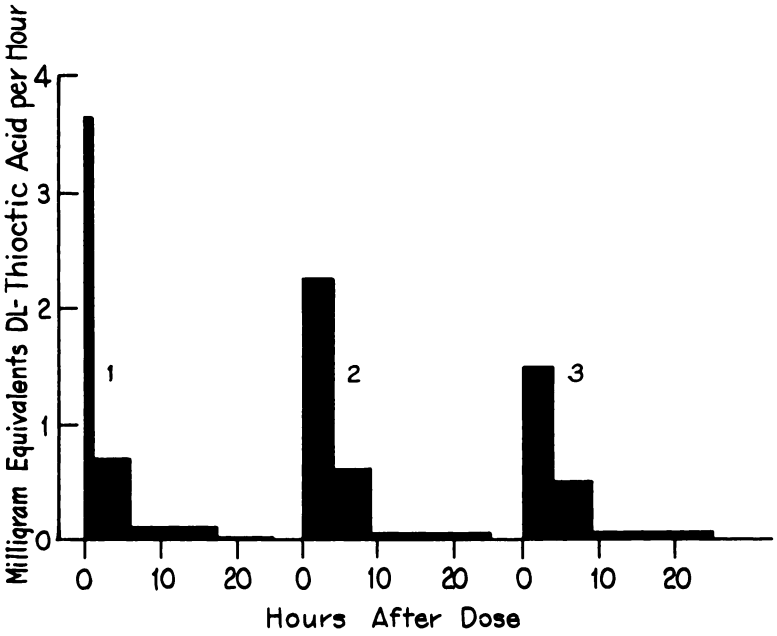

Fig. 1. The urinary excretion of thioctic acid activity after oral administration of DL-thioctic acid with and without thiamine to adult human males. (1) 100 mg DL-thioctic acid. (2) 50 mg DL-thioctic acid (3) 50 mg DL-thioctic acid + 50 mg thiamine hydrochloride.

intravenous, 5.9 per cent; subcutaneous, 5.5 per cent. The size of the dose did not appreciably alter the per cent recovery in the urine, nor was there a marked difference among species.

TABLE IV

Urinary Excretion of Thioctic Acid Activity by Human Adult Males Following Oral Administration of 10 mg of DL-Thioctic Acid

Subject	μg equivalent DL-thioctic acid excreted in 24-hour period		
	Before Dosage	After Dosage	
		Trial 1	Trial 2
1	169	576	1181
2	220	870	760
3	204	756	1051
4	153	567	—
5	156	694	929
6	171	714	—

In adult male humans the average urinary recovery of a 10-mg oral dose of DL-thioctic acid was 6.3 per cent, and the thioctic acid activity of the urine had returned to normal within eight hours after administration of the dose (Fig. 1). When thiamine was simultaneously administered, there was no change in the rate or amount of excretion of thioctic acid activity.

The low recovery of thioctic acid activity in the urine after administration of DL-thioctic acid suggested that storage of the material in the organs might be occurring. Therefore, several organs from rabbits that had received DL-thioctic acid were assayed for their thioctic acid content and compared with those of untreated animals (Table V). No appreciable differences in the thioctic acid content were found.

PROPERTIES OF A NEW FORM OF THIOCTIC ACID IN URINE

The thioctic acid activity in urine was purified about 50-fold by solvent countercurrent distribution (Fig. 2) and chromatographic adsorption on magnesol. Further purification was made difficult by the continuous change in the nature of the material during treatment and storage. Usually the conversion, which was essentially quantitative based on biological activity, was complete in about ten days. Conditions to prevent this were not found. The ultimate conversion

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TABLE V

The Effect of the Administration of DL-Thioctic Acid to Rabbits on the Thioctic Acid Activity in Various Organs

Organ	μg Equivalent DL-thioctic acid per organ	
	Injected	Control
Liver	0.13, 0.11	0.10, 0.08
Kidney	0.09, 0.05	0.09, 0.13
Heart	0.05, 0.10	0.07, 0.12
Lung	0.04, 0.03	0.03, 0.03
Spleen	0.02, 0.02	0.01, 0.01

Two adult female New Zealand rabbits were given 100 mg DL-thioctic acid intravenously three and five days before being sacrificed. Two control animals were untreated. The organs were homogenized and autoclaved three hours in 2.5 N H_2SO_4. The solutions were neutralized and assayed.

