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Contribution to the Study of Veratrine Contraction, with Special Reference to the Action of Quinine.

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Abstract

In this investigation, the veratrine contraction was studied with special reference to the action of quinine, and from the results obtained, the following summary and conclusion are made. 1. Various forms of veratrine contraction were found to be different representations of twitch and veratrine components, corresponding to the concentration of the drug, duration of the action of the drug (time factor), intensity and frequency of stimulations and individual sensitivity of muscles. 2. Veratrine-like contractions were evoked by glycerine and formaldehyde. 3. Veratrine contraction was obviated by lowering the temperature and facilitated by raising it. An intimate relations between the effective minimal dosis of veratrine and temperature were noted. 4. Veratrine contraction was depressed or abolished by calcium or potassium chlorides. Relations between threshold concentration of veratrine and calcium or potassium chlorides were determined. 5. Acidity inhibited the occurrence of veratrine contraction, alkalinity facilitated it. 6. Height of contraction of veratrinised muscle was higher than normal muscle, in twitch as well as in tetanus. Refractory period of veratrine muscle was greatly prolonged and found in the descending phase of veratrine contraction. 7. Quinine obviated veratrine contraction. Veratrine-quinine equilibrium ratio was determined. 8. Atropine inhibited the action of veratrine on muscle. 9. Ergotoxine retarded or depressed the occurrence of veratrine contraction, when applied previously, while if applied afterwards, it exaggerated at first, then alleviated gradually veratrine contraction. 10. Acetylcholine had no effect on veratrine contraction. Eserine, like atropine, inhibited veratrine contraction. 11. Adrenaline caused complete dissociation of twitch and veratrine components. 12. Monoiodoacetic acid had no effect on the occurrence of veratrine contraction. 13. Veratrine had no effect on isolated frog's heart. 14. Veratrine caused increased tonicity and irregular tonic contraction on non-striated muscles (dorsal muscle of leech, intestine and uterus of guinea-pig).

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**Contribution to the Study of Veratrine Contraction,
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Introduction.

A new aspect based on the theory of chemical transmission of nervous impulse originated by *Dale* and his collaborators, brought forwards a remarkable progress to elucidate the mechanisms of nervous impulse and muscular contraction. According to the theory, acetylcholine and its associated substances liberated from nerve-endings are considered to be an effective agent of nervous impulse and evoke muscular contraction. On the other hand, these substances are readily broken down through the action of choline esterase and lose their action.

It is reported recently in the clinical side that an abnormal state of muscular contraction, such as myotonic and myasthenic conditions, though their etiology is not clear, has remarkably been improved therapeutically through the treatment, for example. *Kennedy* and *Wolf* (1936, 1937, 1938), *Kolb*, *Harvey* and *Whitehill* (1937, 1938) treated myotonia congenita et atrophica with quinine and myasthenia gravis with prostigmine and observed that these diseases are influenced favourably by these drugs respectively. Also they observed that quinine aggravates myasthenic symptom and that prostigmine exaggerates myotonic condition. The facts show that these two drugs represent pharmacologic antagonistic action.

Veratrine contraction, though its nature is not yet definitely decided, is generally known as a peculiar form of muscular contraction and is often compared with the condition of myotonic disease (*Howell*, 1937). Since this condition is easily produced at our disposal even in isolated frog's muscle through the poisoning of ve-

ratrine, it is very convenient to study the myotonic condition under simplified state.

The purpose of present investigations is to elucidate the action of quinine and some other substances on veratrine contraction, and to search for the mechanisms of veratrine contraction in the light of chemical transmission theory.

Material and experimental procedure.

For the sake of simplicity of experimental condition, sartorius muscle of *Rana nigromaculata* was isolated and immersed in *Ringer's* solution for a while until it is submitted for the veratrine poisoning. As *Lamm* (1911) pointed out, the methods adopted by many previous authors that injecting veratrine in dorsal lymph-sac of frog and then study the mode of action of the drug upon muscle is unsatisfactory for the purpose to decide precisely the correlation between the action of the drug and its dose. This fact is especially important in order to study the mode of action of veratrine, because the drug, as referred below, shows characteristic manifestations on the manner of muscular contraction so that the author adopted the methods after *Lamm*. In some of the experiments, isolated dorsal muscle of leech, uterus and intestine of guinea-pig, frog's heart were used as the experimental materials.

From the stock solution (0.1 %) of veratrine hydrochloride, solution of the desired concentration was prepared by dilution with *Ringer's* solution before the experiments and thrown away after the experiment is over.

Du Bois Reymond's inductorium was used as stimulating apparatus, of which primary coil is connected to 2 volts battery, and of which secondary coil leads to the stimulating silver-silver chloride electrodes with intercalating short circuit for the purpose to give single opening induction shock. One electrode is fixed to the end of bend glass rod, where one end of muscle is attached, and the another electrode which is bend like hook and connected to enameled fine copper wire, attached to the other end of the muscle and connected to light strow lever through the fine copper wire so as to register the muscular contraction on the smoked rotating drum. Before the stimulation was given, sartorius muscle was lifted from the bathing solution, reimmersed after the stimulation. Maximal stimulation was applied in all the cases. As the manner of veratrine contraction differs according to the interval of stimulation, an interval of 5 or 10 minutes for each stimulation was strictly executed.

Veratrine contraction in general.

Veratrine is composed of at least two alkaloids (cevadine and veratridine) contained in the seeds of *Schoenocaulon officinalis*. Its minimal effective dose for frog fluctuates from 0.05 to 0.0075 mg (*Lissauer, Straub*). It is said that *Rana temporaria* is more sensitive than *Rana esculenta* (*Prévost*).

Since *Kölliker* (1856) discovered that veratrine has a peculiar action on muscular contraction, our knowledge has been greatly enriched by many authors who performed various experiments on this subject. General characteristic feature of contraction evoked by a single stimulation on the veratrinized skeletal muscle, is a rapid and powerful contraction with very slow relaxation accompanying much or less wave-like oscillations. Among them in so-called "one summit type" contraction, the second contraction follows immediately at the summit of the first rapid contraction and does not dissociate in two maxima, while in so-called "two summit type", the second contraction occurs after the summit of primary contraction and the curve shows as a whole two maxima. Between these two forms of veratrine contraction, various modifications of contraction curve can usually be observed. For the sake of convenience, a term, "twitch component" for the rapid contraction and "veratrine component" for the second slow contraction with delayed relaxation are used here. As to the cause of the production of these two types of veratrine contraction, different opinions are offered (see *Böhm*). *A. v. Bezold* insisted that "one summit type" is produced by direct stimulation of muscle, and that "two summit type" is produced by indirect stimulation through nerve, but it is opposed by *Fick* and *Böhm* (1903); *Mostinsky* (1904) and *Lamm* clearly showed by the experiments that in the case of weak poisoning "two summit type" appears and "one summit type" through strong poisoning. Surveying the results obtained by the author it is entirely true in all the cases, as shown in Fig. 1 for example, where in A it shows the change of contraction curve of poisoned muscle under definite concentration of the drug according to the time of immersion in the solution, in B it shows the change of contraction curve under various concentrations of the drug.

In regards to "twitch component", various effects are the same as in the unpoisoned muscle, so that it is not discussed here. "Veratrine component" is however influenced by various conditions, i.e., (1) by internal conditions, sensitivity of muscles to the drug is different from one to another individuals as well as the seasonal variations of sensitivity; (2) by external influencing moments, (a) intensity and

Fig. 1 A. The change of contraction curve according to the duration of immersion of the muscle in veratrine.

(1 : 10^6 veratrine) : (time signal ; 1/5 sec.)

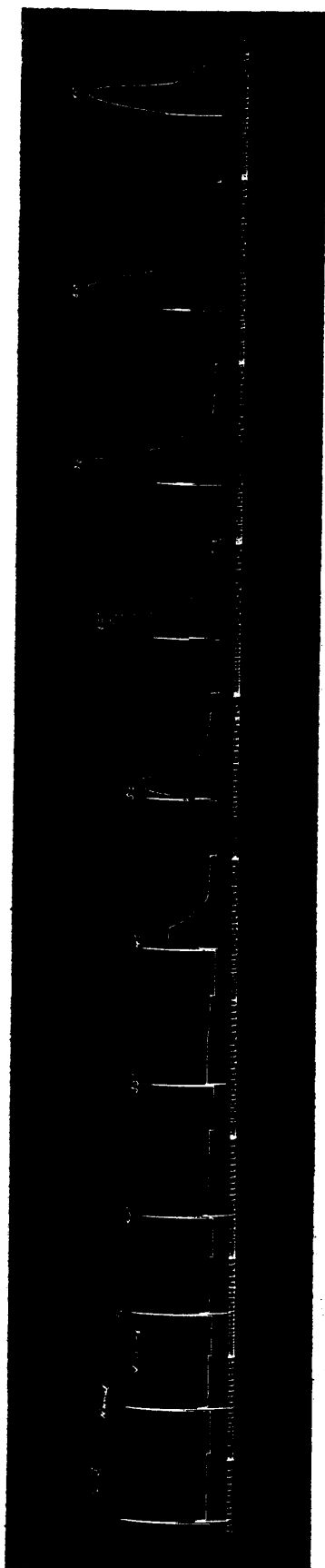
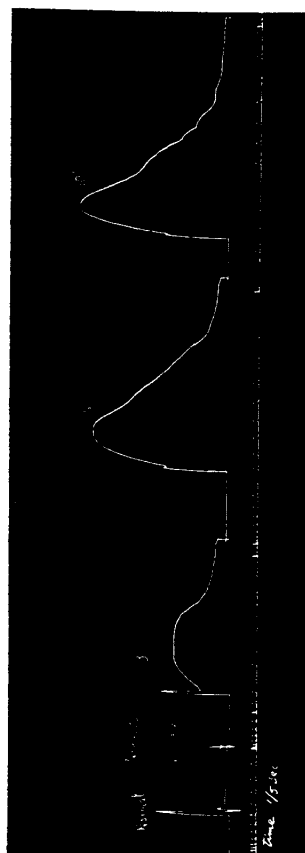
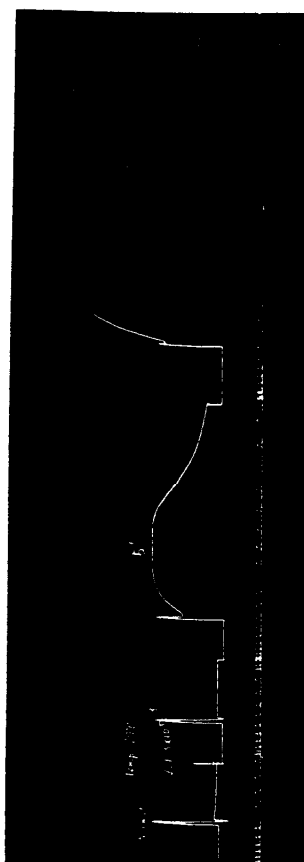


Fig. 1 B. Veratrine curves by different concentrations of the drug.

a. Veratrine concentration (1 : 2×10^5)



b. Veratrine concentration (1 : 5×10^5)



N.B.: Numbers above the contraction curves indicate the time elapsed after the immersion in veratrine solution.

