ON THE CONTRACTION OF MUSCLE, CHIEFLY IN RELATION TO THE PRESENCE OF 'RECEPTIVE' SUBSTANCES. PART IV. THE EFFECT OF CURARI AND OF SOME OTHER SUBSTANCES ON THE NICOTINE RESPONSE OF THE SARTORIUS AND GASTROCNEMIUS MUSCLES OF THE FROG.

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CONTENTS.

In 1905 I[1] showed that in some of the muscles of the fowl there is a certain degree of mutual antagonism in the action of nicotine and of curari, inasmuch as curari, if injected first, prevents nicotine in not too large amount from causing contraction: and if injected in sufficient quantity after nicotine, it decreases or abolishes the contraction. I have also mentioned[2] that in the frog and toad, curari prevents a normally effective dose of nicotine from causing contraction, and that it abolishes the cataleptic condition induced by nicotine, and later[3] that it readily

[1] This *Journal*, XXXIII. p. 387. 1905.

[2] *Proc. Roy. Soc.* B, LXXVIII. p. 187. 1906.

[3] *Arch. Intern. de Physiol.* (*Proc. 7th Physiol. Congress*), v. p. 115. 1907.

prevents the twitchings caused by nicotine in the frog and toad. It was not found to stop the contraction caused by 1 p.c. nicotine.

A preliminary account of further results, some of which are more fully dealt with in this paper, was published a short time ago[1].

The question of the action of curari upon the nicotine contraction has also been dealt with by Boehm[2] in the frog, and by Edmunds and Roth[3] in the fowl. The results of Boehm I refer to on p. 278 and those of Edmunds and Roth on p. 289. I may recall one or two fundamental points with regard to the action of nicotine. Its stimulating effect is due to an action on the muscle, and not to an action on the nerve ending[4]. There is no reason to suppose that its nerve-paralysing effect is not also due to an action on the muscle. Up to ·1 p.c. the stimulating effect is confined to the neural region of each muscle fibre and is of two kinds, one leading to the occurrence of quick contractions more or less conducted throughout the muscle fibres, and the other leading to the occurrence of a slow tonic contraction of a more or less local nature.

I need not recount in full the theory I have put forward to account for these facts. But two points in it must be mentioned. First that two special substances[5] at least (receptive substances) are present in the neural region of the muscle, and that nerve impulses can only cause contraction by acting on a receptive substance. Secondly that the receptive substances form more or less easily dissociable compounds. Thus nicotine in combining with these substances tends on the one hand to prevent them from being influenced by nerve impulses, and on the other tends to cause a breakdown in the contractile substance which leads to contraction. Curari in combining with the receptive substances simply prevents them (and so the contractile substance), from being influenced by nerve stimuli.

I shall use the term receptive substance in describing the phenomena of the action of nicotine and curari, although it belongs as yet to the region of theory, because its use enables the phenomena to be described in the shortest and simplest way. Since nicotine ·25 to 1 p.c. causes a shortening of the muscle both in and outside the neural region, a term

[1] *Proc. Physiol. Soc.* p. lxxi. 1909. (This *Journal*, xxxviii.).

[2] *Arch. f. exp. Path. u. Pharm.* lviii. p. 265. 1908.

[3] *Amer. Journ. Physiol.* xxiii. p. 28. 1908.

[4] Cp. Part III. This *Journal*, xxxvii. p. 285. 1908.

[5] The question whether (on the lines of Bottazzi's theory) the slowly contracting substance is the sarcoplasm, will not be dealt with in this paper.

is required to distinguish the substance so acted on from the receptive substance. I shall speak of it as the general muscle substance.

I have made observations on a considerable number of the muscles of the frog, but chiefly on the sartorius, the flexor carpi radialis and the rectus abdominis. Each of these three has advantages for certain experiments. In this paper I deal mainly with the sartorius, and to a less extent with the gastrocnemius. I may say that the broad results are the same in the other muscles. The general arrangements for taking graphic records have been described in Part II[1]. The Ringer's fluid I have used contained in nearly all cases rather less salts than in my earlier experiments[2]. Both curari and nicotine solutions were made up in Ringer's fluid, and were prepared from stock 1 p.c. solutions kept in well stoppered bottles in the dark.

There are several sources of error in comparing the results obtained in different experiments. The most important is the intrinsic difference in the corresponding muscles of different frogs. In all the experiments given in this paper the frogs used, unless otherwise mentioned, were male English frogs of weight ranging from 18 to 25 grams, usually freshly caught. But considerable differences occur in corresponding muscles of frogs freshly caught and of the same weight; indeed on cutting out the sartorii it may be obvious to the eye that they are different in size and in tint, some being yellower, some whiter than normal. Consequently in drawing conclusions which involve small differences I rely on a comparison of the results on the right and left muscles of the same frog, except where a large number of experiments have been made. The muscles of the two sides do not give a completely accurate basis for comparison. This is especially the case if one muscle is left longer in the body after death than the other, since the irritability of muscles to nicotine diminishes in the body after death, presumably in consequence of the accumulation of acid.

In some recent experiments I find that the difference in the response of the two muscles is less if the muscle preparations are cut out of the body immediately after death and left in Ringer's fluid for some time before they are taken for experiment. In Fig. 1 I give an example of this. The left sartorius (*l*) was placed in Ringer's fluid 6 mins. after death and left in it for 2 hrs. and 22 mins., and then the effect of ·1 p.c. nicotine tried. The right sartorius (*r*) was placed in Ringer's fluid

[1] This *Journal*, xxxvii. p. 165. 1908. The contraction was magnified 8 times.

[2] It consisted of NaCl ·65 %, KCl ·02 %, $CaCl_2$ ·25 %, $NaHCO_3$ ·15 %. The sartorius remained irritable in this for 1½ to 2½ days.

10 mins. after death, and left in it for 2 hrs. and then the effect of ·1 p.c. nicotine tried. Since the muscles are thin, a short stay in Ringer's fluid would probably be sufficient to get rid of the difference caused by one muscle staying longer in the body than the other. This method however did not occur to me till my experiments were finished. The method I have usually employed is to make two pairs of experiments, using for a given observation the muscle first cut out of the body in one pair, and the muscle last cut out in the other pair (cp. Figs. 6, 28, 30). In the Tables, except Table II, the experiments in which the sartorii were taken from the same frog are bracketed.

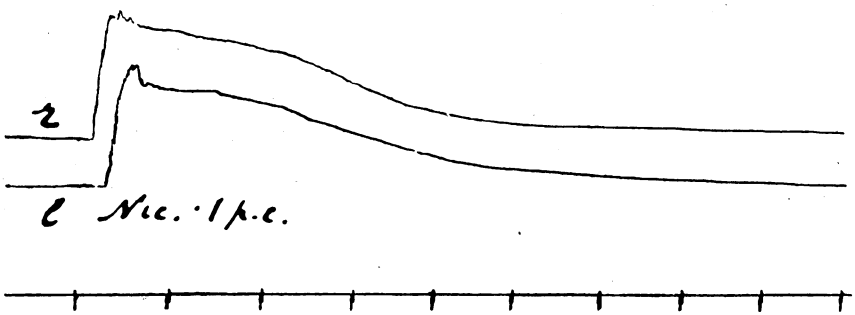

Fig. 1. Sartorius. Description see Text. Load 1½ gram. Time in ½ mins.

A second source of error is the varying temperature at which the experiments are made: other things being equal the response to nicotine increases with the temperature. Most of my experiments were made at a room temperature of 13° to 17° C. I shall mention the temperature when it was 18·5° C. or higher. I have not found any certain difference in the muscles at different seasons of the year except such as can be referred to differences in temperature. A difference in the response to nicotine may occur in the muscles at all seasons.

In determining the degree of tonic contraction caused by nicotine in the sartorius there is sometimes a difficulty in consequence of the twitching. Often it is true, especially in cool weather, the twitching occurs in different fibres at different times, and is not strong enough to appreciably affect the tracing when the muscle is loaded with 1½ to 2 grams. But sometimes, and generally in warm weather, the tracing shows the twitches and the incomplete tetanus simulates tone[1]. This is illustrated in several of the figures, the twitching occurs in an

[1] When a muscle is placed in NaCl ·6, it often remains bent for some minutes after a bout of twitching.

exaggerated form after immersion in NaCl ·6 (cp. Figs. 23, 24). Moreover the sudden fall of the lever after a strong twitch may stretch the muscle and reduce the tone.

Lastly, as I have mentioned in Part I (p. 365), there may be twitching on immersing the muscle in or removing it from a fluid. This however is rare.

In referring in the following pages to the immersion of the muscle in successive solutions of nicotine of increasing strength, it will be understood that the procedure, unless otherwise mentioned, was as follows: the muscle was immersed in each nicotine solution for two minutes, and the interval between running off one solution and pouring in the next was one minute[1].

THE SARTORIUS.

I. THE ACTION OF NICOTINE ON THE NORMAL MUSCLE.

The general behaviour of the sartorius with nicotine has been described in Part I. Since writing that account I have made many further observations, and I give here some additions and corrections.

1. *The forms of curve given by different percentages of nicotine.* The minimal percentage of nicotine which causes a visible contraction by the graphic method (load 1½ grams) is, as I have said earlier (*op. cit.*), about ·001 p.c. but ·001 p.c. does not always cause contraction. The minimal percentage, although in part depending upon the condition of the muscle, in part depends upon the temperature. Thus in warm

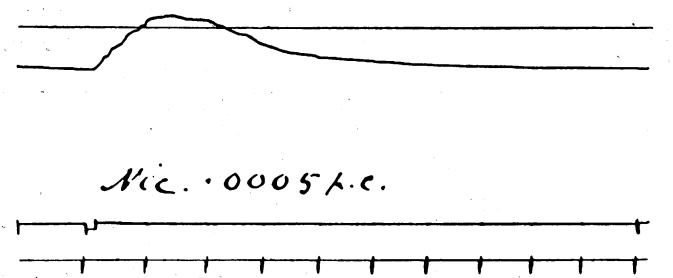

Fig. 2. The sartorius was left in Ringer's fluid at 22°—23° C. for 55 mins. For 50 mins. there was gradual extension, the straight line shows the position of the lever at the beginning. Then the muscle was immersed in nicotine ·0005 p.c. at 22° C. Time in ½ mins. Load 1½ grams.

[1] Usually the muscle was stimulated with a make and break induction shock before applying the first nicotine solution.

summer weather with the fluids at a temperature of 22°—23° C., a contraction may be given with less than ·0005 p.c. (cp. Fig. 2).

The sartorius[1] is not a satisfactory muscle on which to determine the relation between the height of the tonic contraction and the percentage of nicotine since the fibrillar twitches caused simultaneously modify the curve (cp. above p. 238). Nevertheless the main facts are, I think, clear.

With percentages of nicotine which affect the neural region only, *i.e.* ·001 to ·1 p.c., the height of the tonic contraction increases with the percentage. This is shown in Fig. 3.

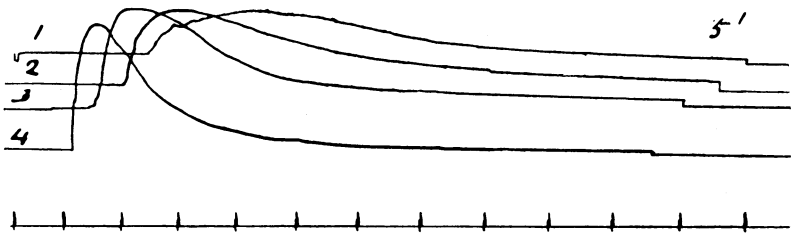

Fig. 3. (1) Right sartorius in ·001 p.c. nicotine. (2) Left sartorius of same frog in ·01 p.c. nicotine. (3) Right sartorius of another frog in ·01 p.c. nicotine. (4) Left sartorius of same frog in ·1 p.c. nicotine. Each muscle was left in Ringer's fluid for 5 mins. before immersion in nicotine. After 5 mins. the drum was stopped for 5 mins. Load 1¾ gram. Time in ½ min. (The difference in the height of contraction of 2 and 3 was probably not due to a difference in the muscles, but to 2 having been left rather longer in the body after death.)

The time which elapses before a fall in the curve begins, is in general less with increase of percentage. Since in each fibre relaxation soon sets in, the height of the contraction depends largely upon the degree to which the fibres are simultaneously affected.

The central fibres will be more quickly affected the higher the percentage of nicotine. Similarly in any one fibre the degree of contraction will be greater if all parts of it are contracted before there is any relaxation. Thus up to a certain limit at any rate the relation mentioned above between the height and duration of contraction and the percentage of nicotine depends upon the rate of action of nicotine on the total receptive substance.

With percentages above ·1, the primary slow contraction has two components, viz. the contraction in the neural region due to stimulation

[1] The rectus abdominis is perhaps the best muscle for the purpose. I shall give an account of this later.

of the receptive substance, and a contraction more or less throughout the muscle, including the neural region. In Part I, I stated that the primary rise was comparatively slight in passing from ·1 to 1 p.c. In later experiments in which the muscles of the two sides of the same frog have been compared, I find that the rise with 1 p.c. is always much greater than with ·5 p.c.[1] (Fig. 4).

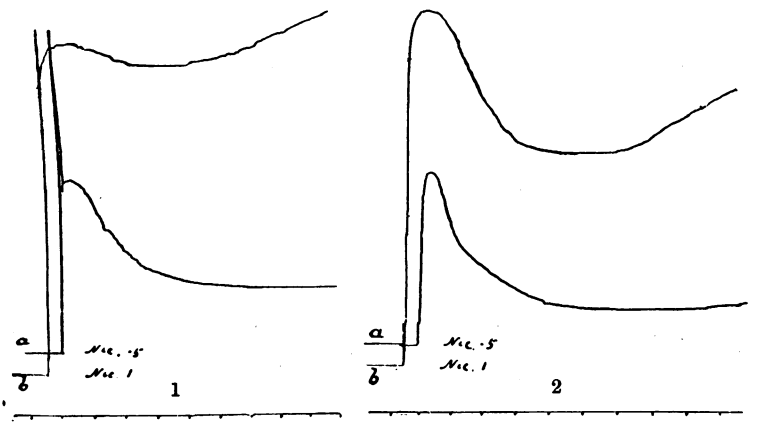

Fig. 4. ×½. (1) Contraction of right and left sartorius (*a*) with ·5 p.c nicotine, (*b*) with 1 p.c. nicotine. Load 1½ grams. (2) A similar experiment with a load of 2½ grams. Time in ½ mins.

In all these cases the height of the contraction is above the average.

But the difference between the height of the primary rise caused by ·1 and by ·5 p.c. nicotine is comparatively slight and if the muscle treated with ·5 p.c. nicotine is taken out of the body about half an hour after that treated with ·1 p.c. nicotine, the decrease in irritability which occurs in the former is often sufficient to make the height of the contractions approximately equal (cp. Fig. 5).

Since ·5 p.c. nicotine acts on the general muscle substance as well as on the receptive substance, the difference between its effect and that of ·1 p.c. nicotine must be due in part if not altogether to this additional action[2]. We have seen above that the increasing effect of nicotine up to ·1 p.c. is largely due to summation of contractions. We may infer then that when the percentage of nicotine is sufficient to cause complete

[1] The error in the earlier experiments was probably partly due to the stronger solutions of nicotine being used several times.

[2] It is possible that the direct action of strong nicotine in the general muscle substance in the neural region reduces the effect due to stimulation of the receptive substance.

summation, differences in rate of action on the receptive substance have little if any effect.

The much greater effect of 1 p.c. than of ·5 p.c. nicotine, I take to be due to its much greater action on the general muscle substance.

The form of the curve depends of course upon the load. Even when the difference is only between 1 and $2\frac{1}{2}$ gr., all parts of the curve are slightly affected but the early part of relaxation more than the rest.

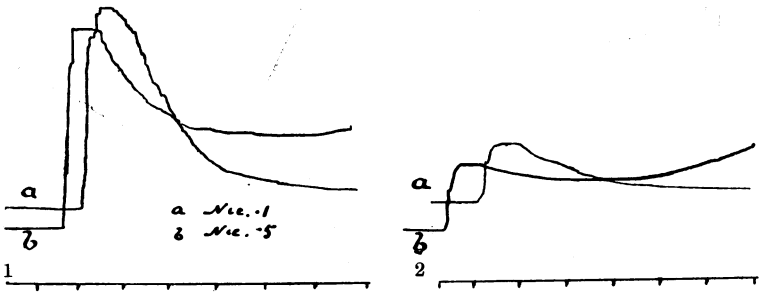

Fig. 5. $\times\frac{2}{3}$. Contraction of right and left sartorius with ·1 p.c. (*a*) and with ·5 p.c. nicotine (*b*). (1) Load $1\frac{1}{2}$ grams, (2) load $2\frac{1}{2}$ grams. The difference in height no doubt partly due to differences in the muscles of the two frogs. Muscles (*b*) were cut out of the body 30 mins. after (*a*), each was left in Ringer's fluid for 5 mins. before immersion in nicotine.

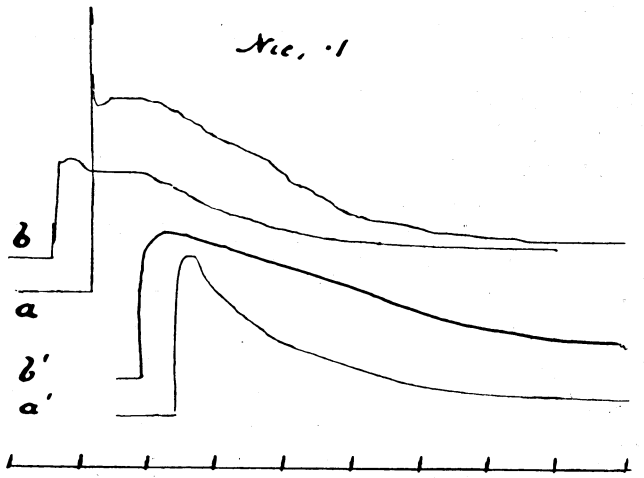

Fig. 6. Two pairs of sartorius muscles immersed in ·1 p.c. nicotine.

a	Load 1	gram.	8′	after pithing	frog.	
b	,, $2\frac{1}{2}$	,,	28′	,,	,,	,,
a′	,, $2\frac{1}{2}$	,,	12′	,,	,,	,,
b′	,, 1	,,	35′	,,	,,	,,

In making the comparison, account must be taken of the decrease of irritability in the muscle left longest in the body. This can be done by using the light load for the muscle first cut out in one experiment, and for that cut out second in another. An example is given in Fig. 6 of the effect of ·1 p.c. nicotine in these circumstances, though it must be mentioned that the difference between *a* and *b* is greater than in the other experiments I have made.

