the blood isopropyl alcohol curve in dogs given 1.0 g per kg intravenously. The predominant effect was a retardation of the rate of decline, and the use of these substances clinically in acute isopropyl alcoholic intoxication does not appear to be practical.

15431

Inhibitory Effect of Thiamine on Vasoconstrictor Action of Nicotine Tested in the Laewen-Trendelenburg Preparation.

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The inhibitory effects of sulfonamides, thiamine and thiazole compounds on the action of nicotine in isolated organs, such as small intestine and striated muscle have been reported previously.[1,2] Recently, investigations have been carried out to determine the effect of thiazole compounds on the blockade action of nicotine on the blood pressure of cats.[3] In view of the probable relationship between tobacco smoking and certain vascular diseases, especially thrombo-angiitis obliterans[4] on the one hand, and the peripheral vasoconstriction induced by nicotine on the other hand, an investigation on the effects of thiamine upon nicotinic vasoconstriction seemed to us of interest. The results reported here deal with such a study.

Methods. All the experiments were carried out using the Laewen-Trendelenburg (L-T) preparation in frogs (*Rana pipiens*). This method is highly sensitive for testing vasomotor substances. Male as well as female frogs were used. Frog Tyrode solution was used as a perfusate. Two Mariotte bottles were connected by a Y-piece with the perfusion system, *i.e.* the rubber tubing leading to the aortic cannula. One contained plain Tyrode solution, the other, dissolved in Tyrode, one of the following substances: crystalline thiamine hydrochloride (Merck), thiazole, pyrimidine, sodium sulfathiazole, thiouracil, para-aminobenzoic acid, nicotinamide. These substances were used at various concentrations. Nicotine in the form of nicotine base† or nicotine salicylate in aqueous solution was used. The injections of the different drugs were made through the rubber tubing into the glass cannula inserted into the abdominal aorta of the frog. All due precautions were used during the injection of drugs to avoid mechanical interference with the perfusion. The vasomotor action of the different drugs was measured in terms of the output variations of drops per minute.

Results. 1. *Control experiments with nicotine.* The injection of a small amount of nicotine (1-10 γ) in the L-T preparation elicited 2 major reactions: (a) Twitchings of the thigh and leg muscles, lasting as a rule from 30 to 60 seconds. (b) A marked reduction (50%-80%) of the outflow from the abdominal vein, as expressed in terms of drops per minute. The duration of the vasoconstrictor effect varied though the average ranged between 15 to 30 minutes until the output returned to its initial level.[5]

A series of experiments was carried out in which nicotine was tested several times in the same preparation. As a rule, the perfused frog became more sensitive to nicotine as the experiment progressed, *i.e.* a

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1 Pick, E. P., Brooks, G. W., and Unna, K., *J. Pharm. and Exp. Therap.*, 1944, **81**, 133.

2 Unna, K., and Pick, E. P., *J. Pharm. and Exp. Therap.*, 1944, **81**, 294.

3 Pick, E. P., and Unna, K., *J. Pharm. and Exp. Therap.*, in press, 1946.

4 Silbert, S., *J. A. M. A.*, 1945, **129**, 5.

† Nicotine Alkaloid Eastman Kodak.

