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ON THE CIRCUMSTANCES WHICH MODIFY THE ACTION OF CAFFEINE AND THEINE UPON VOLUNTARY MUSCLE. By T. LAUDER BRUNTON, M.D., F.R.S. and J. THEODORE CASH, M.D., F.R.S.

The action of caffeine upon voluntary muscle is a subject of especial interest, inasmuch as it affords a marked example of what is sometimes brought as a reproach against pharmacological researches, namely, that different observers sometimes obtain totally different results in experiments with the same drug. At first sight this may appear to indicate that pharmacological experiments are apt to lead to no true and definite result, but this is not the case, for in almost all instances each observer sees a part of the truth, though perhaps not the whole truth, and as Claude Bernard wisely said "it is by investigating the exceptions to any rule, that we are most likely to arrive at the truth."

In the first experiments on the action of caffeine, it was observed to produce tetanus in frogs, but no particular attention was directed to the condition of the voluntary muscles, although in toads a peculiar stiffness was observed before the convulsions appeared. Voit was the first to notice this stiffness in the muscles resembling rigor, but he attributed this not to the direct action of caffeine upon the muscular substance, but to a condition of œdema brought about by dilation of the vessels.

Binz[1] attributed this effect to a tetanus induced by action of caffeine on the central nervous system.

This rigor was again observed by Pratt, and also by Johannsen who was working under Schmiedeberg's direction. Johannsen observed in the frogs with which he experimented, that the muscles became rigid at the place where the caffeine was injected, and this rigidity gradually extended to the rest of the body, but he failed to observe any tetanus. About three years afterwards Aubert arrived at results entirely opposed to those of Johannsen, finding that caffeine

[1] *Archiv. f. Exp. Patholog. u. Pharmakol.* Bd. IX. s. 33.

in the frogs with which he experimented, produced marked tetanus but very slight rigor.

These contradictory results induced Schmiedeberg[1] again to take up the subject, and he found that the discrepancy between the statements of Johannsen and Aubert was to a great extent due to the kind of frog employed by each observer in his experiments, the former having used specimens of *Rana temporaria* and Aubert of *Rana esculenta*. According to Schmiedeberg in *Rana temporaria* caffeine produces muscular rigor without tetanus, the rigor beginning at the place where the poison is applied and extending over the body so gradually, that the muscles first attacked may be completely contracted and rigid, while others may be still slightly irritable. On the other hand, in *Rana esculenta* caffeine frequently produces a violent and continuous reflex tetanus, without any rigidity of muscle other than that dependent on the tetanic contraction. It is only at a late stage of the poisoning, two to three days after the caffeine has been given, that these differences between the two kinds of frogs become equalised, increased reflex action and even tetanic convulsions occurring in *Rana esculenta*, and distinct stiffness of the muscles becoming observable in *Rana temporaria*, although this stiffness never becomes so great as in *Rana temporaria*.

These experiments of Schmiedeberg's seemed completely to have settled the question; more recent observations have shown that the problem is still but partially solved.

It seemed therefore advisable to us to take up again the action of caffeine upon muscle, and subject it to a further examination. In doing this we tried to keep before us the facts that the action which a substance is alleged to have upon a living organism may vary,

(*a*) according to the nature of the drug,

(*b*) according to the nature of the organism,

(*c*) according to the conditions under which the experiments are made (temperature, duration of observation, dose, etc.).

(*a*) The fact that drugs bearing the same name are not always the same in nature, is one cause of the discrepancies sometimes observed in pharmacological researches. Examples of this are afforded by the rarer alkaloids of opium, by the different alkaloids grouped together under the name of aconitine, and by such alkaloids as physostigmine and pilocarpine, which are frequently contaminated with the alkaloids having an antagonistic action, namely, calabarine and jaborine.

[1] *Archiv. f. Exp. Patholog. u. Pharmakol.* Bd. II. s. 62.

It has been usually assumed, since the alkaloids obtained from tea and coffee have been considered identical, that results obtained with one may be applied equally to the other. Recent researches of Dr Mays seem to render this doubtful, for he states that theine does not cause muscular rigidity, even when a muscle is immersed in a solution of it, whilst caffeine does so.

We may so far forestall the results of our experiments as to state that our experience does not entirely coincide with that of Dr Mays, but we have not been able to ascertain, whether the discrepancy between his results and ours is due to difference in the animals employed or to variations in the theine, for it is just possible that there may be differences in the nature and therefore of physiological action in the active principles obtained from different kinds of tea, or even from the same kind of tea by different methods of treatment.

Filehne[1] instituted a careful comparison of the action of xanthine, trimethyl-xanthine, (caffeine) and dimethyl-xanthine (theobromine), together with other related bodies in regard to their general action and their tendency to cause muscular rigor. He found that xanthine caused more marked paralysis of the cord and greater rigor than theobromine.

(*b*) According to the nature of the organism.

The alterations in the effect of caffeine produced by differences in the species of frog employed in experiments, have already been mentioned.

It is well known that variations exist not only in the reaction to drugs but in the irritability, strength, and electrical phenomena of frogs, belonging to the same species at different times of the year, and under varying conditions of nutrition. Thus Kobert[2] found that frogs were unaffected by a dose of caffeine (8 mg.) in November which in February and March always caused poisoning.

The nutrition of animals, expecially as regards the quality and quantity of the inorganic constituents of the body, has been found by us to exercise a marked influence upon the effect of some poisons, such as Barium. The advisability of examining the relative quickness with which rigor was produced in various muscles of the same frog suggested itself to us, as it appeared probable that we might find that the results might not be uniform.

(*c*) Conditions of experimentation.

Under this heading we may include considerations of (*a*) dose,

[1] *Archiv. f. Physiologie.* 1886, p. 72.

[2] Kobert. *Arch. für Exp. Patholog. u. Pharmacolog*, xv. p. 63.

(β) temperature and (γ) mode of application of the drug, and (δ) duration of the observation.

(α) In regard to the action produced by varying quantities of a drug, we may refer to a paper on the modification in the action of aconite, etc. recently published by us[1].

(β) The modifying action of temperature has been largely discussed by Luchsinger and by ourselves.

(γ) The local or general application may influence the action of a drug.

(δ) The necessity of long-continued observation is clearly shown by Schmiedeberg's discovery that it is only after the lapse of two or three days subsequent to the poisoning by caffeine, that the inequality of the symptoms in the two species of frogs becomes equalised.

The Plan of Experiment we generally adopted was to isolate a muscle of a frog, (*Rana temporaria*) and to apply directly to it a solution of caffeine or theine of known strength. We employed muscle chambers which we have already described in a former paper[2].

As it was necessary in most of the experiments to contrast the condition of a poisoned muscle with an unpoisoned one, we employed two of these chambers, each having a capacity of 50 c.c. and each carefully fitted with a thread passing through the capillary tube at the bottom, so that only a few drops of the solution escaped in the course of many hours. A little mercury on the floor of the chamber still further hindered escape. The muscles in these chambers were in connection with levers of equal length, which magnified the actual shortening of the muscle ten or more times. Our recording cylinder, one of M. Verdin's very accurately made pieces of apparatus, rotated once in twenty four hours, the actual movement being through 12 mm. in the hour. This slow rotation we found necessary, in order to register changes in the muscle which sometimes occurred many hours after the introduction of the caffeine solution into the chamber.

As we determined to use $\cdot 7\%$ salt solution as the solvent for caffeine, it seemed necessary in the first instance to make a few observations upon the effect of this alone, not only to ascertain whether the muscle passed of itself into a condition of rigor when suspended in this fluid, but also whether the normal salt solution might cause a

[1] *St Barthol. Hosp. Reports*, Vol. XXII.