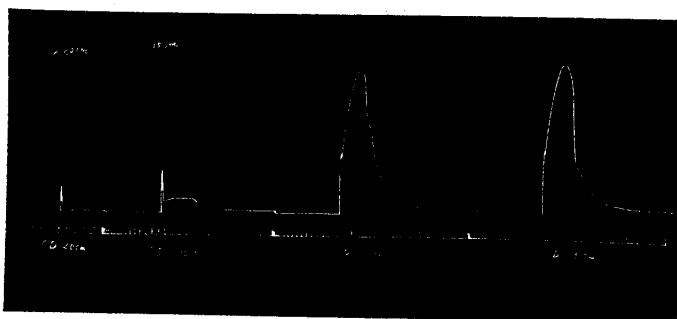
interval of each stimulation, (b) room temperature, (c) concentration of the drug, (d) time of immersion in the solution, (e) composition and reaction of the physiological saline solution, (f) action of various medicaments, etc..

In order to obtain the precise knowledge on the action of veratrine, the recapitulation of experiments, as preliminary experiments, concerning some of these factors is noted below:

(1) Intensity and interval of each stimulation (Fig. 2 and 3).

Intensity of stimulation was varied by the change of coil distance, increasing gradually from weak to stronger stimulation at 1 minute interval. Veratrine contraction is not observed by weak stimulation but appears by stronger stimulation (see Fig. 2). Therefore maximal stimulation is applied throughout the present experiments.

Fig. 2. Effect of the strength of stimulation on veratrine contraction.



N. B.: C.D. denoted the distance of the secondary coil from the primary.

An interval of each stimulation also affects the veratrine component. It becomes less and less marked if the muscle is subjected to repeated contractions, but reappears after short rest (see Fig. 3). For this reason, the interval of at least 5 or 10 minutes between each stimulation was given.

(2) Concentration of the drug and the time of immersion of muscle in the solution.

As to these factors, the results shown in Fig. 1, A and B indicate clearly that "one summit type" and "two summit type" are nothing but two forms of manifestation of the same "twitch component" and "veratrine component".

(3) Minimal effective dose.

According to *Lamm* (1912), threshold of minimal effective dose of veratrine to winter frog is about the twice of that to summer frog,

showing in concentration $1:5 \times 10^6$ to $1:6 \times 10^6$ to the former, $1:10 \times 10^6$ to $1:12 \times 10^6$ to the latter. The present experiments were performed on summer frog and minimal effective dose coincides with that of *Lamm*, showing usually $1:10 \times 10^6$, though the time of manifestation of veratrine contraction differs greatly from one to another individuals. As the concentration of the drug was raised, the time of manifestation of veratrine contraction was remarkably reduced.

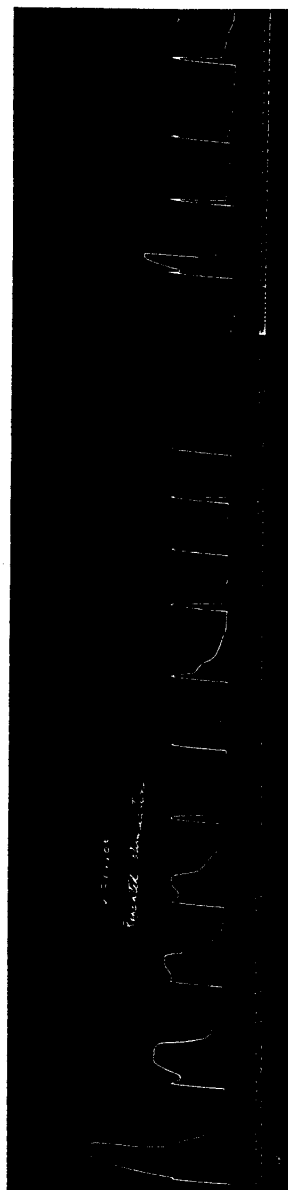
(4) Problem concerning absorption of the drug in muscle.

If the same veratrine solution of known concentration was repeatedly used for many preparations, poisoning action of the solution becomes less and less effective, until at last it becomes ineffective. This fact seems to indicate that veratrine is stored up in muscle substance. *Straub* (1903) demonstrated that many alkaloids are stored up in heart muscle and this occurs most markedly with veratrine. For the above reasons, veratrine solution once applied, was rejected after one administration.

(5) Veratrine-like contraction by other substances.

Besides veratrine, veratrine-like contractions are produced by many other substances (*Bérzar* and *Felter*, 1914; *Bottazzi*, 1901). The author confirmed this fact with formaldehyde and glycerine. Those contraction curves which are presented in Fig. 4 and Fig. 5, are similar with those of veratrine contraction. These contractions similar with veratrine contraction do not occur spontaneously without stimulation, showing sometimes "two summit type", sometimes "one summit type". The only difference from the veratrine action is that the

Fig. 3. Effect of the repeated stimulation on veratrine contraction.
(Time signal; 1/5 sec.)



muscle dies after a short time, even if it is replaced by *Ringer's* solution. The facts that these substances affect cell membrane and protoplasm are an interesting matter.

Fig. 4. Formaldehyde-contraction. (N/200 formaldehyde-Ringer)
(Time signal; 1/5 sec.)

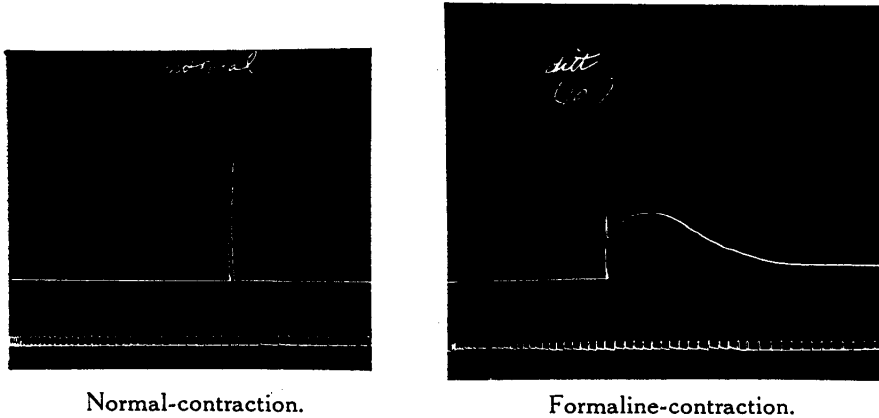
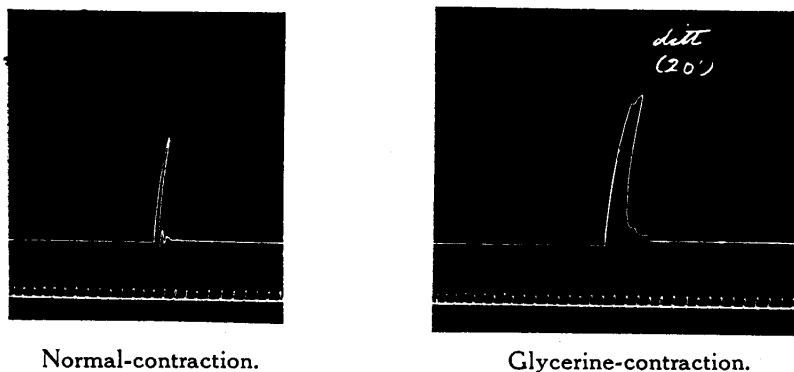


Fig. 5. Glycerine-contraction. (N/2 glycerine-Ringer)
(Time signal; 1/5 sec.)



In regard to the remaining factors, the detail discussions will be presented in the following chapters.

Effect of temperature.

Brunton and *Cash* (1883-84) at first studied the effect of temperature on veratrine contraction. They observed that the altitude of veratrine contraction increases as the temperature of the moist chamber, containing the poisoned muscle was raised. However *Gad*

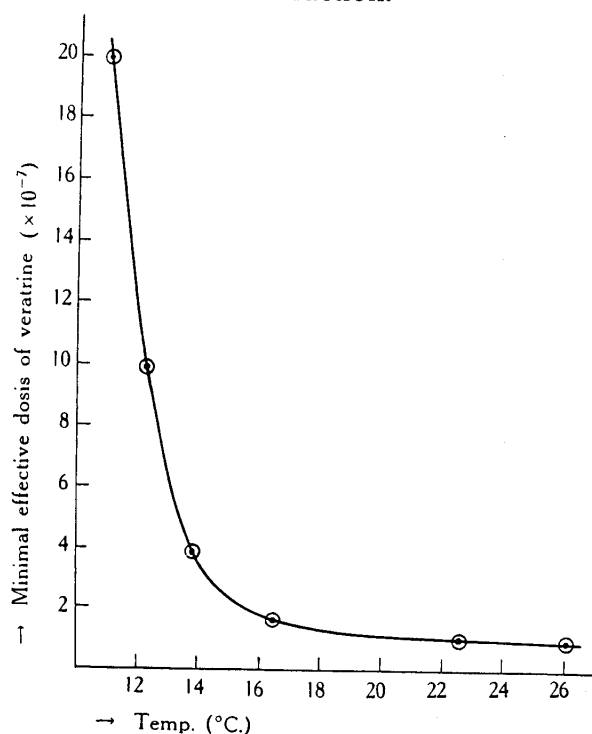
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and *Heymann* opposed and pointed out that is due to the inertia of the recording lever. *Lamm* (1911) investigated in detail the relation between threshold concentration of the drug just to evoke veratrine contraction and temperature, and found that the threshold concentration of the drug must be raised at lower temperature.

The relation between temperature and effective minimal concentration of the drug was investigated in detail also by the author in the following manner. For the purpose to obtain the desired

Fig. 6. Effect of temperature on veratrine contraction.

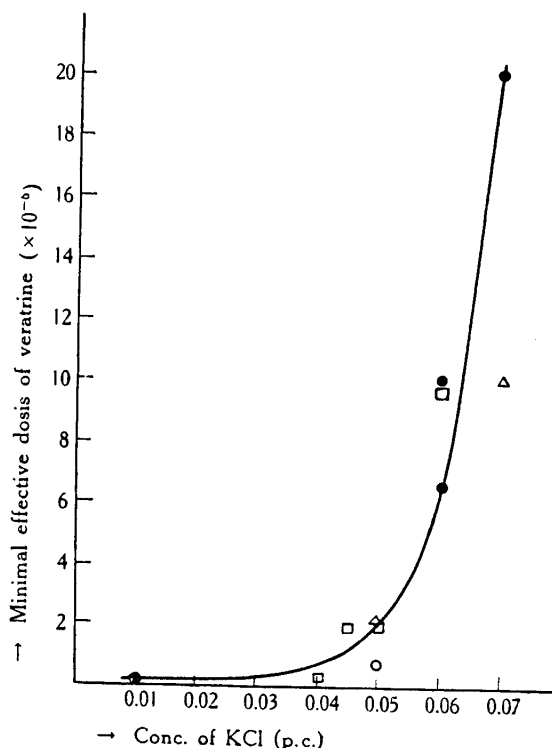


constant temperature *Dewar's* bottle was used. The muscle was introduced in it after poisoned with a definite concentration and attached to the recording lever with enameled wire through a hole of cork stoppage. The maximal opening induction shock was given at usually 5 minutes interval. If veratrine component did not fade away, the temperature was lowered gradually until just it extinguished. Then the temperature was gradually raised at which a first sign of veratrine contraction reappears. Thus the threshold concentration was determined at definite temperature. These results are represented graphically in Fig. 6. It is considered from this fact that

the seasonal variations of minimal effective dose of veratrine action is probably due to the effect of the surrounding temperature.

A more marked influence of temperature is that the time of manifestation of veratrine contraction, after the immersion, is greatly accelerated by raising the temperature and retarded by lowering it. This fact has not been described by any other authors. Moreover, it is worthy to be mentioned that the cooling shortens veratrine component, while the warming lengthens it. So far as the duration of

Fig. 7. Effect of potassium chloride on veratrine contraction.