With a load of only ½ gram, there may be, though there is not always, a prolonged considerable contracture.

When the stronger solutions of nicotine are employed (·5 to 1 p.c.) the fall in the curve soon passes into the rise of nicotine rigor. With ·5 p.c. nicotine (the temperature being under 17° C.) the rigor rise usually begins in 3 to 4 minutes (Fig. 7), with 1 p.c. nicotine it usually begins in about a minute (Fig. 4) and goes much more rapidly than with ·5 p.c. It begins sooner the higher the temperature. There is however considerable variation in different muscles independently of the temperature. The rate of rigor rise is affected by slight variations in the load, thus it is slower with a load of 4 grams than with one of 2 grams. The curve of rigor rise is at first concave to the abscissa but soon becomes convex.

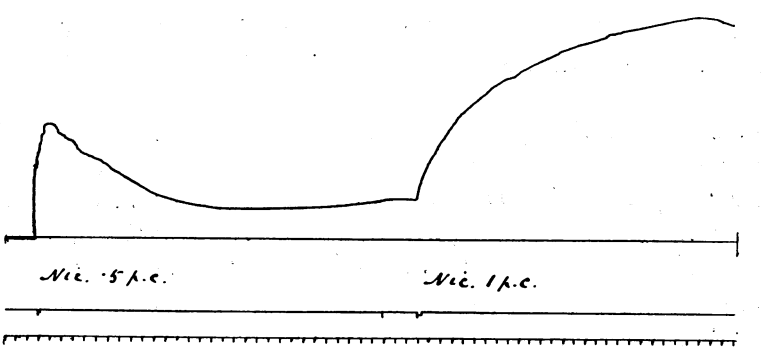

Fig. 7. × ½. Convex curve of rigor rise with 1 p.c. nicotine after the sartorius has been in ·5 p.c. for 5 mins. Load 3½ grams. Time in 10 secs.

The rigor rise is largely if not wholly dependent upon continuous immersion in nicotine; if the nicotine solution is run off, there is a slight fall in the curve although nicotine still adheres to the muscle; how long this lasts I have not determined. A fall in the rigor curve is always obtained by washing out the nicotine with Ringer's fluid (Fig. 8), though the fall is a slow one.

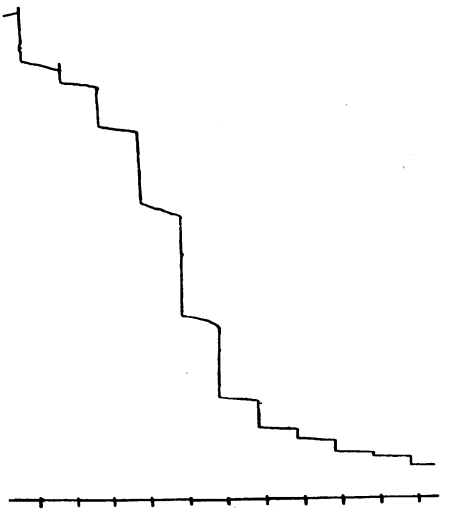

Fig. 8. $\times \frac{2}{3}$. The sartorius had been $4\frac{1}{2}$ minutes in 1 p.c. nicotine. The line of the signal marker shows the position of the lever at the beginning. The drum was then stopped, Ringer's fluid substituted for nicotine, and at the end of 5 mins. a tracing taken for 30 secs. The drum was again stopped, fresh Ringer's fluid added, and at the end of 5 mins., a tracing taken for 30 secs., and so on for 55 mins. Each down-stroke of the marker indicates an interval of 5 mins.

2. *Immersion in successive solutions of nicotine of increasing strength.* The method employed has already been mentioned (p. 239). The height of the contraction caused by a given solution is decreased by the solution previously applied. Nicotine ·001 p.c. even though it causes no contraction may considerably reduce the effect of ·01 p.c. After ·01 p.c. nicotine has caused a contraction, ·1 p.c. causes none, but contractions of increasing height are obtained from ·25, ·5, and 1 p.c. (cp. Figs. in Part I). Since ·1 p.c. nicotine after ·1 p.c. causes no contraction, I take it that the contraction caused by any stronger solution after ·01 or ·1 p.c. is solely due to an action on the general muscle substance. Nicotine ·25 and ·5 p.c. after the more dilute solutions cause a moderately quick primary rise which is usually maintained for 2 to 3 minutes with little or no change (cp. Figs. in Part I), and then passes into the rigor rise.

Nicotine 1 p.c. causes a primary rise which is higher than that caused by any of the diluter solutions; the rise is sometimes followed by a slight fall, sometimes it continues for a short time at a much slower rate than previously and at a slower rate than subsequently. If the time of immersion of the muscle in ·5 p.c. nicotine is decreased, the

primary rise with 1 p.c. nicotine is usually followed by a fall; if it is increased to 5 or 6 minutes, there may be no primary rise with 1 p.c. but only a smooth curve more or less convex to the abscissa (cp. Fig. 7).

3. *The time factor in the action of dilute nicotine.* I have made only a few experiments on the sartorius with regard to this since the flexor carpi radialis and the rectus abdominis, in which the receptive substance is more developed, are better adapted for the purpose.

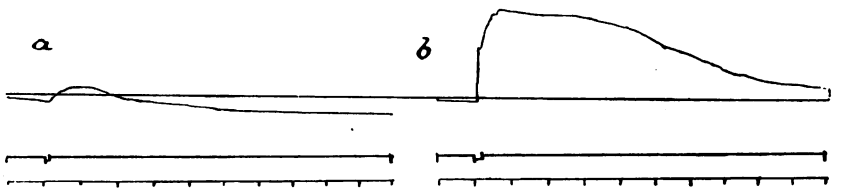

Fig. 9. $\times\frac{2}{3}$. Right sartorius muscle in 25 c.c. nicotine ·0001 p.c. for 24 hrs. then in nicotine ·1 p.c. (*a*). Left sartorius in 25 c.c. Ringer's fluid for 24 hours, then in nicotine ·1 p.c. (*b*). Load $1\frac{1}{2}$ grams. Time in $\frac{1}{2}$ mins.

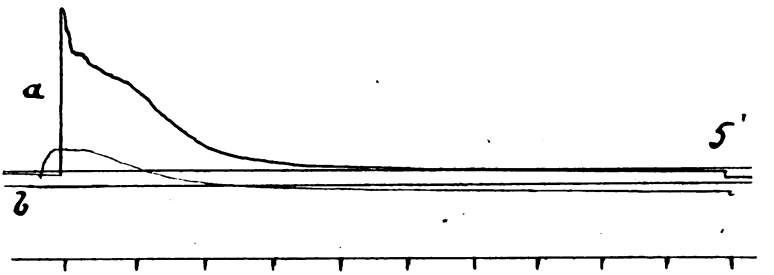

Fig. 10. Right and left sartorius in nicotine ·0001 p.c. (*a*) 1 hour, (*b*) 5 hours, then each in nicotine ·1 p.c. Towards end, drum stopped for 5 mins. Load $1\frac{3}{4}$ grams. Time in $\frac{1}{2}$ mins.

We may take the absence of contraction with ·1 p.c. nicotine as showing a definite stage of action in the receptive substance, and for convenience we may speak of this stage as one of paralysis. We have seen that ·01 p.c. nicotine paralyses (in the sense given above) the receptive substance in 2 to 3 minutes, and that ·001 p.c. in the same time considerably reduces the effect of ·01 p.c. nicotine. If a sartorius is placed for 15 minutes in ·001 p.c. nicotine, and then dropped in ·1 p.c., there is sometimes no effect, sometimes slight twitching without obvious bending of the muscle, *i.e.* the receptive substance is paralysed or nearly paralysed by ·001 p.c. in about a quarter of an hour.

From the results given below in Table I it appears that it takes more than 24 hours for ·0001 p.c. nicotine to paralyse the receptive substance

(Fig. 9, see also Table III B, Exp. 2) and that very little effect is caused in the same time by ·00001 p.c. nicotine. The solutions however may have been a trifle less than the nominal strength as they were made up from a ·1 p.c. solution which had been prepared some days, and not from the stock 1 p.c. solution. In another experiment the action of ·0001 p.c. nicotine was more marked (Fig. 10).

TABLE I.

Effect on the sartorius of prolonged stay in nicotine ·0001 *and* ·00001 *p. c.*

Exp.	Muscle immersed in	Time of immersion of muscle	Height of contraction in mm. with ·1 p.c. nicotine	Remarks
[1] 1	·00001 nicotine	5 hrs.	21	Contraction normal form.
2	·00001 ,,	24 ,,	23	,, ,, ,,
3	·0001 ,,	24 ,,	3	In 50 secs. falls to base line (Fig. 9 *a*).
4	Ringer's fluid	24 ,,	18	Normal form, but rather slower (Fig. 9 *b*).

25 c.c. of each solution was used. Load 1½ grams.

4. *Action of dilute nicotine on the general muscle substance.* Although nicotine up to ·1 p.c. causes no immediate shortening of the muscle by acting on the general muscle substance, a shortening, as I have said in Part I, occurs in time with ·01 and ·1 p.c. and this gradually passes into rigor. The minimal percentage which hastens rigor I have not determined, but we have seen in the preceding section that ·00001 p.c. may have very little effect.

Most of the observations I have made have been on the action of ·1 p.c. nicotine, and the results have varied widely. The variation is partly due to a difference in the muscles; but to a much greater extent to differences of temperature. Thus in winter and spring the sartorius was but little altered from the normal shape and gave a good contraction with induction shocks after a stay of a day in ·1 p.c. nicotine, whilst in some experiments in summer with a room temperature of 22° C., there was rigor in about 6 hours. At this temperature the beginning of the shortening, as shown both by naked eye observation and by the graphic method, began in a quarter to half an hour. Some experiments bearing on this point are given on p. 268. I am inclined to think that at high temperatures, ·1 p.c. nicotine has sometimes a slight immediate effect

[1] As noted in the Introduction, the bracketed experiments in this and in other Tables were made from two sartorii of the same frog.

on the general muscle substance as it also has after a prolonged action of curari (cp. pp. 264, 267).

5. *Naked eye and microscopic observations.* If a fresh sartorius muscle is placed in nicotine, the obviousness of the fibrillar twitches and the time they continue is within limits proportional to the dilution of the nicotine. With ·001 p.c. (in warm weather) the fibrillar twitches go on for 2 to 4 minutes, and there is very little bending or shortening of the muscle to be seen. With ·1 p.c. the muscle becomes bent sharply on itself once or twice, not infrequently first to one surface and then to the other, and rapid local fibrillar twitches are seen for about 30 seconds. The muscle remains bent on itself for a minute or two and then gradually relaxes and becomes again flat but the knee end is a little bent to the lateral side. The time taken for this varies considerably, especially as regards the last stage of relaxation, but usually it is about 5 minutes. This protracted bend of the muscle is always towards the inner surface[1]. It has been shown by Grützner that there is a thin layer of smaller fibres on the outer surface of the muscle. The tonic contraction, then produced by nicotine, is considerably greater in the larger fibres of the inner surface than in the smaller ones of the outer surface. It is rather greater in the fibres of the lateral border of the muscle than in those of the medial border; not infrequently this is shown in the bent condition of the muscle by its being slightly twisted.

The changes in form which the muscle undergoes in passing into rigor in dilute nicotine varies in different cases, but there are two chief varieties (1) the bend on the lateral border at the knee end increases, and this end bends towards the inner surface (the bending may gradually spread $\frac{1}{3}$ to $\frac{1}{2}$ way down the muscle, or it may begin a little past the entrance of the nerve); after this has gone on some time, the pelvic end bends to the outer surface, so that on a side view the muscle is *S* shaped; as these changes are going on there are small bulgings and depressions formed on the whole surface, then the muscle shrinks and takes up the definite rigor shape which is one with a fairly symmetrical bend to the inner surface varying in degree with the degree of rigor[2].

[1] I speak of the surface next the skin as outer surface, the surface next the muscles as the inner surface; the border at which the nerve enters as the medial border, the opposite as the lateral border.

[2] Zenneck (*Pflüger's Arch.* LXXVI. p. 54. 1899) describes the sartorius as nearly always bending towards the inner surface, if it bends at all, when placed in strong solutions of various salts. This no doubt was a rigor shortening.

(2) The muscle in the region of the chief nerve branches, and in the knee end, except the extremity, acquires a series of short folds, usually also being bent to the lateral border, similar short folds come later in the pelvic end and the muscle is more or less twisted; the muscle then passes into the rigor form as given above[1]. If the muscle is placed in 1 p.c. nicotine, the rigor form is reached without the intermediate stages, and the shrinking and final bend of the muscle is greater. There are other minor varieties unnecessary to describe, the essential point is that in the slow rigor caused by ·01 to ·1 p.c. nicotine, the different fibres and to a considerable extent different parts of the fibres are acted on at different rates, and thus cause the muscle to undergo a series of changes of form. The muscle, then, does not consist of two classes of fibres only but of fibres with many shades of difference. This is also indicated by the different vitality of the fibres. If the progress of rigor is observed from time to time under a low power of the microscope, it is found that the rupture of some of the fibres begins comparatively early, at a time when induction shocks still give a twitch of the whole muscle. As rigor proceeds more and more fibres rupture. The ruptured fibres are more numerous on the inner than on the outer surface, but the change is not confined to either surface.

The different appearances shown by the fibres under the microscope are as follows: (*a*) They have close set deepish depressions and bulgings similar to those seen at the angles of much bent fibres, this I have spoken of as crinkling (Part I, p. 358). (*b*) They have regular smooth bends at short intervals giving them a wavy look, but adjoining fibres are in different states. (*c*) The fibres are folded at right angles to the plane of the muscle, all the fibres in one plane being similarly folded; the distance from crest to crest of the folds varies; this is caused when the muscles in the plane below are as a whole contracted; it sometimes occurs on both surfaces owing to greater contraction of the middle layer. (*d*) Some of the fibres have short folds in the plane of the muscle.

Forms (*a*) and (*b*) are best seen on the outer surface; in the early stages of nicotine action they are most marked in the regions known to contain nerve endings, suggesting that the contraction is greater in the neural region and throws the surrounding non-neural regions into folds. But though they are most marked in these regions, they occur early in all parts of the muscle except the extreme ends which are affected much later, so that the difference in the time of beginning change in neural and non-neural regions if it occurs at all can at most be slight. Form (*d*) chiefly occurs on the inner surface, it is caused by some of the fibres being contracted and others not, or by some being contracted more than others. This crinkling and folding of the fibres can be produced in an exaggerated form by stimulating the muscle with not too strong tetanising currents at a stage when the irritability is much diminished; some only of the fibres contract, the intervening ones are thrown into folds; if the current is increased in strength, the intervening fibres also may contract.

[1] This second series of changes occurs in Ringer's fluid, but the rigor shape is less marked.

In dilute nicotine the fibres which rupture show, as a rule, the approaching change by becoming cloudy (those of the inner surface often showing a clear outer margin); after rupture, the ends shrink. In 1 p. c. nicotine the fibres commonly break at the depressions of the crinklings, forming irregular splits which look like very refractive transverse bands. The breaks in the fibres may begin in any part except the extreme ends.

Grützner describes the fibres of the outer surface as containing more protoplasm and as being less transparent than the rest. In the fresh condition I see little difference, but the fibres of the inner surface are as a whole the first to become cloudy. If Grützner's conclusion is correct, it is the less sarcoplasmic fibres which in this case give the strongest tonic contraction with nicotine. In most of the cases I have investigated the reverse is the case.

For some time after the muscle has assumed the rigor form contraction more or less local can be obtained in parts of the muscle by tetanising currents.

In order to observe the last remains of irritability, the currents must be very strong; currents which are too strong to be borne on the lip may produce no effect, though still stronger currents (1 Dan. sec. coil at 0) may give marked contraction. The relaxation is slow and fatigue is rapid, so that the effect soon decreases or ceases. At a certain stage, the stimulus produces no twitch, but if prolonged the whole of the muscle shortens towards the local wheal formed at the electrodes. If the muscle is stretched, a number of fibres are broken, but contraction is much more easily observed, and it occurs in parts which, in the shrunken muscle, appeared inexcitable.

I have not found any constant difference in the time at which the ends and the rest of the fibres lose their irritability, but in the experiments in which I have paid atttention to the position of the fibres retaining their irritability longest, it was found that on the whole the irritability of the outer surface lasted longer than that of the inner, and that the fibres on the lateral border were the last to die.

II. Action of curari and of its salts on the normal muscle.

The curari I have used is the crude substance obtained in gourds. A 1 p.c. solution was made up in Ringer's fluid from time to time and from this the weaker solutions prepared as they were required. Paralysis of the sciatic was produced in about $1\frac{1}{4}$ hours in a frog weighing 20 grams (with the brain destroyed), by injecting 1 c.c. of a ·05 p.c. solution into the dorsal sac, *i.e.* by $\frac{1}{2}$ mgrm.

I have very little new to say in this section on the effect of curari

but it is important to state certain effects as a preliminary to the next section.

Since curari contains salts, and potassium salts prevent dilute nicotine from causing contraction (cp. p. 275), it is necessary to know what effect the salts of curari have on the sartorius.

A portion of the 1 p.c. curari solution was evaporated to dryness, and the residue heated over a Bunsen burner to destroy the organic substances. This was done in two cases, in one the residue was ground with water, and in the other with a little ·1 p.c. HCl which was subsequently neutralized with Na_2CO_3. Water was added to each corresponding to the quantity evaporated, so that the solution contained the salts present in a 1 p.c. solution of curari. No difference was found in the effect of the two solutions.

The sartorius was placed in one of these solutions, undiluted or diluted with Ringer's fluid, for 15 minutes (unless otherwise mentioned), then immersed in nicotine and the contraction, if any, recorded.

The salt solution diluted with three or more volumes of Ringer's fluid had no effect upon the nicotine response: nicotine ·001 p.c. caused a slight contraction in four out of six cases, *i.e.* at least as often and as great as in the normal muscle, and ·01 p.c. nicotine caused the usual contraction. In one experiment the muscle was left in the salt solution diluted with three volumes of Ringer's fluid for 30 minutes; nicotine ·001 p.c. caused a slight contraction.

The solution diluted with an equal volume of Ringer's fluid had no certain effect upon the contraction caused by ·01 p.c. nicotine (·001 p.c. was not tried). In an experiment the muscle was left in the salt solution for 30 minutes instead of 15; in this case the response to ·01 p.c. was diminished.

