

DEVELOPMENT OF HYPERSENSITIVITY TO THIAMINE HYDROCHLORIDE

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APPROXIMATELY 200 cases of allergic or toxic reactions to thiamine have been reported.^{1, 2} These reactions have occurred subsequent to intravenous, subcutaneous, intramuscular, or oral administration of the drug.³⁻⁷ Since thiamine hydrochloride has a molecular weight of 337.3, it would not be expected to possess sensitizing capabilities unless spontaneous conjugation with body protein occurred in vivo. Spontaneous conjugation of this type is believed to account for the antigenicity of other medicinal compounds of low molecular weight.⁸ This study was made in an attempt to induce hypersensitivity to thiamine hydrochloride experimentally in guinea pigs.

MATERIALS AND METHODS

Albino guinea pigs weighing between 300 and 500 grams, with approximately equal numbers of males and females, were used in the animal experiments. They were maintained on a diet of Rockland guinea pig feed containing 410 to 652 mcg. thiamine per 100 grams.

Thiamine hydrochloride was obtained from two sources: Merck* and Laxseed,† the latter preparation containing 0.5 per cent chlorobutanol. Conjugation was attempted by dissolving thiamine in sterile, normal guinea pig serum to a final concentration of 50 mg. per milliliter and incubating the mixture at 4° C. for twenty-four hours. Adjuvant was prepared as follows: three parts paraffin oil, one part Aquaphor, and one part saline (0.85 per cent sodium chloride) containing 50 mg. thiamine.

Complement-fixation and capillary tube precipitin tests were done in the usual manner. Skin tests were performed by both the intradermal route and the scratch technique suggested by Shapero and Gwinner.⁴

RESULTS

To determine the acute toxicity level of thiamine, twenty-seven normal guinea pigs were inoculated intracardially with from 10 mg. to 25 mg. of the drug and ten additional animals received 50 mg. intra-abdominally (Table I). Although the majority of the animals evidenced some reactions, ranging from transient respiratory distress to generalized weakness and death, no significant gross or microscopic pathologic changes were noted. None of the animals receiving 50 mg. thiamine intra-abdominally evidenced any reaction.

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†Laxseed Company, New York, New York.

Twelve normal guinea pigs received seven intra-abdominal injections of 50 mg. thiamine, administered at four- to six-day intervals. Thirteen days after the last injection, six animals received 5 mg. and six received 10 mg. of thiamine intravenously. The guinea pigs receiving the 5 mg. challenge dose displayed no distress. Of the six receiving the 10 mg. injection, one animal had transient respiratory distress of six minutes' duration, with complete recovery.

Twelve guinea pigs received a single intra-abdominal injection of 50 mg. of thiamine contained in water-oil emulsion, as described under "Materials and Methods." Twelve days later, six of these animals were injected intravenously with 10 mg. of thiamine and only one animal evidenced signs of transient respiratory distress. The remaining six animals were subjected to a series of eight subcutaneous injections of 5 mg. of thiamine at four-day intervals. Eleven days following the last inoculation, the animals were challenged with 10 mg. of thiamine intravenously. The animals exhibited no gross signs of physiologic disturbance and all survived.

TABLE I. THE EFFECT OF INTRACARDIAL OR INTRA-ABDOMINAL INJECTION OF THIAMINE HYDROCHLORIDE IN NORMAL GUINEA PIGS

THIAMINE (MG.)	ROUTE	NO. OF ANIMALS	REACTION* (PER CENT)	DEATH (PER CENT)
10	Intracardial	15	73	6.7
15	Intracardial	4	100	0.0
20	Intracardial	4	100	25.0
25	Intracardial	4	100	50.0
50	Intra-abdominal	10	0.0	0.0

*Reaction is defined as signs of toxicity ranging from transient respiratory distress to generalized weakness of ten to sixty minutes' duration, or death.

Six normal guinea pigs were injected intra-abdominally with 50 mg. of thiamine, incubated in normal guinea pig serum. Challenge of these animals after twelve days with 10 mg. of thiamine intravenously did not elicit an observable reaction. Subsequently, these animals received four intra-abdominal injections of 50 mg. thiamine at weekly intervals, were allowed to rest for ten days, and were then challenged by the intravenous injection of 10 mg. of thiamine. Three of the animals coughed occasionally within five minutes of the injection, but no other symptoms were noted.

Capillary tube precipitin tests on the serum specimens from all experimental animals did not reveal detectable antibodies, although concentrations of thiamine in excess of 5 mg. per milliliter produced a light precipitate with serum from both normal and thiamine-injected animals. Similarly, complement-fixation tests were negative.