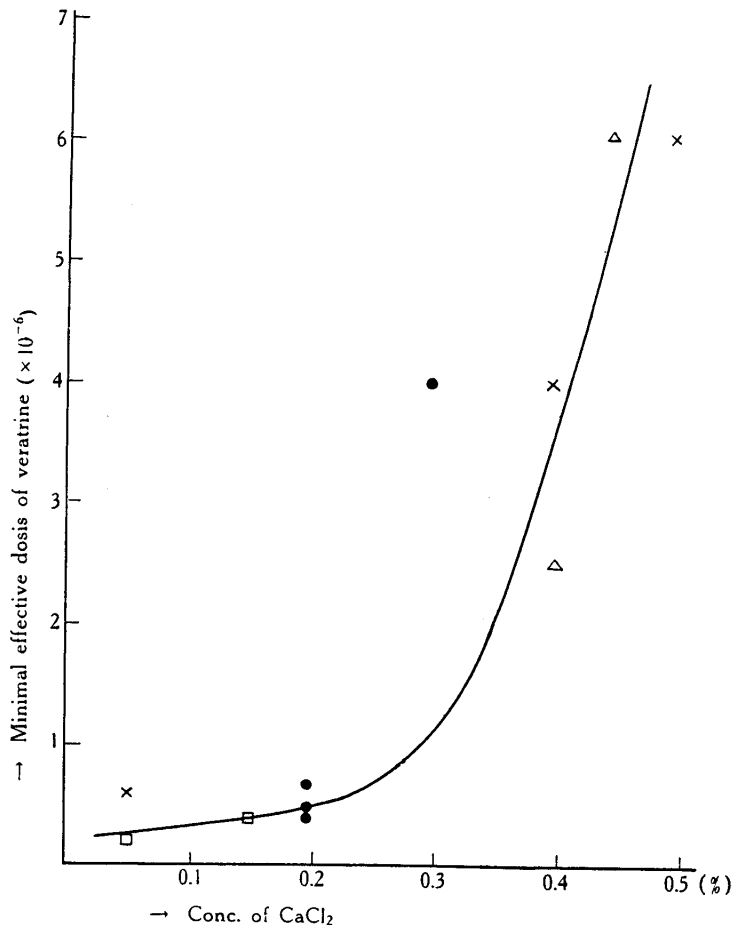


muscular contraction is concerned, the change of temperature has contradictory effect upon these "twitch and veratrine components". For example, in the case poisoned by $1:10^6$ veratrine, minimal immersion time at which the first sign of veratrine contraction appears, lies within 1 minute at 30°C ., while at low temperature, viz. at 20°C ., first sign of veratrine contraction appears after 5 minutes of immersion. Even by this time, veratrine component appears very feeble compared with 1 minute immersion at 30°C . From such a remarkable influence of temperature, it can be concluded that the action of veratrine to muscle is chemical in nature.

*Effect of potassium and calcium chlorides on
veratrine contraction.*

Three constituents of physiological saline solution, namely sodium, potassium and calcium chlorides, have in part antagonistic action and maintain physiological condition of the tissue under the

Fig. 8. Effect of calcium chloride on veratrine contraction.



definite composition. Among them, sodium chloride increases permeability, while calcium and potassium chlorides decrease it, in consolidating the cell membrane or the colloidal state of protoplasm. The fact that calcium and potassium chlorides have great influence on veratrine contraction is a very interesting phenomenon. It is discovered by *Ringer* (1884-85) on heart muscle and by *Buchanan* (1899) on skeletal muscle that potassium chloride acts as antagonist

to veratrine contraction. Later *Bottazzi* (1901), *Lamm* (1911) and *v. Frey* (1912) performed the experiments on this subject concerning potassium and calcium chlorides.

As in the case of determining the effect of temperature on veratrine contraction, sartorius muscle had been previously poisoned by a definite veratrine concentration, then potassium or calcium chloride was added in the veratrine-*Ringer's* solution until the added salts abolish totally the veratrine component. When the veratrine component extinguished, this solution was replaced with veratrine-*Ringer* and confirmed that the veratrine component reappears. As a troublesome matter, potash or lime salt does not reveal its action immediately after the administration, a considerable time must be therefore awaited in order to ascertain the antagonistic effect decisively. Fig. 7 and 8, which recorded the results of a number of experiments, show considerable variations. In these figures, the abscissae shows concentration of potassium or calcium chloride in per cent, and the ordinate, the liminal concentrations of the drug at which veratrine contraction is exactly abolished by the concentration of these ions.

Influence of the reaction of Ringer's solution.

As the fourth factor which affects the manifestation of veratrine contraction, the reaction of the surrounding medium can be pointed out in which the muscle is bathed. Sodium carbonate and sodium hydroxide were used as alkali, lactic acid as acid, in order to alter the reaction of veratrine-*Ringer's* solution. The absolute amount of added alkali or acid was so small that the change of pH of *Ringer's* solution after the addition of alkali or acid could not be detected by the "Tôyô pH testing paper".

A slight alkalinity greatly accentuates the manifestation of veratrine contraction but the acidity on the contrary depresses it. Moreover, the alkalinity diminishes the minimal effective dose, while the acidity increases it. These phenomena are noted in Fig. 9 A and B.

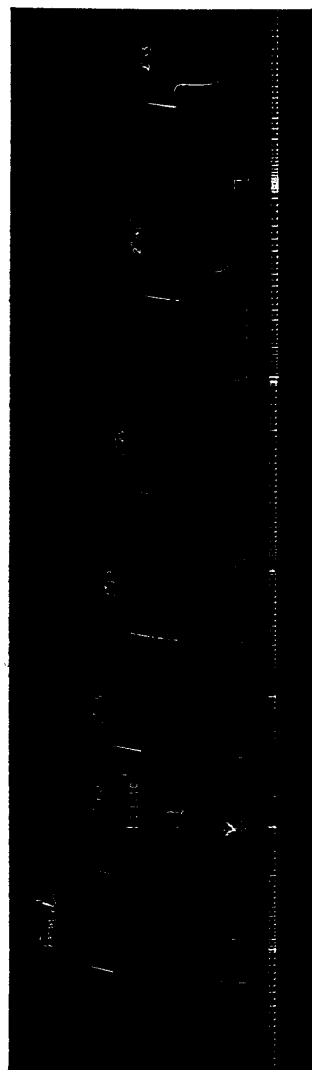
In studying the effect of various medicaments on veratrine contraction, it should be carefully considered that the reaction of the surrounding medium has such marked influence on veratrine contraction which complicates the results in evaluating the action of these medicaments, as considered below.

As an explanation of such influence of alkalinity and acidity, *Lamm* (1912) assumed as follows; i.e., the strength of veratrine poisoning is determined by the degree of dissociation of this alkaloid, insisting alkalinity increases the dissociation and strengthens the

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Fig. 9 A. Influence of alkalinity on veratrine contraction.



20/TR
Temp. 25.5°C

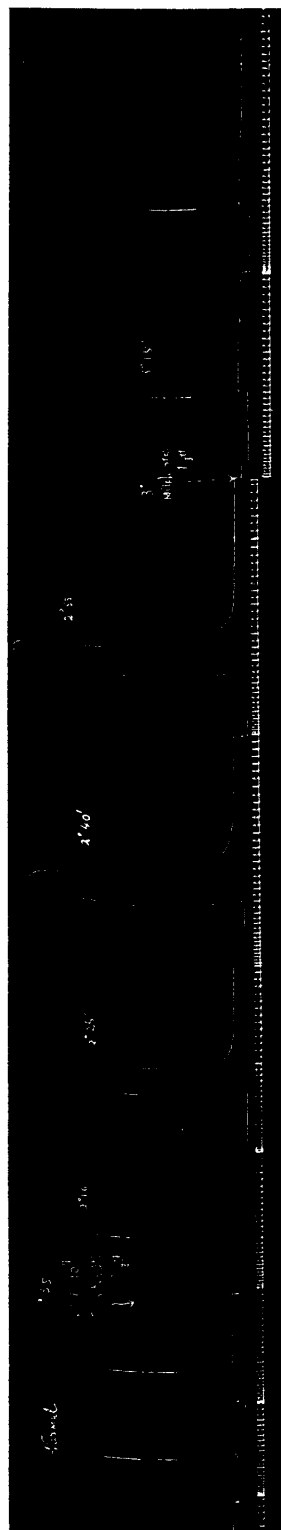
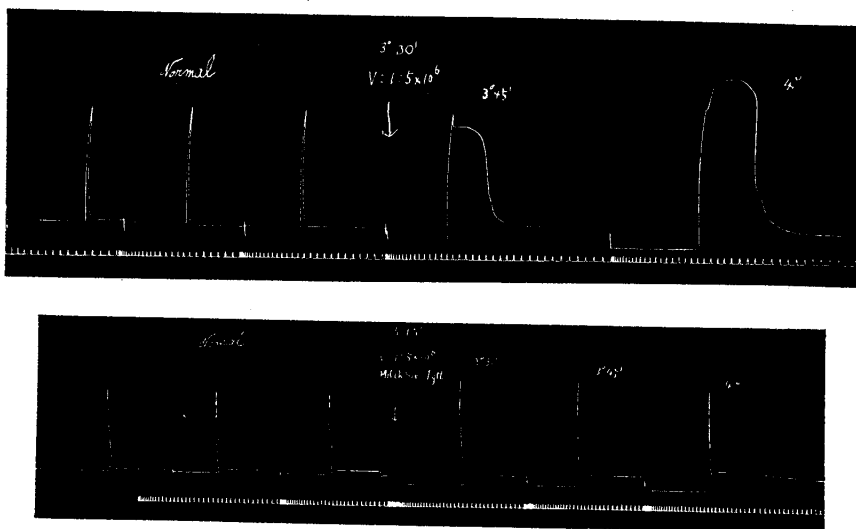


Fig. 9 B. Influence of acidity on veratrine contraction.



Upper figure: Contraction curve of sartorius muscle poisoned by veratrine.

Lower figure: Contraction curve of sartorius muscle poisoned by veratrine added one drop of lactic acid.