The undiluted solution was applied to the muscle for 30 mins., this diminished the response to ·01 p.c. solution, but had no certain effect on the response to ·1 p.c. nicotine. In the two experiments in which ·001 p.c. nicotine was tried, there was no response. The excitability of the muscle to single induction shocks appeared to be more reduced than the excitability to nicotine.

These results show that the salts in a 1 p.c. solution of curari in half-an-hour have little effect on the response of the sartorius muscle to ·1 p.c. nicotine, though they reduce the excitability to more dilute solutions; and that the salts in a ·25 p.c. solution of curari have in the same time no influence on the nicotine response appreciable by the method used.

When a sartorius is placed in curari ·1 to 1 p.c., made up in Ringer's fluid, there is a decrease in its irritability to induction shocks, which comes on sooner and develops more quickly the stronger the solution of curari[1]. Local irritability is not completely lost for 1 to 2 days. The effect, however, may be due to the salts in it, and as the solution is hypertonic, some change would be expected in prolonged action.

The experiments were made in two ways. (*a*) A graphic record was taken, the vessel containing the muscle was filled with a given percentage of curari, left for a given time, then poured off and the muscle stimulated directly with induction shocks, the electrodes being at opposite ends of the muscle; the curari solution poured back, left for another period, stimulated, and so on for several hours. By this method there is a rapid decrease in the height of the contractions in a few minutes even with dilute curari, mainly due to paralysis of the receptive substance. After this there is a slow fall in the height of the contractions and stronger and stronger stimuli are necessary to produce more than a small contraction. The rate of reduction of irritability increases with increase in the percentage of curari from ·1 to ·5 p.c.

(*b*) (March.) Muscles were placed in curari solutions ·1, ·25, ·5 p.c. and 1 p.c., taken out at intervals, placed on glass, and stimulated at different points with induction shocks, the electrodes being about 2 millimetres apart, and the contraction observed with a dissecting microscope. In all the irritability lasted more than a day, but the reduction of irritability was greater the greater the percentage of curari, it was rapid in ·5 to 1 p.c. curari and was accompanied by some shrinking. The decrease of irritability is accompanied by a decrease in conduction, so that the contraction becomes more local. At a later stage there is local contraction only, whatever the strength of the induction shock, and the contraction is polar; the make contraction is equal to or stronger than the break contraction, probably as M. and Mme Lapicque point out because the make current is of longer duration. Fatigue and exhaustion occur rapidly. At this stage there are many ruptured fibres.

III. Effect of curari on the contractions caused by nicotine.

1. *Action of nicotine up to* ·1 *p.c. on the curarised muscle.*

Since the immediate contraction caused by nicotine up to ·1 p.c. is confined to the neural region, the first point to determine is the percentage of curari which prevents the diluter solutions of nicotine from having an effect. The muscle was immersed in the curari solution, usually before, sometimes after, fixing in the apparatus; it was then washed for 5 to 10 secs. in Ringer's fluid, and finally nicotine poured into the vessel containing it. Successive nicotine solutions were usually applied (cp. p. 239), but the effect of curari is of course best determined by the response of the muscle to the first solution (cp. p. 244).

The results with curari ·0001 to ·001 p.c. are given in Table II.

[1] According to M. and Mme Lapicque (*C. R. Soc. Biol.* p. 991. 1906) the irritability decreases gradually without any sudden break.

TABLE II.

Effect on the action of nicotine ·01 to ·1 p.c. caused by placing the sartorius for 15 to 30 minutes in a solution of curari.

The load in all cases was 1½ to 2 grams. The Exps. were made in all seasons except spring.

Exp.	Temp. of room	Percentage of curari	Number of minutes in curari	Effect of nicotine ·01 p.c.	Effect of nicotine ·1 p.c.
1	—	·0001	15	—	Slight gradual rise of 5 mm. (Fig. 12.)
2	—	·0001	15	Rise 3 mm. and slight twitches	Rise ·5 mm.
3	—	·0001	30	Slow rise ·5 mm.	Slow rise ·5 mm.
4	—	·0001	30	—	Rise 3·5 mm. with one or two slight twitches.
5	—	·0001	30	—	Slow rise 1 mm.
6	—	·0005	15	—	0
7	—	·0005	15	—	Slow rise 1 mm.
8	—	·0005	15	0	Slow rise 1 mm.
9	—	·0005	30	—	Twitch 4 mm. at beginning, no rise or fall after 20 secs.
10	—	·0005	30	—	Slight twitches with rise 3 mm.*
11	—	·0005	30	—	Slow rise ·5 mm.*
12	—	·00075	15	0	No effect for 1 min., then slow rise 1 mm.*
13	—	·00075	15	—	Twitches, gradually increase then decrease. (Fig. 14.)
14	—	·00075	30	—	Rise 1 mm.
15	—	·00075	30	0	0*
16 & 17	—	·001	15	0	0
18	—	·001	30	0	0
19	18° C.	·001	15	—	0
20	18·5° C.	·001	15	—	1 No fall in 3 mins. Frog 32 grams.
21	19° C.	·001	15	—	0 Rise of 11 mm. in ½ an hr.
22	19° C.	·001	15	—	1 Rise in first 5 secs. (no fall), slowly passing into rigor rise. (Fig. 11.)
23 & 24	20° C.	·001	15	—	0 (Fig. 15 *a*.)
25	22° C.	·001	15	nic. ·05 1	— Fall below base line in 2¾ mins.†
26	22° C.	·001	60	½	— Fall below base line in 4 mins.†
27	22° C.	·001	15	0	0 Curari and nicotine solutions at 22° C.
28	22° C.	·001	15	nic. ·075 0	
29	22° C.	·001	22½ hrs.	0	Muscle shrunken, apparently dead, slowly stretches for several hours.

* Slight twitch on running off the nicotine.

† The lever was falling before adding nicotine. At high temperatures the muscle slowly stretches with a light weight and it may take half-an-hour or more before a constant length is attained. (Cp. Fig. 1.)

The experiments show that nicotine ·01 to ·1 p.c. has commonly no effect after the muscle has soaked in ·001 p.c. curari for 15 minutes, and when there is an effect, it is reduced to minimal proportions. Indeed since the slight occasional effect occurred when the temperature was abnormally high for frog's muscle, and the contraction reached its maximum in 5 to 10 secs. (cp. Fig. 11), it is probable that the rise was due to an action in the general muscle substance. One might expect

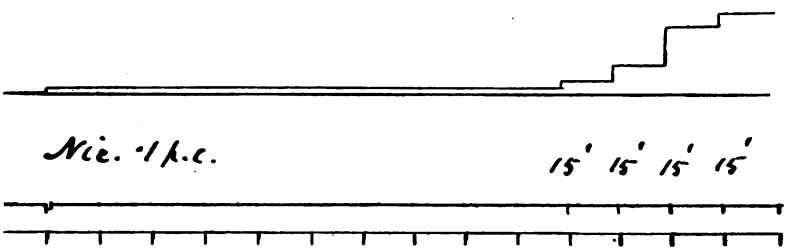

Fig. 11. × ½. Sartorius in ·001 p.c. 15 mins., then in nicotine ·1 p.c. Time in 10 secs. The steps in the tracing occurred in intervals of 15 mins. each.

that if the unstretched muscle were placed in ·1 p.c. nicotine, contraction, too weak to be marked in the graphic method, would be visible to the eye. I have made a few observations by this method. The sartorius was placed in ·001 p.c. curari for 15 mins., rinsed in Ringer's fluid, and then placed in 20 c.c. of ·1 p.c. nicotine. The results were much as by the graphic method. In warm weather (19° to 22° C.), there was no visible effect in four experiments, in cold weather in one out of four cases there was slight brief twitching.

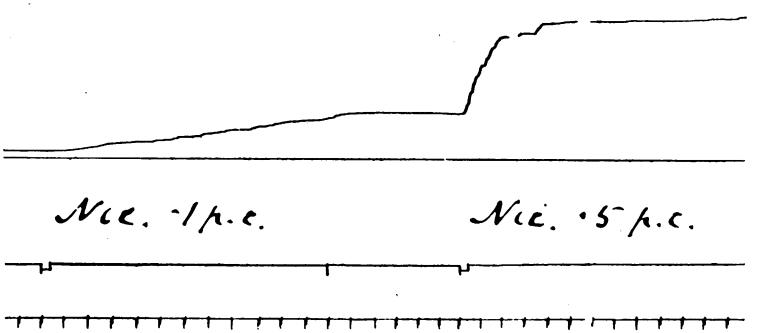

Fig. 12. Sartorius in curari ·0001 p.c. 15 mins., then in nicotine ·1 p.c. and ·5 p.c. The curve shows a slow, gradual rise with ·1 p.c. nicotine. Load 2½ grams. Time in 10 secs.

I have also made observations under a low power of the microscope of the effect of flooding the muscle with ·1 p.c. nicotine after a stay of 15 minutes in ·001 p.c. curari. Usually there was no change, but once or twice (in winter) the fibres became slightly crinkled in places, indicating slight local contraction (cp. Part I, p. 358).

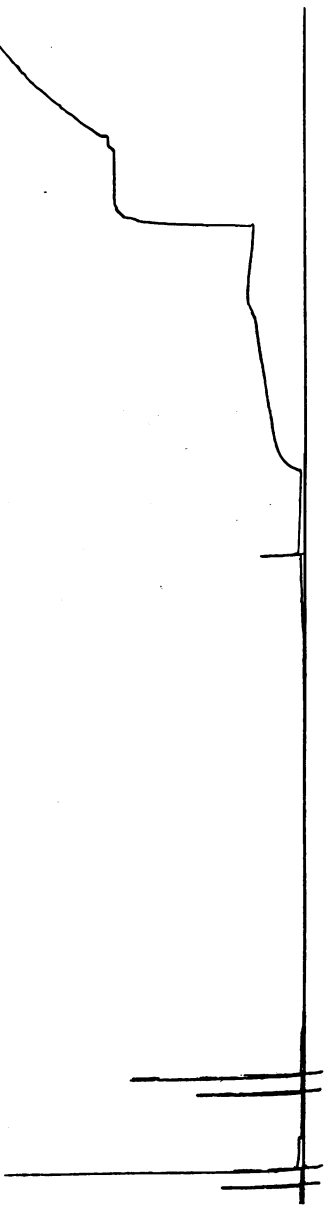

Fig. 13. ×⅔. Effect of successive solutions in nicotine of increasing percentage after a stay of 15 mins. in curari ·00075 p.c. The muscle was stimulated with electrodes at the two ends of the muscle before and after immersion in curari; the immersion occurred at *C*. Time in 10 secs. Load 1½ grams.

It will be seen also from Table II that ·0001 p.c. curari applied for 15 mins. very considerably reduces the effect of nicotine ·01 and ·1 p.c. and that a less percentage of curari is required to stop ·01 than to stop ·1 p.c. from causing contraction. It is certain then that a much smaller percentage of curari than ·0001 will stop the effect of ·001 p.c. nicotine. In the normal muscle, the tonic contraction attains its maximum with ·1 p.c. nicotine in about 20 secs. and in about 30 secs. with ·01 p.c. In all cases in Exps. 1 to 13 in which nicotine caused tonic contraction not too much obscured by twitches, the maximum height was more slowly attained than

normal. Fig. 12 (Exp. 1 of Table II) gives the best example of this. Normally after the muscle has been for 2 mins. (and probably much less) in ·01 p.c. nicotine, ·1 p.c. has no effect. In the curarised muscle at a certain stage (Exps. 2, 3, 8, 12) in similar circumstances a slight slow contraction is caused by ·1 p.c. nicotine (Fig. 13). Lastly in the few cases in which marked twitches were caused after curari by ·1 p.c. nicotine, the twitches instead of being as normal greatest at the beginning of the curve, were small at first and gradually increased (Fig. 14). All three facts afford proof that the nicotine gradually replaces the curari.

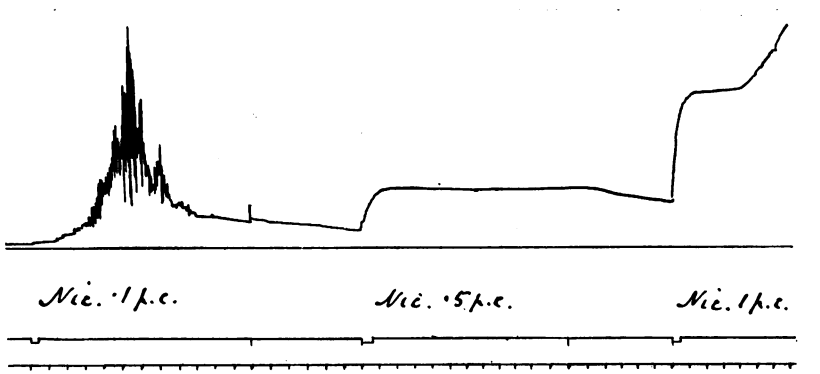

Fig. 14. ×⅔. Sartorius 15 mins. in ·00075 p.c. curari. The figure shows the strong twitches occasionally obtained with dilute nicotine and their gradual increase and decrease. Time in 10 secs. Load 1½ grams.

The occurrence of a displacement of curari by nicotine was tested in another way. The sartorius of a frog was placed in ·001 p.c. curari for 15 mins., in Ringer's fluid containing nicotine ·01 p.c. for 30 mins., and then a tracing taken of the effect of ·1 p.c. nicotine, a slight rise (1 mm.) followed by a like fall was obtained. Now we have seen that ·01 p.c. nicotine applied to a fresh muscle for about 2 mins. prevents ·1 p.c. nicotine from having an effect; I take it then that in the experiment given above the ·01 p.c. nicotine combined with the receptive substance as it was dissociated by the Ringer's fluid (cp. p. 262), and that the contraction obtained with ·1 p.c. nicotine was due to its displacing curari from its combination.

The experiments give some evidence that the paralysis of the receptive substance causing twitching, and of that causing tonic contraction, takes place at slightly different times. In most of them, when nicotine had any effect, it caused a trifling tonic contraction without any indication of twitching, in some there were slight twitches, in one (Exp. 13 and Fig. 14) there were many and strong twitches, so that the degree of tone could not be judged. In two other experiments

a similar tracing was obtained. These were incidental experiments. I wished to determine whether a ·2 p.c. solution of curari made up 9 days previously had lost any of its strength. It had been kept in a test tube plugged with cotton wool. From this a ·001 solution was made (*a*), and also a ·001 p.c. solution from the stock 1 p.c. solution (*b*). Two frogs were taken and a sartorius muscle from each placed in each of the curari solutions and after 15 mins. a graphic record was taken of the effect of ·1 p.c. nicotine. The nicotine had no effect on the muscles placed in (*b*), these are Exps. 23 and 24 of Table II. Both muscles placed in (*a*) gave rapid twitches, in one case lasting 50 secs. and in the other about 90 secs. and when the twitches were over there was very little tone. The

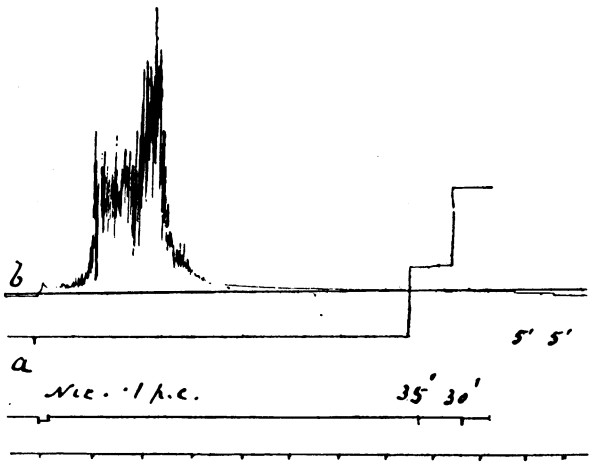

Fig. 15. $\times\frac{3}{4}$. (*a*) Right sartorius in curari ·001 p.c. 15 mins. (*b*) Left sartorius in curari of slightly less percentage. Both treated with ·1 p.c. nicotine. Load 1½ grams. Time in ½ mins.

more marked case is given in Fig. 15. In this connection I may recall that in the muscles of torpid frogs, and in muscles observed some time after death, punctiform application of ·1 p.c. nicotine frequently gives tone without twitching (cp. Part I, p. 355).

In the preceding experiments the muscle was only left in each nicotine solution for 2 mins. On the theory that nicotine displaces curari some action might be expected if a longer time were allowed. Since however as we have seen (p. 240) the height of the contraction depends upon the rate of action of nicotine on the receptive substance a sufficiently slow substitution of nicotine for curari would cause no contraction detectible by the graphic method. For such an experiment

the flexor carpi radialis and the rectus abdominis muscles are better adapted. By the graphic method, when no contraction is caused in a curarised sartorius by nicotine in the first 15 secs., I have not obtained a rise followed by a fall in longer stay in nicotine. In time, depending upon the percentage of nicotine, a rise occurs, but in the cases in which I have continued the tracing still longer, it was clear that the rise was the beginning of the rigor rise (cp. Fig. 15 *a*). The result tends to show that if nicotine ·1 p.c. does not in a deeply curarized muscle when the curari is not washed out, affect the receptive substance in 2 minutes to the degree necessary to cause a rise in the tracing it will not do so in a detectible manner in any time.

I have made also a few experiments in which the muscle was placed in fluid and so free to contract. As said above (p. 247) a muscle placed in ·1 p.c. nicotine straightens out in a few minutes, and after an interval the bending or folding which passes into rigor comes on. In the experiments following the bending or folding came on much earlier in the curarised or partly curarised muscle.

Exp. Temp. 22° C. Four frogs were taken. The left sartorii were put in ·1 p.c. nicotine as controls. The right sartorii were treated as follows: (1) in curari ·001 p.c. 15 mins., then in nicotine ·1 p.c.; (2) curari ·0001 p.c.+·1 p.c. nicotine; (3) curari ·001 p.c.+·1 p.c. nicotine; (4) curari ·01 p.c.+·1 p.c. nicotine. The changes of form began earlier and progressed faster up to 1½ hours in (2) to (4) than in their controls. In (2) the change of form began rather later (40 mins.) and in another 10 mins. the muscle was nearly doubled up, whilst the control was still flat. The later changes were not observed, but in 6 hours they all had assumed the rigor form. I have not however found this result to be constant (cp. Table VIII, p. 268).

