

VITAMIN B₁ IS NOT A SYSTEMIC MOSQUITO REPELLENT IN MAN

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Shannon (1943) was the first and only investigator to report that orally ingested thiamine chloride (Vitamin B₁) protected humans from mosquito bites. Wilson *et al.* (1944) and Kingscote (1958) subsequently re-examined the claim and failed to confirm it. Wilson's findings were confirmed by tests undertaken by the United States Dept. of Agriculture. Nevertheless, in recent years we received numerous communications from scientists suggesting that thiamine chloride, when ingested protects against mosquito bites. A recent Medical Letter endorsement stimulated further interest. We, therefore, decided to study this drug in a controlled trial in man.

METHODS AND MATERIALS

Thiamine chloride tablets were given orally to volunteers at a dose of 50 mg., three times daily for three days. They were then tested for mosquito attractancy by the Probing Time (PT50) method (Khan *et al.* 1965). Six female *Aedes aegypti* (L.) 8 to 10 days old and previously fed on 5 per cent sugar solution only were placed in a cage with a plexiglass frame (5 × 5 × 1.5 cu.) covered with 20 mesh nylon net. The cage was positioned 1 cm. above the forearm and the time when half the number of mosquitoes were probing on the bottom was recorded (PT50). Another subject who did not take thiamine chloride was tested as control.

The same subjects were then tested in biting experiments. Ten mosquitoes were placed in a plastic cylinder 14 cm. high and 5 cm. in diameter and covered at the ends with 20 mesh net. The cylinder was placed on the forearm of the subject and the mosquitoes that engorged in three minutes were counted.

To avoid the possibility that either the dose of thiamine chloride given was too small or the experimental design not realistic for this problem because mosquitoes were brought too close to the skin artificially, we administered with a double-blind technique thiamine chloride to volunteers at 200 mg. three times a day for two days, and an identical placebo to an equal number of persons to serve as controls. On the morning of the third day, 30 minutes after ingesting another 200 mgs. of thiamine chloride, test subjects and a matched control were seated in a 9 × 19 × 9 ft. room on chairs three feet apart with their shirts off. Their legs were protected against mosquito bites by plastic bags. The room was closed; all possible exits for mosquitoes were checked and sealed. One hundred female *A. aegypti* previously fed on sugar-water only were then released into the room. An observer collected mosquitoes feeding on

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each subject for 10 minutes with an aspirator tube. For the next pair of volunteers, fresh mosquitoes were released in the room to make up for those mosquitoes that had fed previously. In this way subjects on thiamine chloride were tested directly against a control. Both Negroes as well as Caucasians were tested, but only members of the same race were paired.

We also tested thiamine chloride by applying it directly on the skin at 2 mg./sq. in. in aqueous solution. Mosquito attraction was tested by the PT50 method; first, immediately after the application, and again after 30 minutes.

TABLE I.—Mean PT50 in seconds and the per cent of mosquitoes biting on subjects given 50 mg. of thiamine chloride three times a day for three days.

		Subjects				
		1	2	3	4*	control
PT50†	34.3±13	29.2±4	26.0±5	8.4±1	40.8±12
% biting	...	50	50	75	100	75

†Each figure is a mean of 10 replicates ± S.E.

*Significantly more attractive ($P < 0.05$). The subject was perhaps more attractive than others to start with (c.f. Khan *et al.* 1965). We do not interpret his greater attractiveness as a result of thiamine chloride ingestion.

Besides testing thiamine chloride we also tested Tixtak, a preparation received from Vegeholm, Sweden, manufactured by Cernelle Ltd. (in Swedish AB Cernelle). The literature accompanying the tablets said that it was a pollen extract preparation and contained B-vitamins and pollen yeast. We did not administer this to man but gave tablets to two guinea pigs at four tablets per Kgm weight. A third guinea pig of equal weight served as control. The guinea pigs on Tixtak were bitten by mosquitoes as avidly as the control.

RESULTS

Table 1 lists the attractancy of individuals receiving thiamine compared to a control subject. The measure of attractancy was the probing time 50 (PT50), *i.e.* the time required for half of a group of mosquitoes in a small cage to probe toward the skin. If the treated subjects became less attractive with thiamine, it would have taken longer for the mosquitoes to probe. The subjects were also exposed to biting conditions. If the subjects were less attractive with thiamine, fewer mosquitoes would be expected to bite. In neither experiment was there evidence of less attraction in thiamine treated subjects.

TABLE 2.—Number of mosquitoes biting on 9 subjects given 200 mg. of thiamine chloride three times a day for two days compared with 9 controls in paired tests.