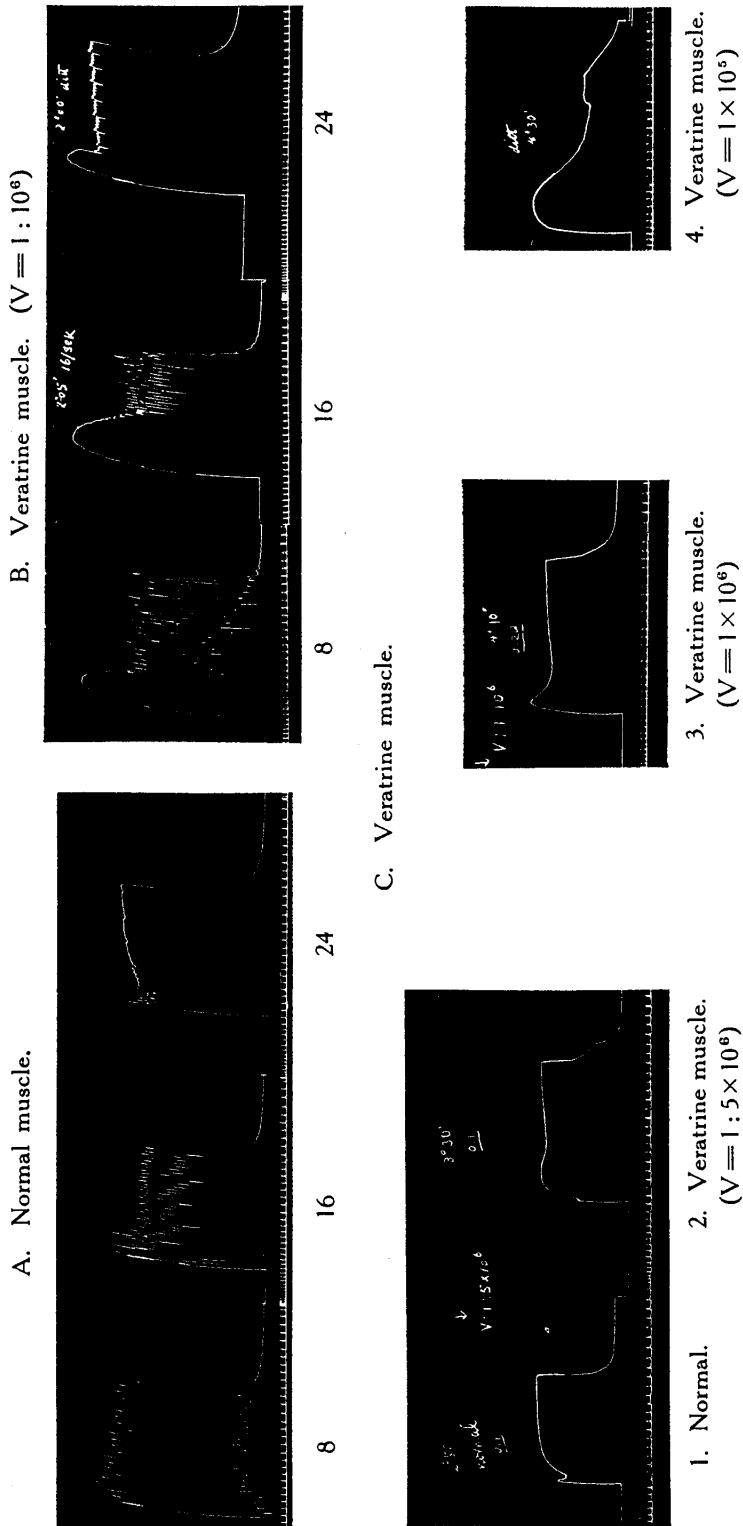
action of the drug, while acidity acts reversely. Another aspect of the cause, which the present author should like to emphasize, is that the reaction of the surrounding medium affects the cell membrane of muscular tissue in the reversed direction according to alkaline or acid, and this causes the difference of veratrine action.

Effect of repetitive stimulations.

Veratrine muscle is often compared, in its form of contraction, with heart muscle (Müller, 1908). Were it truly so, it had not been expected to obtain tetanic contraction in veratrine muscle, because it is the case of heart muscle. On the contrary, some authors reported that veratrine muscle can be tetanized as in normal muscle, while others considered that veratrine contraction is, in its nature, tetanus (Lamm, Hoffmann, 1912, et al.). The present author has previously confirmed that veratrine component is greatly modified by repeated stimulations (see Fig. 3). What contraction the veratrine muscle would make, if stimulated by frequently repeated stimulations of varying numbers per sec., is the present object of investigation in this chapter.

The devices of repetitive stimulations of varying numbers were fulfilled through condenser discharge (0.1 μ F.) with the coupling, "cylindre à cames" of *Lapicque*. By this method the tetanic con-

Fig. 10. Effect of repetitive stimulations on veratrine muscle.



Explanation of the Figures: Numbers subscribed to the curves are the frequency of stimulation per sec. A, the contraction curves of normal muscle; B, those of the same muscle under veratrine poisoning (veratrine concentration $1 : 10^6$); C, tetanic contractions at fusion of frequent stimulation. Tetanic contractions of veratrine muscle are higher than those of the normal muscle, especially in an earlier stage of tetanic contractions. By strong poisoning (veratrine concentration $1 : 10^5$), the curve shows rapid decline.

traction curves of veratrine muscle were compared with those of the normal muscle.

As seen in the Figures, it is clear that fusion frequency of each contractions in veratrine muscle must be reduced, especially in the earlier stage of tetanus, where the tetanus-like contraction of veratrine muscle, induced by the earlier stimuli, is predominant. More clear distinction of each contraction by repetitive stimulations appeared at the descending phase, and thereafter the veratrine muscle responds at each stimulation as in the normal muscle. So it can be concluded that the refractory period of veratrine muscle greatly prolonged, so that in frequent stimulations after effective stimulus, some numbers of later stimuli have no effect. These relations may be clearly seen in the curves of less frequent stimulations. Veratrine muscle is said to represent the same magnitude of contraction height as the tetanic contraction of normal muscle. In this experiment, however, tetanic curves of veratrine muscle show rather higher magnitude than the normal muscle, especially in an earlier stage of tetanus. In case of strong poisoning, the tetanic curve faded away rapidly.

Action of quinine on veratrine contraction.

(1) General action of quinine on muscle.

Quinine in moderate concentration stimulates muscle, increasing at first, but rapidly decreasing the muscular activity. It is considered that the working point of quinine lies in muscle substance itself, because curare does not interfere. This action is not due to the increase of potential energy in muscle, but due to the abnormal utilization of materials, as it goes into fatigue rapidly.

On heart muscle, as on skeletal muscle, quinine increases transiently its activity. According to *Senta*, gaseous exchange of muscles is reduced even by the application of dilute quinine solution. *Dongen* (1938) considered that the curative action of quinine on heart fibrillation is attributed to the lengthening of refractory period and of transmission of excitatory process.

(2) History of the introduction of quinine in the treatment of myotonic diseases.

As the discoverer of quinine treatment in myotonia, *A. Wolf* (1938) stated as follows; "with *Walker's* discovery (1935) that prostigmine alleviates the muscular weakness of myasthenia gravis and *Wolf's* finding (1936) that quinine abolishes the myotonus of congenital and atrophic myotonia, new impetus was given to the study of the pathophysiology of these disorders. The clinical observation that prostigmine exaggerates myasthenia established a relation of

opposition in response to drugs that accentuated the obvious contrast between these muscular states. It is this contrast that makes appropriate a comparative evaluation of their symptomatic response to pharmacologic antagonists”.

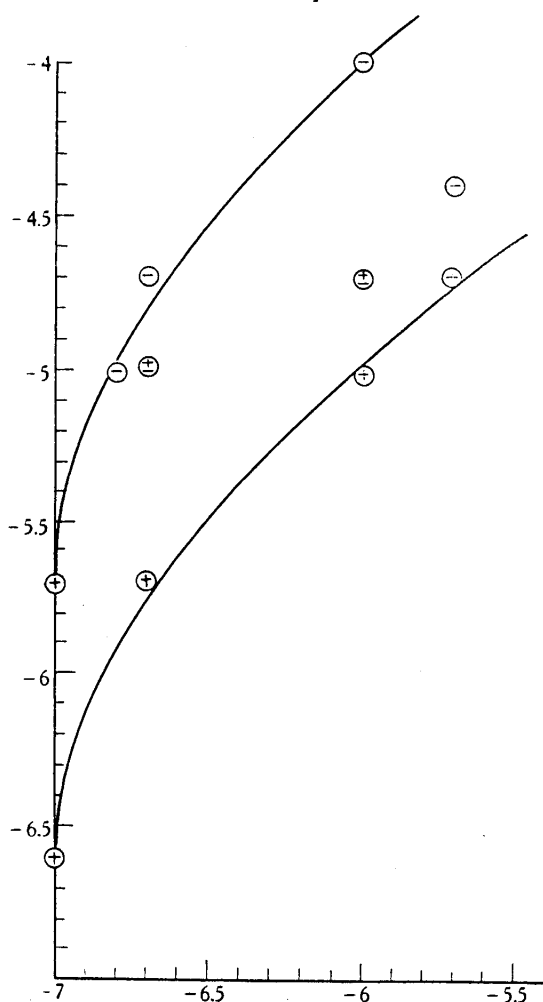
From what considerations he introduced quinine treatment is not clear. But, it is at least certain from the above description that he intended to give explanations concerning the working mechanisms of quinine on muscle in the light of new chemical transmission theory.

(3) Action of quinine on veratrine contraction.

So far as the author is aware, none has investigated on the problem that quinine abolishes veratrine contraction. As referred in the introduction, the prime motive of the present investigation lies in *Wolf's* discovery that quinine abolishes myotonic disorders.

Stock solution (0.1 %) of quinine hydrochloride was prepared and placed in dark place to avoid the decomposition through light. Before use, it was diluted to the desired concentration according to the experimentation. After observing that sartorius muscle had been fully veratrinised, quinine solution was then added in the veratrine-*Ringer's* solution in which the muscle was immersed. If the veratrine contraction did not disappear, quinine concentration must be raised gradually until the veratrine contraction is exactly abolished. Most tedious

Fig. 11. Veratrine-quinine relations.



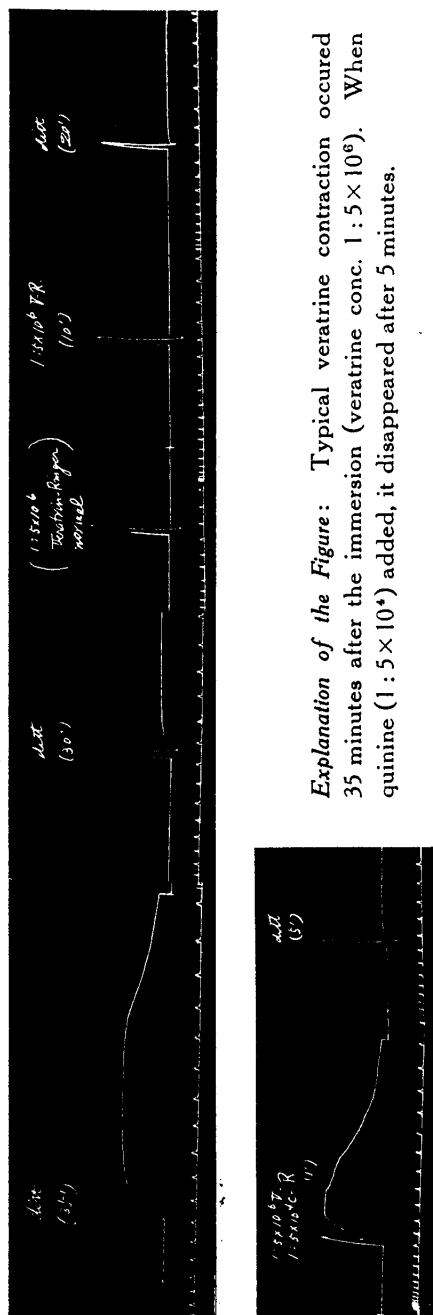
N. B. Ordinate : Liminal concentration of quinine
dosis in log.

Abscissa : Veratrine concentration in log.

circumstances in these experimental procedures is that quinine is not efficacious directly after the addition but reveals its action slowly, so

that a considerable time must be awaited in order to ascertain whether it is enough, by the added quinine concentration, to annihilate the veratrine contraction or not. For such reasons, quantitative determinations of veratrine-quinine relations, i. e., the minimal effective concentration of quinine which abolishes veratrine contraction of muscle under definite veratrine poisoning, fluctuates from one to another experiments. Therefore for the sake of convenience, it is shown in Fig. 11 the liminal quinine concentrations at which veratrine contraction just disappeared ($\ominus$) and those at which minimal veratrine contractions still existed ($\oplus$). In Fig. 12, well marked tracings of the effect of quinine on veratrine contraction are given. Although quinine or veratrine in strong concentration does not cause muscle death when applied separately, but it occurs when both of them are given simultaneously. For example, quinine in concentration $1:10^4$ and veratrine $1:10^5$ were applied simultaneously, muscle death occurred within 40 minutes.