[2] "The relation between chemical constitution and physiological action." *Phil. Trans.* Pt. I. 1884.

condition of oedema, such as was regarded by Voit as being a probable cause of contraction and stiffness occurring in the muscles of frogs poisoned by caffeine. As we had previously exposed muscles to the action of the normal salt solution for many hours without seeing any fibrillation or apparent tendency to rigor, we began at once by leaving the gastrocnemii of frogs exposed in the manner we have indicated for twenty-four hours and longer, and we found that neither did they show any perceptible fibrillation, nor did any rigor causing contraction of the muscle usually result during this time. In most instances the muscles were found to be still irritable at the end of twenty-four hours, in a few cases all irritability had disappeared. We have continued the experiment up to three and four days, when decomposition had developed itself, without meeting with any muscular shortening which would have been indicated by the elevation of our levers. These levers exercised a traction of about 3 grms. upon the muscle when no additional burden was suspended.

The caffeine we employed was obtained from Schuchardt in Görlitz. The theine was a pure specimen from Messrs. Hopkins and Williams.

Amount of caffeine producing rigor.

We have stated that a solution of caffeine in salt solution was employed by us; in addition to this we added a small quantity of salicylate of sodium which greatly assists the solubility of caffeine. Our usual solution was constituted as follows:—·4 grm. caffeine, ·2 grm. salicylate of sodium, 100 c.c. ·7 % salt solution.

When the muscle had been introduced into the chamber, which had been previously thoroughly cleansed by distilled water and salt solution, a definite number of c.cs. of this strong caffeine solution was measured into a finely graduated 10 c.c. measure, the solutions and the washings of the first measure transferred to a larger one, and diluted with salt solution up to 50 c.c. The whole of this fluid was transferred by means of the funnel and indiarubber connecting tube to the muscle cylinder, and a mark on the drum was made recording the time of the addition. In many experiments the muscle was immersed in salt solution for from 30 minutes to 4 hours before the introduction of the caffeine mixture.

The second muscle chamber, the lever of which wrote from the same side exactly beneath the other, was filled in a similar manner with the

control fluid, and the experiment then went of itself for twenty-four hours or longer as was desired.

Control experiments we made, convinced us that the quantity of salicylate of soda employed had no active part in promoting the occurrence of rigor.

We will consider in the first instance the effect of dose both in the case of theine and caffeine. For purposes of comparison we have tabulated several experiments which yield information on this point. The length of time before contraction begins and the total amount of contraction, were used as the best means of estimating the effect of the drug upon the muscle.

If this table be examined closely the fact becomes evident that the action is very irregular between similar muscles of different individuals for the same dose, and deviates though to a much smaller extent between companion muscles in the same animal. Even when the time of the commencement of the rigor is the same for the two muscles under examination, the speed at which the maximum contraction is reached, and its absolute altitude, may show considerable variation. Thus in experiment F, the rigor commences simultaneously, but its maximum is reached by one muscle 75′ before it is by the other, and its extent is 17 mm. greater on the curve (1·7 mm. actual measurement in the muscle) than it is in the case of its neighbour.

Experiment G shows us that an amount of caffeine greater than was employed in experiment D may altogether fail to produce rigor, whilst in the latter a strong rigor commenced in four hours reached an altitude of 64 mm. (6·4) and was maintained during fourteen hours.

Again contrasting A and C. The same fact will be observed in the case of theine, which in the former causes a marked rigor in 4 hrs. 30 mins., whilst no effect is observed in the latter.

That a small dose is not really more active than a larger one when companion muscles of the same animal are contrasted, is shown by a large number of experiments. It was invariably found that if two companion gastrocnemii were subjected to the action of solutions of caffeine, of different strength, that the greater effects in the way of rigor were always produced by the stronger solution. The gastrocnemius, again contrasting companion muscles, which began first to contract, almost invariably developed the greatest degree of contraction and attained it sooner than the other.

The table we have given appears to show a somewhat greater activity of theine than of caffeine, but the variation in action between

the two is much less than the variation in the action of either substance upon frogs of the same species, selected at random at the same time of year. To demonstrate conclusively that a difference does exist, we must contrast this action upon companion muscles of the same animal between which there are much smaller variations in reaction. On looking

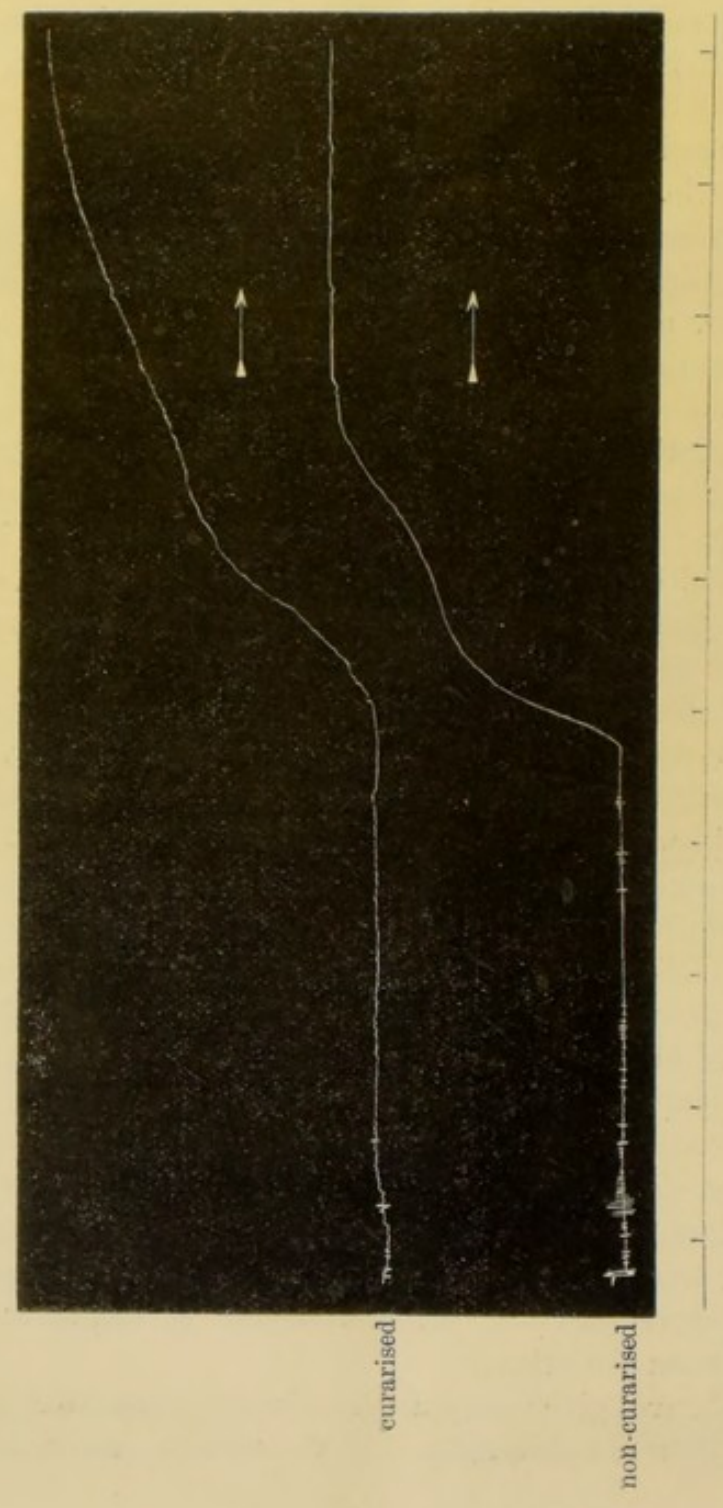

FIG. 1. A. Action of theine on the curarised and non-curarised gastrocnemius of the frog. The inequalities in the horizontal lines are chiefly caused by the vibrations of the floor of the laboratory. Speed of drum (applicable to all curves) 12 mm. per hour, as indicated by marks on line below figure.

over a large number of experiments, we have found several in which a certain strength of theine solution produced a marked, though never a very powerful rigor of the muscle, but in which the companion muscle having been subjected to the action of an exactly equal quantity of caffeine showed no rigor whatever. (It must be remembered that at present we are only contrasting the action of the two specimens of alkaloids obtained from the sources named.)