5 Handovski, H., and Pick, E. P., *Arch. f. exp. Path. u. Pharmakol.*, 1913, **71**, 89.

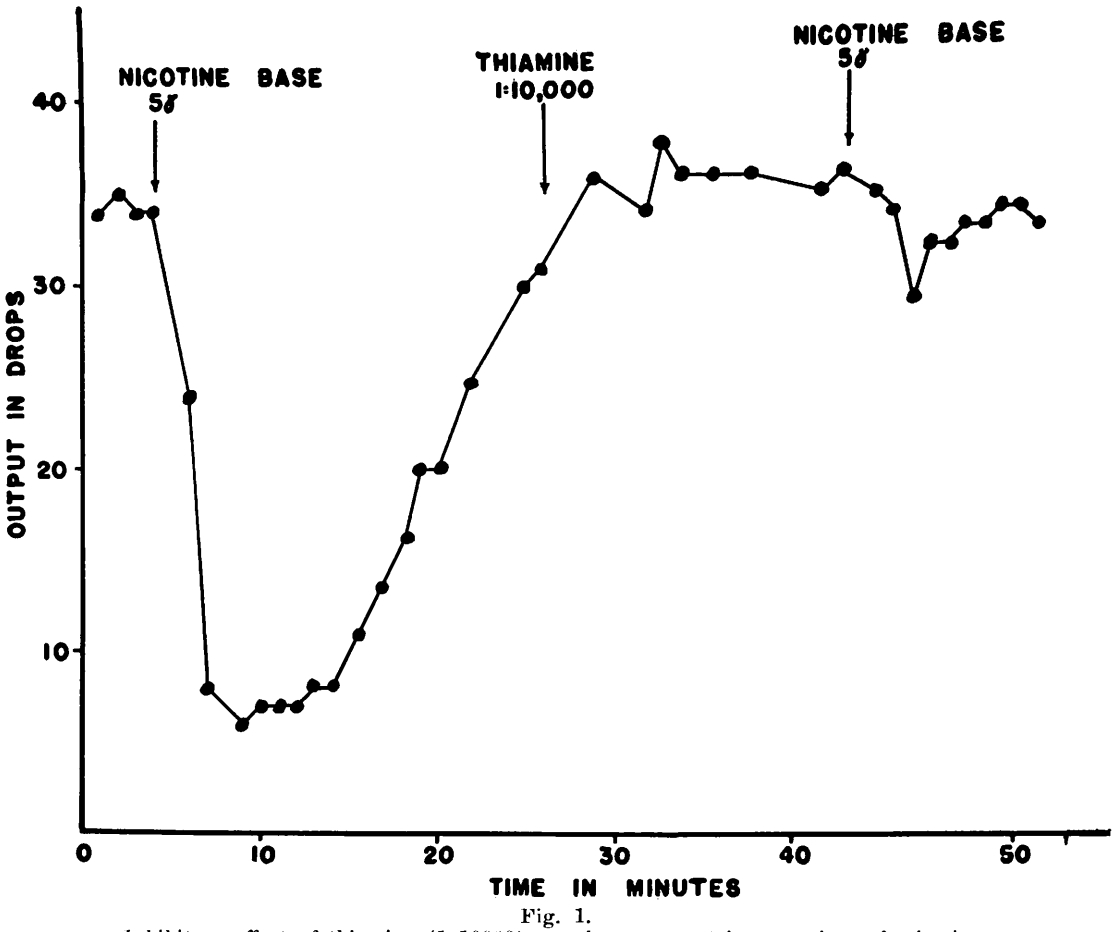

Fig. 1.
Inhibitory effect of thiamine (1:10000) on the vasoconstrictor action of nicotine.

small dose induced a more marked vasoconstriction. In some rare instances, however, a tolerance to nicotine took place. It was found that some frogs exhibit individual variations as to the doses of nicotine used, and some were entirely refractory to the usual small doses. In view of the above facts in the following series of experiments each individual preparation was first tested for its sensitivity to nicotine. Preparations exhibiting reduced reactivity to small amounts (1-10 γ) of nicotine were discarded. The best results obtained were those with nicotine base, which was used in most of the present work.

2. *Inhibitory effect of thiamine on nicotine.* The perfusion of the hind legs of the frog with a solution of thiamine hydrochloride in the concentration ranging from 1:10000 to 4:10000 completely inhibited the action of nicotine in most cases (Fig. 1). More dilute solution of thiamine was effective, although to a lesser degree, in preventing the nicotine vasoconstriction.