The short summary of the experiments given in Table II shows little difference in the effect of curari whether the muscle was left in it for 15 or for 30 minutes, but an examination of the curves seemed to show

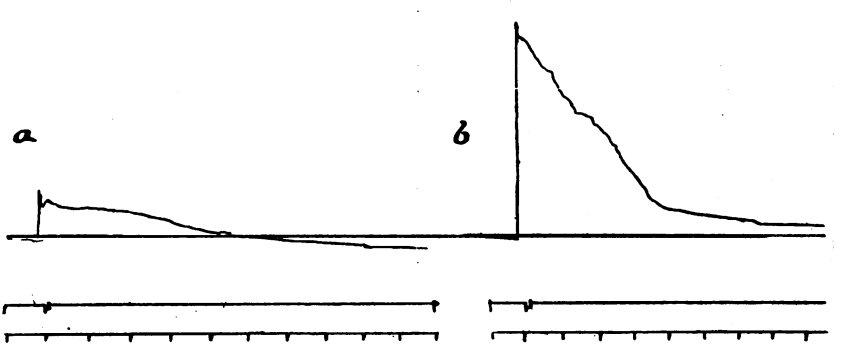

Fig. 16. × ⅕. Right sartorius in 25 c.c. ·00001 p.c. curari 24 hours, then in nicotine ·1 (*a*). Left sartorius in 25 c.c. Ringer's fluid for 24 hours, then in nicotine ·1 (*b*). Load 1½ grams. Time in ½ mins.

that the effect was rather greater in the latter than in the former case. I have accordingly tried the effect of a more prolonged stay in the diluter solutions.

TABLE III.

Effect on nicotine contraction of prolonging the immersion of the sartorius in curari.

Load 1½ grams. 20 c.c. of each solution was used except in *b* 2.

Series *a*. Room temp. 20° C.

Exp.	Solution	Time in solution in hours	Rise with ·1 p.c. nicotine.	Remarks
1	Curari ·0001 p.c.	¼	6 mm.	Slight twitches, in 1½ mins. fall below base line.
2	,, ,,	2	0.	In 3 mins. fall below base line.
3	,, ,,	4	1½ mm.	Fall below base line in 2½ mins.
4	Ringer	4½	31 mm.	Not reach base line in 5 mins.
5	Curari ·00001 p.c.	6	0.	Slow fall.
6	,, ,,	24	0.	Fall, rather more than in 5.

The fall below the base line was going on before immersing the muscle in nicotine.

Series *b*. Temp. 19° C.

1	Curari ·0001 p.c.	6	28 mm.	Slight twitch at beginning.
2	½ c. nicotine ·0001 p.c.	24	10 mm.	Slow rise, slight twitches only.
3	Curari ·0001 p.c.	24	5 mm.	Primary twitch to 8 mm. falls below base line in 2 mins.
4	Ringer	24	35 mm.	Primary twitch, more rapid fall than usual.

Both curari and nicotine may have been a trifle less than the nominal strength as they were not made up from the stock 1 p.c. solution.

Series *c*. Room temp. 22° C.

1	Curari ·00001 p.c.	¼	33.	
2	,, ,,	2	35.	More prolonged than 1.
3	,, ,,	4	36.	
4	,, ,,	6	31.	More prolonged than 3.
5	,, ,,	24	4.	Fall below base line in 1⅔ mins.
6	Ringer's fluid	24	20.	Less prolonged than 1 to 4.

Series *d*. Room temp. 22° C.

1	Curari ·00001 p.c.	½	35.	More twitching in 1 than in 2; contracture at end of 5 mins., 4 mm. in 1 and 5 mm. in 2.
2	Ringer's fluid	½	39.	

It will be seen from these experiments that curari ·0001 p.c. does not paralyse the receptive substance in a quarter of an hour, but does so in 2 hours, and that ·00001 p.c. curari does not completely paralyse the receptive substance in 24 hours (Fig. 16). The differences shown

in the early stages with ·00001 p.c. curari are probably due to differences in the muscles of the two sides.

It appears then that the rate of paralysis of the receptive substance decreases very rapidly with decrease in the percentage of curari, as we have seen above (p. 246) is also the case with nicotine.

2. *Duration of the curari effect.*

In the preceding experiments the curarised muscle was left a short time only in Ringer's fluid. Some experiments were also made to see whether the effect of curari would disappear after a longer stay in the salt solution. Muscles were placed for 15 mins. in curari, rinsed in Ringer's fluid, and each then placed in 30 c.c. of the fluid, and left in it for ½ to 24 hours before being tested with ·1 p.c. nicotine. (Table IV.)

TABLE IV.

Duration of curari paralysis.

(Load 1½ grams.)

Series *a*.	Percentage of curari	Time in curari in mins.	Time in Ringer's fluid in hours	Effect of nicotine ·1 p.c.
1	·001	15	½	Slight twitch on immersion, with contracture; no further effect in 5 mins.
2	·001	15	½	Slow rise, ·5 mm.
3	·001	15	½	Considerable twitching, lasting 1½ mins. and some tone, the effect was much greater than in Fig. 17.
4	·01	15	½	Twitch 1 mm. on immersion, after 1 min. slow rise to 1·5 mm.
5	·01	15	½	0. (Slight twitch on running off nicotine.)
6	·001	15	½	Fig. 17 *a*.
7	·001	15	2	Fig. 17 *b*.
8	·001	15	6¾	Fig. 17 *c*.
9	·001	15	24	Fig. 17 *d*.

Series *b*. Four sartorii were placed in ·001 p.c. nicotine for 15 mins., then in Ringer's fluid for 1, 2, 3 and 4 hrs. respectively, and then in nicotine ·1 p.c. In all there were some twitches and bending up of the muscles on placing in nicotine, but no certain difference in degree in the several muscles.

There is then a decrease of the curari paralysis when the muscle is left in Ringer's fluid (Fig. 17)[1], the decrease is more rapid at first, and then progresses very slowly so that even at the end of a day, the paralysing effect is still considerable.

[1] The return of twitching has varied much more than that of tone so far as the latter could be judged.

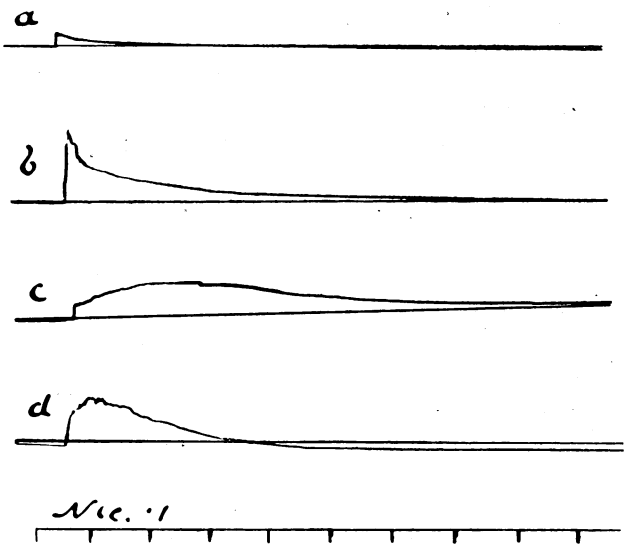

Fig. 17. 4 sartorii in curari ·001 p.c. 15 mins., then in Ringer's fluid (*a*) ½ hr., (*b*) 2 hrs., (*c*) 6¾ hrs., (*d*) 24 hrs. The tracings show the contraction with nicotine ·1 p.c. (cp. Table IV. Exps. 6—9). Load 1½ grams. Time in ½ mins.

We have seen above that the effect of curari increases from ·00001 p.c. up to ·001, and that after the latter, ·1 p.c. nicotine does not cause contraction. The increasing effect might be due to more and more complete saturation, or (on the theory that the combination formed with the receptive substance depends upon the relative concentration of curari and nicotine) to the concentration of the curari in the muscle spaces being greater the greater the percentage of curari. In order to throw some light on this point, pairs of sartorius muscles were placed in curari solutions of different percentage, for the same time, left in Ringer's fluid for the same time, and then the effect of ·1 p.c. nicotine noted.

TABLE V.

Duration of curari paralysis with varying percentages.

Series *a*. Fig. 18. Pairs of sartorii were placed for 15 mins. in curari as follows:

1	·0005 p.c.	3	·001 p.c.	5	·001 p.c.	7	·01 p.c.
2	·01 ,,	4	·01 ,,	6	·1 ,,	8	1·00 ,,

Each was then placed in Ringer's fluid for an hour, and then a graphic record taken of the effect of ·1 p.c. nicotine; 1 gave a normal curve except that the twitching was rather greater, 2 and 5 gave contractions of the same type, viz. twitches lasting 20 to 30 secs.

followed by a slight tonic rise. The tonic rise was very slight in 3, but the first twitch was stronger than in 5.

2, 4 and 7 gave curves of the same type, viz. a rise accompanied by twitches lasting 20 to 30 secs., without any subsequent tonic rise (7, not given in Fig. 18, was like 4 but rather less), 6 and 8 gave a mere trace of rise, barely perceptible in 6.

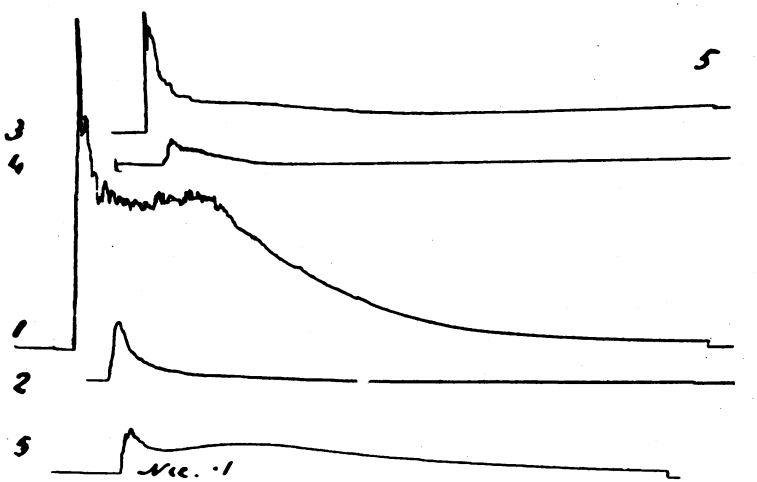

Fig. 18. $\times \frac{1}{3}$. Curarised muscles washed out with Ringer's fluid; then a tracing taken of the effect of ·1 p.c. nicotine (see *a*, Table V). The tracing was taken for 5 mins. and then the drum stopped for 5 mins.

Series *b*. Fig. 19. The sartorii were placed for 15 mins. in curari as follows:

1	·001	p.c.	3	·1	p.c.
2	·01	,,	4	1·0	,,

Then rinsed in Ringer's fluid, and placed in Ringer's fluid (renewed at the end of each hour) in a shaking machine, 1 for $4\frac{3}{4}$ hrs., 2 for 5 hrs., 3 for 5 hrs., 4 for $5\frac{1}{4}$ hrs. A tracing was then taken of the effect of ·1 p.c. nicotine. The result in 1 and 2 is shown in Fig. 19. It will be noticed that in this case a greater contraction was obtained from the muscle treated with ·01 p.c. curari, this was a $\frac{1}{4}$ hr. longer in the Ringer's fluid, but the result indicates that prolonged washing out tends to equalize the condition of the muscles. In 3 there was a very slight rise, soon over. In 4 there was no effect.

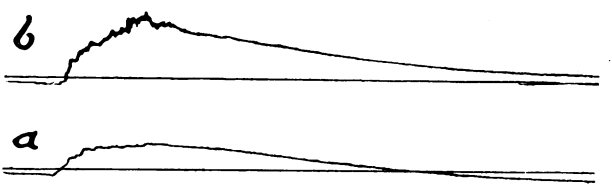

Fig. 19. Right and left sartorius muscles (*a*) 15 mins. in curari ·001 p.c.; and $4\frac{3}{4}$ hrs. in Ringer's fluid, (*b*) 15 mins. in ·01 p.c. and 5 hours in Ringer's fluid. Effect of nicotine ·1 p.c. The tracing occupied 5 mins.

The experiments show that the duration of the effect increases with the percentage of curari from ·0005 up to 1 p.c. In series *a* the maximum effect was obtained with ·1, but in series *b* a difference between 1 and ·1 p.c. was perceptible. In view of the fact that ·001 p.c. is sufficient to paralyse the receptive substance if the muscle is not washed out, it is not probable that the differences in duration of effect with increasing percentages of curari are due to any large extent to differences of saturation[1]. On the other hand it is fairly certain that the curari free in the muscle spaces was reduced to a minimal quantity after the treatment in Ringer's fluid series *b*, and probably in series *a*, whether the solution originally used was ·001 or 1 p.c. I shall give later some evidence that curari is adsorbed by tissues in proportion to its percentage, and the simplest explanation of the results seems to me to be as follows: the receptive substance is saturated in 15 minutes with curari of ·001 or any higher percentage; but more curari is adsorbed the higher the percentage. When the muscle is left in Ringer's fluid there is on the one hand a gradual dissociation of the curari compound (ions or salts probably taking its place). There is also a slow setting free of curari from its adsorbed condition, the free curari tends to delay the dissociation of curari compound. On adding nicotine the adsorbed curari is set free at a faster rate (nicotine taking its place) and this cuts short the action of nicotine on the receptive substance.

On this view the differences would diminish after more prolonged washing out of the curari, and that this is the case is indicated by ser. *b*, Exps. 1, 2 in Table V (Fig. 19). I propose to make further experiments on the matter.

3. *Effect of curari on the action of* ·5 *to* 1 *p.c. nicotine.*

Solutions of nicotine ·25 to 1 p.c. cause as I have said an immediate shortening of the muscle by acting on the general muscle substance as well as by acting on the receptive substance of the neural region. There are two questions on the effect of curari to be determined in this connection, first whether any contraction is caused by nicotine antagonizing the curari and acting on the receptive substance, and secondly whether curari alters the action of nicotine on the general muscle substance.

[1] The determination of varying degrees of saturation presents difficulties, and I shall return to it in a later paper.

TABLE VI.

Effect of ·5 p.c. nicotine on the curarised sartorius.

The muscle was stimulated with a make and a break induction shock before the immersion in nicotine. The load was 2·5 grams. The height of the secondary rise, unless otherwise mentioned, was measured from the lowest point of the curve after the primary rise to the point attained at the end of 20 minutes from giving nicotine.

Series *a*. Dec.

Exp.	Percentage of curari	Time in curari in mins.	Primary rise in mm.	Extent of subsequent fall	Height of secondary rise	
1	·001	15	9·5	0	42·5	
2	·001	15	10	0	36	
3	·01	15	10·5	·5	39	
4	·1	15	9·5	1	22·5	
5	1·0	15	12	1	45	
6	1·0	120	37·5	0	37·5[1]	
7	1·0	120	32	3	7[2]	
8	1·0	120	40	14	11[3]	Fig. 21.

[1] Rise taken after 15 mins., slight fall in next 5 mins. then no rise or fall for 10 mins. [2] Rise taken after 10 mins. [3] Rise after 15 mins.

Series *b*. March. Some of the muscles were placed in Ringer's fluid instead of curari, this is indicated by 'Ringer' in column 2. In all cases the time of stay in the fluid was 15 mins. Load 2½ grams.

Exp.	Percentage of curari	Primary rise in mm.	Fall in mm.	Secondary rise	Total rise	
1	Ringer	24·5	16	16·5	25	Fig. 20 *a*.
2	·0001	11	5	25·5	31·5	Fig. 20 *b*.
3	Ringer	22	10·5	70·5	82	
4	·0001	14	1	61	74	
5	Ringer	38	28	58	68	
6	·001	9·5	0	53	62·5	
7	Ringer	27	19·5	36·5	44	
8	·001	4	0	29·5	33·5	
9	·01	6·5	0	45·5	52	
10	·1	8·5	0	45	53·5	
11	·01	6·5	0	70·5	77	
12	·1	8·5	0	70·5	79	
13	Ringer	32	15·5	74·5	91	

It is clear from these experiments that ·0001 p.c. curari greatly reduces the primary contraction caused by ·5 p.c. nicotine, *i.e.* if any immediate antagonism occurs it is only slight. Exp. 1 gives some indication that there is a slight slow antagonism since there is a greater secondary rise after curari than after Ringer's fluid, but no stress can be placed on a single experiment. On comparing the effect of ·0001 p.c.

and ·001 p.c. curari it appears that the latter has a somewhat greater effect in reducing the nicotine contraction; this may reasonably be attributed to the paralysis of the receptive substance by ·001 p.c. curari being more complete. Allowing for this, a very small part of the rise can be due to an antagonising action; and as the secondary rise is less after ·001 p.c. than after Ringer's fluid there is no evidence of a slow antagonising action. I conclude then that when the receptive substance

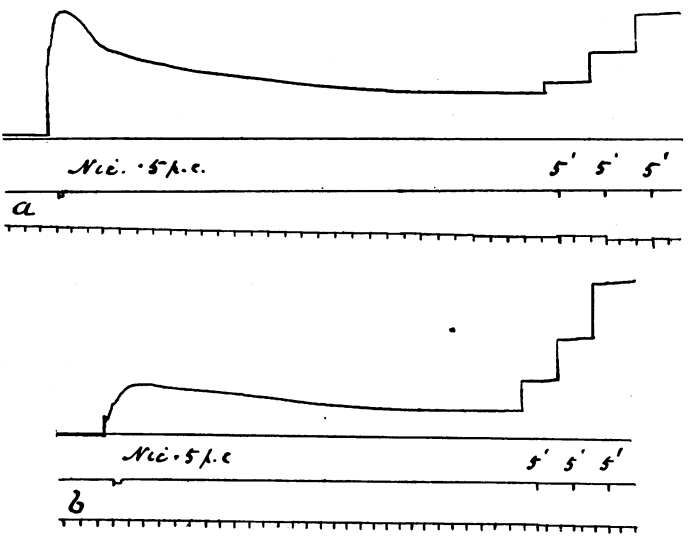

Fig. 20. ×⅔. Effect of nicotine ·5 p.c. on (*a*) right sartorius placed in Ringer's fluid, and on (*b*) left sartorius placed in curari ·0001 p.c. for 15 mins. (cp. Table VI *b*, Exps. 1 and 2). Load 2½ grams. Time in 10 secs.

has been paralysed as regards ·1 p.c. nicotine, ·5 p.c. nicotine has extremely little if any antagonising action on the curari paralysis in the time taken by the primary contraction. The experiments give no evidence in this case of a slow substitution of curari by nicotine but it is not likely that if this occurred it would be appreciable.