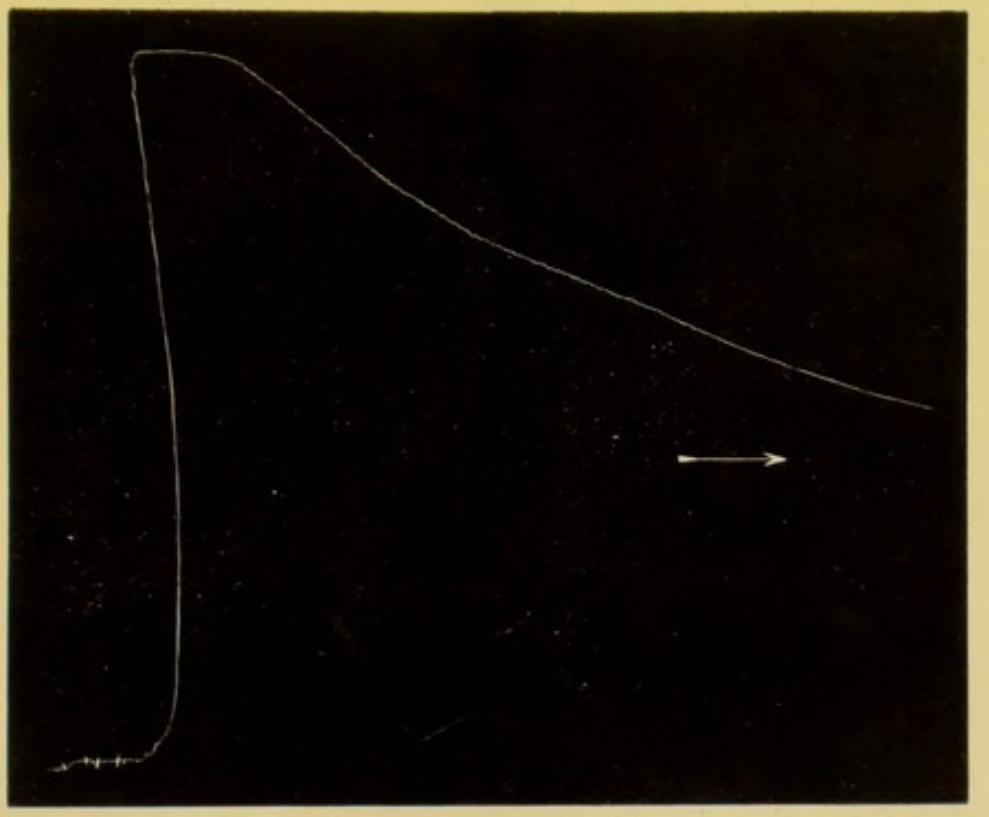

FIG. 2. B. Action of caffeine on the gastrocnemius of the frog. Weight suspended 10 grammes in all. Lever multiplies 10 times.

A. Fig. 1.

Gastroc. Weight 20 grms.	Upper curve. Curarised muscle.	·028 Theine. ·014 Salicylate of Soda.	Rigor commences in 4 hrs. 40 mins. Maximum reached in 10 hrs. 33 mins. Altitude 33 mm.	Each showed a notch or break in the ascent.
Gastroc. Weight 20 grms.	Lower curve. Not curarised.	50 c.c. ·7 % Salt solution.	Rigor commences in 4 hrs. 30 mins. Maximum reached in 7 hrs. 30 mins. Altitude 28 mm.	

B. Fig. 2. Rana Temporaria of 17 grammes.

Gastroc. left. Weight 20 grms.	Neither curarised.	0·04 Caffeine. ·032 Salicylate of Soda.	Rigor commences in 30 mins. Greatest altitude 58 mm.	In 50 mins. after maximum rigor, lengthening begins. In 12 hrs. rigor 24 mm. In 36 hrs. rigor 15 mm.
Gastroc. right. Weight 20 grms.		50 c.c. ·7 % Salt solution.	Rigor commences in 20 mins. Greatest altitude 70 mm.	In 50 mins. after maximum rigor lengthening begins. In 12 hrs. has fallen to 38 mm. In 24 hrs. has fallen to 33 mm.

C. Frog of 15 grammes. (Cold weather.)

Gastroc. left. Weight 10 grms.*	·036 Theine. ·032 Salicylate of Soda. 50 c.c. ·7 % Salt solution.	This experiment was continued for a week. In this case no rigor of either muscle occurred. Both muscles elongated.	
Gastroc. right. Weight 10 grms.			

D. Frog of 35 grammes.

Gastroc.	Curarised.	·032 Caffeine. ·016 Salicylate of Soda.	Commencing rigor 2 hrs. 10 mins. Altitude 75 mm.	For 14 hrs. the maximal rigor maintained.
Gastroc.	Not curarised.	50 c.c. Salt solution.	Commencing rigor 4 hrs. Altitude 64 mm. Maximum attained 4 hrs. 48 mins.	

* Where not otherwise stated the total weight was 10 grms. and the lever multiplies the actual shortening of the muscle ten times.

E.

Gastrocnemius.	Not curarised.	0·04 Caffeine. ·03 Salicylate of Soda.	Commences to rise in 2 hrs. ,, ,, sharply 4 hrs. Maximal rigor 80 mm. ,, reached in 5 hrs. 30 mins.	Rigor maintained.

F. Rana Temporaria of 24 grammes' weight.

Left gastrocnemius.	·04 Theine. ·02 Salicylate of Sodium.	Begins to contract 4 hrs. 20 mins. Maximum reached in 6 hrs. Greatest altitude 49 (÷ 10).	In 24 hrs. it has fallen to 37 mm.
Right ,,	50 c.c. ·7 % Salt solution.	Begins to contract 4 hrs. 20 mins. Maximum reached in 4 hrs. 45 mins. Greatest contraction 66 mins. (÷ 10).	In 24 hrs. it has fallen to 50 mm.

G. Rana Temporaria of 13 grammes.

Left gastrocnemius. Right ,, Weight 20 grms.	·04 Caffeine. ·02 Salicylate of Sodium. 50 c.c. Salt solution.	No rigor caused in 24 hrs.

H. Fig. 3. Gastrocnemius of Rana Temporaria.

Left gastrocnemius.	·08 Caffeine. ·04 Salicylate of Sodium. 50 c.c. Salt solution.	Rigor rapid but maximum not attained for 100 mins. Greatest altitude 54 mm.
Right ,,	·12 Caffeine. ·06 Salicylate of Sodium. 50 c.c. Salt solution.	Rigor. Instantaneous contraction. Greatest altitude 82 mm., reached in 60 mins.