3. *Effective amount of thiamine retained in the tissues.* The perfusion with thiamine was usually allowed to run for 5 to 10 minutes before the tests were performed. Repeated nicotine injections showed that the vessels failed to constrict and the striated muscles failed to twitch. Since the L-T preparation is an open perfusion, it is self-evident that a large amount of the thiamine was passing only through the vessels and was being excreted. Only a partial amount would be retained therefore in the tissues. In order to evaluate the actual amount of thiamine fixed by the tissues we have

TABLE I.

Exp. No.	Thiamine input (γ) (10 min.)	Thiamine output (γ) (10 min.)	Amount retained (γ)	% retained
50	412	355	57	13.8
51	226	188	38	16.8

measured, in 2 perfusion experiments, fluorometrically, the input and output of thiamine over a period of 10 minutes. The difference thus evaluated (38-57 γ) represented the actual amount of thiamine fixed by the tissues. As can be seen from the following table (Table I) only from 13.8% to 16.8% of the perfused thiamine was retained and, of course, a very small part of it might be linked selectively on the specific nervous or muscle elements.

4. *Action of the individual moieties of the thiamine molecule on nicotine.* The 2 moieties of the thiamine molecule, 4-methyl-5-hydroxyethylthiazole and 2-methyl-5-ethoxymethyl-6-aminopyrimidine obtained in pure form‡ were tested individually. Thiazole in concentrations of 1 to 4 per 10000 cc of Tyrode solution, inhibited, partially or completely, the muscular twitchings and the vasoconstriction due to the action of nicotine (Fig. 2). The pyrimidine moiety, on the other hand, failed to counteract the effects of nicotine in the concentrations of 2 to 4 per 10000 cc of Tyrode.

5. *Action of related substances containing a thiazole or pyrimidine group on nicotine.* (a) In contrast to thiamine, sodium sulfathiazole antagonized the action of nicotine only in a relatively high concentration (4:1000), which was approximately 10 times as high as that of thiamine. Smaller doses (.1 to 2 per 1000) inhibited the nicotine action only slightly or not at all. It is noteworthy that in the proper concentration sulfathiazole exhibited not only an inhibitory effect on the vasomotor activity of nicotine but also abolished the muscular twitchings of the hind legs of the frog.

In connection with sulfathiazole, para-aminobenzoic acid was tested with no inhibitory effect. The same statement is true for nicotinamide.

(b) Thiouracil, which is a pyrimidine, used in concentrations of 1 to 4 per 10000 did not inhibit the action of nicotine.

Discussion. Nicotine used in the L-T preparation as test object, acts on both striated and smooth muscle. Thiamine, through its thiazole group, blocks these 2 actions. After excision of both sympathetic chains and resection of all spinal nerves of the hind legs, the injection of small amounts of nicotine (1-10 γ) still induces the same degree of twitching and vasoconstriction as before. This fact seems to indicate that the site of action of nicotine in the L-T preparation is not in the autonomic ganglia but more peripheral. It is well known that *striated muscle* can be stimulated by applying nicotine to it. "The twitchings are rarely, if ever, produced except from the region of the nerve endings." (Langley).[6]

As to the *smooth muscle* of the vascular bed, the site of action of *nicotine* seems to be either at the post-ganglionic nerve endings or at the neuro-effector cells. This would be in agreement with Stoehr's work on mammalian vessels[7] and with Krogh's work on the frog vessels,[8] according to the latter it is extremely improbable that local ganglion cells exist in the vessels of the extremities. On the other hand, since *thiamine* as shown previously[2] has an affinity for synapses, it is to be assumed that it is fixed at the myoneural junctions and thus blocks the action of nicotine.

Thiazole is the active group of the molecule, while pyrimidine is ineffective. These findings are in agreement with previous investigations on isolated smooth and striated muscles.[1,2] The intimate mechanism of the

‡ These substances were obtained through the courtesy of Dr. W. H. Engels of the Merck Research Laboratory, Rahway, N. J.

