[Chem. Pharm. Bull. 17(8)1605—1610(1969)]

UDC 615.324.015 : 597.54 : 615.217.2.015

Antagonistic Phenomena between Crystalline Tetrodotoxin and Guanidines on Sympathetic Nervous Systems[1]

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(Received December 13, 1968)

The antagonistic phenomena between crystalline tetrodotoxin and guanidine in the sympathetic nerve–smooth muscle preparations were investigated and the following results were obtained.

In the hypogastric nerve–vas deferens preparation of the guinea–pig, the contractions of the vas deferens induced by the electrical stimulation of the pre- and post–ganglionic fiber of the hypogastric nerve were suppressed after the administrations of crystalline tetrodotoxin 5×10^{-8} M and procaine 10^{-4} M, and the suppression by tetrodotoxin was antagonized by guanidine 10^{-3} M or methylguanidine 10^{-3} M, and that by procaine was only temporally antagonized. These antagonistic phenomena were observed also on the guinea–pig trachea preparation and the nictitating membrane of cat.

In the hypogastric nerve–vas deferens preparation of the guinea–pig, moreover, the contraction which could not be observed in the absence of Na^+ in the external solution was recovered by applying guanidine into the solution.

From these results, it is concluded that tetrodotoxin and guanidine or methylguanidine would be antagonistic at the presynaptic site of sympathetic nerve ending.

Authors have already reported the action of crystalline tetrodotoxin on sympathetic nervous systems using the nictitating membrane of cat, the vas deferens of guinea–pig and isolated rabbit ileum,[3] and it could be concluded that the sites of action were the ganglion and the nerve ending and not smooth muscle itself, and that the action on the nerve ending might be more excellent. The observation which tetrodotoxin did not affect on the smooth muscle itself was reported by Kuriyama, *et al.*,[4] Ohga and Nakazato,[5] Narahashi, *et al.* [6] and Nonomura, *et al.*[7]

It has been reported that the mechanism of action of tetrodotoxin on the skeletal muscle preparation was the selective suppression of Na^+ permeability at the excitable membranes of the nerve and muscle.[6,8] And it was presumed that the suppression of Na^+ permeability would occur since Na-channel is inhibited by the guanidino group which tetrodotoxin possesses.[9] The authors reported a temporary antagonistic phenomenon between tetrodotoxin and guanidine or methylguanidine in the skeletal muscle preparation.[10] According to the experiment of Tasaki, *et al.*[11] in the giant axon of squid, in the case which guanidinium ion

1) This work was reported at Meeting of Tohoku Branch, Pharmaceutical Society of Japan, Sendai, July 1968.
2) Location: *Aobayama, Sendai.*
3) H. Ozawa and K. Sugawara, *Japan. J. Pharmacol.*, **17**, 287 (1967).
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was included in Na-free perfusion medium an action potential was observed. Moreover, it is suggested that guanidine passes through Na-channel of the membrane. From these points, in the present paper, authors investigated the antagonistic phenomena between guanidine and tetrodotoxin on sympathetic nervous system, and compared with those between guanidine and procaine, which suppresses the permeability of both Na^+ and K^+.

Methods and Materials

1. Guinea-pig Hypogastric Nerve-Vas Deferens Preparation——After the male guinea-pigs (250—500 g) were stunned and bled out, the vas deferens was dissected together with the hypogastric nerve. The preparation was suspended in a 10 ml bath containing a Tyrode solution of the following composition: sodium chloride, 137 mM; potassium chloride, 2.7 mM; calcium chloride, 1.8 mM; magnesium chloride, 1.1 mM; sodium bicarbonate, 11.9 mM; sodium dihydrogenphosphate, 0.4 mM; and glucose, 5.6 mM. The contraction of the vas deferens induced by the electrical stimulation of the hypogsatric nerve was recorded on a smoked paper with an isotonic writing lever. The electrical stimulation was applied every 3 min at a frequency of 50 cps with 1 msec duration and at supramaximal voltage for 3 sec. The stimulation of the postganglionic fiber of the hypogastric nerve was carried out transmurally according to the method of Birmingham.[12] In this case, the electrical stimulation was applied at a frequency of 20 cps with 1 msec duration and at 90—120 V for 3 sec. During the experiments, the bath was maintained at 37° and aerated with 95% O_2+5% CO_2. Na^+-free solution was prepared by substituting with an equivalent Li^+ instead of Na^+ and K^+-free solution by substituting with an equivalent Na^+ instead of K^+.