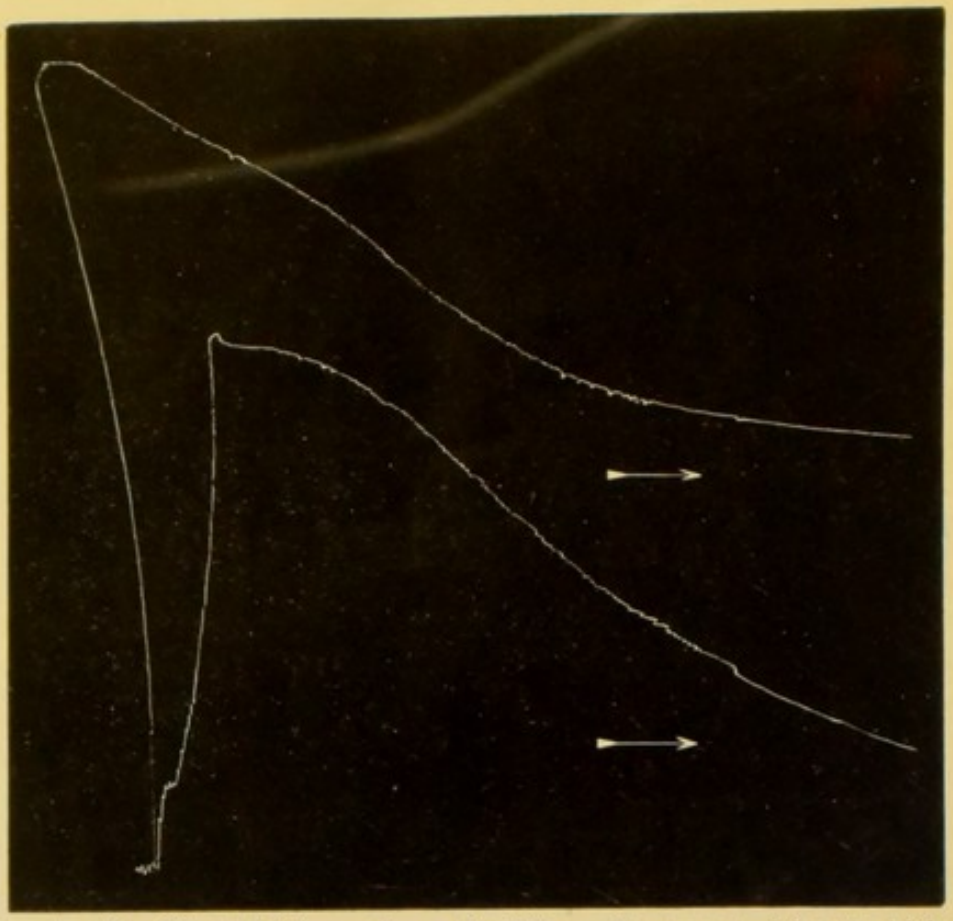

FIG. 3. H. Effect of different doses of caffeine. The muscle giving the higher curve received ·12 grm caffeine and the lower ·08 caffeine.

Effect of curare.

In pegged frogs the iliac vessels on one side were ligatured, and small doses of curare injected into the dorsal lymph sac. After curarisation was more or less advanced, preparations were made of the curarised and non-curarised gastrocnemius respectively, and each was exposed to the action of an equal amount of caffeine. The results obtained were somewhat irregular.

Incomplete curarisation seemed to have no effect in shortening or lengthening the time at which rigor commenced, though in two instances the subsequent rigor attained its maximum later, and the contraction was greater than (A) in the other muscles.

After complete curarisation ·036 grammes theine in 100 c.c. salt solution, the rigor of the curarised muscle commenced in 100 minutes, as contrasted with 3 hours 20 minutes for the non-curarised. The maximal shortening was more rapidly attained, and its extent was greater in the former instance than in the latter.

Contrast between various Muscles of the same frog.

The muscles employed were the gastrocnemius, triceps, sartorius.

We instituted these experiments, not only in order to test the effect of solutions applied locally to various muscles which might probably be differently affected by the immersion, according to the thickness of the investing fascia, etc., but also in order to obtain a contrast of the rigor curve furnished by them.

With regard to the latter point, we found that the rigor of the triceps was rapidly developed, rose quickly to its maximum, and was both extensive and strongly maintained.

The gastrocnemius showed (vide all the former experiments also) a slower development of rigor, and a relatively later maximal contraction which was not so extensive as that of the triceps. It is worthy of remark, that on these points there is some correspondence between the simple contraction curve of these muscles and their rigor curves.

A notch or depression indicating a pause in the development of rigor is not unfrequently seen in the case of the gastrocnemius, to one instance of this we have already called attention.

The sartorius contracts rapidly when it begins to pass into rigor, its maximum is soon attained, the maximal shortening which is never very great, even under the burden of the lever only (weight 3 grammes), persists for some hours, 6 to 8, and then relaxation commences.

As regards the time of commencing rigor, we must refer to the contrast notes of the experiments quoted which show that considerable variation exists.

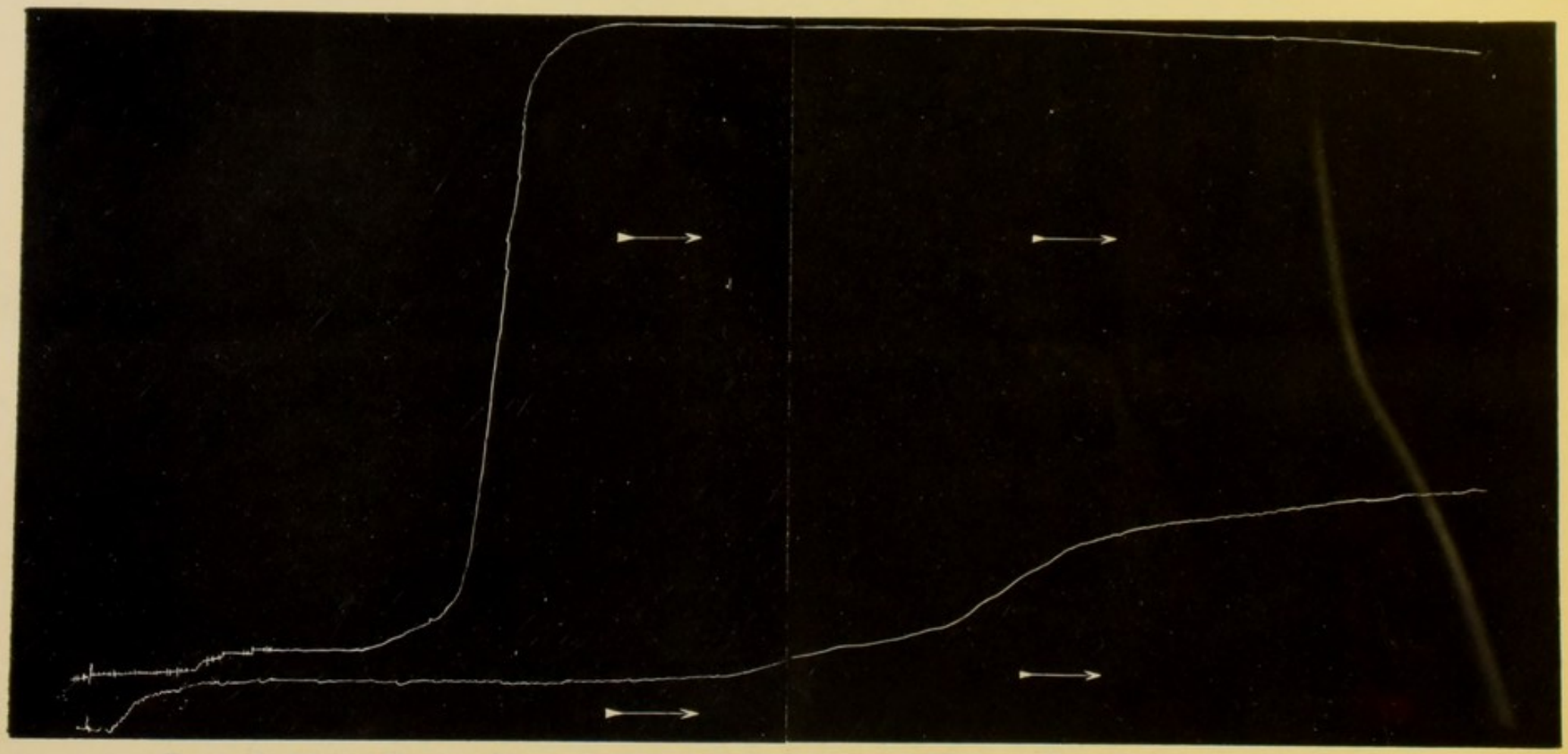

FIG. 4. I. Effect of Theïne on different muscles. The lower curve is that of the gastrocnemius, the upper that of the triceps.