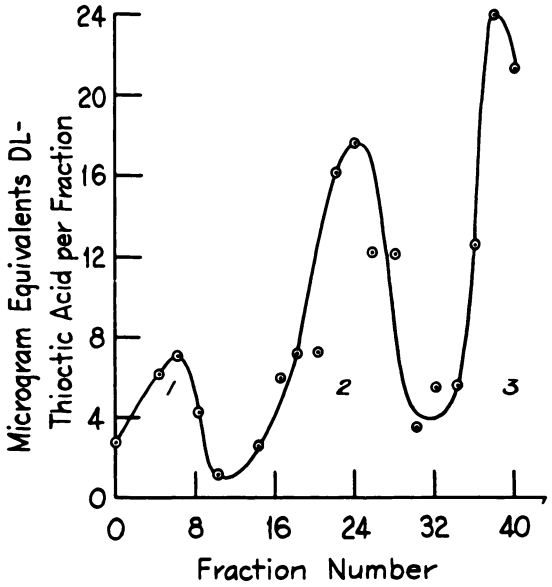

Fig. 2. Solvent counter-current distribution of urinary thioctic acid activity. System: n-butanol—0.5 M phosphate pH 3. Peak 3 (K = 10) is thioctic acid. Peak 2 (K = 1.7) is an unidentified form of thioctic in fresh urine which changes during storage to thioctic acid and a second unidentified form of thioctic acid, peak 1 (K = 0.2).

products appeared to be thioctic acid and a smaller amount of thioctic acid monoxide. These were identified by paper chromatography (Table VI) and by solvent counter-current distribution in the system of 0.5 M phosphate pH 7.3—chloroform. The distribution coefficients (K = concentration in organic phase ÷ concentration in aqueous phase) of

TABLE VI

R_f Values of the Thioctic Acid Activity in Urine and Thioctic Acid after Certain Chemical Treatments

Sample and Treatment	R_f Values for Thioctic Acid Activity		
DL-thioctic acid monoxide	—	—	0.82
DL-thioctic acid	—	0.68, 0.84	
DL-thioctic acid + 10 N HCl, 120° C, 6 hr.	—	0.72	0.89
DL-thioctic acid + 6 N NaOH, 120° C, 3 hr.	—	0.62	—
Fresh urine	0.34	—	—
Fresh urine + 10 N HCl, 120° C, 6 hr.	—	—	0.80
Fresh urine + 6 N NaOH, 120° C, 3 hr.	0.29	—	0.79 (trace)
Urine stored 4°, 8 days	0.40, 0.54, 0.69		
DL-dihydrothioctic acid	—	0.62, 0.77	
Purified thioctic acid activity from urine in liquid NH_3	0.32	—	—
Purified thioctic acid activity from urine in liquid NH_3 + sodium	—	0.66, 0.80	

thioctic acid and thioctic acid monoxide in this system are 0.5 and 0.1, respectively. After several days' storage of urine the major portion of the thioctic acid activity had a K of 0.6 and a small amount with K = 0.15. In stored samples of urine a second unidentified form of thioctic acid was found (Fig. 2, K = 0.2 and Table VI, R_f = 0.54) which was characterized by its slight distribution into organic solvents. Whether this was a monoxide of the unidentified form of thioctic acid in fresh urine and gave rise to the thioctic acid monoxide on ultimate conversion or whether it was an intermediate in the conversion to thioctic acid was not determined.

