STUDIES ON THE ALLERGY-LIKE FOOD POISONING ASSOCIATED WITH PUTREFACTION OF MARINE PRODUCTS

IV. EFFECT OF TRIMETHYLAMINE AND TRIMETHYLAMINE OXIDE ADDED TO HISTAMINE OF "SAURINE" UPON THEIR OWN PHYSIOLOGICAL ACTION

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INTRODUCTION

Miyaki et al⁽¹⁾ pointed out that the main causative agents of some allergy-like food poisoning might be unusual amount of histamine and other cooperative factors contained in the incriminated material. They presumed that such biogenic amines as agmatine, phosphorylcholine or methylguanidine would play synergistic action to histamine in decreasing its lethal dose for mice. Hayashi⁽²⁾, using uterus of guinea pigs, in his subsequent experiment, described the action of trimethylamine (TMA) or trimethylamine oxide (TMAO) to enhance the action of histamine, and he also reported a similar observation by means of blood pressure method using cats when TMA, TMAO or phosphorylcholine was added to histamine.

The present paper deals with the effect of TMA or TMAO added to "saurine" or histamine upon their respective physiological action tested on the contractility of intestine or uterus of guinea pigs, shock symptoms in guinea pigs, skin reaction in rabbits and lethal dose for mice (LD_{50}).

EXPERIMENTALS

Exp. 1. Tests on the Contractility of Intestine and Uterus of Guinea Pigs

a. Method i) "Saurine" solution was prepared by the same procedure as described in our previous paper, III. Four tenths cc of "saurine" was found corresponding to 2γ of histamine in its action to contract intestine of guinea pigs by Magnus' method.

ii) The following four test-solutions were used.

	Concent. of original solution
TMA (Wako Pure Chemicals Co.)	10 ⁻¹ M
TMAO	"
Histamine (Merck Co.)	"
Histidine (Takeda Chem. Co.)	"

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iii) Contraction test using uterus or intestine of guinea pigs:

Contraction of uterus or intestine was determined by Magnus' method. The technique adopted by Hayashi⁽²⁾ was as follows: Using uterus of guinea pigs, threshold value, the maximum concentration of no visible contraction, was deter-

Fig. 1. The effect of TMA or TMAO added to histamine or "saurine" tested on the contraction of uterus of guinea pigs

H: histamine S: "saurine"

Table 1. Summarized results on the effect of TMA or TMAO added to histamine or "saurine" shown in those kymogram of Fig. 1

Kymogram	Test solution	Synergism	Rate of increase	Method
A	H, 1γ+TMAO 10 ⁻⁵ M, 0.2cc	+		I
B	H, 2.5γ+TMAO 10 ⁻⁵ M, 0.5cc	+	<10%	II
C	H, 1γ+TMA 10 ⁻⁵ M, 0.1cc	-		I
D	H, 2γ+TMA 10 ⁻⁵ M, 0.4cc	-	0%	II
E	S, 0.2cc+TMA 10 ⁻⁵ M, 0.2cc	+		I
F	S, 0.3cc+TMAO 10 ⁻⁵ M, 0.2cc	+		I
G	S, 0.1cc+TMAO 10 ⁻⁵ M, 0.1cc	+	<5%	II

Note: H: histamine, S: "saurine"
Method I: qualitative method, II: quantitative

mined prior to the examination. Then, after adding appropriate concentration of other kind of amine to the threshold value of histamine, change in contraction of uterus was observed. In this respect, this procedure might be regarded as a qualitative method. In this paper, a quantitative method using intestine as well as uterus were employed. The method was detailed in our paper I⁽³⁾.

b. *Results* i) Synergistic action judged by the results using uterus:

As shown in Fig. 1 and Table 1, sometimes synergistic action of TMA or TMAO added to either histamine or "saurine" was observed to some extent (see A, B, E, F and G in Fig. 1), and sometimes no action was recorded in the same experiment (see C and D in Fig. 1). However, even when positive synergistic action was observed, the rate of increase in the action of histamine or "saurine" was less than 10% (which seemed to be within the range of error). Sometimes, strong spontaneous contraction occurred during experiment. These irregular results might have been due mainly to the nature of contraction of uterus.

ii) Synergism judging from the contractility of intestine

—quantitative method—

TMA + Histamine

No appreciable contraction of intestine was observed with 1 cc of 10^{-2} M TMA only, but slight action was recorded with 0.3 cc of 10^{-1} M. As shown in Table 2, no significant synergism was detected with TMA plus histamine, except-