Fig. 12. Tracings showing the effect of quinine on veratrine contraction.



Explanation of the Figure: Typical veratrine contraction occurred 35 minutes after the immersion (veratrine conc. $1:5 \times 10^5$). When quinine ($1:5 \times 10^4$) added, it disappeared after 5 minutes.

In order to obtain the precise knowledge in the working mechanisms of quinine on veratrine contraction, measurements were made on some tracings as to

the duration and the height of muscular contractions of normal and veratrinised muscle (twitch and veratrine components) before and after quinine application. The results, as given in the Table, indicate that quinine increases the height of twitch component and decreases the height as well as the duration of veratrine component. As a proof that the depressing action of quinine is not due to the inactivation of veratrine through chemical combination with it, a typical veratrine contraction can be obtained again if quinine removed.

Table.

	Duration of contraction (sec.)	Height of contraction (mm.)	Remarks
1) Normal muscle	0.113	17.3	
Veratrine-muscle ($V = 1 : 5 \times 10^6$)	0.465	19.9 (12.4)	
Veratrine-quinine-muscle ($Q = 1 : 10^5$)	0.192	20.6	After 50 min.
2) Normal muscle	0.104	19.9	
Veratrine-muscle ($V = 1 : 5 \times 10^6$)	0.110 (0.986) 0.094 (0.769)	11.9 (6.0) 11.0 (14.9)	After 40 min. After 50 min.
Veratrine-quinine-muscle ($Q = 1 : 5 \times 10^4$)	0.095	14.3	Veratrine comp. disappeared
3) Normal muscle	0.127	16.0	
Veratrine-muscle ($V = 1 : 2 \times 10^6$)	0.414	14.9 (14.2)	
Veratrine-quinine-muscle ($Q = 1 : 10^5$)	0.126	19.5	
4) Normal muscle	0.174	20.4	
Veratrine-muscle ($V = 1 : 10^6$)	0.393	20.4 (23.8)	
Veratrine-quinine-muscle ($Q = 1 : 10^5$)	0.298	20.7 (23.4)	
Veratrine-quinine-muscle ($Q = 1 : 5 \times 10^4$)	0.242	22.7 (22.7)	After 10 min.
Veratrine-quinine-muscle ($Q = 1 : 2 \times 10^4$)	0.149	27.8	After 45 min.

N. B.: Numbers in parenthesis represent those of veratrine component.

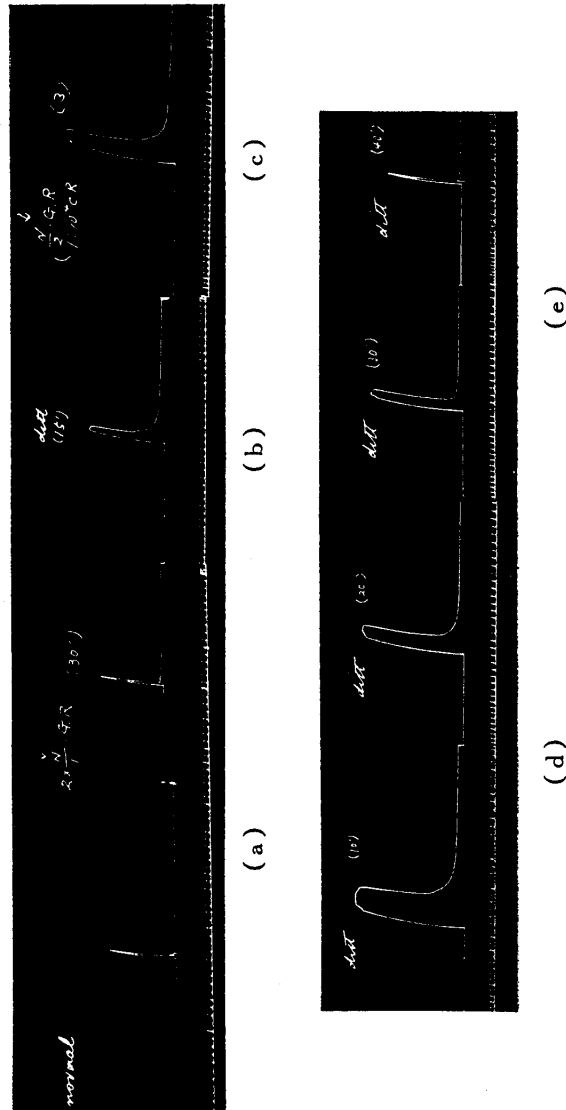
(4) Action of quinine on other veratrine-like contractions.

Quinine also abolishes veratrine-like contractions produced by formaldehyde and glycerine, as it does in veratrine muscle. These results are presented in Fig. 13.

(5) Action of quinine on muscle fatigue.

It is considered from the above various experiments that quinine inhibits generally the contracture causing moment. What relations has it with muscle fatigue? As muscle fatigue goes on, it contracts slowly and the contraction curve does not return to the base-line (fatigue contracture!).

Fig. 13. Effect of quinine on glycerine contraction.

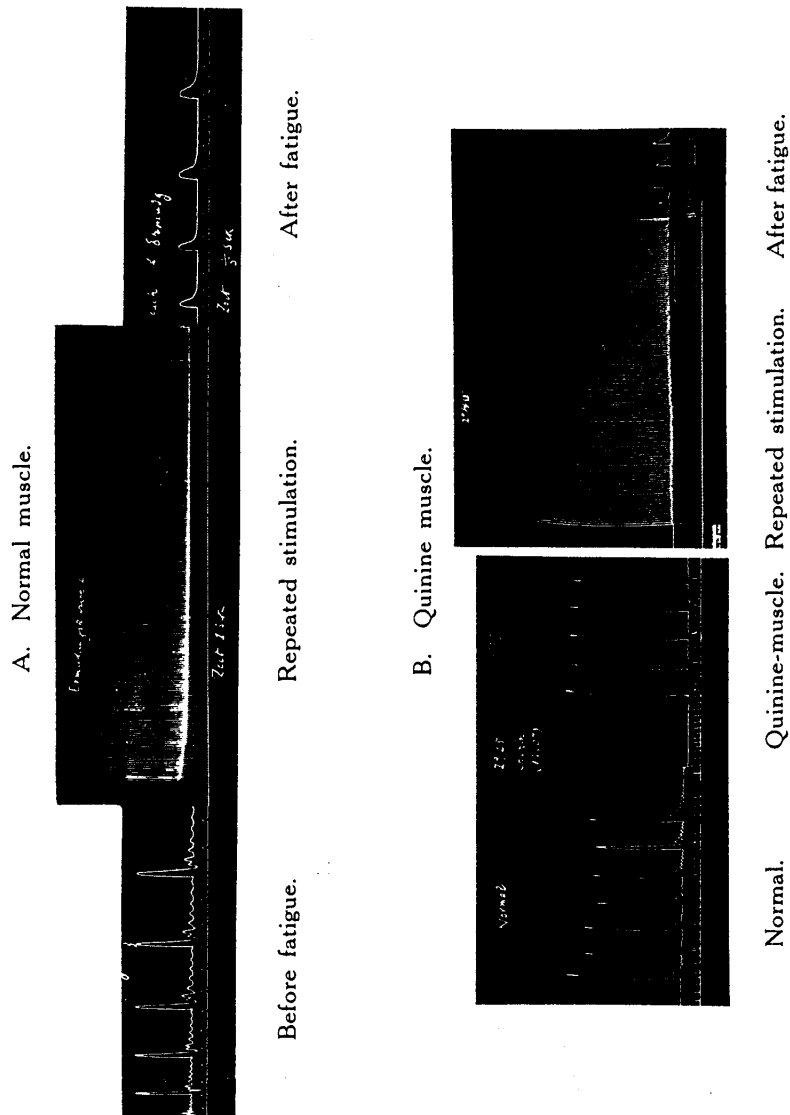


Explanation of the Figure: a, b, c, d and e are a series of one experiment. After 15 min. glycerine contraction occurred (b). Then quinine ($1 : 10^4$) added (c). Contracture disappeared 40 min. after quinine application (e).

Although fatigue sets in rapidly in quinine muscle, quinine seems to depress also the occurrence of fatigue contracture (see Fig. 14). Quinine concentrations down to $1 : 5 \times 10^4$ have little effect on fatigue

contracture. Also fatigue curve does not differ from those of normal muscle.

Fig. 14. Effect of quinine on muscle fatigue.



Effect of some other medicaments on veratrine contraction.

It is said that ether (*Müller*) and strophantine (*Langley*) besides above mentioned antagonists also abolish veratrine contraction. From the point of view of chemical transmission theory *Kennedy* and *Wolf* (1938) found that prostigmine aggravates myotonus and quinine exaggerates myasthenia. On the other hand, acetylcholine

alleviates the symptom of myasthenia. Referring to such considerations, the following experiments were made concerning the action of poisons which affect the nerve-endings of autonomic nervous

system on veratrine contraction, in order to criticize the propositions offered by these authors, in regards to the genesis of the above diseases.

Most medicaments used by the author belong to alkaloide group which naturally are susceptible to dissolve in water only under the combined form with acid. Acid influences so remarkably veratrine contraction that it must be taken into account in evaluating the action of the drug, whether it is attributable to the action of acid or to that of the drug.

(1) Action of atropine.

That atropine paralyses the nerve-endings of parasympathetic and makes ineffective the action of acetylcholine, is a well known fact. But it exercises no influence on myotonic diseases. The atropine concentration which was used by the author was usually from $1:10^4$ to $1:5 \times 10^4$.

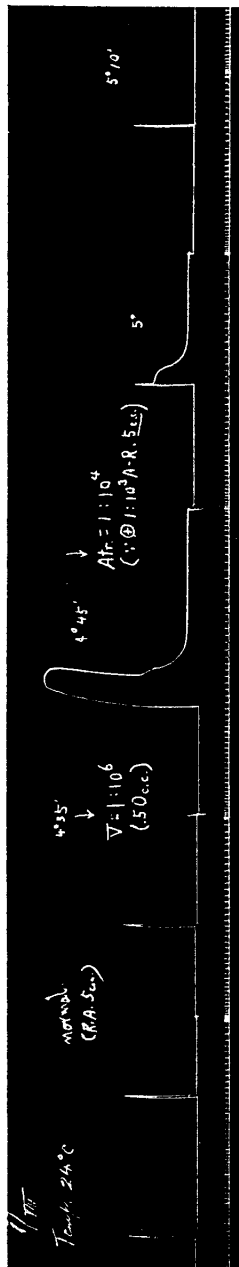
On previously atropinised muscle, veratrine contraction is more or less suppressed according to the mutual concentrations of the two drugs. When it was applied on previously veratrinised muscle, atropine also decreases or annihilates veratrine contraction (see Fig. 15). Atropine concentration down to $1:10^5$ seems to have little or no effect.

(2) Action of ergotoxine.

Ergotoxine is known as a paralyzing medicament of the sym-

pathetic nerve-endings. The drug acts on frog's muscle stimulating at first and then weakening in such a manner as to cause fatigue easily.

Fig. 15. Action of atropine on veratrine contraction.