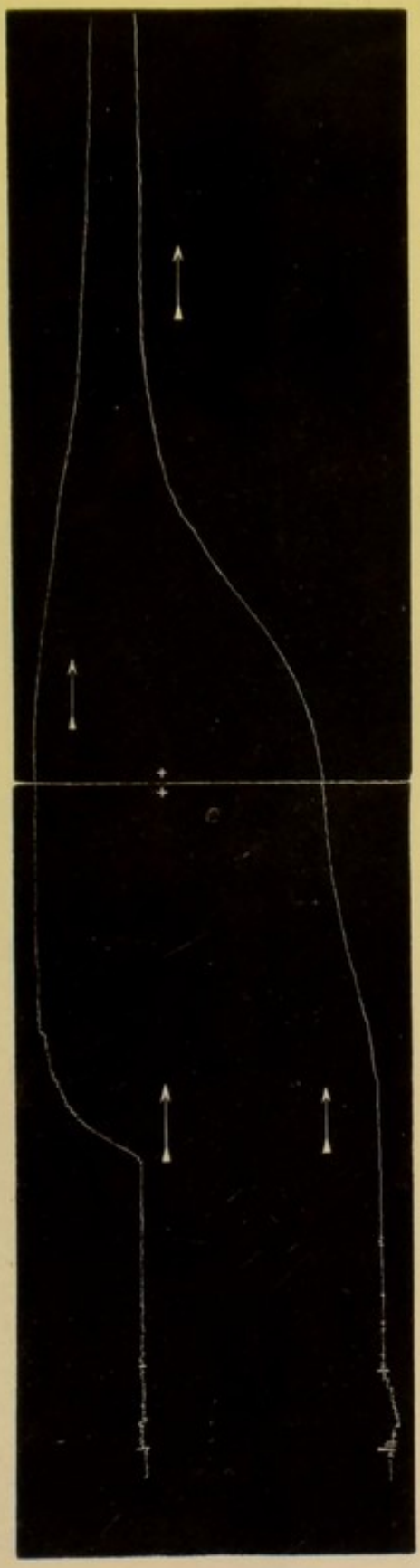

FIG. 5. K. Effect of Caffeine upon different muscles. The upper curve is that of a sartorius and the lower of a gastrocnemius.

I. Fig. 4. Rana Temporaria of 35 grammes' weight.

Gastrocnemius.	(Lower curve.)	·028 Theine. ·014 Salicylate of Soda. 50 c.c. Salt solution.	Began to rise in 20 mins. Attained a height of 6 mm. in 1¼ hr. Remained nearly at the same height till 7 hrs. after the application of the caffeine, then began to rise again. At the end of 15 hrs. had attained a height of 32 mm., at which it continued to the end of 24 hrs.
Triceps.	(Upper curve.)	·028 Theine. ·014 Salicylate of Soda. 50 c.c. Salt solution.	Began to rise in 1¼ hr. Rose in ½ an hr. more to 4 mm. at which it remained until 3 hrs. after the application of the theine, when it again began to rise slowly for an hour, and then rapidly. At the end of 8 hrs. it had attained its maximum of 86 mm., it remained almost constant for 6 hrs. and then slowly fell, until at the end of 24 hrs. it had come down to 72.

K. Fig. 5. Rana Temporaria of 30 grammes' weight. (Deeply curarised.)

Gastrocnemius. Weighted with lever + 10 grammes (13 grms.).	·028 Theine. ·014 Salicylate of Soda. 50 c.c. Salt solution.	For 4 hrs. there was no rise of the lever, at the end of that time, it began slowly to rise showing a notch. At the end of 11 hrs. it had attained its maximum of 26 mms., and remained at that height till the end of 24 hrs.
Sartorius loaded with the lever alone.	·028 Theine. ·014 Salicylate of Soda. 50 c.c. Salt solution.	No rise till the end of 2¾ hrs. when rise began quickly, reached its maximum of 12 mms. in 4 hrs., remained constant till the end of 7½ hrs., when it began slowly to fall. At the end of 10 hrs. had fallen to 8, and thereafter the decline became more gradual.

Effect of Acids and Alkalies, etc. on Caffeine Rigor.

Many important investigations which have been made touching the changes produced in the irritability of striated and unstriated muscular fibre by acids and alkalis, suggested to us the importance of combining the caffeine solution with these antagonising agencies, in order that any modification of the rigor curve which they might produce could be examined. The results obtained were constant and striking. Lactic acid increases the rapidity of the production of rigor in weak caffeine and theine solutions—not only in contrast with the normal muscle, but also when compared with a corresponding muscle suspended in a solution of caffeine of the same strength but rendered alkaline by KHO. The amount of lactic acid employed was usually ·01 (one centigramme) to the 50 c.c. of caffeine mixture admitted to contact with the muscle. We do not lose sight of the fact that not only do these solutions act specifically on the muscle itself, but they may act by combination with the ingredients of the caffeine solution, and also by so altering the fascial covering of the muscle as to render the action of the caffeine weaker or more energetic.

The experiments necessary for the elucidation of the questions under this heading may be divided into three series:

(1) The action of lactic acid alone. Lactic solution (of the strength usually employed) contrasted with the action of a certain dose of caffeine or theine.

(2) The action of lactic acid caffeine solution contrasted with caffeine solution.

(3) The action of lactic acid caffeine solution contrasted with potash caffeine solution.

M.

Gastrocnemius of a healthy frog of 25 grammes.	·01 Lactic acid (Puris.) in 50 c.c. salt solution ·7 per cent.	A faint indication of commencing contraction in 8 hrs. 40 mins. Maximum attained in 1 hr. 4 mm. (÷ 10).
Companion.	Theine ·048. Salicylate of soda ·015. Salt solution 50 c.c. Lactic acid ·01.	Rigor commenced in 5 hrs. Maximum 73 mm. (÷ 10) attained in 10 hrs. and long maintained.

It is evident in this case that though lactic acid applied in salt solution may show an increased tendency to cause rigor when compared

with the latter alone, yet the effect produced by such a dose as was administered is very slight and tardy in making its appearance.

(2) Lactic acid theine solution contrasted with theine solution.