Table 2. Histamine added to TMA

Histamine	TMA	Contraction of intestine (shown in equivalent histamine)	Synergism
—	10^{-2} M, 1cc	—	
—	10^{-1} M, 0.3 „	weak	
2 γ	10^{-8} M, 0.5 „	2 γ	—
„	10^{-7} M, „	2 $\gamma \sim 2.1 \gamma$	—
„	10^{-6} M, „	2 γ	—
„	10^{-5} M, „	„	—
„	10^{-4} M, „	„	—
„	10^{-3} M, „	„	—

The effect of pretreatment of intestine with TMA

TMA	Histamine	Contraction of intestine (shown in equivalent histamine)	Synergism
10^{-7} M, 0.5cc	1.5 γ	1.5 γ	—
10^{-6} M, „	2 „	2 „	—
10^{-5} M, „	2 „	2 „	—

ing a case with 0.5 cc of 10^{-7} M. In this case, however, the rate of increase was within 5% which might be within the range of error. Increase in the rate of contraction of the intestine could hardly be detectable when the intestine had been pretreated with TMA, allowed to stand for 1 minute, and then histamine was added.

Histidine+Histamine

Histidine has no activity to contract intestine of guinea pigs (0.5 cc of 10^{-1} M solution). Addition of histidine to histamine resulted in no detectable change to decrease or increase in its contractility of intestine (see Table 3).

Table 3. Histidine added to histamine

Histamine	Histidine	Contraction of intestine (shown in equivalent histamine)	Synergism
—	10^{-1} M, 0.5cc	—	
2 γ	10^{-7} M, 1cc	2 γ	—
"	10^{-6} M, "	"	—
"	10^{-5} M, "	"	—
"	10^{-4} M, "	"	—
1 γ	10^{-7} M, "	1 γ	—
"	10^{-6} M, "	"	—
"	10^{-5} M, "	"	—

TMAO+Histamine

One half cc of 10^{-1} M TMAO did not affect intestine to any measurable extent. Results with the mixture consisted of TMAO and histamine are shown in the upper column of Table 4, which indicates negative synergism. Using

Table 4. TMAO added to histamine

TMAO	Histamine	Contraction of intestine (shown in equivalent histamine)	Synergism
10^{-1} M, 0.5cc	—	—	
10^{-5} M, 0.7cc	1 γ	1 γ	—
" , 0.1cc	2 γ	2 γ	—
10^{-8} M, 1 cc	1 γ	1 γ	—
" , 0.5cc	"	"	—
" , "	2 γ	2 γ	—
Pretreatment with TMAO			
10^{-5} M, 0.3cc	1 min. after 2 γ	2 γ	—
10^{-8} M, 0.5cc	" "	"	—

uterus, Hayashi⁽²⁾ pointed out that a synergistic effect of TMAO to histamine was detectable at the concentration of 10^{-5} M, whereas it acted either synergistic or inhibitory when the addition was made in different sequences or in different amounts to histamine. However, quantitative method using intestine carried out by the present authors disclosed that no significant increase or decrease was observed in the contractility of histamine even when the intestine was pretreated with TMAO.

TMA+“*Saurine*”

As shown in Table 5, the contractility of “saurine” was not enhanced by the addition of TMA, agreeing with the results with TMA plus histamine.

Table 5. TMA added to “saurine”

TMA	“saurine”	Contraction of intestine (shown in equivalent histamine)	Synergism
10 ⁻² M, 0.9cc	0.3cc	1.5γ	—
10 ⁻³ M, „	„	„	—
10 ⁻⁴ M, „	„	„	—
10 ⁻⁵ M, „	„	„	—
10 ⁻⁶ M, „	„	„	—
10 ⁻⁷ M, „	„	„	—

Three tenths cc of “saurine” is equivalent to 1.5 γ of histamine

TMAO+“*Saurine*”

No synergistic effect was detected by the addition of TMAO to “saurine” on its contractility of intestine. Results obtained are shown in Table 6.

Table 6. TMAO added to “saurine”

TMAO	saurine	Contraction of intestine (shown in equivalent histamine)	Synergism
10 ⁻² M, 0.9cc	0.4cc	2γ	—
10 ⁻³ M, „	„	„	—
10 ⁻⁴ M, „	„	„	—
10 ⁻⁵ M, „	„	„	—
10 ⁻⁶ M, „	„	„	—
10 ⁻⁷ M, „	„	„	—

Four tenths cc of “saurine” is equivalent to 2 γ of histamine

Histidine+“*Saurine*”

Addition of histidine to “saurine” resulted in negative synergism in its contractility of intestine (see Table 7).