The new form of thioctic acid in fresh urine was stable to autoclaving in strong alkali but was changed to thioctic acid during autoclaving in strong acids (Table VI). By sodium reduction in liquid ammonia it was converted probably to dihydrothioctic acid which during development on paper was oxidized to thioctic acid and thioctic acid monoxide (Table VI). The biological activity remained unchanged for *C. bovis* and increased for *S. faecalis* during sodium reduction in the same manner as occurred during storage. The microgram equivalents of DL-thioctic acid before and after re-

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duction, respectively, were as follows: *C. bovis*, 165 and 134; *S. faecalis*, 0 and 121. These data suggest that the thioctic acid in urine was conjugated through a bond which was labile to acid and sodium reduction but which was stable to alkali. Such a linkage probably would not involve the carboxyl of thioctic acid but instead one or both of the sulfur atoms.

Thiamine and thioctic acid are both involved as coenzymes in the biological oxidation of pyruvic acid and α-ketoglutaric acid. The availability of thioctic acid makes possible a new experimental approach to clinical states which are characterized by elevations in blood keto-acids, such as hepatic coma,[17,19] parenchymal liver disease,[18] and patients who have received fructose by infusion[18]. The present investigation indicates that a pathway may exist for the metabolism of thioctic acid in normal subjects.

SUMMARY

The urine of several animal species, including man, contains an unidentified form of thioctic acid that supports the growth of *Corynebacterium bovis* but not *Streptococcus faecalis* on a propionate medium. The thioctic acid activity of the urine showed an increase followed by a return to normal during the first 24 hours after administration of DL-thioctic acid by several routes. Following this administration, the recovery of thioctic acid activity from the 24-hour urine ranged from 0.6 to 10 per cent of the dose administered, all in the form of the unidentified conjugate. The simultaneous administration of thiamine with DL-thioctic acid to man did not alter the thioctic acid activity in the urine. No storage of an injected dose in several of the viscera was detected. The thioctic acid activity in urine is conjugated probably through one or both of the sulfur atoms in a bond which is labile to acid or to sodium reduction but is stable to alkali. The use of thioctic acid in patients with elevations in blood keto-acids is discussed briefly.

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Perspective in Research

"Research is not done only in the laboratory. It may be conducted at the bedside, in the operating room, and in the field of public health and epidemiology. It is not the place that counts. It is the perspective. It is the original mind asking a question and designing an experiment to find the answer. It should not be pebble picking—it should be the building of magnificent castles. I have no sympathy for the view that any kind of research is good. Research should advance a fundamental concept or it should have an obvious practical value and early application. Research is not gadgeteering. It is the pursuit of ideas. With the development of elaborate tools, like flame photometers, ultracentrifuges, angiocardiographs, ballistocardiographs, electron microscopes, electrophoresis, and isotopes, there has developed a small group who play their instruments for all they are worth. Having acquired the equipment, the buildings, the technicians, and the project grants, they go madly searching for ideas. They scrutinize grant applications if they are on research-allocation committees, they scan published reports, they rush to meetings, they write to their friends, and they ask visiting colleagues for ideas. This is obviously all wrong. Research should start from ideas, and then tools should be obtained, not vice versa."

—L. N. Katz. *J. A. M. A.* 160: 1137, 1956.

Call to Nonconformity

"I have given you the simple practical grounds for allowing scientists to be awkward, but I believe also that imaginatively and intellectually this is equally important. The sense of intellectual heresy is the lifeblood of our civilization. And the heresy of scientists cannot be confined to their science. Newton was thoroughly and rightly contrary in science, and he was also a thorough heretic in religious matters. For the same reason, people like Oppenheimer and Einstein are found to associate with such unreliable characters. You cannot say to scientists: 'When you get into the laboratory at nine in the morning you are going to become a dissenter; and when you go out at five-thirty you are going to become a citizen who touches his cap and is politically sound.' The intellect is not divided into these simple categories."

—J. Bronowski. *Bull. Atomic Scientists* 12: 13, 20, 1956.

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