2. Guinea-pig Trachea Preparation——The experiment was carried out according to the method of Foster.[13] A cannula was tied into each end of the trachea and a long platinum wire electrode was passed up through the lower cannula and tracheal lumen until its end lay in the upper cannula. The preparation was fitted into a 20 ml bath containing Tyrode solution at 37° and aerated with 95% O_2+5% CO_2. The other platinum electrode lay in the bath opposite the tracheal muscle. The electrical stimulation was applied every 10 min at a frequency of 10 cps with 0.4 msec duration and at 60 V for 30 sec. Since the relaxant response was object of study, atropine (4×10^{-7} g/ml) was included in all Tyrode solution which came into contact with trachea.

3. Cervical Sympathetic Nerve-Nictitating Membrane of the Cat——The cats (2—4 kg) both sexes were anaesthetized with 1.4 g/kg urethane subcutaneously. After tracheal cannulation, and cannulation of one femoral vein for intravenous administration of drugs, cats were appropriately prepared for recording. Blood pressure was recorded through the mercury manometer from one femoral artery. The contractions of one nictitating membrane were recorded on a smoked paper with an isotonic writing lever. The contractions were induced at 3 min intervals by the stimulations of the ipsilateral pre- and post-ganglionic cervical sympathetic nerves at a frequency of 20 cps with 1 msec duration and at supramaximal voltage. The direct injection of drugs to ganglion was carried out according to the method of Morrison and Paton[14] and Trendelenburg. In this method, a polyethylene cannula was inserted into the lingual artery, and the drug was injected retrogradely into the carotid artery through the cannula. During intraarterial injection, the external carotid artery was occluded with a clip. The drugs were usually injected in a volume of 0.3 ml.

Materials——The drugs used in these experiments were following: crystalline tetrodotoxin (C.TET.), procaine hydrochloride, guanidine sulfate (G), and methylguanidine sulfate (MG).

Results

1. Guinea-pig Hypogastric Nerve-Vas Deferens Preparation

The contraction of the vas deferens induced by the electrical stimulation of the preganglionic fiber of the hypogastric nerve was completely blocked by 5×10^{-8} M of C.TET. 30 min after the administration (Fig. 1a). After the contraction was suppressed about 50% by C. TET., G 10^{-3} M was administered in the bath, so that the height of the contraction was recovered to a previous level. This shows an antagonism between C.TET. and G (Fig. 1b). Also, the suppression of the vas deferens induced by C.TET. was antagonized by 10^{-3} M of MG (Fig.1c). But when G and MG were administered after the contraction was completely block-

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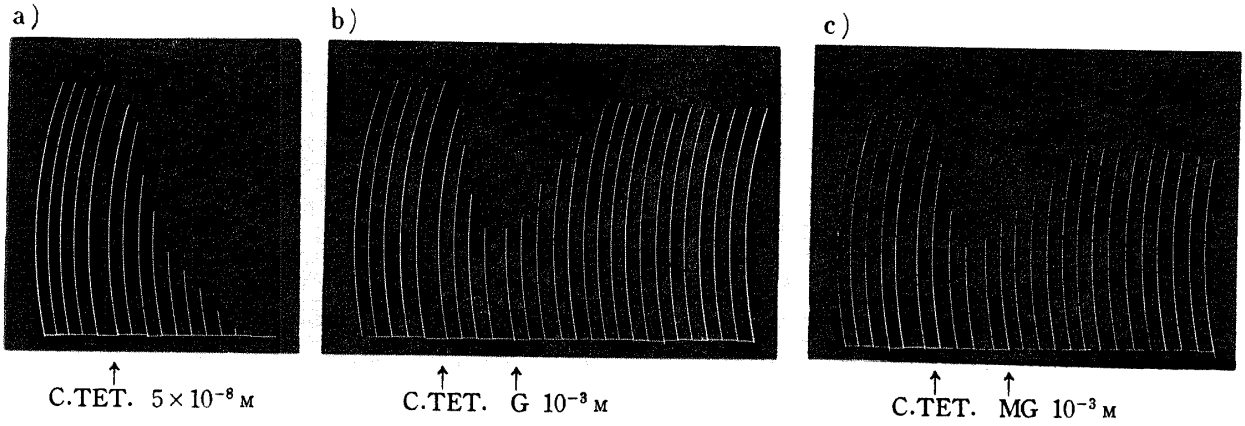