Table 7. Histidine added to saurine

Histidine	saurine	Contraction of intestine (shown in equivalent histamine)	Synergism
10 ⁻¹ M, 0.5cc		—	
10 ⁻³ M, 0.9cc	0.2cc	1γ	—
10 ⁻⁴ M, „	„	„	—
10 ⁻⁵ M, „	„	„	—
10 ⁻⁶ M, „	„	„	—
10 ⁻⁷ M, „	„	„	—

Two tenths cc of “saurine” is equivalent to 1 γ of histamine

Histamine+“*Saurine*”

Additive effect between histamine and “saurine” has been reported in our paper III; re-examination on the mixture of both solutions rendered our finding more valid, in which the rate of contraction of the mixture of both solutions agreed with that of total of the rate of contraction of each solution. These results seem to prove the presence of an additive effect and the absence of synergism between them.

Table 8. Effect observed between histamine and “saurine”

Histamine	Saurine	Contraction of intestine (shown in equivalent histamine)	Effect
1 γ	0.4cc	2 γ	additive
1 „	0.2cc	1.5	„
1 „	0.2cc	„	„

Four tenths cc of “saurine” is equivalent to 1 γ of histamine

Exp. 2. Effect TMA or TMAO Added to Histamine or “Saurine” Tested on Shock Symptoms in Guinea Pigs

a. Method The minimum lethal dose of histamine or “saurine” by intravenous injection was determined with guinea pigs prior to the examination; then TMA or TMAO was added in various concentrations to the threshold value of

Table 9. Effect of TMA or TMAO added to histamine
(shock symptoms in guinea pigs)

No.	Body wt (g)	histamine (γ)	TMA (γ)	TMAO (γ)	Shock symptoms observed
1	420	50			++ survived
2	490	100			## „
3	565	200			## „
4	490	300			died after 30''
5	535	300			### survived
6	605	500			died after 15''
7	480	200	5		## survived
8	560	100	„		## „
9	635	50	„		## „
10	670	200	50		## „
11	700	200		5	## „
12	470	200		10	died after 5'
13	455	200		50	## survived

+ Urination (observed in all animal tested on)

++ Sway

Inversion

More severe symptoms developed

histamine or "saurine"; the latter value was expressed in equivalent histamine estimated by Magnus's method. Each test solution was simultaneously injected intravenously into guinea pigs and symptoms developed were recorded.

b. Results Results thus obtained are shown in Tables 9 and 10. In no case, significant influence upon the lethal dose of histamine or "saurine" was observed when TMA or TMAO was added to them, excepting a case in which an injection with 200 γ of histamine containing 10 γ of TMAO brought to death. However, a typical acute death by shock with histamine is generally considered to occur 30 seconds or 1 minute after the injection. Whereas, in the case

Table 10. Effect of TMA or TMAO added "saurine"
(shock symptoms in guinea pigs)

No.	Body wt (g)	saurine (γ)	TMA (γ)	TMAO (γ)	shock symptoms observed
1	565	500			died
2	660	400			"
3	565	200			## survived
4	550	200			died
5	660	150			### survived
6	635	200		20	died
7	540	150		15	## survived
8	550	200	15		### "
9	690	150	"		## "

Dosages of "saurine" injected are shown in equivalent histamine:

Inversion

More severe symptoms developed

described above, the death happened 5 minutes after the injection and other guinea pig injected with 200 γ of histamine containing 50 γ of TMAO did survived. Therefore, the death described in the above might have been due to not the synergism but to an accident.