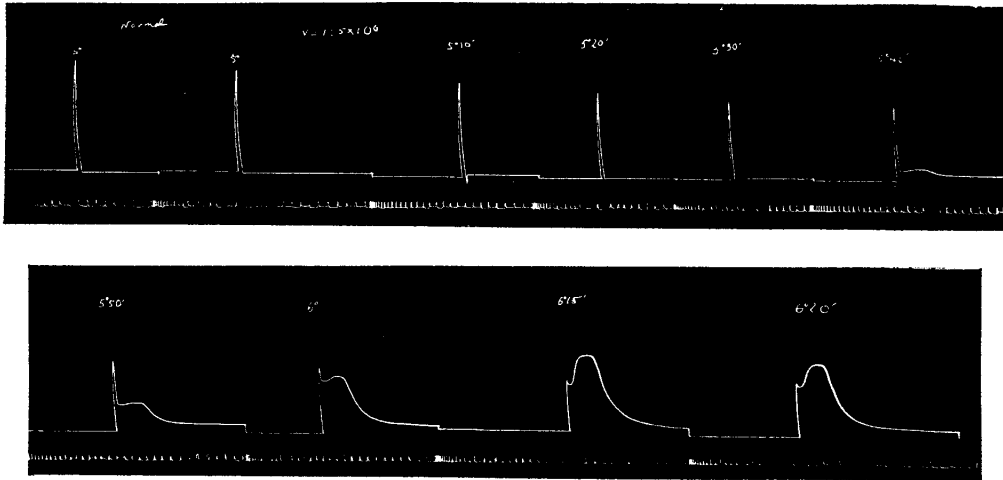
Explanation of the Figure: Atropine ($1:10^4$) was applied on previously fully veratrinised muscle (veratrine $1:10^4$). Veratrine contraction fairly disappeared 25 min. after atropine application.

traction, with Special Reference to the Action of Quinine.

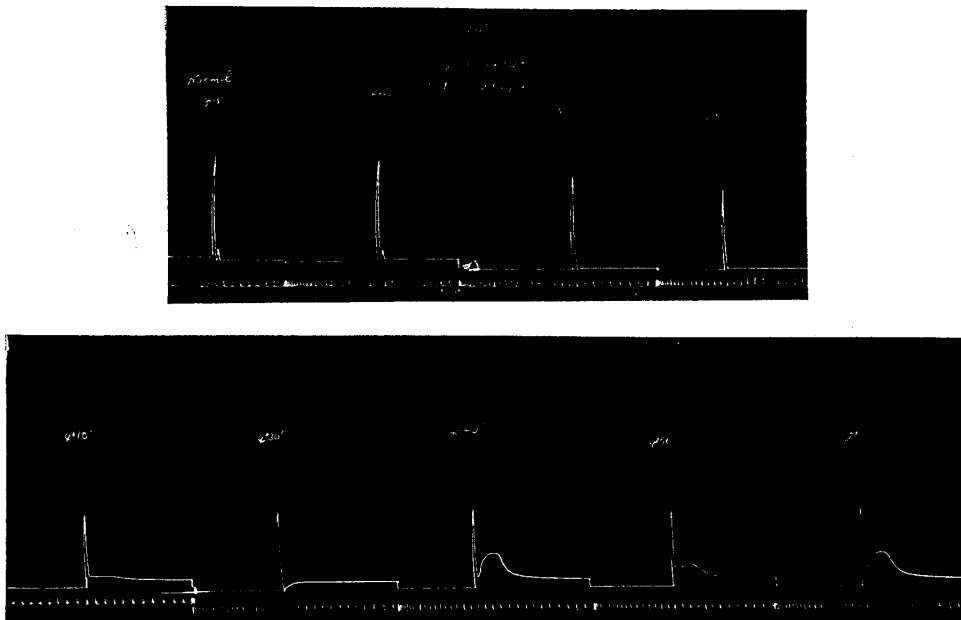
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Fig. 16 A. Example showing the effect of ergotoxine on veratrine contraction when simultaneously administered.

- a. Course of the manifestation of veratrine contraction (veratrine concentration $1:5 \times 10^6$) was reproduced as control. Veratrine contraction appeared in 40 min. after the administration, and increased its manifestation as time progressed.

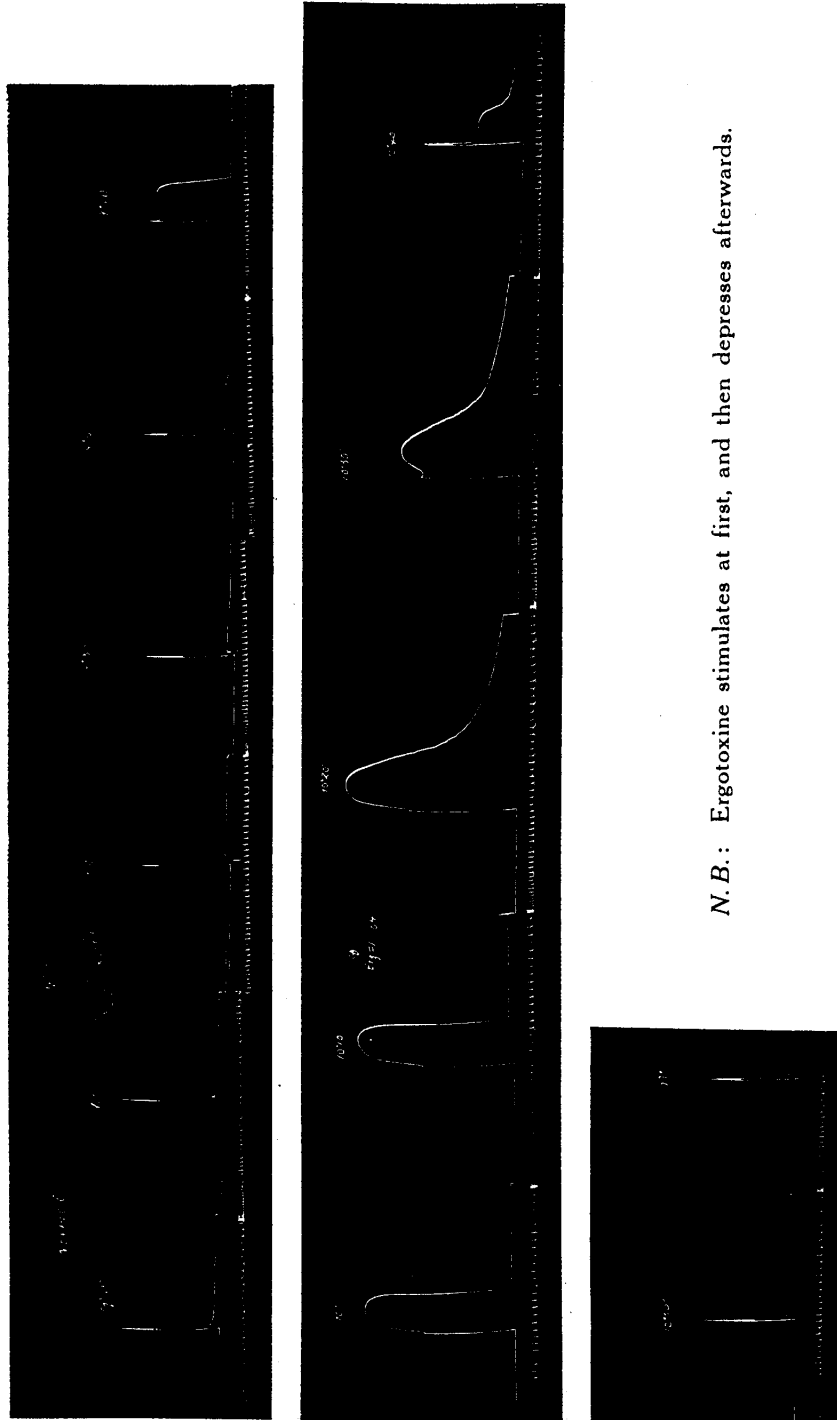


- b. Veratrine ($1:5 \times 10^6$) and ergotoxine ($1:2.5 \times 10^4$) were simultaneously added. Occurrence of veratrine contraction was retarded. The height of veratrine contraction did not increase as time elapsed.



Contraction curve shows much prolongation, especially in descending phase, and becomes to have the character of fatigue contracture.

Fig. 16 B. Example showing the effect of ergotoxine on previously veratrinised muscle.



N.B.: Ergotoxine stimulates at first, and then depresses afterwards.

The action of ergotoxine on veratrine contraction was investigated by the same experimental procedures as cited above. As ergotoxine itself does not dissolve in water, chemical equivalent dose of tartaric acid was added. Also the drug dissolves in a solution of alkaline medium. The ergotoxine concentration used, was usually $1:10^4$, dissolving both in acid and alkaline medium. Though the action of ergotoxine was feeble and inconsistent, the following results were obtained:

(a) When ergotoxine had been previously or simultaneously applied, the occurrence of veratrine contraction was suppressed or remarkably retarded. (b) When ergotoxine was applied on previously veratrinised muscle, the drug exaggerated veratrine contraction at first, then alleviated gradually until it abolished the contraction. Veratrine contraction was generally retarded by the administration of ergotoxine (see Fig. 16).

Through the parallel experiments regarding the action of acid or alkali under the same concentrations, it was proved that these effects of ergotoxine are not due to the action of acid or alkali, in which ergotoxine was dissolved.

(3) Action of acetylcholine and eserine.

It is believed that acetylcholine is liberated from nerve-endings of various nerve fibres and reveals various actions according to the function of the nerve to the organ. According to the new aspect originated by *Dale* and his colleagues, acetylcholine is the transmitter of motor nerve impulse causing muscular contraction. Also the drug has long been known as "Vagusstoff", as the transmitter of vagus nerve impulse. Considering from the fact that atropine suppresses veratrine contraction and that acetylcholine alleviates myasthenia, it is supposed that acetylcholine facilitates the occurrence of veratrine contraction. However it has been confirmed by the author's experiments that the drug has no effect.

Eserine and its associated substances inhibit the action of choline esterase on acetylcholine and increase the sensitivity of muscle to acetylcholine so that in respects to the action of acetylcholine, eserine is the antagonist to atropine. Whilst eserine, like atropine, inhibits or decreases the manifestation of veratrine action as shown in Fig. 17.

(4) Action of adrenaline.

Adrenaline, as acetylcholine in parasympathetic, is the transmitter of sympathetic nervous impulse. What influence adrenaline exercises on veratrine contraction, is an interesting problem.

Adrenaline in concentration $1:2.5 \times 10^4$ was applied on veratrine muscle (veratrine concentration $1:5 \times 10^6$). Then veratrine compo-

ment not only increased the height, but also appeared after the twitch contraction had returned to the base-line. It is common in ordinary veratrine contractions, where veratrine component is higher represented than twitch component, that veratrine component begins at

Fig. 17. Example showing the effect of eserine on veratrine contraction.