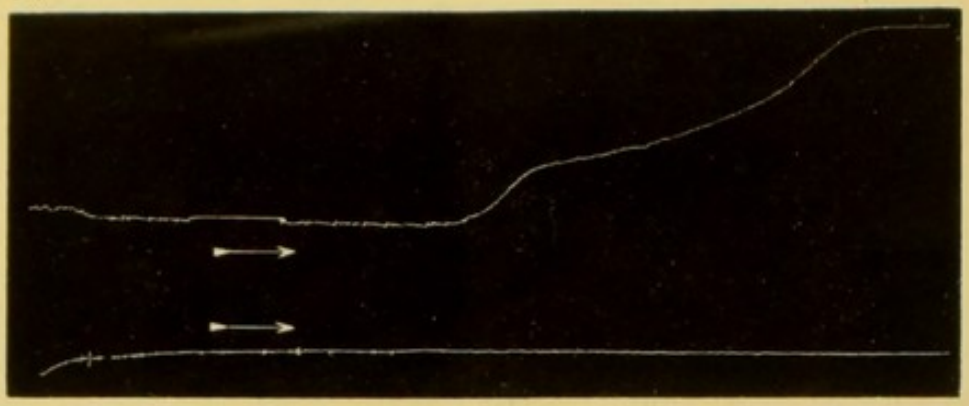

FIG. 6. N. Effect of lactic acid on the action of theine. The upper curve shows the action of theine together with lactic acid on the gastrocnemius of a frog. The lower shows the action of theine alone on the companion muscle.

N. (Fig. 6).

Gastrocnemius of normal frog.	Immersed in theine solution, as in M, the same quantity of lactic acid ·01 being present.	After slight preliminary relaxation rigor commenced in 3 hrs. 10 min. Attained its maximum of 20 mm. in 6 hrs. 40 min. and muscle remaining contracted to this extent for 24 hrs.
Companion.	Same theine solution but no lactic acid added.	No rigor whatever produced in course of 24 hrs.

The result of these two experiments is that lactic acid and theine together produce much greater contraction than lactic acid alone, and more than theine, alone.

(3) Lactic acid caffeine solution contrasted with potash caffeine solution.

As we shall have to show presently that alkalies retard the caffeine effect, it is to be expected that between caffeine solutions containing alkalies and acids a marked variation should occur, and this is in fact the case. The acid rigor is more rapid, it develops more and its maximum is relatively greater.

O.

Gastrocnemius of *Rana temporaria.*	·032 Theine. ·015 Salicylate of soda. 50 c.c. Salt solution. ·01 KHO.	Rigor commences slowly in 9 hrs., its maximal being 6·5 mm.

Companion Gastrocnemius.	Same theine solution + ·01 lactic acid.	Rigor commences rapidly 3 hrs. 5 min., its maximal attained being 13·5 mm.

The muscle exposed to the alkaline theine solution thus passed into a feeble rigor 6 hours after the acid muscle had begun to pass rapidly into a moderately firm one.

Modification of the Caffeine rigor by potash:

(1) Potash and salt solution as contrasted with salt solution.
(2) ,, caffeine ,, ,, caffeine ,,
(3) ,, ,, ,, ,, lactic acid ,,

P.

(1) With the quantities of potash employed ·005 to ·01 grm. per 50 c.c. no rigor was induced, and as the contrast muscle suspended in salt solution did not in the experiments made pass into rigor, we cannot say that the advent of rigor was actually delayed or prevented.

(2) P.

Gastrocnemius of frog of 30 grms. (upper).	·028 Theine. ·017 Salicylate soda. 50 c.c. Salt solution. ·01 KHO.	Rigor commenced in 13 hrs. Maximum 5 mm. (÷ 10) attained in 2 hrs. 15 min.
Companion muscle to above (lower).	Theine solution same, but no potash.	Rigor commenced in 9 hrs. 40 mins. Maximum 55 mm. attained in 1 hr. 50 mins.

Q.

Gastrocnemius of small healthy frog.	·032 Caffeine. ·02 Salicylate soda. 50 c.c. Salt solution. ·005 grm. KHO.	In course of 24 hrs. not the least rigor produced.
Companion gastrocnemius (lower).	Same caffeine solution without potash.	Shortening commenced in 7 hrs. 15 mins., attained a maximum of 6 mm.

(3) For a consideration of this question we must refer to what has been already stated with regard to Lactic acid.

Guanidine.

As is well known from the researches of Gergens, Baumann[1], Putzeys and Swaen[2], guanidine causes active fibrillation, lasting for

[1] *Pflüger's Archiv*, XII., p. 205.
[2] *Bull. de l'Acad. Roy. d. Sciences, de Belgique*, 1876, No. 4, p. 813.

a considerable time in curarised or uncurarised muscle exposed to its action. During this period of activity we have noticed that the muscle usually does not relax fully under the ordinary weight (10 to 20 grammes), with which it is burdened, but remains in a slightly shortened condition. The possibility of rigor being accelerated by this tendency to increased tonicity, or by the active exertion the muscle undergoes during its rapidly repeated spontaneous contractions, made it a matter of interest to determine what effect guanidine would have when employed in conjunction with caffeine.

We found that it did not produce rigor when applied in salt solution alone in doses of ·005 per 50 c.c., which did not cause spontaneous contractions, or of ·02 per 50 c.c., which caused contractions. Rigor caused by theine is apparently postponed for two hours by the addition of ·05 guanidine.

When we contrasted the effect of guanidine and of barium, which likewise causes spontaneous contraction, the interesting result appeared that the rigor of the former (succeeding a much larger amount of work and at the same time producing a feebler tonic contraction) is much later than that of the latter, when they are mixed with equal amounts of theine.

The following experiment illustrates this point.

R.

Rana esculenta. Gastrocnemius muscle.	·032 Theine. ·017 Salicylate soda. 50 c.c. Salt solution. $BaCl_2$ ·05.	Shortening of muscle during action (contractions which continue for 50 mins.) 13 mm. Rigor commences 7 hrs. 10 mins. Maximum 13 mm. (÷ 10) well sustained.
Companion muscle.	Same theine solution. Guanidine ·05.	Shortening of muscle 6 mm. during spontaneous contraction, which continues very actively for 1 hr. Rigor commences 15 hr. 45 mins. Maximum was 18 mm. when experiment terminated in 20 hrs. 45 mins.

Chloride of Potassium and Chloride of Calcium.

The addition of a large dose ·1 of these salts to theine solution, showed that the accompanying rigor of this substance was relatively accelerated by calcium salts. The muscle suspended in the theine

solution to which ·1 of the chloride of calcium had been added, began to pass into rigor in 4 hrs., whilst the other to which ·1 chloride of potassium had been added showed no sign of rigor till 8 hrs. 15 mins. The rigor in the case of the calcium solution was much more rapid and extensive than in the other.

One peculiarity which we have observed in the caffeine curve, or we must say more strictly in that of theine[1], has interested us so much that, though we cannot as yet offer a full explanation of it, it seems to deserve special mention. It is the occurrence of a rhythmical contraction in the muscle exposed to the action of theine solutions of certain strength. This rhythm is so slow that unless we had employed an unusually slow rate of movement in our recording apparatus (one revolution of the cylinder in 24 hours) its occurrence would probably have escaped us entirely.

The following points will serve to describe this phenomenon.

(1) The rhythm is usually produced by smaller doses of theine which do not cause a marked rigor.

(2) It may however occur at the commencement of a pseudo-rigor or of a contraction which develops into a lasting rigor, or on the relaxation of a pseudo-rigor (see curve 9).

(3) The rhythm is most rapid at the commencement of its occurrence and slower towards its termination. To this rule we have only seen three or four exceptions.

We have observed it as frequent as 20 relaxations and contractions in the hour, immediately after the application of the theine solution.

(4) The total extent of this contraction and relaxation is small. From valley to summit it seldom exceeds 2 mm. in all; the lever multiplying 10 times would reduce the actual movement therefore to one-fifth of a millimetre.