*Exp. 3. Effect of TMA or TMAO Added to Histamine or "Saurine" Tested
in Skin Reaction with Rabbits*

a. Method After depilation of the backs of rabbits, 0.1 cc of each test solution was injected intracutaneously. Original test solutions were those 500 γ /cc each of histamine and "saurine" (equivalent histamine estimated by Magnus'

Table 11. Concentration of test solution and the amount of TMA or TMAO added to the test solution

Concent. of histamine or "saurine"	31 γ	15.5 γ	7.75 γ	3.87 γ	1.94 γ	0.97 γ
TMA or TMAO	$3.1 \times 10^{-3}M$	$1.55 \times 10^{-3}M$	$7.75 \times 10^{-4}M$	$3.87 \times 10^{-4}M$	$1.94 \times 10^{-4}M$	$9.7 \times 10^{-5}M$

method) and 0.5×10^{-1} M each of TMA and TMAO. The constituent parts of each test solution injected are shown in Table 11. The activity of the test solution to develop skin reaction was observed immediately after, 15 minutes and 3 hours after the injection.

b. Results As shown in Table 12, distinct redness and wale were observed with higher concentration than 7.5γ /cc of histamine and "saurine" as well, whereas, the addition of TMA or TMAO to both substances did not enhance their activity. At higher dilutions of the test solution, no significant difference in skin reaction was detected between those solution containing TMA or TMAO and those of control solution. Thus, the addition of TMA or TMAO did not increase the effect of histamine or "saurine" to develop skin reaction in rabbits.

Table 12. Effect of TMA or TMAO added to histamine or "saurine"
(Skin reaction in rabbits)

Test solution	Period	Concentration of histamine or "saurine"					
		31 γ	15.5 γ	7.75 γ	3.87 γ	1.94 γ	0.97 γ
Histamine	Instant	R Q	R Q	R Q	Q	Q	Q
	15' after	R Q	R Q	R Q	q	q	—
	3 hr. after	Q	Q	Q	—	—	—
Histamine +TMA	Instant	R Q	R Q	R Q	Q	Q	Q
	15'	R Q	R Q	R Q	Q	Q	Q
	3 hr.	Q	Q	Q	q	q	—
Histamine +TMAO	Instant	R Q	R Q	R Q	Q	Q	Q
	15'	R Q	R Q	R Q	Q	Q	Q
	3 hr.	Q	Q	q	—	—	—
"Saurine"	Instant	R Q	R Q	R Q	$\pm$ Q	Q	Q
	15'	R Q	R Q	R Q	q	q	—
	3 hr.	Q	Q	Q	q	q	—
"Saurine" +TMA	Instant	R Q	R Q	R Q	q	q	q
	15'	R Q	R Q	R Q	q	q	—
	3 hr.	Q	Q	Q	q	—	—
"Saurine" +TMAO	Instant	R Q	R Q	R Q	$\pm$ Q	Q	Q
	15'	R	R	R	q	q	—
	3 hr.	Q	Q	Q	q	—	—

Note: R; redness (Rötung)

Q; wale (Quaddel)

q; small-sized wale

Concentrations of "saurine" are expressed in equivalent histamine by means of Magnus' method.

Exp. 4. Effect of TMA or TMAO Added to Histamine or "Saurine" Observed from the Lethal Dose for Mice (LD_{50})

Being insensitive or having a high tolerance to biogenic amines, mouse is considered to be unsuitable for the examination for their toxicity. Meanwhile, synergistic action of some biogenic amines to histamine in decreasing its lethal dose for mice was reported by Miyaki *et al*⁽¹⁾ Therefore, an attempt was made to know the effect of TMA or TMAO added to histamine or "saurine" by testing the lethal dose for mice (LD_{50}).

a. *Method* The lethal dose of histamine and the effect of the addition of TMA or TMAO upon it were examined by intraperitoneal injection into mice. Several groups, each consisted of six mice weighing 16 ± 2 g, were injected with one test solution. At first, the lethal dose (LD_{50}) of histamine-2 HCl (Merck) was determined by means of Van der Werden's method, and subsequently, the lethal dose of histamine containing 0.5×10^{-4} M each of TMA or TMAO was estimated.

b. *Results* The LD_{50} of each test solution is as follows:

Histamine	31.9 ± 1.01 mg/mouse (Van der Werden)
Histamine+TMA	35 mg/mouse (Behrens-Kaerber)
Histamine+TMAO	36.5 ± 1.03 mg/mouse (Van der Werden)

From the results tabulated in the above, it is obvious that the addition of TMA or TMAO to histamine caused no significant change in the lethal dose of histamine.

DISCUSSION

Miyaki et al⁽¹⁾ presumed that the causative agents of some outbreak of allergy-like food poisoning should be attributable to the presence of unusual amount of histamine and some synergistic factors such as other kind of putrefactive amines which cooperate with histamine in the responsible foodstuff. Subsequently, they stated that TMA, TMAO, phosphorylcholine, agmatine etc. are such substances. In the first place, the authors attempted to learn the effect of TMA or TMAO added to "saurine" or histamine (as control) upon its own physiological action, but failed to show synergism between them.