The effect of ·01 and ·1 p.c. curari it will be convenient to take after that of 1 p.c. It will be seen from Table VI series *a* that the sartorius after being placed in 1 p.c. curari for 15 minutes gave an appreciably higher primary rise with ·5 p.c. nicotine than when curari ·001 to ·1 p.c. had been used, and that the rise very greatly increased when the muscle remained in the solution for 2 hours (Fig. 21). In all these experiments on prolonged curari action the irritability to induction shocks was very

slight. The secondary rise was decreased but had a different form in each experiment. Obviously the prolonged stay in the curari had injured the muscle; the effect however may be due to the salts of curari and not to curarine.

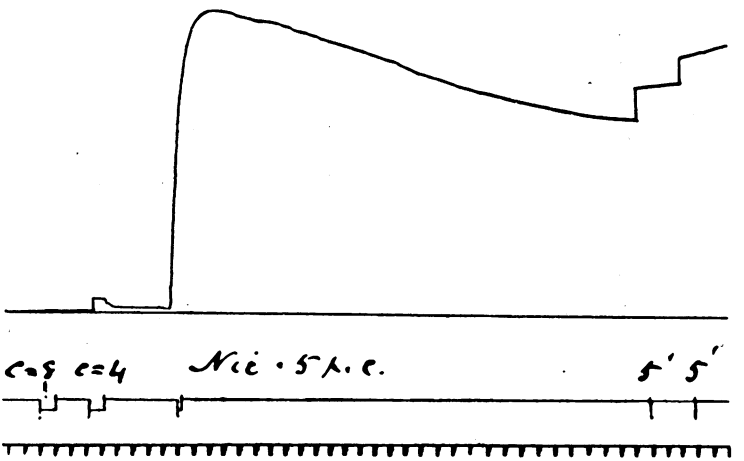

Fig. 21. Effect of ·5 p.c. nicotine on sartorius which had been placed in 1 p.c. curari for 2 hours. Load 2½ grams. Time in 10 secs. (cp. Table VI *a*, Exp. 8).

Taking now the effect of solutions of curari neither very dilute nor very strong, we see that in ser. *a* there was no appreciable difference between the effect of ·001 and of ·1 p.c. Allowing for the fact that the muscles were taken from different frogs, it is still obvious that curari up to ·1 p.c. has in 15 minutes very little effect on the general muscle substance. In ser. *b* there is a slight difference in the primary rise after ·01 and ·1 p.c. nicotine in both of the experiments made. The difference in this case is I think genuine and not due either to differences in the muscles or in the mode of handling them. In that case it must either be due to nicotine antagonising the curari slightly after ·01 curari and not after ·1, or to ·1 p.c. curari slightly increasing the nicotine effect, as we have seen stronger solutions do. I take it to be due to the latter. For when the muscle is left 2 to 3 hours in ·005 to ·1 p.c. nicotine, or in 1 p.c. for a shorter period, ·1 p.c. nicotine generally causes an immediate rise in the tracing and this in some cases passes without a plateau into rigor.

I have made a few similar experiments with 1 p.c. curari.

TABLE VII.

Effect of curari on the contraction caused by 1 *p.c. nicotine.*

Exp.	Percentage of curari	Time in curari in mins.	Primary rise in mm.	Total rise in 15 mins.	
1	·001	15	30·5	92	
2	·01	15	19	87	
3	·01	15	29	83	
4	·1	15	31·5	73·5	
5	·1	15	33	110	Fig. 22 *a*
6	·5	15	40	111	Fig. 22 *b*.
7	1·0	120	45 [1]	46	

[1] Followed by fall of 11 mm., the electrical irritability was very slight.

				Fall	Total rise in 5 mins.
8 [2]	·001	30	41·5	4·5	85
9	·01	30	37	3	85

[2] 8 was placed in Ringer's fluid for 30 mins., and 9 for 40 mins. They were immersed in ·1 p.c. nicotine for 2 mins. before putting in 1 p.c., this had no effect. The rise in 8 was quicker than that in 9.

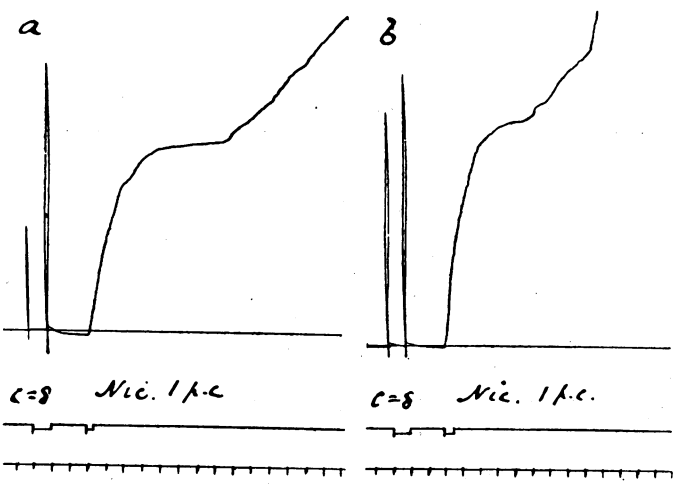

Fig. 22. ×$\frac{2}{3}$. Effect of ·1 p.c. nicotine on (*a*) right sartorius placed in curari ·1 p.c. for 15 mins. and on (*b*) left sartorius placed in curari ·5 p.c. for 15 mins. (cp. Table VII, Exps. 5 and 6). Load 2½ grams.

The general results it will be seen are similar to those with ·5 p.c. nicotine. Curari above a certain percentage—apparently beginning about ·1 p.c.—increases the primary rise caused by 1 p.c. nicotine; and it causes in proportion to the concentration and to the duration of action a decrease in the secondary rise and a great reduction in irritability to

electrical stimuli. In Exps. 1 and 2 the primary nicotine rise was much greater after ·001 p.c. than after ·01 p.c. curari, but as the rise after ·01 p.c. was less than usual, the difference may have been more or less due to a difference in the muscles. In Exps. 8 and 9 the muscles were cut out of the body within a few minutes of one another and placed in Ringer's fluid for about half an hour. Nicotine 1 p.c. caused a distinctly quicker and a somewhat higher rise followed by a rather greater fall in the muscle which had been treated with ·001 p.c. curari. The results indicate that there is a partial immediate antagonising of the action of ·001 p.c. curari by 1 p.c. nicotine.

The primary nicotine rise is slightly greater after ·1 than after ·01 p.c. curari: this may have been due to a difference in the muscles of the two sides, or, as I have suggested above in speaking of the effect of ·5 p.c. nicotine, mark the beginning action of curari upon the general muscle substance.

In a few experiments in which ·25 p.c. curari was used, curari was also added to the successive nicotine solutions to make ·25 p.c.; the contraction with 1 p.c. nicotine was not appreciably altered.

In my preliminary account I stated that ·5 to 1 p.c. nicotine caused a greater primary rise after curari than after dilute nicotine and I attributed this to the contraction in the neural region of the muscle caused by dilute nicotine lowering the irritability in this region. The conclusion was based upon the results of a large number of experiments, but they were made for the most part on different frogs. In some subsequent experiments in which the right and left sartorius of the same frog have been compared, I have not obtained constant results and further experiments on the point are required.

4. *Effect of curari after nicotine.*

I have shown earlier that in the coraco-radialis and flexor carpi radialis muscles[1], curari applied a few minutes after dilute nicotine causes relaxation or increases the rate of that going on. Since then I have experimented both on these and on other muscles in the frog. In all cases when curari is applied soon enough after dilute nicotine, there is a decrease in the effect of the nicotine; shown by a more rapid rate of relaxation, and in certain conditions by a decrease in the height of contraction. When curari is applied late during the residual contraction there is little or no effect.

[1] Tracings of these effects were shown at the Intern. Physiol. Congress in 1907.

The time after immersion in nicotine at which curari increases the rate of relaxation varies widely in different muscles; it depends also upon the percentage of nicotine and the duration of the contraction. In the sartorius after ·1 p.c. nicotine, curari applied even for as short a time as 20 secs. after the nicotine may have no effect in hastening the rate of relaxation. The flexor carpi radialis and the rectus abdominis muscles have a wider range of response to nicotine, and the contraction is higher and more prolonged; in these muscles some relaxation is obtained with curari for more than ¼ of an hour after immersion in nicotine. I reserve a detailed consideration of the effects till a later time; but the general conclusions to be drawn from the experiments are I think clear. These are, first that curari in all cases puts an end to the stimulating action of nicotine, and secondly that it has little and perhaps no effect upon the state which is caused by the stimulation. In other words curari paralyses the receptive substance but (in the percentage given in these experiments) has little or no action on the general muscle substance whether this is at rest or contracted (cp. also p. 290).

5. *Effect of curari on the survival irritability in dilute nicotine.*

In a few experiments made in winter, the irritability of curarised sartorii lasted longer in ·01 to ·1 p.c. nicotine than that of the uncurarised muscles. I have made further experiments on the point. There were two exceptions out of seven cases, so that the possibility that the difference found in survival irritability is simply due to differences in the muscles in the two sides, is not excluded. The later experiments were as follows:

TABLE VIII.

Series *a*. Temp. 13° to 11° C.

1	Curari ·001 p.c. 15′, then nicotine ·1 p.c.	
2	Ringer's fluid 15′	,, ,, ,,
3	Curari ·01 p.c. 15′	,, ,, ,,
4	Ringer's fluid 15′	,, ,, ,,
5	Nicotine ·1 p.c.	
6	Curari ·001 p.c. + nicotine ·1 p.c.	

5 hours. 1 and 2. Little change in either, but a trifle more in 2 (change is in length, in curve to lateral border, and in bend at knee end).

3 and 4. Fold in nerve region, and bent at the fold, 4 much more than 3.

5 and 6. Nearly normal shape, 6 a trifle shorter.

TABLE VIII (*cont.*).

21½ to 23½ *hours.* 2 is rather more shrunken than 1, has nearly assumed rigor form. 1 has a double bend and is more irritable than 2.

3 and 4 have both nearly complete rigor form; 3 only traces of irritability; 4 the same, except on lateral border where better irritability.

5 and 6 have both nearly complete rigor form; 5 has slight irritability on lateral border, 6 not irritable.

Series *b*. Room temp. 18° C.

1 Nicotine ·01 p.c. for ½ hour, then in nicotine ·1 p.c.
2 Curari ·002 p.c. ,, ,, ,, ,,

3 Nicotine ·1 p.c.
4 Curari ·001 p.c. + nicotine ·1 p.c.

5 Nicotine ·1 p.c. made up in NaCl ·7 p.c.
6 Ringer's fluid.

At the end of 19 hours, the condition was as follows:

1 Partly shrunken, much folded, irritability slight.
2 Flat, slightly bent at knee end, irritability much better than in (1).
3 Rigor form, but not opaque white; irritability trace only.
4 Partly shrunken, with some folds; irritability better than in (2).
5 Rigor form, more shrunken and more opaque than (3), irritability gone.
6 Flat, little if at all shrunken, irritability fair, distinctly better than in 4.

Series *c*. Room temp. 22° C.

1 Ringer's fluid 15′, then nicotine ·025.
2 Curari ·005 15′, then nicotine ·025.

3 Nicotine ·025.
4 Curari ·005 and nicotine ·025.

22 *hours.* 1 and 2 in rigor form, irritability slight, a trifle better in 2 than in 1.

3 and 4, folded and somewhat shrunken, irritability better than in 1 and 2, and better in 4 than in 3.

6. *The primary effect of mixtures of nicotine and curari.*

From the point of view of testing how far tissues take up nicotine after curari poisoning (cp. p. 284) it is important to know the degree of contraction, if any, which is caused in muscles by different mixtures of the two poisons. In order to determine this, mixtures containing different proportions of nicotine and curari were dropped over the muscles and a graphic record taken. I give here the experiments on the sartorius.

TABLE IX.

Series *a*. July. Load 1½ grams.

1	Curari	·0005	+	nicotine	·1.	The first three at the beginning gave a violent twitch of
2	,,	·002	+	,,	·1.	7 or more cms., small twitches for a minute or two and
3	,,	·02	+	,,	·1.	residual tone of 3 to 7 mm. at the end of 10 mins.
4	,,	·2	+	,,	·1.	(4) gave slight twitch and tone (rise of 5 mm.), the lever reached the base line in 3 mins. and then fell slightly below it.

Series *b*. Aug. Load 1½ grams.

						Rise
1	Curari	·0005	+	nicotine	·01.	2 mm., reaches base line in 4 mins.
2	,,	·002	+	,,	·01.	2½ mm., 1½ above base line in 5 mins.
3	,,	·02	+	,,	·01.	1½ mm., fall below base line in 3½ mins.
4	,,	·2	+	,,	·01.	0, fall below base line in 3 mins.

Thus nicotine ·01 can be detected in the presence of curari up to about ·02 p.c., and nicotine ·1 p.c. in the presence of curari ·2 p.c. The arm and abdominal muscles as will be shown at a later time are much more delicate indicators.

7. *The effect of nicotine in the early stage of paralysis of nerve effect by curari.*

On the theory I have put forward the nerve impulse affects primarily the receptive substance. It has long been known[1] that the tonic action of nicotine in the arm muscles begins before the nerves are paralysed. For on injecting nicotine under the skin of a frog, the cataleptic condition of the arm muscles is obvious at a time when voluntary movement of the arms still occurs (cp. also Part II p. 203).

A more interesting point is the condition of the receptive substance as regards nicotine stimulation, when electrical stimulation by way of the nerves has no effect. This I have investigated in the following manner. The brain of a frog is destroyed and curari injected into the dorsal sac; when reflex action ceases, the nerve to the muscle to be observed is stimulated with strong interrupted currents. If this has no effect, nicotine is applied locally to the muscle or it is cut out, and either placed in nicotine ·01 or ·1 p.c. and the effect observed with the eye or a graphic record taken.

The paralysis of the nicotine twitchings is nearly synchronous with the paralysis of nerve effect but as a rule outlasts it a little. This I

[1] Cp. Langley and Dickinson. This *Journal*, I. p. 270. 1890.

have observed in the sub-maxillary muscle, the thigh muscles, the pectoral-abdominal, and the transverse and oblique abdominal muscles, but the twitchings were always much less than normal. In some cases, and more frequently in the thigh muscles than in the others, there were no twitchings in an early stage of paralysis of the nerve endings, but it is to be remembered that in some frogs the uncurarised thigh muscles show very little twitching. The time of paralysis of the receptive substance for tonic contraction is markedly different in different muscles. In the sartorius it outlasts very little the twitchings, *i.e.* in this muscle —and apparently in other thigh muscles—the paralysis of the nicotine tonic contraction is nearly synchronous with the paralysis of nerve effect. It usually lasts a little longer in the sub-maxillary muscle, and longer still in the digital muscles. The contraction of the digital muscles is easily seen by placing the leg, after removal of the skin, in ·1 p.c. nicotine. After a dose of nicotine considerably larger than is necessary to paralyse the nerve effect, a graphic record of the flexor carpi radialis and of the rectus abdominis treated with ·01 p.c. nicotine shows a fair contraction in the former, and a good contraction in the latter. A similar result on the gastrocnemius is mentioned below (p. 281). After a large dose of curari and sufficient time of action none of the muscles give any contraction with nicotine.

Thus as regards the quick conducted contraction, the effect of nicotine outlasts the nerve effect but a short time, and probably only in fresh irritable muscles. It is clear that the nerve impulse is a much more effective stimulus than nicotine on the receptive substance for quick conducted contractions. As regards the receptive substance causing the tonic contraction we have no definite evidence that this can be stimulated by nerve impulses, and we have seen that in some muscles stimulation of the motor nerves with tetanising currents causes no contraction at a time when the receptive substance responds fairly readily to nicotine. It is possible then that the 'tonic' receptive substance is only put in action by some metabolic products carried to it in the blood, and this might help to account for the prolonged contraction of the arm muscles of the frog during the breeding season. But there are several facts, and especially the localization of the receptive substance in the region of the nerve endings, which make it unsafe at present to conclude that nervous impulses are without effect. We may however conclude that if the nerve impulses set up by tetanising currents do affect it, their paralysis by curari is but a stage in the progressive paralysis of the receptive substance.

IV. Effect of neutral salts on the nicotine contraction.

On this subject I have only made sufficient experiments to form some idea whether different salts have a different action on the receptive substance, on the primary rise caused by 1 p.c. nicotine and on the secondary (rigor) rise. The salts, the effect of which was tried, were the chlorides of Na, K, Ca and Mg. The usual method was as follows: the sartorius was fixed in the apparatus and stimulated with a make and a break induction shock giving a sub-maximal response, the currents passing through the muscle from end to end; the vessel containing the muscle was filled with the salt solution for a given time, as a rule 15 mins., the solution run off and the muscle again stimulated. Finally the nicotine solutions, as a rule successive solutions of increasing strength, were added. By the method used it is not likely that the induction shocks remained constant, but I have no doubt they were sufficiently constant to show any considerable variation in the condition of the muscle, moreover the results agree in the main with those of previous observers[1]. It was only desired to compare broadly the response of the muscle to induction shocks with that to nicotine.

NaCl. Sodium chloride was used in ·6 p.c. solution in distilled water and the muscle left in it for 15 mins. The response to induction shocks was much increased and usually much prolonged (cp. Figs. 23, 24). Running the solution in and out of the vessel constantly caused a twitch of the muscle of considerable height.

Ringer (This *Journal*, VIII. p. 292. 1887) noticed that after perfusion of a frog with ·6 p.c. NaCl solution the period of relaxation was enormously prolonged, and that there was sometimes great increase in the height of contraction. Locke (*Pflüger's Arch.* LIV. p. 501. 1893) found at certain seasons of the year that strong induction shocks passed through a sartorius which had been soaked for some time in NaCl ·6 p.c. caused a high tetaniform contraction lasting several seconds. The absence of effect when it occurred he was inclined to attribute to a higher temperature. In the Exp. mentioned below made in August, and in which all the fluids were at 22° C., the contraction obtained with a weak induction shock after treatment with NaCl ·6 p.c. was like that given in Fig. 23. Mines (This *Journal*, XXXVII. p. 408. 1908), passing the currents through a small portion of the muscle, finds that the irritability to induction shocks is not affected by treating the sartorius for a few minutes with NaCl ·7 p.c., but that after prolonged treatment the contraction was often much increased for the first stimulus.