Fig. 1. Effect of C. TET. on Hypogastric Nerve–Vas Deferens Preparation of Guinea–pig

Electrical stimulation to preganglionic fiber was applied at 50 cps, 1 msec, supramaximal voltage.
Drugs were administered at arrow.
a) C. TET. 5×10^{-8}M, control
b) G 10^{-3}M after administration of C. TET. 5×10^{-8}M
c) MG 10^{-3}M after administration of C. TET. 5×10^{-8}M

ed by C.TET., the contration could not be recovered. The contraction of the vas deferens induced by the electrical stimulation on the preganglionic fiber of the hypogastric nerve was blocked by procaine 10^{-4}M as well as by C.TET., but the block was only temporally recovered by G and MG (Fig. 2). As shown in Fig. 3, also, the suppression of the contraction of the vas deferens induced by the postganglionic stimulation of the hypogastric nerve was antagonized by G and MG.

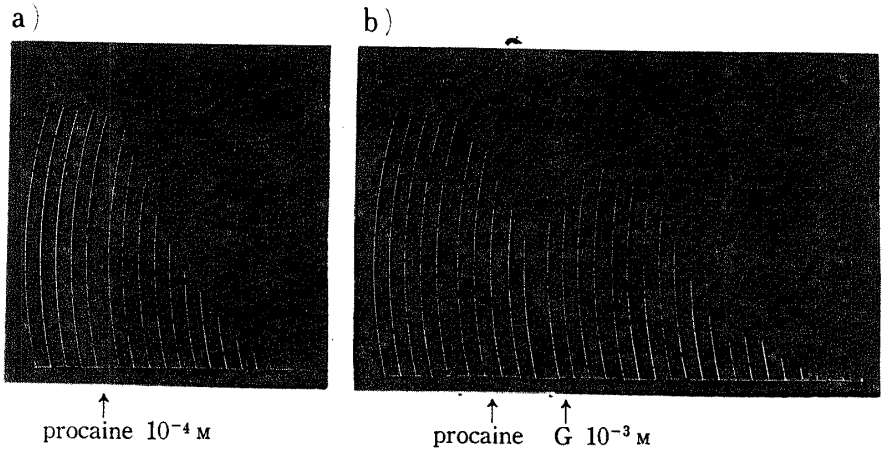

Fig. 2. Effect of Procaine on Hypogastric Nerve–Vas Deferens Preparation of Guinea–pig

Electrical stimulation to preganglionic fiber was applied at 50 cps, 1 msec, supramaximal voltage.
Drugs were administered at arrow.
a) procaine 10^{-4}M, control
b) G 10^{-3}M after administration of procaine 10^{-4}M

The contraction of the vas deferens induced by the electrical stimulation of the hypogastric nerve was reduced together with the decrease of Na^+ and could not be observed in the absence of Na^+ in the external solution. However, if G was included in external solution containing a low concentration of Na^+, the contraction was recovered (Fig. 4). When 10 mM of G was included in external solution of 40 mM of Na^+, the response was almost completely recovered.

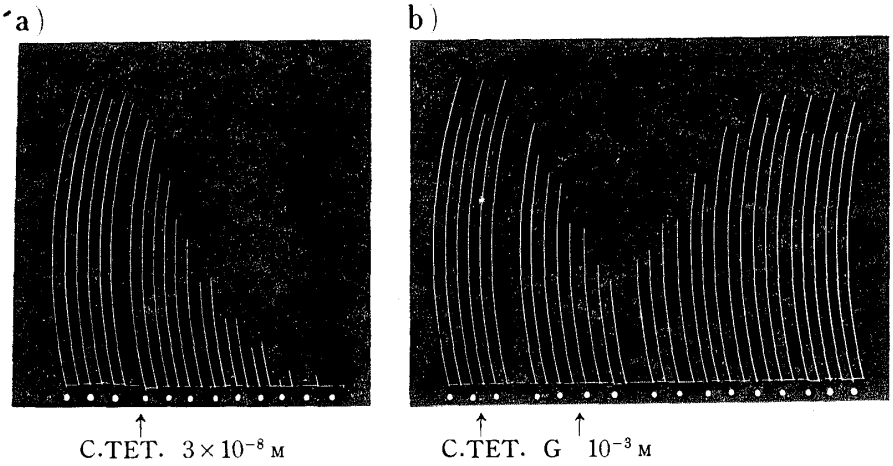

Fig. 3. Effect of C. TET. on Hypogastirc Nerve–Vas Deferens Preparation of Guinea–pig