Twelve normal animals and twenty-two survivors of the sensitizing experiments were skin tested by both the intradermal route and the scratch technique. The results are shown in Table II. It was apparent that some degree of tolerance to thiamine was evinced in animals having previously received the drug. Scratch tests with the three drug concentrations in both groups of

animals failed to give uniform results. Only four normal and five thiamine-injected animals gave local reactions with the 25 mg. per milliliter preparation.

TABLE II. THE EFFECT OF 0.1 ML. INTRADERMAL INJECTION OF THIAMINE IN NORMAL AND PREVIOUSLY INJECTED GUINEA PIGS

CONCENTRATION OF THIAMINE (MG./ML.)	NORMAL ANIMALS		ANIMALS PREVIOUSLY INJECTED WITH THIAMINE	
	NO.	REACTION* (PER CENT)	NO.	REACTION* (PER CENT)
25.0	12	100.0	22	77.2
2.5	12	41.7	22	0.0
0.25	12	0.0	22	0.0

*Reaction was a rapid appearing, blanched urticarial wheal surrounded by an erythematous area.

DISCUSSION

Although we were able to show the development of a degree of tolerance to thiamine in the guinea pig following repeated injections, we were unable to demonstrate the establishment of a hypersensitive state. We could not demonstrate the formation of an antigenic conjugate of thiamine and serum proteins. Precipitating and complement-fixing antibodies were not demonstrable with the techniques employed.

In 1942, while skin testing human patients, Kalz⁹ observed that thiamine was a primary urticariogenic agent. Our observations reaffirm this finding, with the additional observation that guinea pigs possess a higher threshold of reactivity than man. Animals previously injected with thiamine evinced the acquisition of some tolerance by reacting to a lesser degree, or not at all, to concentrations that elicited wheals of fairly consistent diameter in normal animals. The scratch test suggested by Shapero and Gwinner⁴ failed to yield uniform results.

SUMMARY

1. We have been unable to demonstrate the development of a hypersensitive state toward thiamine hydrochloride in the guinea pig, employing multiple injections of thiamine, thiamine plus adjuvant, or thiamine incubated with normal guinea pig serum.

2. Precipitating or complement-fixing antibodies could not be demonstrated in animals injected with thiamine, although antibodies similar to those described by Loiseleur and Levy¹⁰ may be present.

3. Multiple injections of thiamine elevated the threshold of skin reactivity of guinea pigs in a manner similar to that described by Haley and Flesher,¹¹ who used rabbits.

REFERENCES

1. Weigard, C. G.: Reactions Attributed to Administration of Thiamin Chloride, *Geriatrics* 5: 274, 1950.
2. Jaros, S. H., Wnuck, A. L., and deBeer, E. J.: Thiamine Intolerance, *Ann. Allergy* 10: 291, 1952.
3. Reingold, I. M., and Webb, F. R.: Sudden Death Following Intravenous Injection of Thiamine Hydrochloride, *J. A. M. A.* 130: 491, 1946.
4. Shapero, W., and Gwinner, M. W.: Sensitivity to Thiamine Hydrochloride, *Ann. Allergy* 5: 349, 1947.

5. Armanino, L. P., and Scott, W. S., Jr.: Anaphylactic Shock Following Intravenous Administration of Thiamine Chloride, *California Med.* **72**: 178, 1950.
6. Stiles, M. H.: Hypersensitivity to Thiamin Chloride, With a Note on Sensitivity to Pyridoxine Hydrochloride, *J. ALLERGY* **12**: 507, 1941.
7. Mills, C. A.: Thiamine Overdosage and Toxicity, *J. A. M. A.* **116**: 2101, 1941.
8. Gell, P. G. H., Harington, C. R., and Rivers, R. P.: The Antigenic Function of Simple Chemical Compounds: Production of Precipitins in Rabbits, *Brit. J. Exper. Path.* **27**: 267, 1946.
9. Kalz, F.: Thiamine Hydrochloride—An Obligate Wheal Producing Agent, *J. Invest Dermat.* **5**: 135, 1942.
10. Loiseleur, J., and Levy, M.: La Specificite des Anticorps Consécutifs à L'injection Directe de Molécules Organiques de Faible Poids Moléculaire, *Ann. Inst. Pasteur* **73**: 116, 1947.
11. Haley, T. J., and Flesher, A. M.: A Toxicity Study of Thiamine Hydrochloride, *Science* **104**: 567, 1946.