Test Subjects (Average)				Control (Average)			
Age (years)	Weight (lbs.)	Height	% mosq. biting	Age (years)	Weight (lbs.)	Height	% mosq. biting
39.1	175.3	5' 11"	50.0	34.4	167.2	5' 10"	38.8
(8)*	(22)	(1")	(18)	(10)	(25)	(0.5")	(16)

*Figures in parentheses are standard deviations.

Table II presents the per cent of mosquitoes biting the control versus the thiamine treated subjects in the paired comparison study. Since the mosquitoes were free flying in the room, this represents the closest approximation of a laboratory model to field conditions. There was no significant difference in number of bites between treated and control subjects.

If there were mosquito protective qualities to thiamine, topical application might provide a convenient method of use. Table III data demonstrates lack of effectiveness of the thiamine topically applied (utilizing the probing time technique).

TABLE 3.—PT50 in seconds on the forearm of a subject before and after the topical application of thiamine chloride at 2 mg/sq. inch.*

Before application	Immediately after application	30 mins. after application
11.6±2 —	12.3±1 (100)	14.0±2 (88)

*Figures are means of 20 replicates ±S.E. Figures in parentheses are the per cent of mosquitoes engorged when applied to the skin for four minutes.

DISCUSSION

Shannon (1943) based his claim for thiamine chloride on 10 reported case histories which consisted of three adults, four children and three babies. On taking thiamine chloride at 80-120 mg. on the first day and about 10 mg. per day thereafter his patients got either complete protection from mosquito bites or got fewer bites and less itching. He further claimed thiamine chloride prevented the formation of the papule completely at the site of the bites. He does not, however, mention the total number of persons who tried thiamine chloride and the percentage who received protection from mosquito bites. Wilson *et al.* (1944) repeated Shannon's experiments with negative results. Their subject in one experiment ingested 505 mg. of thiamine chloride in three days but failed to repel mosquitoes (*A. aegypti*). The thiamine blood level of the subject when he had ingested 385 mg. of the vitamin was 6.9 mg./100 cc. In another experiment their subject ingested 100 gm. of thiamine chloride and exercised to gross sweating, but was bitten by the mosquitoes readily. In all their experiments neither the biting by the mosquitoes nor the subject's reaction to the bites differed materially from the control. Our results (Tables I and II) also show that thiamine chloride when given at as high a dose as 600 mg. per day for three days does not protect against mosquito bites. Furthermore, it did not in any way, as far as we could ascertain, reduce itching or wheal formation. Nor did its application on the skin deter mosquitoes from probing or biting (Table III).

In experiments with Tixtak, no significant difference was found in the number of mosquitoes feeding on guinea pigs given Tixtak compared to those on control.

It is unfortunate that Shannon (1943) does not give any information regarding the mosquito species in the field against which his patients received protection. Other private communications making similar claims for thiamine chloride also suffer from the same defect, and are based on second or third hand information rather than on data collected in controlled experiments under supervision.

The experiments conducted on Tixtak in Sweden were supervised by a tourist guide and the tablets at a dose of four per person per day were administered to a group of 36 tourists, the guide and a bus driver. The guide reported that after two hours 29 members of the group obtained complete protection against midges. No further and better controlled studies were conducted. A preparation of such promise certainly needed a more thorough and intensive and extensive investigation. We, therefore, consider it important to publish our data so that, firstly, any false hope that might have been raised by such reports be quelled and, secondly, to suggest that any future reporting to the same effect be supported with more concrete and scientific

evidence, especially regarding the range of effective dose, the per cent of population that received such protection, the quality of protection received, *i.e.* absolute or partial, the ecological and ambient conditions during the test period and the mosquito and other insect species against which it gave protection.

SUMMARY

Thiamine chloride (vitamin B₁) was tested as a systemic repellent against the yellow fever mosquito—*Aedes aegypti* (L.). Mosquito probing and biting were used as criteria. In probing experiments mosquitoes were exposed in a small cage to the forearm of test subjects and the time was recorded when half the number of mosquitoes were probing towards the skin on the bottom of the cage (PT50). No significant differences were observed in the probing time on the test subjects compared to that on the controls. In the biting experiments a subject who had ingested large doses of thiamine chloride and another identical placebo, sat with their shirts off three feet apart in a large room with free flying mosquitoes. The mosquitoes feeding on each subject were then collected for 10 minutes by an observer. No significant differences were found in the attraction of the nine pairs so tested. Thiamine chloride also did not reduce probing or biting when applied topically on the forearm nor did it reduce itching or wheal formation after mosquito bites. It is concluded that thiamine chloride is ineffective as a systemic mosquito repellent in man. Recommendations are made suggesting criteria necessary for demonstrating efficacy for proposed systemic repellents.

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