Electrical stimulation were applied to pre- (white dot) and post-ganglionic fiber.
condition of stimulation: pre; 50 cps, 1 msec, supramaximal voltage
post; 20 cps, 1 msec, 90—120 V
Drugs were administered at arrow.
a) C. TET. 3×10^{-8}M, control
b) G 10^{-3}M after administration of C. TET. 3×10^{-8}M

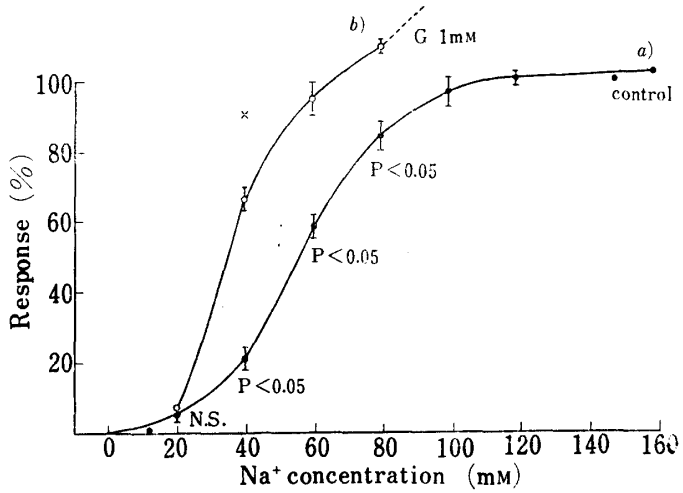

Fig. 4. Relation between G and Na^+ in Tyrode Solution (Hypogastric Nerve-Vas Deferens Preparation of Guinea-pig)

Vertical bars represent the standard errors of mean. Probabilities were obtained by t test for paired data.
N.S.=not significant ($P>0.05$).
a) response at each concentration of Na^+ in Tyrode solution
b) response in solution containing G 1 mM under condition of *a*)
×: response of G 10 mM in solution containing Na^+ 40 mM

2. Guinea-pig Trachea Preparation

As shown in Fig. 5, the dilation of the trachea induced by the transmural electrical stimulation was suppressed by C.TET. 10^{-8}M or procaine 10^{-4}M as well as the response in the vas deferens. The suppression by C.TET. was antagonized, and that by procaine was temporally antagonized by G 10^{-3}M or MG 10^{-3}M.

3. Cervical Sympathetic Nerve-Nictitating Membrane of the Cat

In the cervical sympathetic nerve–nictitating membrane preparation of the cat, when C.TET. 8 μg was intraarterially injected into the ganglion through a lingual artery, the con-