(5) Whilst at first the contractions and relaxations have nearly equal time values, as the phenomenon progresses the relaxation becomes relatively more rapid and the contraction slower; the contraction (or we will call it the return to the previous length) occupying a longer proportion of time. The condition of normal contraction is preserved when the phenomenon has continued some hours, for it may be an hour or

[1] Since writing the above we have obtained the same or a very similar effect with a specimen of Xanthin (Schuchard) and a specimen of Caffeine from raw coffee beans kindly given to us by Dr B. Paul, and prepared by himself. Although this rhythm is characteristic of the action of the caffeine group we believe that we have produced it by other agencies. The results we obtained must furnish subject for another paper.

more; then on this suddenly supervenes a moderately rapid extension, which is completed within 5 minutes; this in its turn is succeeded by a very short pause in extension, and contraction then follows.

Whether this muscular rhythm is related to the slow contraction and relaxation lasting for many days, which Prof. Brown-Séquard[1] has observed in the muscle of the rabbit during cadaveric rigidity, we cannot positively state, there are however many features of difference between the phenomena.

(6) The duration of the phenomenon is sometimes very considerable, though at whatever time it manifests itself the phases are very closely similar in character. We have the greater rapidity of the alternating lengthenings and shortenings, in the first instance succeeded by great prolongation of the pauses in contraction terminated by a comparatively rapid extension. It is no unfrequent thing for the phenomenon to continue 16 hours, and we have on one occasion observed the final lengthening and contraction no less than 31 hours after the first made its appearance.

(7) There was some variation with regard to the dose of theine necessary to provoke the occurrence of this phenomenon.

(8) The experiments were necessarily conducted through the night as well as in the day, but we were unable to associate any high or particularly low temperature of the laboratory with the production of the rhythm. Some of the nights on which it was recorded were cold and frosty, others again mild, the temperature however never falls very low at night in the laboratory, owing to the heat remaining in steam pipes running in one of the walls of the room.

We shall now describe three experiments in detail, and for the sake of convenience in the special description of these, we shall abandon the schedule form in which previous results have been given.

S. (Fig. 7).

Companion gastrocnemii of a healthy frog of 21 grammes were taken. The animal had been obtained 10 days previously and kept five days in the laboratory before the experiment. To one muscle in the glass chamber was added the following solution:—·028 Theine, ·014 Soda salicylate in 50 c.c. normal (7) Salt solution. To the other ·028 Caffeine, ·014 Soda salicylate in 50 c.c. (7) Salt solution.

We may dismiss the second experiment by saying that in the course of

[1] *Comptes Rendus*, Tome CI. and CV.

24 hours no rigor, rhythm or lengthening was observed, the result being purely negative.

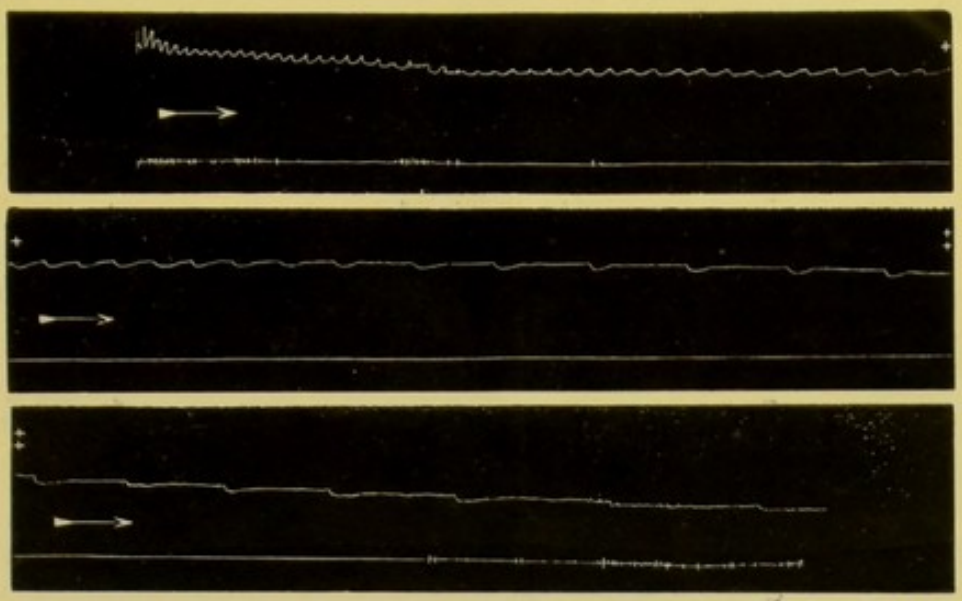

FIG. 7. S. Rhythmical contractions of gastrocnemius muscle produced by theine. The curve has been divided into three pieces for convenience of printing. The marks + and $\overset{+}{+}$ show where the curves join each other. Weight of lever 3 grms.

With the former however the result was very different. Within 5 minutes of applying the solution a contraction 2·5 mm. (lever multiplies 10 times) occurred, this was rapidly succeeded by a relaxation and then by a regular series of shortenings and relaxations. At the same time the basal line tended to fall with regard to the permanent abscissæ.

The speed of rhythm and the relationship to the basal line may be given in hours as follows:

0—1 hour	11·5	contractions and relaxations.		
1—2 hours	9	,,	,,	– 1 mm. below basal line.
2—3	7	,,	,,	– 1·5 mm. below.
3—4	6	,,	,,	2 ,, ,,
4—5	5	,,	,,	2 ,, ,,
5—6	4·5	,,	,,	2 ,, ,,
6—7	4	,,	,,	2 ,, ,,
7—8	3·5	,,	,,	1·75 ,, ,,
8—9	2·5	,,	,,	1·5 ,, ,,
9–10	1·75	,,	,,	2 ,, ,,
10–14	5	,,	,,	2·5 ,, ,,
14–18	4·75	,,	,,	4 ,, ,,
18–21	3·5	,,	,,	4·5 ,, ,,

The day and night throughout which this experiment was in progress were cold but not frosty.

It will be observed that although the curve is entirely sub-abscissal there is at first a relaxation, then a distinct rise, and finally at the end of the record a more marked relaxation. In this instance then there is no evidence of positive shortening of the muscle and nothing approaching a condition of rigor.

T. (Fig. 8).

For the second experiment we have chosen a curve which illustrates a different rhythm and a slight permanent contraction of the muscle which

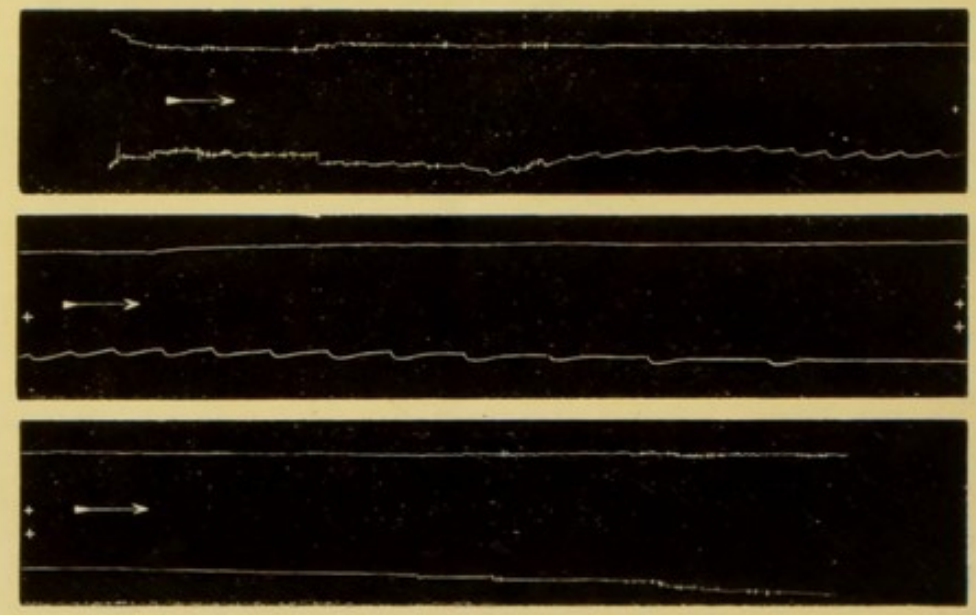

Fig. 8. T. Comparative action of theine and caffeine. The curve has been divided into three parts for convenience of printing and the marks + and $\frac{+}{+}$ indicate where the parts are continuous. The upper line in each is the caffeine curve and the lower the theine curve.

was not seen in S. The frog the muscles of which we employed was an animal of 20 grammes weight caught 10 days previously, and kept in the laboratory for some days.