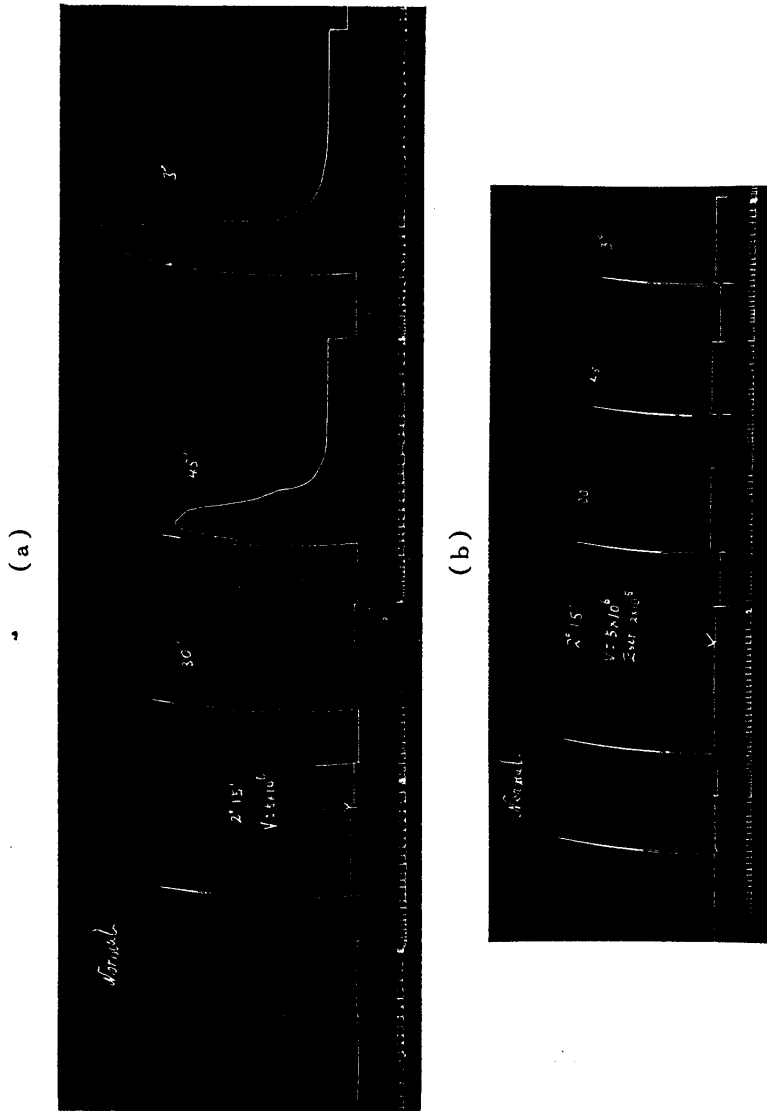


Fig. 18. Action of adrenaline on veratrine contraction.

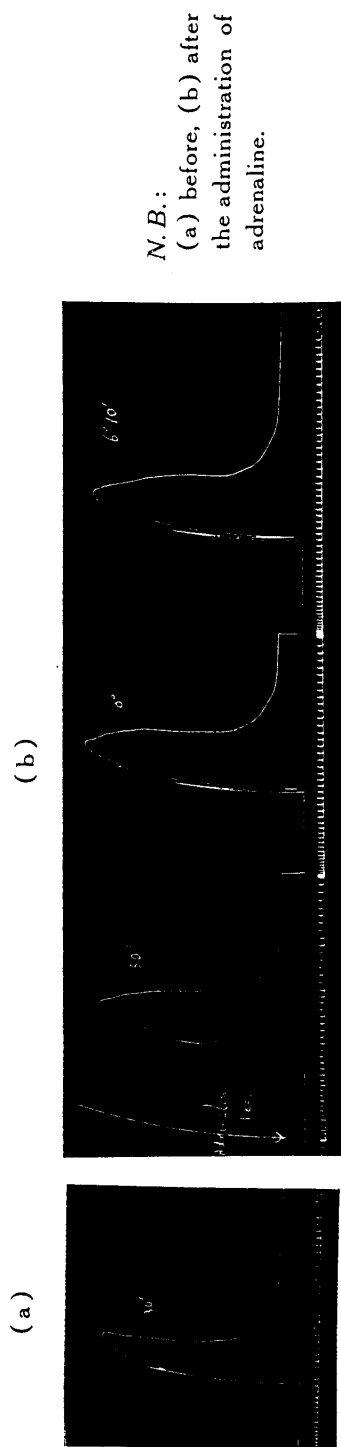
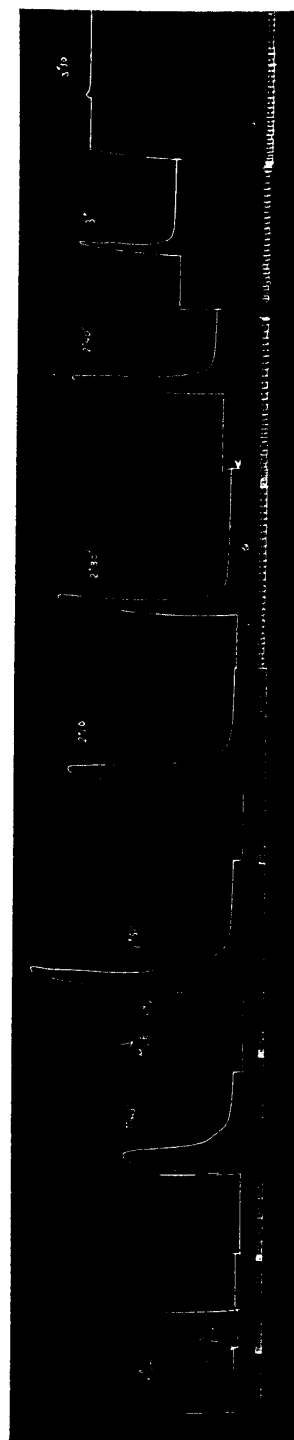


Fig. 19. Effect of monoiodoacetic acid on veratrine contraction.



(5) Action of monoiodoacetic acid.

Hartree and Hill (1922) concluded from their experiments on the heat production of the veratrine contraction that the liberation of energy in the veratrine contraction bears, qualitatively and quantitatively, the same relation to the mechanical response of normal muscle under tetanic contraction. As the basis of such phenomenon, it is natural that they attributed it to the abnormal liberation of lactic acid, which is induced by a shock and continues for a while in veratrine contraction, where it occurs momentarily in the normal muscle.

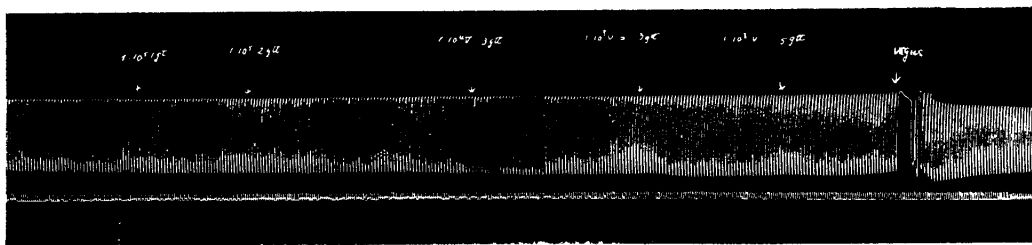
Monoiodoacetic acid inhibits the liberation of lactic acid in muscular contraction (*Lundsgaard*, 1930). Then what effect has veratrine on monoiodo-muscle? The experimental procedures used are as the same with previous experiments. Concentration of monoiodoacetic acid, exactly neutralised with sodium carbonate, was usually 1:104. Muscles bathed in this solution are fully poisoned after 30 minutes.

Veratrine contraction also occurs on monoiodo-muscle, as on normal one. When monoiodoacetic acid was administered afterwards, transitory increase of the veratrine component was noted which is considered to be due to the slight alkalinity of sodium carbonate, used to neutralise monoiodoacetic acid. The only difference from the normal muscle is that veratrine muscle became rigor rather rapidly (see Fig. 19).

Action of veratrine on heart muscle and non-striated muscles.

Veratrine, even in strong concentrations, exercises little effect on these muscles, as compared with skeletal muscles. The following experiments were made by the author on frog's heart muscle, uterus and intestine of guinea-pig, and dorsal muscle of leech.

Fig. 20. Tracings of isolated frog's heart beats.



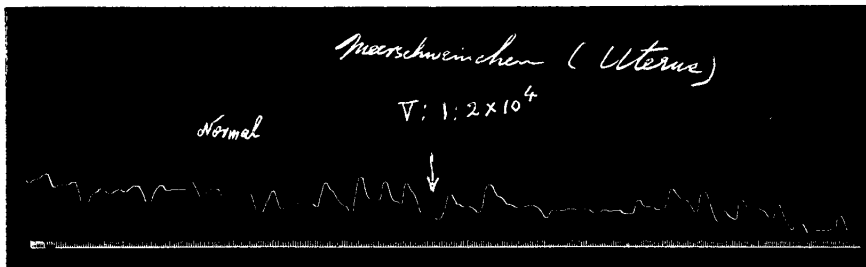
N. B.: The drug are added at the points indicated by arrows.

(1) Action of veratrine on frog's heart.

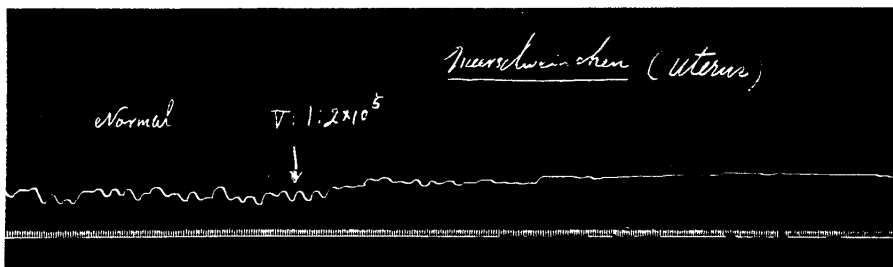
According to the *Straub's* method, frog's heart with vagus nerve was perfused with *Ringer's* solution. Veratrine of known concentration was added drop by drop in the perfusing *Ringer's* solution. The heart muscle is not affected in its frequency and amplitude of contraction. Stimulation of vagus is also effective (see Fig. 20).

Fig. 21. Tracings of uterus movements of guinea-pig.

a. Veratrine concentration. ($1:2 \times 10^4$)



b. Veratrine concentration. ($1:2 \times 10^5$)



(2) Action of veratrine on uterus and intestine of guinea-pig.

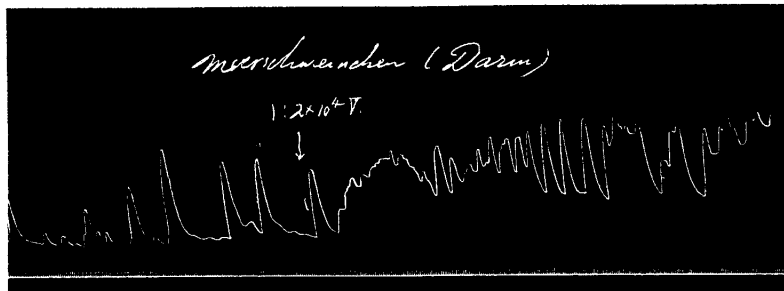
Movements of isolated uterus and intestine are recorded on rotating kymographion, after the suspension method of *Magnus*. Rhythms of spontaneous movements and tonic condition of the muscles were observed when veratrine was added in the bathing medium. The results are shown in Fig. 21 and 22. It seems to increase the muscle tonus through veratrine administration.

(3) Action of veratrine on leech dorsal muscle.

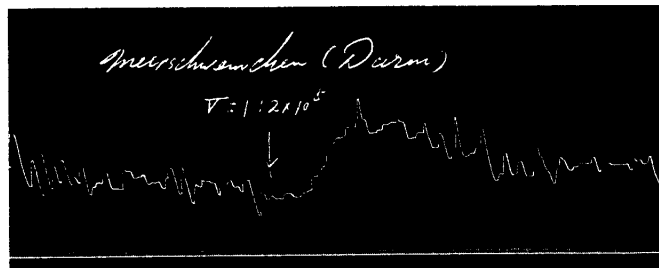
Leech dorsal muscle was carefully prepared and attached to the recording lever. After awaiting it had attained constant state under

Fig. 22. Tracings of in intestine movements of guinea-pig.