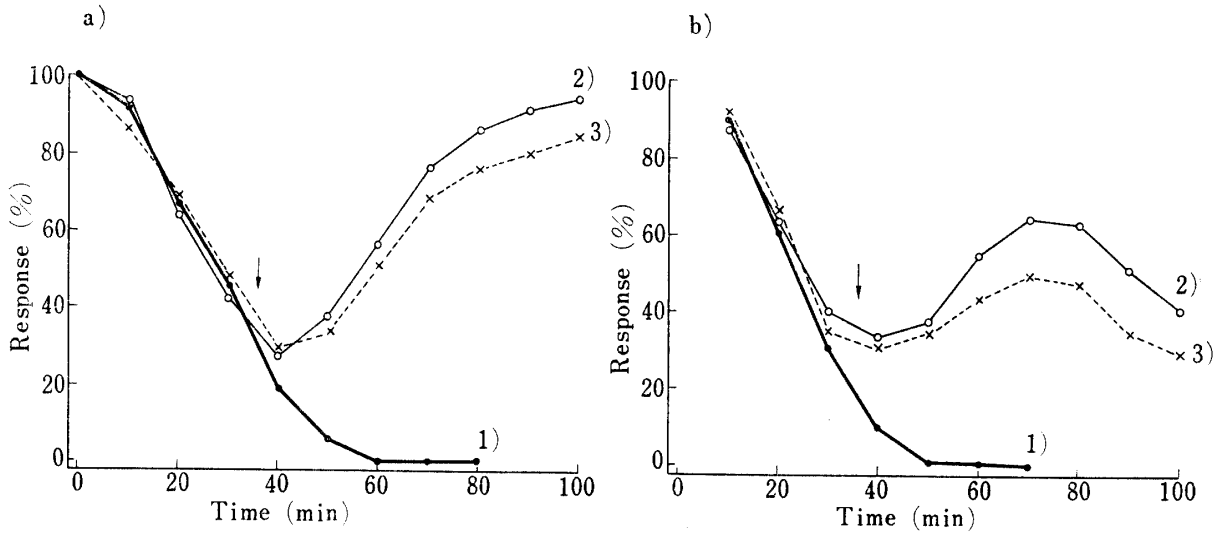

Fig. 5. Effects of C. TET. and Procaine on Trachea Preparation of Guinea-pig

Electrical stimulation was applied transmurally at 10 cps, 0.4 msec, 60 V.
a) effect of C. TET
1) C. TET. 10^{-8}M, control
2) and 3) after administration of C. TET. 10^{-8}M, G 10^{-3}M and MG 10^{-3}M were administered at arrow, respectively.
b) effect of procaine
1) procaine 10^{-4}M, control
2) and 3) after administration of procaine 10^{-4}M, G 10^{-3}M and MG 10^{-3}M were administered at arrow, respectively.

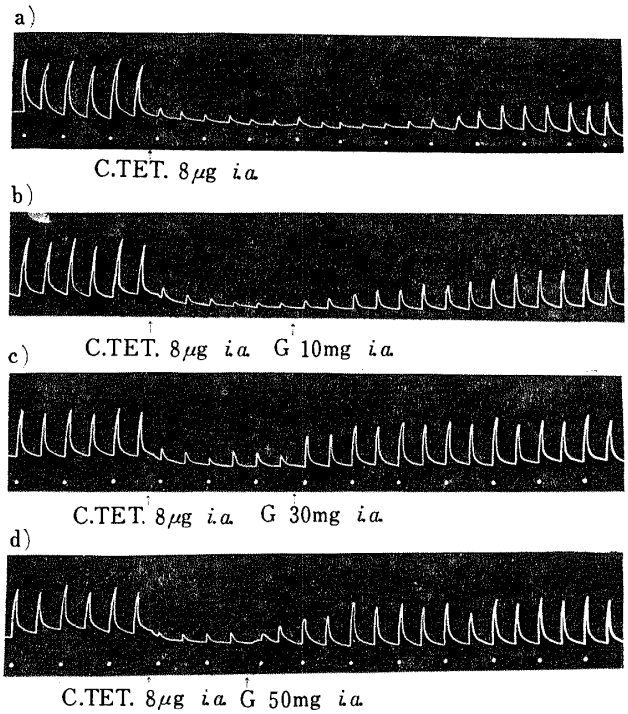

Fig. 6. Effect of C.TET. on the Contraction of the Nictitating Membrane of Anaesthetized Cat

Pre- (white dot) and post-ganglionic stimulations were applied at 20 cps, 1 msec, superamaximal voltage.
Drugs were administered intraarterially at arrow.
a) C.TET. 8 μg *i.a.*, control
b)—d) G 10 mg, 30 mg, 50 mg *i.a.*, respectively, after administration of C. TET. 8 μg *i.a.*