The solutions we employed were exactly similar to those used in experiment S. The only result observed in the case of the muscle suspended in caffeine solution, was a very slight tonic contraction commencing 8 hours 15 minutes after immersion, and amounting to 1·25.

With the muscle suspended in theine the case was different, faint contractions and relaxations soon making their appearance after the admission of the solution (containing ·027 grm. theine) to the muscle chamber. As it occurred to us that tension exerted at the time when a contraction or shortening would follow in course might modify the rhythm, we kept the lever steadily depressed for 2 minutes during such a time. On releasing

the lever it was found that the rhythm continued, though the muscle seemed to have extended itself slightly.

After application of solution.	Rhythmical contraction.	Change of basal line.
0—2 hours	6	0. Slight rise at first reduced by mechanical extension.
2—4	8·5	2
4—6	7·75	2·5
6—8	6	3
8—10	4·5	3
10—12	3	2·75
13—14	2·25. Contracted slightly and remained shortened.	3

The third experiment (Fig. 9) which completes the number to which we must confine ourselves, has been already referred to as remarkable

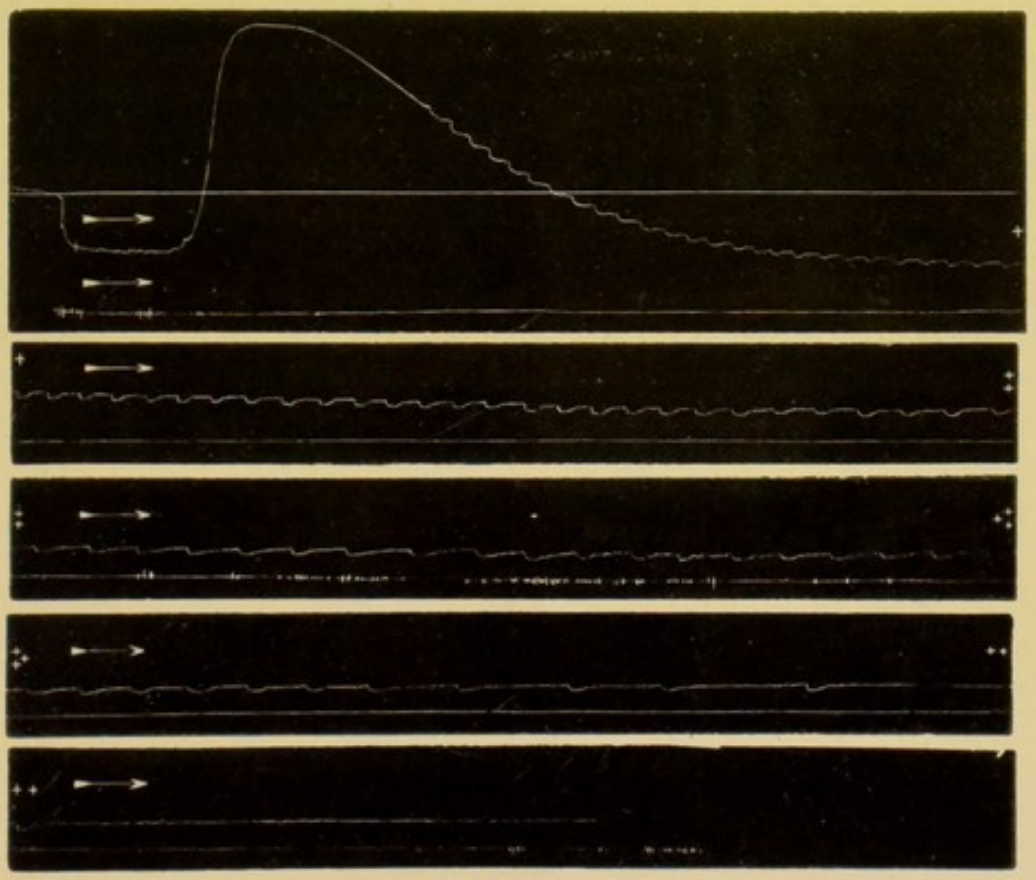

Fig. 9. U. Showing elongation of muscle with rhythmic contraction produced by theine. The straight line in the middle of the first curve indicates the position of the lever with the muscle at its normal length.

for the time of duration of the rhythm provoked by theine. It has further a very remarkable feature, namely the production of a rigor, or better, a pseudo-rigor lasting for some 2 hours, and then relaxing, a

very well marked rhythm marking its appearance during the relaxation and lasting in all for 31 hours, its speed constantly declining after the manner already indicated.

Immediately upon the introduction of the theine solution a marked elongation of the muscle occurred, so that the base line cuts the pseudo-rigor curve both at its commencement and at its relaxation, the rhythm succeeding all occurs in a condition of extension of the muscle.

An extension is usually caused by adding salt solution alone to a muscle suspended in a moist chamber.

U.

Small *Rana temporaria* of 17 grammes well nourished. Had been kept some days in the laboratory prior to the experiment.

To one of the gastrocnemii was added 50 c.c. salt solution containing ·032 theine with soda salicylate, to the other an equal amount of caffeine. The muscle in the chamber containing the latter did not exhibit any rigor or rhythm during the 40 hours for which the experiment lasted.

Immediately on applying the theine solution the lever descended for 6 mm. (lever multiplies by 6). The relaxation remained for one hour, and was succeeded by a rise which attained its maximum 45 minutes after its commencement, its greatest altitude being 23 mm. or 17 mm. above the original basal line.

After the maintenance of the maximum for 30 minutes, relaxation commenced slowly, and at the end of the 4th hour from the commencement of the experiment, rhythm commenced. At the end of 5 hours 15 minutes the abscissæ was cut by the descending lever which thereafter remained below it.

The occurrence of Rhythm.	Speed of Rhythm.	The occurrence of Rhythm.	Speed of Rhythm.
0—2 hours	11 waves	16—18 hours	4 waves
2—4	10·5	18—20	4
4—6	8·5	20—22	4·5
6—8	7	22—24	4·75
8—10	7	24—26	3
10—12	6	26—28	2
12—14	5	28—30	1
14—16	4·75	30	

The slight acceleration at the 20th to the 24th hours, is the only instance to be observed in this experiment out of series in the uniform decline in the speed of rhythm.

Although we have much enlarged the scope of this research by examining other specimens of caffeine as well as xanthine, alloxan etc., we think it advisable to publish the above notes—an abstract of which was read at a meeting of the Royal Society, March 29, 1887—and to reserve the further discussion of the subject to another paper. More especially as we have been already, for so long, obliged to postpone this communication.