On immersing the muscle in ·001 p.c. nicotine, there was a fairly strong contraction followed by a series of twitches (Fig. 23) lasting 2 or

[1] Observations on the effect of Na, K, and Ca salts in electrical irritability by a more accurate method have recently been made by Mines (This *Journal*, XXXVII. p. 408. 1908).

more minutes. Subsequent ·01 p.c. had a variable effect, once renewing the twitches, once having a slight effect (Fig. 23), once having no effect; the result apparently depended partly on the state of the muscle, and partly on the temperature. Nicotine ·1 after ·01 had no effect. When all the fluids were warmed to 22° C. (one Exp. only), the twitchings with ·001 p.c. were sooner over and subsequent ·01 had no effect. Nicotine 1 p.c. after the ·1 p.c. caused a contraction higher than normal except in

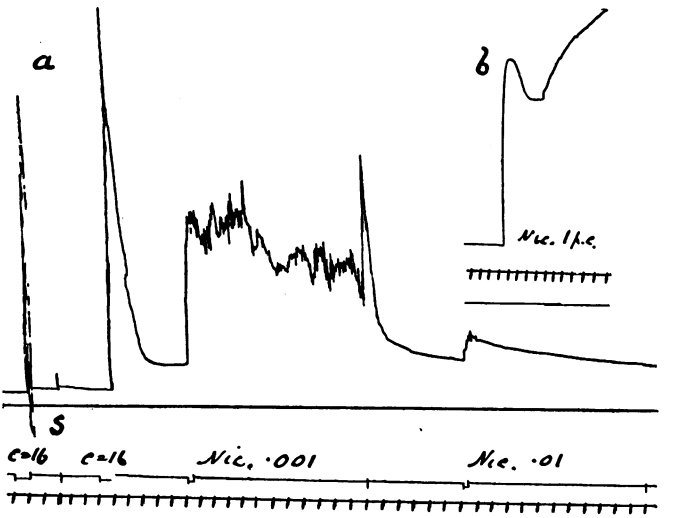

Fig. 23. ×⅔. Effect of NaCl on the nicotine contractions:

(*a*) Left sartorius. At *S* the muscle was placed in ·6 NaCl for 15 mins. Nicotine ·1 p.c. after the ·01 p.c. had no effect. Load 1½ grams. Time in 10 secs.

(*b*) Right sartorius. All fluids at 22° C. Effect of 1 p.c. nicotine after ·001, ·01, and ·1 p.c.

the experiment in which the fluids were warmed to 22° C. The effect in this case is given in Fig. 23 *b* as it shows a marked fall after the primary rise. On immersing the muscle in ·1 p.c. nicotine, there was a much larger first contraction, with numerous small twitches on a rapidly descending curve (Fig. 24). Subsequent 1 p.c. caused a contraction much higher than normal.

With ·5 p.c. nicotine the first part of the curve was like that with ·1 p.c., but the fall stopped about half way, and subsequent 1 p.c. gave a convex rigor rise, with slight depressions as from irregular shrinking.

With 1 p.c. nicotine there was a much larger rise, with small twitches on the fall; the curve was similar to that in Fig. 4 *a* but the top was less rounded.

The twitches with ·001 to ·1 p.c. nicotine did not occur when the muscle was placed for 15 minutes in ·01 p.c. curari before being placed in the salt solution[1], but the rise caused by 1 p.c. nicotine was greater than normal.

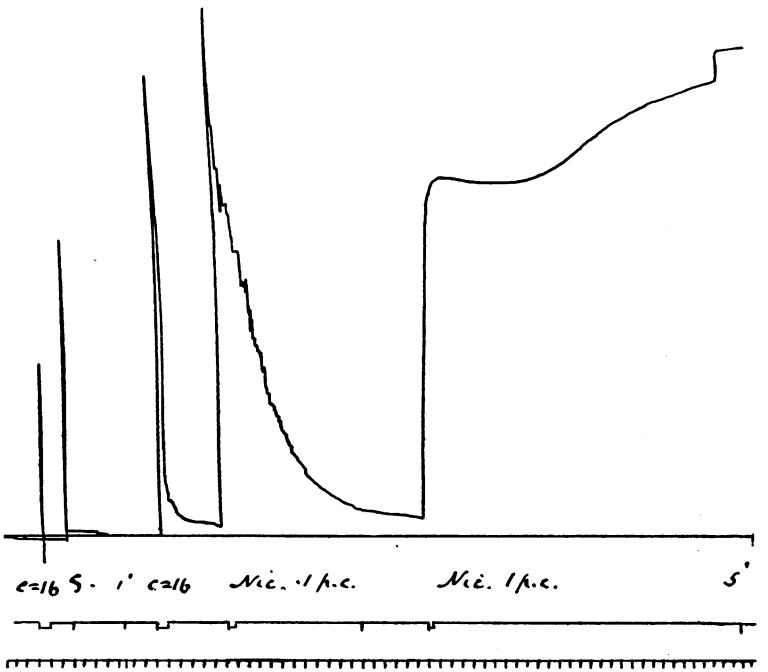

Fig. 24. ×⅔. Sartorius immersed in NaCl ·6 p.c. 15 mins., at *S* the contraction occurred on running off the solution. Muscle stimulated with secondary coil at 16 cm. before and after immersion in NaCl. Immersed in ·1 p.c. and then in 1 p.c. nicotine. Load 1½ grams. Time in 10 secs.

[1] There may of course be twitchings in the NaCl solution. Ringer and others have shown that dilute curari does not stop the twitchings in NaCl solution. Mines (*op. cit.* p. 416) finds that ·2 p.c. curari prevents them from occurring, but that the salts contained in the curari act in the same way.

Previous treatment with dilute nicotine (15 to 30 minutes in ·1 p.c.) makes little difference to the twitchings caused by placing the sartorius in NaCl ·6 p.c. In some early experiments in which dilute nicotine was made up in NaCl ·6 instead of in Ringer's fluid, the twitchings soon stopped in nicotine ·1 to ·012—though contractions began on transferring to pure NaCl ·6 p.c. Since then I have made a few more experiments. On placing the muscle in nicotine ·01 p.c. in NaCl ·6, twitchings occurred though to a less extent than in the control; they could however be readily induced by moving the muscle about or by electrical excitation, and on excitation they occurred after 24 hours. When the muscle was placed in nicotine ·1 p.c. in NaCl ·6 p.c. the twitchings stopped in a few minutes. It may be noticed that the twitchings in NaCl ·6, except perhaps at first, are often local and not conducted throughout the fibres affected.

The most decided effect then of a short stay in sodium chloride is to increase the nicotine twitchings. The twitchings are due to an action in the receptive substance since they are readily stopped by curari. Whether the immersion in sodium chloride increases the irritability of the receptive substance or simply the response of the muscle the experiments do not definitely decide. The response of the general muscle substance to ·5 and 1 p.c. nicotine was it is true in nearly all cases distinctly higher (cp. Fig. 24) than is usual in similar circumstances when the muscle is not treated with sodium chloride, but no comparison was made of the two muscles of one frog, one with and the other without immersion in the salt solution.

The experiments give no evidence that sodium chloride increases the tonic contraction caused by nicotine. The curve may keep well above the base line, but when this occurs, it appears to be due to the repeated twitchings; when the twitchings cease, the curve is below the usual level rather than above it (cp. Fig. 24). This question I shall consider in dealing with the arm and abdominal muscles in which the twitchings are either absent or too feeble to affect the tracing.

The other salts were not used in pure solutions. A ·5 p.c. solution was made up in Ringer's fluid.

KCl. The muscle was left in the potassium chloride solution 15, 30, 60, and 90 minutes. It is known that KCl in this strength rapidly destroys the electrical irritability. Even after 15 minutes' stay in the

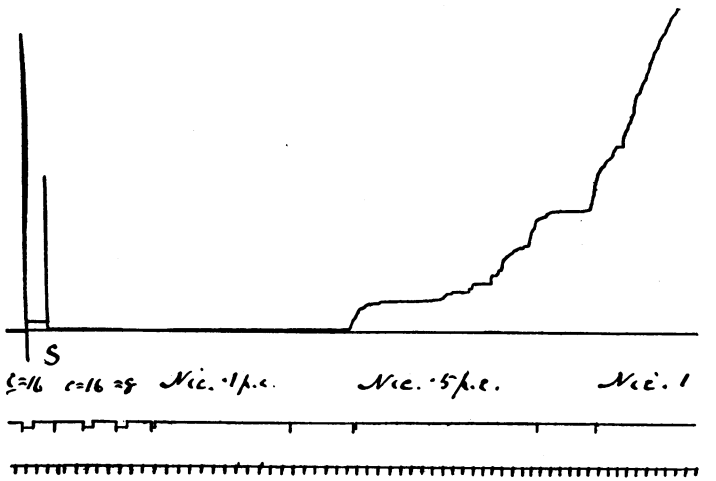

Fig. 25. ×⅔. Sartorius stimulated with secondary coil at 16 before immersion for 15 mins. in ·5 p.c. KCl in Ringer's fluid, and with secondary coil at 16 and at 8 afterwards. Nicotine ·1, ·5 and 1 p.c. Load 2½ grams. Time in 10 secs.

solution, induction shocks with the secondary coil at 8 cm. caused no contraction although a contraction of several centimetres had previously been obtained with the coil at 16 cm. (Fig. 25), ·1 p.c. nicotine had no effect, ·5 p.c. nicotine caused a slight rise, and 1 p.c. a greater one, irregular at first (cp. Fig. 25) then smooth and convex to the abscissa. The height of the rise in 15 mins. with 1 p.c. nicotine decreased with increased duration of the action of KCl.

Since muscle contains *K* salts it would be expected that a strong extract of muscle would paralyse the receptive substance and injure the irritability of the muscle. This was found to be the case.

Four grams of arm muscles were taken and ground with 4 c.c. of Ringer's fluid, boiled, a drop of acetic acid added when boiling, filtered, and a drop of Na_2CO_3 added to neutralize =(*a*) 1·5 c.c. the filtrate was dried, and burnt, and the ash ground up with 1·5 c.c. of distilled water=(*b*). In each fluid (*a*) and (*b*) a sartorius muscle was left for 15 min.; the one in (*b*) gave slight twitches. Each muscle was then placed in the apparatus. The irritability to strong induction shocks was very slight; nicotine ·1 p.c. caused no contraction, the contractions with ·5 p.c. and 1 p.c. nicotine were greatly reduced, the muscle treated with (*a*) gave with 1 p.c. nicotine a convex curve only and no primary rise.

$CaCl_2$. The effect of calcium chloride (·5 p.c. in Ringer's fluid) for 15, 60, and 110 minutes was tried.

After 15 mins. the irritability to induction shocks was reduced, but a contraction was still obtained with the secondary coil at 16 cm.; nicotine ·1 p.c. caused a rise of 1·5 mm. in the first 20 secs. and then no further rise or fall (Fig. 26); the rise with ·5 p.c. nicotine was fairly normal, that with 1 p.c. became markedly convex in a few minutes and was higher than normal. After 60 mins. a slight contraction was obtained with the secondary coil at 12; nicotine ·1 caused a similar rise to that in the previous experiment but only of

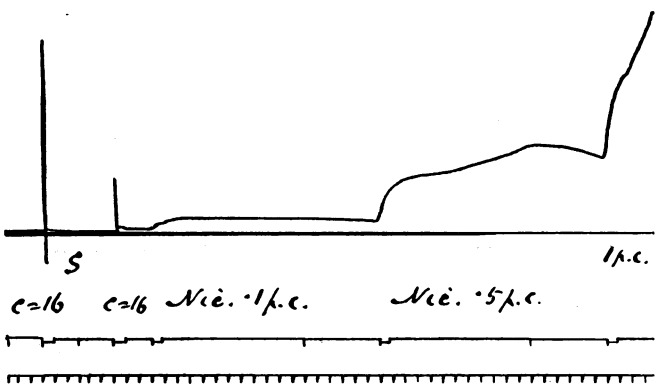

Fig. 26. ×¾. Sartorius stimulated with secondary coil at 16, before and after 15 mins. in $CaCl_2$ ·5 p.c. in Ringer's fluid (at *S* in the tracing). Immersed in nicotine ·1, ·5 and 1 p.c. Load 2½ grams. Time in 10 secs.

·5 mm.; nicotine ·5 and 1 p.c. had much the same effect as before. After 100 mins. the irritability to induction shocks was further decreased; nicotine ·1 p.c. had no effect; ·5 and 1 p.c. had much less effect than in the other experiments.

These experiments show that excess of $CaCl_2$ in Ringer's fluid whilst reducing the electrical irritability of the general muscle substance reduces[1] also and in time abolishes the effect of the receptive substance on it. How far this is due to a direct action on the receptive substance is not easy to determine. The primary rise with ·5 and 1 p.c. nicotine was not increased, but the rigor rise with 1 p.c. appeared to begin earlier and reach a greater height.

Two experiments were made in which the muscle was placed in ·5 p.c. $CaCl_2$ in Ringer's fluid for 15 mins. and then transferred to a vessel containing ·01 p.c. nicotine made up in Ringer's fluid. In neither case were there twitchings or any obvious contraction. After 4 hours they were placed in NaCl ·6 p.c. and in time twitching and bending of the muscles commenced.

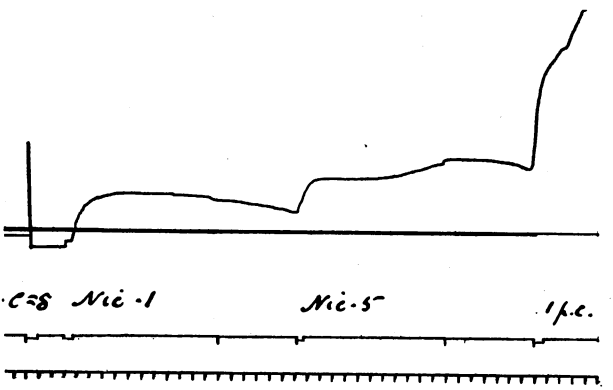

Fig. 27. $\times\frac{3}{4}$. Sartorius in $MgCl_2$ ·5 p.c. in Ringer's fluid, for 1 hour. Load $2\frac{1}{2}$ grams.

$MgCl_2$. ·5 p.c. in Ringer's solution even in an hour had no great effect upon the contraction given by ·1 p.c. nicotine (Fig. 27) although it considerably reduced the irritability to induction shocks. The rise with 1 p.c. nicotine had, as after KCl and $CaCl_2$, a marked tendency to the convex form. In this case then the general muscle substance appeared to be more affected than the receptive substance.

[1] The form of the curve with ·1 p.c. nicotine (cp. Fig. 26) suggests an action on the general muscle substance, if that is so the receptive substance is paralysed early and the response of the general muscle substance is increased.

The Gastrocnemius.

The nicotine contraction of the gastrocnemius muscle and the effect of curari on it.

In the few experiments on the gastrocnemius referred to in Part I[1], p. 366, the contraction caused by dilute nicotine began slowly to fall soon after the maximum height of contraction had been obtained. In consequence I regarded the gastrocnemius as belonging to the same class as the sartorius in its response to nicotine. Boehm in his account of the effect of nicotine (and of other substances) on the gastrocnemius of *R. esc.* gives one curve (op. cit. Fig. 4) of a somewhat similar kind to those I had obtained, but in another figure (op. cit. Fig. 3) the rise is followed by a nearly straight line. In some later experiments (Part II[2], p. 211) I obtained a plateau to the curve with a light weight, but a curve slowly falling from the maximum with a heavier weight, if the muscle was small, but still showing a plateau if the muscle was large. Nevertheless I was still inclined to consider that the fibres of the muscle respond to nicotine in the same way as the sartorius. In Part III I gave figures (Figs. 4 and 5) of the curves of contraction of the normal and degenerated gastrocnemius; in the former there was a prolonged plateau, but not in the latter.

The question of the degree of resemblance of the gastrocnemius and sartorius muscles is of no importance for my purposes, and I should not have taken further trouble in the matter but that some of the results obtained by Boehm on the curarised muscle are not, in the form they are stated, in accordance with those I have described. This is chiefly due to his not distinguishing the nicotine contraction caused by an action in the receptive substance from that caused by an action on the general muscle substance.

The response of the gastrocnemius to nicotine decreases more rapidly when left in the body after death than does that of the sartorius, and this must always be taken into account (cp. p. 237 and Fig. 36).

Boehm did not find that the form of the curve was affected by the load. No doubt this was not intended to refer to considerable differences in load, but Boehm does not mention the limits he tried. In *Rana temp.* there is a marked difference in the curve with a load of $1\frac{1}{2}$ and a load of 8 grams. In Fig. 28 it is seen that the maximum height is

[1] This *Journal*, xxxvi. 1907. [2] *Ibid.* xxxvii. 1908.

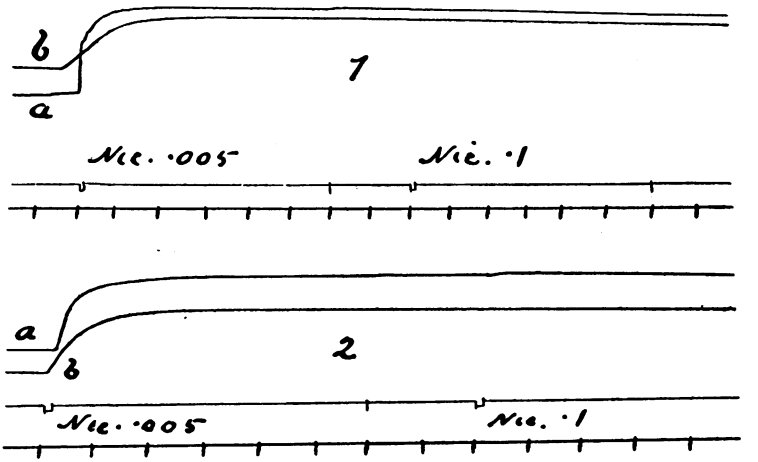

Fig. 28. ×⅔. 1. (*a*) Left gastrocnemius, load 1½ grams. (*b*) Right gastrocnemius, load 8 grams. Muscle (*a*) was cut out first.

2. A similar experiment but muscle (*b*) was cut out first.

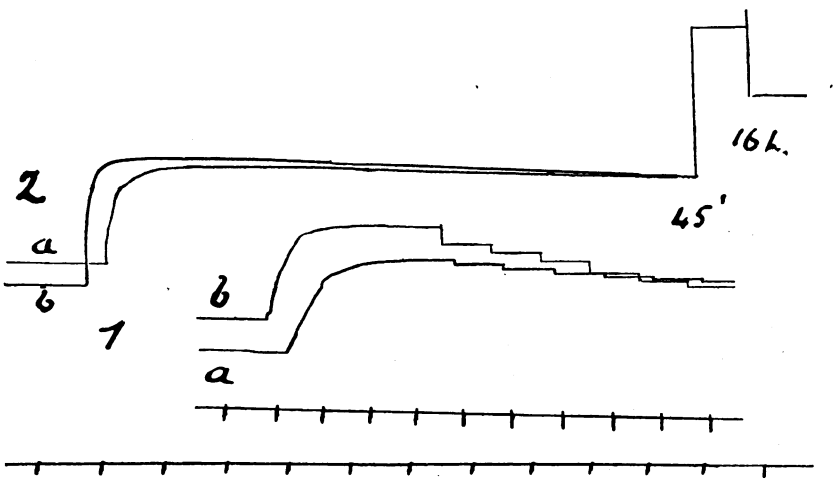

Fig. 29. Gastrocnemius. Load 5 grams. Time in ½ mins.