Using uterus of guinea pigs, sometimes synergistic action of TMA or TMAO added to either histamine or "saurine" was observed to certain extent, and sometimes no action was recorded in the same experiment. Moreover, sometimes strong spontaneous contraction occurred during experiment. These irregular results might have been due mainly to the nature of uterus. However, even when positive synergism occurred, the rate of increase in the action of histamine or "saurine" is small. On the contrary, no synergistic action was observed in the experiment using intestine of guinea pig. Moreover, the addition of TMA or TMAO resulted in no observable change in the action of histamine or "saurine" as tested on shock symptoms in guinea pigs, skin reaction in rabbits and the lethal dose for mice (LD_{50}). Therefore, the presence of TMA or TMAO, if any, could not be regarded to stand as a cooperative factor to either histamine or "saurine" in a food responsible for allergy-like food poisoning. Of course, our experiment was restricted in the addition of TMA or TMAO, consequently, the invalidity of TMA or TMAO as a cooperative factor to histamine or "saurine" does not mean the denial of synergism of the whole putrefactive amines. Therefore, the authors wish to extend our experiment to other kinds of amine in future.

Generally, the content of TMAO in fish muscle is considered^(4, 5) to be rich in white muscle of such species as cod, haddock, seabream (*Pagrus cardinalis*)

and so forth, especially, remarkable amount of TMAO is contained in the muscle of elasmobranchs, on the other hand, poor in such fishes having reddish muscle. In these muscle, however, a large amount of histidine⁽⁶⁾ in free state is contained as compared with that in white muscle, from which such harmful biogenic amine as histamine may be produced by bacteria during the course of putrefaction. The outbreaks of allergy-like food poisoning have been restricted to the consumption of fishes with reddish muscle, so far, therefore, the amount of TMAO contained in the food may not be playing an important role in the outbreak of such poisoning.

As for the amount of TMA contained in the responsible food of outbreaks, the authors' analytical data revealed that about 10–13 mg% of TMA–N was estimated and trace or slight amount of TMAO was detected in each incriminated food. These findings may suggest that TMAO contained in fish flesh has already been reduced to TMA by bacterial action and the presence of TMAO in such slight amount might not play an important role in the outbreak of allergy-like food poisoning when observed from the actual view points, even though a strong synergistic action to histamine it may have.

SUMMARY AND CONCLUSION

The effect of trimethylamine (TMA) or trimethylamine oxide (TMAO) added to histamine or "saurine" (an autonomic nervous poison like histamine recently isolated by the authors) was examined by means of Magnus' method using intestine or uterus of guinea pigs and, further, by shock symptoms in guinea pigs, skin reaction in rabbits and the lethal dose for mice (LD_{50}).

Results obtained are as follows:

1. Using uterus, sometimes synergistic action of TMA or TMAO to either histamine or "saurine" was observed to certain extent, and sometimes no action was recorded in the same experiment. These irregular results might have been due mainly to the nature of uterus. However, even when positive synergism was observed, the rate of increase in the action of histamine or "saurine" was less than 10%.

2. In no case, synergistic action was observed in the experiment using intestine. Results obtained are summarized as follows:

Concent. of histamine or "saurine"		Range of concent. of test solution in M at which no synergistic action occurred	
Histamine	2 γ	TMA	0.5×10^{-3} – $0.5 \times 10^{-8} M$
"	"	TMAO	0.1×10^{-5} – $0.5 \times 10^{-8} M$
"	"	Histidine	1×10^{-4} – $1 \times 10^{-7} M$
"Saurine"			
0.3 cc (1.5 γ equivalent histamine)		TMA	0.8×10^{-2} – $0.5 \times 10^{-7} M$
0.4 " (2 " " " ")		TMAO	0.9×10^{-2} – $0.9 \times 10^{-7} M$
0.2 " (1 " " " ")		Histidine	0.9×10^{-3} – $0.9 \times 10^{-7} M$

3. The addition of TMA or TMAO to histamine or "saurine" resulted in no appreciable change in the action tested by shock symptoms in guinea pigs, skin reaction in rabbits and the lethal dose for mice (LD_{50}).

4. Therefore, the presence of TMA or TMAO, if any, could not be regarded to stand as a cooperative factor to either histamine or "saurine" in a foodstuff responsible for allergy-like food poisoning.

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