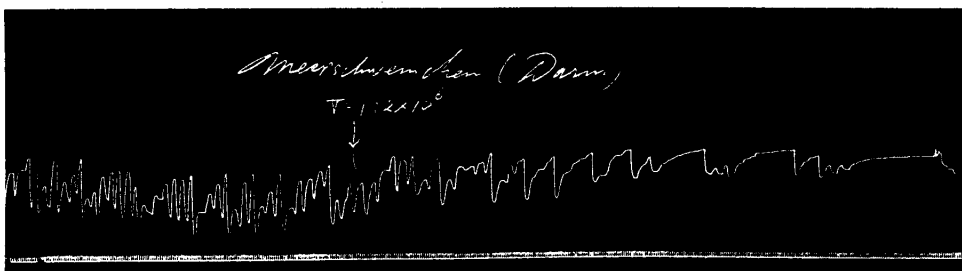
a. Veratrine concentration. ($1:2 \times 10^4$)



b. Veratrine concentration. ($1:2 \times 10^5$)



c. Veratrine concentration. ($1:2 \times 10^6$)



definite tension, maximal single induction shock was applied, and its contraction curve was recorded. Then veratrine from $1:5 \times 10^6$ to $1:10^6$ was applied. Leech muscle, if not stimulated, had steady state

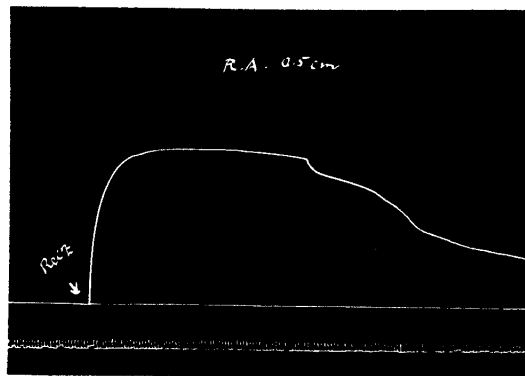
traction, with Special Reference to the Action of Quinine.

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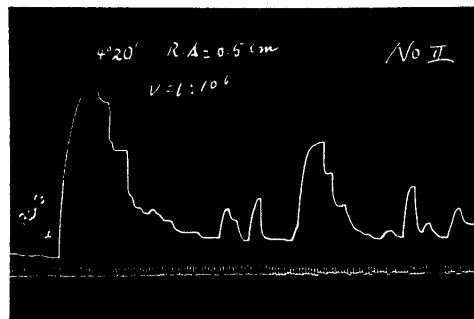
as normal muscle. But, if stimulated, it showed spontaneous movements in the descending phase of contraction and it continues thereafter (see Fig. 23).

Fig. 23. Tracings of the contraction of leech dorsal muscle.

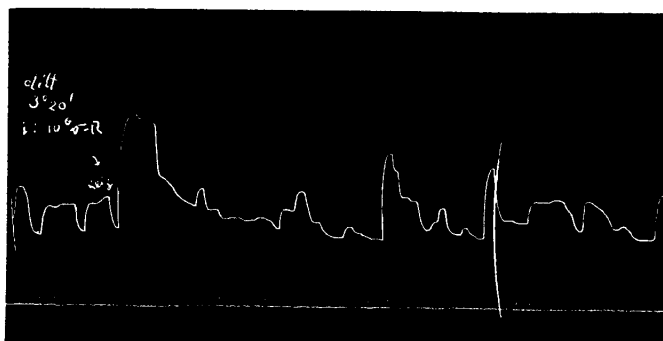
a. Normal contraction.



b. Contraction of veratrinised muscle.



c. Spontaneous movements after stimulation.



Discussion.

It is generally accepted that the action point of veratrine lies in muscle substance itself and not in nerve fibre or nerve-endings. Various hypothesis have been offered on the genesis of veratrine contraction, but they are only useful for explaining the representation of veratrine contraction, and not alluded to the working mechanism of the drug. *Bottazzi's* sarcoplasma hypothesis (1901; 1897), the most convincing one, explains the veratrine contraction, assuming muscle has two sorts of contractile element, i.e., the one is muscle fibril contracts rapidly and the other, sarcoplasma which causes tonic slow contraction, and veratrine affects the latter specially. But his hypothesis, similar with others, does not give an answer to the question why veratrine exercises such specific action on sarcoplasma.

Considering from the fact that veratrine contraction is profoundly influenced by the change of temperature, the working mechanisms of veratrine must be chemical in its nature. The mode of chemical combination of veratrine with muscle substance, whether it combines with sarcoplasma or sarcolemma, is, as *Straub* (1903) suggested, perhaps some sorts of absorption. However, the absorption is a physical phenomenon, and therefore is not so much influenced by temperature and it must be assumed that this absorption is a process by which veratrine and muscle substance are brought into combination, and the essential point of veratrine action lies in the mode of combination, upon which temperature greatly influences. Such assumption may be accepted considering from the fact that veratrine action has latent period after the absorption until it reveals its full action, and that veratrine contraction disappears through repeated stimulations and reappears after some time of rest. From the latter case, it can be imagined that the chemical combination of veratrine with muscle substance is a dissociable one, provoking dissociation through stimulation and combining again after rest.

Veratrine contraction is not absolutely specific to the action of veratrine, and it is also produced by many other organic and inorganic substances, not infrequently observed even under physiological condition (*Tiegel's* contracture!). *Tiegel's* contracture (1876) differs from other like-contracture in the point that it is facilitated at low temperature, and in this respect it is compared with myotonic diseases. This differentiation of the effect of temperature on the similar contracture forms, provides the explanation of the working mechanism of veratrine. Therefore the present author postulates that veratrine contraction occurs only under special mode of combination of veratrine with muscle substance, and sarcoplasma becomes sus-

ceptible after single shock of stimulation, which is insensible under ordinary condition, so as to cause abnormal breakdown of materials. *Lamm* (1912) demonstrated such chemical compound from the etherial extract of veratrinised muscle. Under such supposition the results obtained here may be clearly understood.

Potash and lime salts abolish or depress veratrine contraction, according to their variable mutual concentrations. This depressing action is not manifested until a considerable time elapsed after the administration. The latency of the action is a curious matter in veratrine contraction. It is not the case of these ions which directly influence cell membrane or protoplasm, because they pass freely through it. The author assumes therefore that these ions disturb the balance of veratrine-muscle compound. As a proof for this assumption, veratrine contraction promptly reappears, if these ions are removed.

Different explanations are offered regarding the influence of acid or alkali on veratrine contraction. *Lamm* considered, as stated above, that the action of veratrine is determined by the degree of dissociation of this alkaloid, while *Straub* considered that the physiological action of veratrine is directly proportional to the absorption of the drug in muscle, and that the abolishing action of chloroform and CO₂ is due to the decreased capability of absorption of muscle. The author is unable to decide which of these explanations holds true. But it is at least conceivable from the results obtained here, in which acid clearly obviated the veratrine contraction evoked by simultaneous administration of veratrine and alkali, that the chemical compound of veratrine and muscle substance is sensitive to the reaction of the surrounding medium, and readily decomposed in acid reaction.

Our knowledge in respects to the action of quinine on muscle is very meager. Skeletal muscle of frog is injured by strong concentration. But in dilute concentration, as *Santesson* (1892) and *Schtschepotjew* (1878) confirmed, quinine increases the excitability and activity of muscle. The site of quinine action lies in muscle and not in nerve.

Clinical studies of *Kennedy* and *Wolf* (1936; 1937; 1938), *Smith* (1937), *Kolb*, *Harvey* and *Whitehill* (1938) on myotonic diseases with quinine treatment afford nothing to the mechanisms of the quinine action. It is clear from the experiments here that quinine obviates the contracture in general, regardless through what procedures was it evoked. If all contractures, as *Bottazzi* assumed, are evoked by sarcoplasmic activity, quinine inhibits this activity and so abolishes also veratrine contraction. The author cannot decide however from his experiments where quinine acts to the chain of contracture

causing processes. In an isolated sartorius muscle, it is not conceivable, as *Wolf* and *Kennedy* assumed, that "myotonic phenomena are in all likelihood due to accumulation of acetylcholine or to an insufficient concentration of choline esterase at the motor end-plate." Further they continued, "yet it is difficult to imagine how myotonus could be due to excess acetylcholine, when atropine, an antagonist of acetylcholine, has little or no effect on the disease. May be the parasympathetic stuff at the motor end-plate or in muscle in this disease is somehow unaffected by atropine." On the contrary, atropine and eserine depress or abolish veratrine contraction, while acetylcholine does not interfere with it. Since these pharmacologic antagonists have the same effect, it indicates veratrine contraction has nothing to do with parasympathetic stuff.

On the other hand, adrenaline and ergotoxine are antagonists to the sympathetic. These actions on veratrine contraction are somewhat different. The author does not know what explanations may be given to it. Yet it is more justified to consider, apart from the problem of autonomic nervous system, that these alkaloides, as *Bottazzi's* experiments indicated, act on protoplasm directly.

Veratrine contraction also occurs in monoiodo-muscle, where lactic acid formation is absent. Therefore, there exists no direct relation between the occurrence of veratrine contraction and maintenance of mechanical performance and lactic acid formation, as *Hartree* and *Hill* (1922) assumed. Abnormal exhaustion of material in veratrinised muscle, accelerates the occurrence of monoiodo-contraction.

Ringer (1884-85) observed on heart muscle that veratrine causes irregular contraction and reduces the natural beats gradually until it undergoes arrest in diastole. Also he observed that potassium chloride and veratrine are reciprocally antagonistic. But the author failed to observe such veratrine action on isolated frog's heart.

With regards to the veratrine action on non-striated muscle, the results obtained here are similar with those of *Bottazzi* (1901), i.e., (1) increased tonicity, (2) strong rhythmic contraction and (3) irregular contraction are observed. An interesting matter is that the nerve-free leech dorsal muscle becomes to show rhythmic contractions after stimulation under veratrine poisoning.

Summary and Conclusion.

In this investigation, the veratrine contraction was studied with special reference to the action of quinine, and from the results obtained, the following summary and conclusion are made.

1. Various forms of veratrine contraction were found to be different representations of twitch and veratrine components, corresponding to the concentration of the drug, duration of the action of the drug (time factor), intensity and frequency of stimulations and individual sensitivity of muscles.
2. Veratrine-like contractions were evoked by glycerine and formaldehyde.
3. Veratrine contraction was obviated by lowering the temperature and facilitated by raising it. An intimate relations between the effective minimal dosis of veratrine and temperature were noted.
4. Veratrine contraction was depressed or abolished by calcium or potassium chlorides. Relations between threshold concentration of veratrine and calcium or potassium chlorides were determined.
5. Acidity inhibited the occurrence of veratrine contraction, alkalinity facilitated it.
6. Height of contraction of veratrinised muscle was higher than normal muscle, in twitch as well as in tetanus. Refractory period of veratrine muscle was greatly prolonged and found in the descending phase of veratrine contraction.
7. Quinine obviated veratrine contraction. Veratrine-quinine equilibrium ratio was determined.
8. Atropine inhibited the action of veratrine on muscle.
9. Ergotoxine retarded or depressed the occurrence of veratrine contraction, when applied previously, while if applied afterwards, it exaggerated at first, then alleviated gradually veratrine contraction.
10. Acetylcholine had no effect on veratrine contraction. Eserine, like atropine, inhibited veratrine contraction.
11. Adrenaline caused complete dissociation of twitch and veratrine components.
12. Moniodoacetic acid had no effect on the occurrence of veratrine contraction.
13. Veratrine had no effect on isolated frog's heart.
14. Veratrine caused increased tonicity and irregular tonic contraction on non-striated muscles (dorsal muscle of leech, intestine and uterus of guinea-pig).

In conclusion, I wish to express my sincere thanks to Prof. Oinuma for his kind suggestions and advices during the investigation.

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