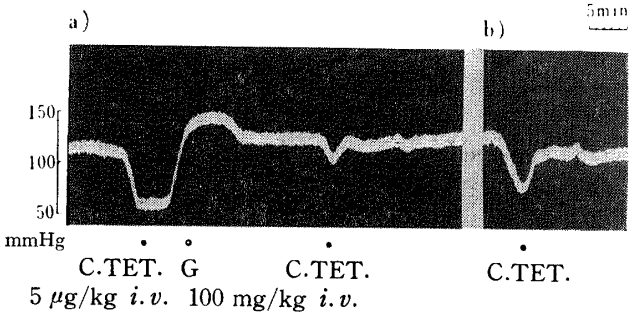

Fig. 7. Effect of C. TET. on the Blood Pressure of Anaesthetized Cat

Drugs were administered intravenously at dots.
a) C.TET. 5 μg/kg *i.v.* and G 100 mg/kg *i.v.*
d) 1 hr after administration of G

tractions of the nictitating membrane induced by the stimulations of pre- and post-ganglionic fibers were suppressed almost the same extent (Fig. 6a), and the suppression was continued for about 40 min. During this suppression, when 10, 30 and 50 mg of G were intraarterially injected, the recovery became more rapid depending on the increase of the dose of G, that is, G antagonized to the suppression by C.TET. (Fig. 6b—d). On the blood pressure of the cat, also, as shown in Fig. 7, the depressor effect induced by the intravenous injection of C. TET. 5 μg/kg was antagonized by that of G 100 mg/kg.

Discussion

It has been demonstrated that the inhibitory action of C.TET. on the contraction of skeletal muscle preparations is due to the selective suppression of Na^+ permeability at the excitable membrane,[6,8] and that the mechanism of action is the occupation of Na-channel by guanidinium group of C.TET.[9] On the basis of these reports, Moore, *et al.*[16] supposed the relation between guanidinium ion of C.TET. and the number of Na-channel using the nerves of legs of *Homarus americanus*. It was assumed that the inhibitory action of C.TET. on the contraction would be antagonized by G, and so far various antagonistic phenomena have been reported. However, since C.TET. has a complicated action, the temporary antagonism is no more than grasped.[10,17,18]

Now, in the smooth muscle preparations innervated by the sympathetic nerve, C.TET. affects selectively on the nerve ending without affecting on the smooth muscle itself.[3,4,5,6,7]

In the present paper, the inhibitory actions of C.TET. on the guinea-pig hypogastric nerve-vas deferens preparation, on trachea preparation and on the cervical sympathetic nerve-nictitating membrane of the cat were antagonized by G and MG. Thus, the inhibitory action of C.TET. which suppresses selectively the permeability of Na^+ was antagonized by G and MG. Whereas, the inhibitory action of a local anaesthetic, procaine, which suppresses the permeability of both Na^+ and K^+, was only temporally antagonized by G and MG. Therefore, it could be considered that the action of G is highly dependent on Na^+. On the vas deferens preparation, moreover, when guanidinium ion was included in perfusion medium instead of Na^+, the muscle which could not be contracted by the electrical stimulation in Na-free solution was recovered. And also, the inhibitory action of C.TET. was not antagonized by high Na^+. From these results, it is assumed that since the guanidinium group of C.TET. which is occupying Na-channel at the membrane of the sympathetic nerve ending was perhaps removed by G or MG for some reason C.TET. and G or MG would be antagonistic.

However, the presence of ganglion is a problem one should not ignore because it is considered that G and MG have stimulating action to ganglion. Barzaghi, *et al.*[19] have observed that G has stimulating action to ganglion since when the contraction of the nictitating membrane induced by the electrical stimulation of the preganglionic fiber of the cervical sympathetic nerve was suppressed with ganglionic blocking agent pempidine, the suppression was recovered by 100 mg of G. In the present paper, the contraction of the nictitating membrane induced by the stimulation of the postganglionic fiber was recovered as well as that induced by the stimulation of the preganglionic fiber. Therefore, it is considered that G acts not only the ganglion but also the sympathetic nerve ending, and that the action to the latter is more excellent.

Burn and Rand[20] have described the dependence of cholinergic mechanism in sympathetic nerve. That is, they concluded that the release of adrenergic transmitter would be mediated by cholinergic mechanism at sympathetic nerve. Otsuka and Endo[21] concluded that G would accelerate ACh release at cholinergic nerve ending. If it is assumed that C.TET. suppresses cholinergic mechanism in sympathetic nerve, it seems to be quite all right to consider that G antagonizes to C.TET. at cholinergic mechanism. But since it is considered that G would accelerate the release of adrenergic transmitter at sympathetic nerve, these facts are very complicated and involve many problems to be solved.

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