[6] Langley, T. N., *J. Physiol.*, 1907-1908, **36**, 347.

[7] Stoehr, P., Jr., *Mikrosk. Anat. d. veget. Nervensyst.*, Berlin, Springer, 1928, 67.

[8] Krogh, A., *The Anat. and Physiol. of Capillaries*, Yale University Press, 1924, 81.

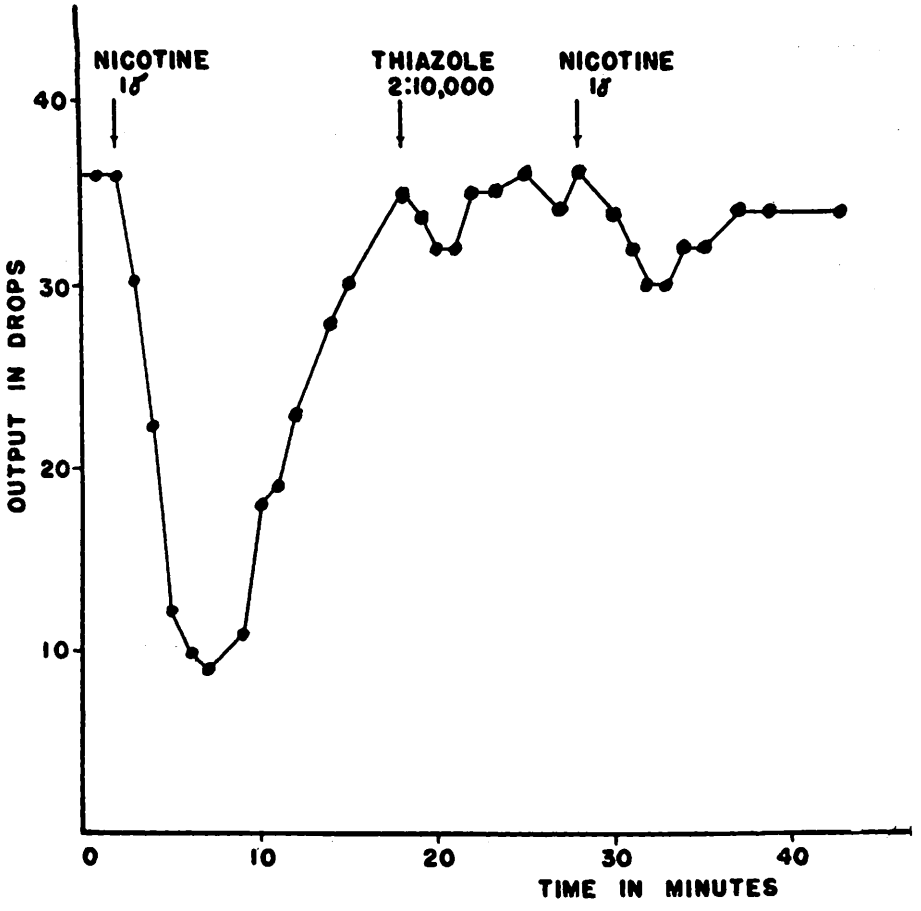

Fig. 2.
Inhibitory effect of thiazole (2:10000) on the vasoconstrictor action of nicotine.

antagonism between thiazole and nicotine was not investigated here.

Summary. 1. Thiamine, in very small concentrations (1:10000), inhibits the vasoconstrictor action of nicotine in the frog vessels. 2. The inhibiting action of thiamine is linked with the thiazole moiety of the molecule, the pyrimidine group being ineffective. 3. Thiouracil, nicotinamide, paraaminobenzoic acid do not inhibit the action of nicotine; sulfathiazole has an inhibiting effect only in a relatively high concentration (4:1000). 4. The site of action of thiamine seems to be at the myoneural junction in the striated muscles and at the postganglionic nerve endings or in the muscle elements of the vessel walls in the smooth muscles.