1. (*a*) Left muscle in nicotine ·005 p.c.
   (*b*) Right muscle in nicotine ·1 p.c.

The steps in the curve mark intervals of 10 mins. each.

2. (*a*) Left muscle in nicotine ·1 p.c.
   (*b*) Right muscle in nicotine 1 p.c.

In (*b*) the drum was stopped for 45 mins. and then for 16 hours.

Muscles (*b*) were taken out of the body after (*a*), so that the rise in (*b*) is somewhat less than normal.

more slowly attained and is less with a load of 8 grams; this load as is well known is very small compared to that which the gastrocnemius can lift. There is also a marked difference in the curves with a load of 10 grams and one of 20 grams. It is clear that in increasing the load from 0, the form of the curve will soon be affected.

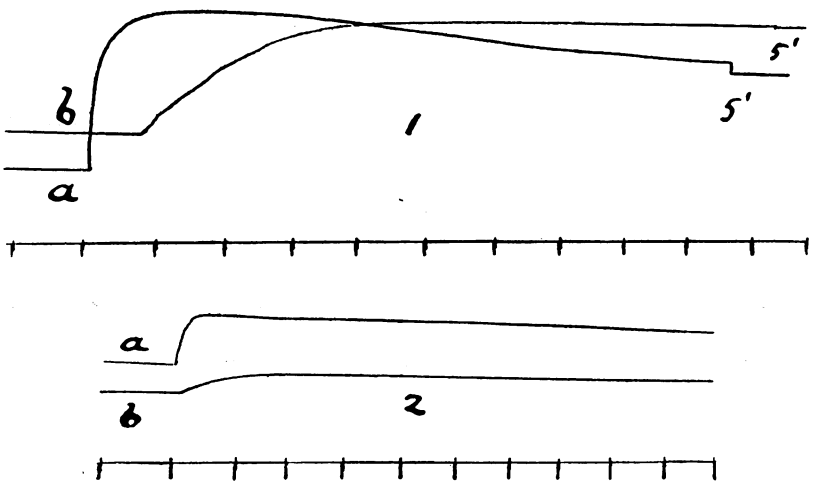

Fig. 30. Load 8 grams.

1. (*a*) Right gastrocnemius in nicotine ·1 p.c. (*b*) Left gastrocnemius in nicotine ·005 p.c. (*a*) was taken out of the body before (*b*).

2. A similar experiment on a small frog (weight 12 grams), but (*b*) was taken out of the body before (*a*).

In each case the interval between (*a*) and (*b*) was 25 minutes.

Boehm noticed that in the gastrocnemius of *R. esc.*, ·005 p.c. nicotine causes a contraction which is not much weaker than that caused by ·1 p.c. In *Rana temp.* I find that with a load of 5 grams there may be no difference in the height of contraction with ·005 and ·1 p.c., but the rate of rise is slower with the lower percentage (Fig. 29). With a load of 8 grams, the rise is much slower with ·005 p.c. than ·1 and the height is less (Fig. 30). According to Boehm the rate of contraction only varies when very dilute solutions of nicotine are used. I find that whilst with a load of 5 grams the increment in the rate of rise decreases from the weakest effective nicotine solution up to ·1 p.c.; 1 p.c. nicotine distinctly increases the rate of rise (Fig. 29) and increases somewhat the height of contraction.

It will be noticed in the curves that the sooner the maximum height is attained, *i.e.* the greater the percentage of nicotine, the sooner the

fall begins. A similar phenomenon has already been shown in the flexor carpi radialis (Part II, p. 178) and the sartorius (cp. above Fig. 3).

When the muscle is immersed in successive solutions of nicotine of increasing strength, there is little or no distinction of primary and secondary rise as there is in the sartorius (cp. Figs. 4 and 5, Part III). The rise though somewhat quicker at first, is not (with a load up to 8 grams) followed by a fall before the rigor rise. It will be noticed in Fig. 28 that ·1 p.c. nicotine after ·005 p.c. causes no rise in the curve, this indicates that the ·005 p.c. solution had paralysed the receptive substance, and that ·1 p.c. did not stimulate the general muscle substance.

Boehm states that the gastrocnemius contracts with nicotine if curari is injected into the frog, though it does not contract if the muscle is cut out and soaked in curari. I find that if ·5 c.c. of 1 p.c. curari is injected into the dorsal sac of a frog with the brain destroyed, and it is left for an hour, nicotine ·005 p.c. has no effect, but ·1 p.c. causes a slow contraction (Fig. 31). After injection of 1 c.c. of 1 p.c. curari,

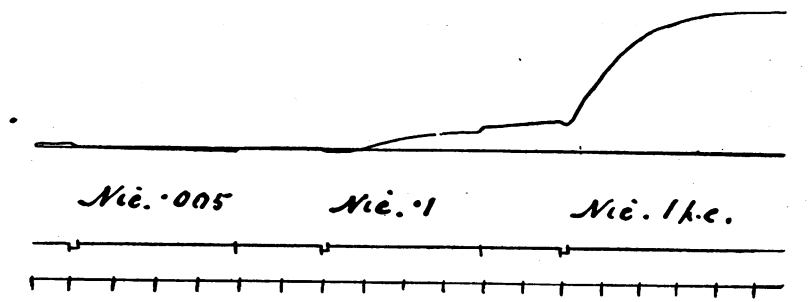

Fig. 31. × $\frac{4}{5}$. Effect of nicotine on the gastrocnemius muscle of a curarised frog. Brain destroyed, ·5 c.c. 1 p.c. curari injected into dorsal sac, muscles taken after 1 hour. Load 1½ grams.

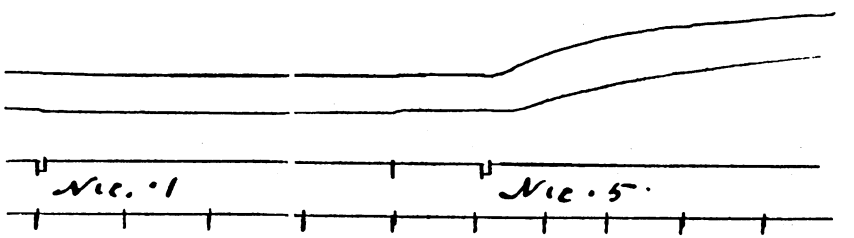

Fig. 32. Right and left gastrocnemius muscles, 2 hours after injection of 3 c.c. 1 p.c. curari. Load 1½ grams. Time in ½ mins. There is an interval of 3 mins. between the two parts of the tracing.

·1 p.c. nicotine gives a mere trace of contraction with a load of 8 grams but still gives one with a load of 1½ grams. After injection of 1 c.c. of 1 p.c. curari into the dorsal sac and of 2 c.c. into the abdominal cavity, the muscle being taken after 2 hours, ·1 p.c. nicotine has no effect, but a contraction is obtained with ·5 p.c. and with 1 p.c. (Fig 32).

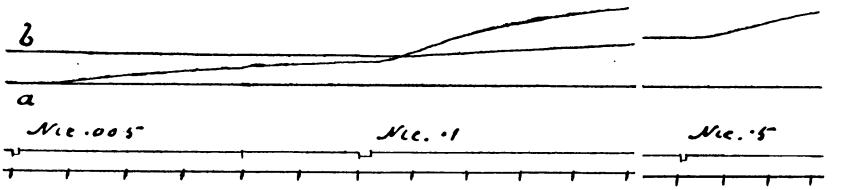

Fig. 33. ×⅔. (*a*) Left gastrocnemius in curari ·001 p.c. ½ hr. (*b*) Right gastrocnemius in curari ·01 p.c. 1 hour. Load 1½ grams. Time in ½ mins. Between the two pieces is an interval of 3 mins.

On soaking the cut out muscle in curari, the same gradual abolition of the effect of nicotine is observed. In Fig. 33 the gastrocnemius of one side (*a*) was soaked in ·001 p.c. curari for ½ hour, and the gastrocnemius of the other side (*b*) in ·01 p.c. curari for an hour. In the former case nicotine ·005 p.c. caused contraction, in the latter even ·1 p.c. had a mere trace of effect.

In another experiment the right gastrocnemius was left in ·1 p.c. curari for 15 mins. and the left gastrocnemius for 60 mins. (Fig. 34). Nicotine ·1 p.c. had no effect in either case; and the effect of ·5 and of 1 p.c. was practically the same in the two cases.

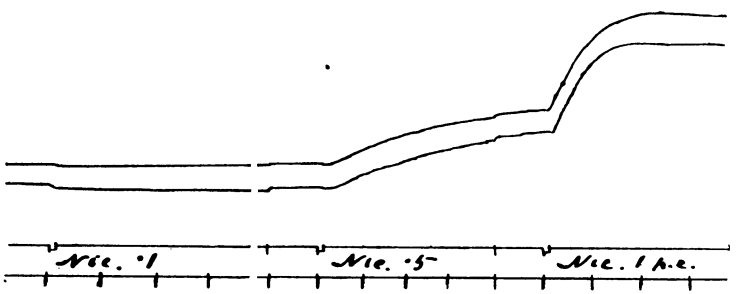

Fig. 34. ×½. Right and left gastrocnemius muscles soaked in ·1 p.c. curari (*a*) for 15 mins., (*b*) for 60 mins.; then immersed in nicotine. Load 1½ grms. Time in ½ mins.

The statement of Boehm then only holds for certain strengths of curari and nicotine. After injection of a sufficient dose of curari the gastrocnemius does not contract with nicotine up to ·1 p.c. but does

with ·5 and with 1 p.c.; and after immersion of the muscle in curari, a contraction is still obtained with ·5 and with 1 p.c. nicotine.

In the foregoing experiments, all the muscles gave the more prolonged form of curve spoken of above. It is clear that in these cases the gastrocnemius differs in type from the sartorius and more nearly resembles the coraco-radialis (cp. Part I, p. 376) and the flexor carpi radialis (cp. Part II). Its receptive substance requires a much higher percentage for complete paralysis than is the case with the sartorius. The difference in this respect in different muscles of the frog I have mentioned in my Preliminary Communication[1].

I have also pointed out that the greater the amount of curari required to paralyse the receptive substance in a muscle, the smaller the percentage of nicotine which will stimulate it. In accordance with this rule, the gastrocnemius gives a fairly good, though slow, contraction with ·0001 p.c. nicotine (Fig. 35). We have seen that in the sartorius the fibres do not all respond equally with nicotine. It is possible that

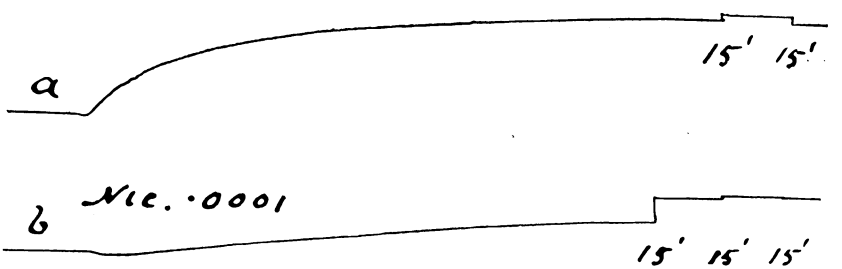

Fig. 35. (*a*) Left gastrocnemius taken out of body 7 minutes after death. (*b*) Right gastrocnemius taken out of body 1 hr. after death. Each immersed in nicotine ·0001 p.c. The steps at the end of the curve mark intervals of 15 mins. in the tracing. The time from immersing in nicotine to the first stop was 5 mins. Load 1½ grams.

in the gastrocnemius, there are a varying number of fibres of the type of the sartorius; that their presence accounts for the early partial fall which, as I have said at the beginning of this section, is sometimes observed.

Boehm found in the gastrocnemius that curari sometimes, but not constantly, increased the rate of relaxation, and he states that it is not obtained if nicotine above ·05 p.c. is used. I take it that curari always causes relaxation after nicotine, if the contraction is being maintained at the time by stimulation of the receptive substance. I have obtained a distinct fall after ·1 p.c. nicotine (Fig. 36).

[1] This *Journal*, XXXVIII. 1909 (*Proc. Physiol. Soc.* p. lxxi).

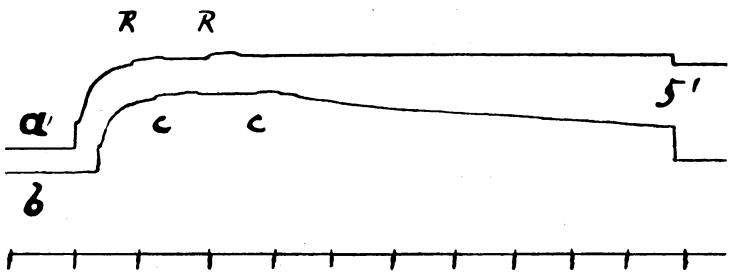

Fig. 36. Right and left gastrocnemius, (*b*) was taken 24 mins. after (*a*). The vessel containing the muscle was first filled with nicotine ·1 p.c. After 30 secs., this was run off, and Ringer's fluid poured in (*a*), and curari ·1 p.c. in (*b*). In 30 to 45 more secs. the fluid in each was poured off and renewed.

THE ABSORPTION OF NICOTINE AND CURARI BY MUSCLE AND OTHER TISSUES.

I have said earlier that the sartorius gives a slight tonic contraction with about ·001 p.c. nicotine, the flexor carpi radialis with about ·0001 p.c. and the rectus abdominis with about ·00001 p.c.[1] The effect of the nicotine in each case is prevented by a definite amount of curari. The muscles then form a series which can be used to determine the amount of nicotine or curari contained in a tissue extract provided allowance is made for any stimulating or paralysing action of the other substances in the extract. A brief account of some of the experiments I have made on these lines may be given here, though I shall treat the matter more fully at a later time.

The tissue extract was made by placing the tissue in Ringer's fluid (generally in the proportion of 1 gram of tissue to 10 c.c. of fluid), boiling, grinding—mincing if necessary—boiling again and filtering. In testing this for nicotine a portion of fibrate is dropped over the rectus abdominis muscle arranged for giving a graphic record, the fluid is collected in a watch glass placed under the muscle, and sucked up with a pipette, dropped in the muscle again and so on for 5 mins. The extract of a fresh unpoisoned tissue 1 in 10 causes a trace of contraction only. In testing it for curari a sartorius muscle is immersed in it for 15 to 30 minutes, and then a graphic record taken of the effect on the muscle of ·1 p.c. nicotine.

Proceeding in this way I find that striated muscle, unstriated muscle, liver and kidney on being placed in a nicotine solution for 15 mins. and

[1] *Proc. Physiol. Soc.* p. lxxi. 1909 (This *Journal*, xxxviii.).

then in Ringer's fluid for 30 mins. all contain an easily detectible amount of nicotine. If the tissue is boiled before being placed in nicotine, very much less is taken up.

The amount taken up increases with the percentage of nicotine in the solution in which the tissue is placed, except perhaps with very dilute solutions.

The amount taken up from a ·001 to ·01 p.c. solution is approximately the same for all the tissues tried.

The slowness of the extraction with Ringer's fluid I take to mean that the nicotine is in a state of 'adsorption.'

Curari does not prevent muscles from taking up nicotine.

Since nicotine action, as we have seen, depends more on the percentage than on the total amount, the fact that it is adsorbed by tissues generally must very greatly reduce its action on the receptive substance, and so protect the specially sensitive tissues.

I have made fewer observations on curari but the general results have been the same, though various tissues not known to be specially acted on by curari apparently took up more curari than either the sartorius or the rectus abdominis.

Summary and Conclusions.

Unless otherwise mentioned the results were obtained on the sartorius muscle.

The minimal percentage of nicotine which causes twitchings and slow tonic contraction is about ·001 p.c. at 13°—17° C.; the percentage decreases as the temperature increases.

The height of the tonic contraction increases with increase of percentage from ·001 to ·1 p.c. nicotine; the difference in height is but slight in passing from ·1 to ·5 p.c., but is large in passing from ·5 to 1 p.c.

Other things being equal, the quicker the maximum height is attained, the sooner the fall sets in; with ·5 to 1 p.c. nicotine the fall soon passes into the slow rise of rigor, the curve of rigor when established is convex to the abscissa. The rise depends upon continuous immersion in nicotine, and a slow fall is obtained by transferring the muscle to Ringer's fluid.

Dilute nicotine decreases the effect of a stronger solution; after brief action of ·01 p.c. or longer action of ·001 p.c., ·1 p.c. nicotine has no

immediate effect and the immediate effect of 1 p.c. is much reduced. Nicotine ·0001 p.c. has a very slow paralysing action.

Nicotine ·01—·1 p.c. in time causes a slow rigor rise, the time depending greatly on the temperature. Sometimes, apparently, at 22°—24° C., ·1 p.c. nicotine causes a slight immediate rise by an action on general muscle substance, and this slowly passes into the rigor rise. Local contraction can be obtained by strong electrical stimuli in a late stage of the rigor rise.

The muscle fibres differ greatly in their vitality and they differ also in their reaction to nicotine; the fibres of the inner surface give a stronger tonic contraction than those of the outer surface, and the rigor contraction is greater in them.

The salts contained in a 1 p.c. solution of curari, acting on the muscle for 30 mins., do not prevent the effect of ·1 p.c. nicotine, but they have some action since they reduce the effect of ·01 p.c. nicotine. The salts contained in a ·25 p.c. solution of curari have in 30 mins. no appreciable effect. Curari ·25 to 1 p.c. gradually reduces in proportion to the percentage the electrical excitability of the muscle.

On immersing the muscle in curari for 15 mins. and then in nicotine it is found that a much less percentage of curari than ·0001 will prevent the effect of ·001 p.c. nicotine; as the percentage of curari is increased, a higher percentage of nicotine is required to cause contraction. It is on a similar relation of the amount of paralysing poison to the amount of stimulating poison that most physiological antagonisms obtained in the body rest. From a comparison of the effects of curari and nicotine on the different muscles of the frog, we may infer that the range of physiological antagonism between any stimulating and paralysing poison is greater the greater the responsiveness of the receptive substance to the stimulating poison.

After a certain percentage of curari, evidence of antagonistic action is less obvious. Thus after the sartorius has been soaked for 15 mins. in ·001 p.c. curari, ·1 p.c. nicotine has no immediate effect upon the receptive substance, nor does ·5 p.c. appear to have any. But so far as the experiments go, 1 p.c. has a slight immediate effect. This I take to be due to the substitution of nicotine for curari in the combination formed with the receptive substance. Below ·001 p.c. curari there is evidence that a slow substitution takes place.

The paralysis by curari of the twitchings and of the tonic contraction caused by nicotine does not necessarily occur at the same time.

The time taken to paralyse the receptive substance to ·1 p.c. nicotine rapidly increases with percentages below ·0001.

The curari paralysis diminishes on leaving the muscle in Ringer's fluid, but does not disappear in a day. The rate of disappearance decreases as the percentage of curari increases.

No evidence was obtained of an action of curari up to about ·1 p.c. in 15 mins. on the general muscle substance, but on longer stay in ·1 p.c. or in stronger solutions, the primary rise with ·5 to 1 p.c. nicotine increases, there is a slight primary effect with ·1 p.c., and the secondary rise is slower.

Curari given after nicotine stops the stimulating action of the latter, but has little if any effect upon the tonic contraction caused by the previous stimulus, *i.e.* it paralyses the receptive substance but has little if any effect on the general muscle substance whether this is at rest or contracted.

Curari ·001 p.c. was not found to have any certain effect on the slow loss of irritability caused by ·1 p.c. nicotine.

When mixtures of curari and nicotine are poured over a muscle, a slight contraction is obtained with ·1 p.c. nicotine in the presence of ·2 p.c. curari, and with ·01 p.c. nicotine in the presence of about ·02 p.c. curari.

On injecting curari into a frog, the effect of nicotine on the twitching ceases to be obtainable at about the time when contraction can no longer be obtained by nerve stimulation; but sometimes the former slightly outlasts the latter. The time at which tonic contraction ceases to be obtainable varies in different muscles; in the sartorius it is approximately the same as that at which paralysis of the nerve occurs, though usually it is a little later; in the flexor carpi radialis, the rectus abdominis and the gastrocnemius it is considerably later.

NaCl ·6 p.c. greatly increases the twitchings caused by nicotine, especially ·001 p.c.; no evidence was obtained that it increases the tonic contraction. Previous immersion for 30 mins. in ·1 p.c. nicotine does not prevent the occurrence of twitches in NaCl ·6, but it decreases them. KCl ·5 p.c. in Ringer's fluid soon prevents nicotine ·1 p.c. from causing contraction. A similar effect is produced by a strong solution of the salts of muscle. $CaCl_2$ ·5 p.c. in Ringer's fluid also prevents the action of ·1 p.c. nicotine, but more slowly.

$MgCl_2$ ·5 p.c. in Ringer's fluid does not in an hour prevent the effect of ·1 p.c. nicotine, though it considerably reduces the electrical irritability.

In the gastrocnemius muscle the general results are the same as in the sartorius, but the muscle responds to a much smaller percentage of nicotine, and its contraction is more prolonged. Since the duration of the plateau varies in different frogs, there is probably in the muscle a varying admixture of different kinds of muscle fibres.

Nicotine is adsorbed by all tissues; the amount taken up increases with the percentage in the fluid in which the muscle is placed. In the body this probably tends to decrease the poisonous action. Curari does not prevent the adsorption, and so far as the experiments go, curari is similarly adsorbed.

Nicotine ·25 to 1 p.c., after the receptive substance has been paralysed[1], frequently gives in the sartorius a curve in which there is a fall after the primary rise (cp. Fig. 23 *b*). Both the rise and the subsequent fall indicate an action on some special molecules in the general muscle substance, different from those which are acted on in producing the secondary (rigor) rise. I have previously suggested that the receptive radicle is present but in more stable chemical combination throughout the general muscle substance. The fact mentioned above favours this suggestion, on the other hand I have not found that curari within wide limits has any influence on the substance which gives the primary rise with 1 p.c. nicotine.

The experiments I think tend to support the theory that nicotine and curari form dissociable compounds with the receptive substance. The chief results may be stated in the terms of this theory:

The height of the contraction caused by the combination of nicotine with the whole of the receptive substance depends upon the rate of combination and on the duration of the resulting contraction; this dependence is chiefly, if not altogether, due to the degree of summation of contraction in all the muscle molecules. The saturation point is quickly reached in the sartorius with ·01 p.c. nicotine and then ·1 p.c nicotine has no effect. If combination is slow enough, and the duration of contraction in each muscle molecule brief enough, complete saturation of the receptive substance may occur without any visible contraction.

When the receptive substance is saturated with curari, and in some cases before complete saturation, nerve impulses cannot affect it.

The substitution of nicotine for curari in a curarised muscle is delayed by adsorbed curari.

[1] After the receptive substance has been paralysed by curari, 1 p.c. may cause some contraction by turning out the curari and stimulating the receptive substance. There would naturally be some fall after this contraction.

There appears to be a lower limit to the concentration of nicotine and of curari, which can saturate the receptive substance; this implies that the latter is combined with something of very weak affinity for it, but of just sufficient affinity to prevent poisons below a certain concentration from combining with it. The most probable combination of the receptive substance is with an ion, or a molecule, of neutral salt. On this view the action of poisons depends not only upon their relative affinities for the receptive substance, and on their relative concentration, but also on the relative affinity and concentration of certain ions or salt molecules.

CRITICAL REMARKS.

In the preceding account I have taken it for granted that nicotine and curari act on the same substance because it seems to me that no other explanation can be given of the facts. It is true that Magnus[1] a short time ago argued that the phenomena of mutual antagonism gives no basis for drawing any conclusions as to the point of action of poisons. But Magnus deals only with the theory that the specific action of poisons is one on the nerve endings, he does not consider my theory of the presence of more than one receptive substance in the cell. I need then only point out that if his argument is just, it affords a reason in addition for rejecting the theory of the specific nature of nerve endings.

Experiments on the fowl have been made by Edmunds and Roth. When their experiments were the same as mine they obtained the same results except that in long denervated muscles they obtained, in every case but one, less relaxation of nicotine contraction by injecting curari. They tried much more frequently than I did the effect of curari after nicotine and they found that from about the third day onwards curari had (with the one exception mentioned above) a mere trace of effect on the nicotine contraction. Their conclusion from this is that curari does not antagonise the action of nicotine in the denervated muscle. This conclusion is different from mine, which was that curari antagonised nicotine in the denervated as well as in the normal muscle, though a larger amount of curari was required. Edmunds and Roth quote two of my experiments as being in favour of their view. They have however overlooked what these experiments were designed to test and what they in fact show. I had pointed out that there were three ways of testing the antagonism of curari for nicotine.

[1] *Pflüger's Arch.* CXXIII. p. 99. 1908.

(1) By giving curari primarily and seeing whether a normally effective dose of nicotine had its normal effect.

(2) By interpolating an injection of curari between two doses of nicotine, the second being given at a sufficient interval after the first to ensure that without curari it would produce an effect nearly if not quite as large as the first.

(3) By giving curari after nicotine.

These experiments of mine which are referred to by Edmunds and Roth were made on the second plan, not as supposed by them on the third. The experiments clearly showed that the injecting of curari greatly reduced the effect of the second dose of nicotine, *i.e.* that curari antagonised the action of nicotine. This result, combined with the slight effect obtained by plan 3, showed, though I did not appreciate it till later, that curari whilst preventing the stimulating action of nicotine does not, or only to a slight extent, undo the effects of the stimulus when this has been delivered.

The difference in the behaviour of the normal and of the denervated muscle as regards the amount of curari required to antagonise the nicotine action, I attributed to an increased irritability of the muscle to nicotine. This view Edmunds and Roth support, and give further evidence that the irritability is in fact increased. But the decreased effect of curari *after* nicotine in the denervated muscle is due in part to another factor, viz. a decrease in what may be called the relaxing power of the general muscle substance. An increase in contracture is a known feature of atrophying muscle.

Edmunds and Roth find in the denervated muscle a similar antagonism between curari and physostigmine on the muscles of the fowl to that which exists between curari and nicotine, adding thus another instance to the list of those which show that the action of poisons supposed to act specially on nerve endings is fundamentally the same whether nerve endings are normal or degenerated. The parallelism which exists in the behaviour of the muscles of fowl, and the muscles of the frog which respond to nicotine, shows I think definitely that in the former as in the latter the action of curari, nicotine and physostigmine is not on the general muscle substance but on the receptive substance.

Whilst speaking of Edmund and Roth's experiments I may note that they refer to Heidenhain[1] as if he had advocated the view that nicotine and curari do not act in motor nerve endings in the tongue

[1] Heidenhain. *Arch. f.* (*Anat. u.*) *Physiol.* 1883. Supp. p. 133.

muscles but on the 'sole' of the nerve endings. Heidenhain gave this view, as one of two alternatives[1], to account for the persistence of nicotine and curari action on the tongue muscles, after section of the hypoglossal nerve. The explanation of the phenomena observed after section of the hypoglossal nerve was at that time and to a certain extent still is obscure. But on the theory I have given, the phenomena are readily accounted for. I take the spontaneous twitchings which occur shortly after section of the hypoglossal as due to chemical stimulation of the receptive substance increased in excitability by nerve section, and the continuance of the nicotine and curari action as another instance that these substances act on the receptive substance and not on nerve endings.

Latterly some observers have suggested that variation in the permeability of the cell, or of the solvent power of its limiting layer (on the lines of Overton's and H. Meyer's results), may account for the specific action of poisons. The suggestions are not sufficiently formulated to make it worth while to consider them. But one definite suggestion has been made by Straub[1] in relation to the action of muscarine and atropine on the heart, and this it is necessary to discuss.

Straub finds in Aplysia that muscarine is stored up in the heart muscle; that a certain percentage in the outside fluid is required to produce inhibitory change; that if the muscarine in the surrounding fluid be removed, the heart at once begins to beat, and that after a certain amount has been absorbed the heart beats spontaneously and is unaffected by muscarine whatever quantity the fluid surrounding it contains. He suggests that inhibition is caused by the physical process of the passage of muscarine through the limiting layer of the cell and that when it has passed this layer it cannot cause inhibition. But Straub himself finds that this does not necessarily hold in the case of the frog's heart, inasmuch as inhibition may continue when he thinks absorption must have ceased. It is most unlikely that the laws of inhibition are different in these two cases, so that if Straub's conclusion for the frog is correct, a theory which is based on the assumption that inhibition is caused by absorption must be inadequate. On the chemical theory the muscarine must pass into the cell before it can combine, so that in general the inhibition will be produced during absorption, but on the chemical theory the inhibition is not necessarily restricted to the time

[1] Heidenhain says "Ohne für jetzt zwischen beiden Möglichkeiten, die eine kritische Erwägung der Thatsachen im Auge zu behalten hat, entscheiden zu wollen...." *Op. cit.* p. 166.

during which absorption takes plate, *i.e.* it is more in accordance with the facts so far as they are known. Straub as mentioned showed that in aplysia, the heart after absorbing a certain amount of muscarine was no longer affected although the percentage of muscarine in contact with the heart was greatly increased. If as he supposes, the absorption is due to a concentration difference inside and outside the cell, there seems no reason why a stage should be reached, apart from damage to the cell, at which this difference should cease to act. The aplysian heart beats normally at the stage at which concentration difference is supposed to have no effect. On the chemical theory the result can be readily explained, the receptive substance sets up a stimulus by combination; when it is saturated further muscarine has of course no effect.

Straub does not explain the mechanism by which absorption causes inhibition, nor why strychnine, which he finds is absorbed, does not cause inhibition. There are also other difficulties when the hypothesis is extended to meet other cases, such as the frequent paralysis of nerve effect by stimulating poisons, and the fact that the same substance, for example adrenalin, sometimes causes contraction and sometimes inhibition within the same class of tissue cells.

Straub has also dealt with the question of the mutual antagonism of poisons. He found in the Selachian heart that atropine though it did not prevent muscarine from being absorbed, delayed the rate of absorption. He suggests that atropine alters the limiting membrane, so that it is a worse solvent for muscarine and thus reduces the rate of muscarine absorption below the threshold velocity. An objection may be made to this view on the ground of the experiment itself, the reduction which atropine caused in the amount of muscarine absorbed was not great, and it seems to me insufficient to account for the total absence of inhibitory action. However this may be, the result is much more naturally accounted for by the hypothesis that atropine combines with the receptive substance and in consequence prevents muscarine from having any effect.

In the case of nicotine and curari Straub's hypothesis is I think inconsistent with the phenomena. In the sartorius muscle of the frog nicotine ·005 to ·1 p.c. causes a slow contraction when applied to the neural region of the muscle fibre but not when applied elsewhere. This, as we have seen, is an effect on the cell and not on the nerve ending. Nicotine ·25 to ·5 p.c. causes contraction in other parts of the muscle, but the contraction is of much less intensity than that caused by more dilute nicotine in the neural region and so can be distinguished from it. Curari ·001 p.c. prevents a contraction being obtained by nicotine ·1 p.c. but

curari ·25 p.c. does not appreciably affect the contraction caused by nicotine ·5 p.c. in the non-neural region. If Straub's hypothesis is applied to this case it follows that ·001 p.c. curari so alters the limiting layer of the muscle fibre that nicotine ·1 p.c. is practically unabsorbed, and yet that it alters it so little that ·5 p.c. nicotine is absorbed at a rate indistinguishable from normal. It might perhaps be said that the limiting layer is different in the neural and non-neural region, but this would be no way out of the difficulty. For after curari ·001, nicotine ·25 to ·5 p.c. produces at least as great a contraction in the neural region as it produces elsewhere, and this contraction is not diminished by increasing the curari up to ·25 p.c.

Another hypothesis has recently been put forward by Dixon and Hamill as far as regards the action of vegetable drugs. They suggest that vegetable drugs set free a hormone which combines with a receptive substance in the cell. In two essential points, viz. the existence of a receptive substance and the production of activity by a chemical combination with the receptive substance, this view is the same as mine. It differs in that the poison instead of itself combining with the receptive substance sets free from something else another body—an organic body comparable to secretin and adrenalin—which combines with the receptive substance. They consider that the same hormone is set free by nerve stimuli.

We may consider first the reasons they allege against the theory that vegetable drugs combine directly. The reasons are two (1) that strychnine when ground up with spinal cord can be extracted from the emulsion. This is directed against an argument used by Ehrlich in the case of toxin receptors, but it obviously has no force against the view I have given above, any more than the fact that oxygen can be extracted from oxy-hæmoglobin is evidence that oxygen does not combine with hæmoglobin. (2) The second is that a spinal cord emulsion has no specific effect in diminishing the physiological action of strychnine. This is based on their observation that a spinal cord emulsion does not diminish the lethal dose of strychnine more than it is diminished by other colloids. The explanation I think is that all colloids adsorb poisons, and that in the emulsion the adsorbed part is much larger than the chemically combined, so that the reduction of lethal action is approximately equal with a variety of colloids. A delicate test is then required to detect the difference due to chemical combination, and the injection of a given quantity per kilogram of body weight into different animals can hardly be called a delicate method.

Further the argument assumes that the receptive substance can combine after death. It is possible that the receptive substance becomes altered on the death of the cell so that it no longer combines with poisons.

The hypothesis of Dixon and Hamill is more complicated than that which I have given, since it requires the presence of an additional substance in the cell capable of liberating a hormone. And it is less complete since it does not explain how the hormone is liberated. They say that drugs 'like enzymes and catalytic agents take no part of a chemical nature in the ultimate changes,' but they do not say that drugs are catalytic agents. A theory based on catalysis (though it would be simpler without the supposition of hormones) might find some support from the adsorption phenomena I have described; but it has grave difficulties, chief amongst them perhaps that of attributing an anti-catalytic agency to curari and other similar poisons.

On Dixon and Hamill's hypothesis adrenalin and the active substance of other internal secretions correspond to the primary products caused by nerve impulses, and not, as generally held, to the final products. It may fairly be argued that if in glands and muscle, the primary products are produced by nerve impulses, there would be a specially developed nerve supply in those tissues in which the formation of such products are specially developed. In fact there is no clear evidence that the glands of internal secretion are influenced by nerve impulses at all.

Dixon and Hamill's view of the method of action of secretin also differs from mine. They find that secretin combines chemically with a pro-zymogen in the pancreas and converts it either into zymogen or into enzyme, and they consider that in doing this it sets up secretion. It seems to me unlikely that secretion is caused by this process, and more probable that it is due to the combination of secretin with a receptive substance (an atom-group) of the living protoplasm.

In this paper I have not discussed my hypothesis that the receptive substance is an atom-group of the protoplasm, but I may say a word or two about it.

I assume that all protoplasm has a number of atom-groups which can combine with this or that chemical substance. The degree to which these are essential parts of the molecule varies, but two groups may be distinguished.

In one group the linkage of the atom-groups is such that they can be combined so as to be thrown out of gear (and probably split off), without injury to the rest of the molecule. Their chemical combination may however lead to those changes in the whole molecule normally associated with its function. These are the receptive atom-groups. In less differentiated cells the existence of such atom-groups is indicated by the formation of anti-bodies. In more differentiated cells, a special

development of receptive atom-groups takes place and it is in these that secretin, adrenalin, curari, nicotine etc. act. When a nerve fibre ends in connection with the cell, the receptive atom-groups are in some cases, and perhaps in all, more or less localised in the region of the nerve endings.

In the other group, the linkage of the atom-groups is such that they cannot be split off or combined without causing serious alteration in the whole molecule. These are essential to the proper working of the mechanism, and they may be called the fundamental atom-groups.

Whilst we can refer a large number of chemical actions to one or other of these two groups, there are some, for example the action of salts and ions on the general muscle substance, which cannot at present be definitely referred to either, and some, for example that of digitalis on the heart, in which the combination seems to aid the mechanism.

This hypothesis is an extension of Ehrlich's side chain hypothesis, though it has been reached by entirely different experiments and by a different line of argument. In 1906 I suggested that the action of drugs might be referred to a combination with side chain radicles. At that time Ehrlich had not brought these bodies into his scheme. Since then he has done so. The evidence he gives is based on the action of various arsenic compounds on the trypanosomes of sleeping sickness. The particular protoplasmic groups which combine with chemical bodies of the drug type he calls chemo-receptors. It will be noticed from what I have said above that the chemo-receptors of Ehrlich include atom-groups which do not belong to my receptive class. For the combination of arsenic compounds with the chemo-receptors of the trypanosomes leads to the destruction of the organism, *i.e.* the atom-groups acted on are fundamental and not receptive.