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Effects of Drugs on Frog's Ventricle

by

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OF THE INFLUENCE OF CERTAIN DRUGS ON THE PERIOD OF DIMINISHED EXCITABILITY.

By SYDNEY RINGER, M.D., and HARRINGTON SAINSBURY, M.D. Plate XXI.

In a series of experiments on the comparative effect of certain of the salts of potassium, sodium and ammonium on the activity of the Frog's ventricle, the results of which were given in a paper read before the Medico-Chirurgical Society, June 13, 1882, the effect of continuous faradization applied to the ventricle was described shortly. This effect was much modified by potassium hydrate and potassium salts generally, so much so, and so distinctively that the modification was quite characteristic of these salts.

The effect of continuous faradization applied to the undrugged ventricle is, it will be remembered, to cause what appears to be a blending or fusion of neighbouring contractions. A necessary provision for this is that the intensity of the current and the frequency of interruption be sufficiently great; these being given, the above-mentioned fusion occurs and constitutes continuous spasm. The completeness of this fusion is dependent, caet. par., on the intensity of the current.

Given such fusion, in response to continuous faradization, potassium salts, thrown into the circulation, gradually lessen the fusion and, as the dose increases, remove it completely; finally in the later stages the effect is as follows:—even though at the time spontaneous beats of good value and of good frequency are happening continuous faradization abolishes them completely so long as it is applied; on its cessation spontaneous beats after a longer or shorter interval recur. This result may be reproduced again and again, it is quite definite and constant.

It was toward the elucidation of at least one part of the potash action, viz. its lessening of the above-mentioned fusion, that the present experiments were undertaken. M. Marey[1] it will be remembered

[1] *Physiologie Expérimentale*, Vol. II. p. 81, 1876.

applied the term "tetanus" to the fusion resulting from a rapid succession of electric stimuli thrown into the ventricle. The explanation given by him of this phenomenon is involved in the present subject, it may therefore with advantage be restated. Marey, in the first place, shewed that the excitability of the heart was not the same during the whole period of a beat, i.e., during the period comprising both systole and diastole. Thus he demonstrated that a minimal stimulus applied during systole was ineffectual whilst the same applied during diastole was responded to by contraction. The period of diminished excitability he named the "refractory phase," and he shewed that its duration was dependent on the strength of the stimulus applied during the same; the stronger the stimulus, the less the duration, till, with sufficient strength of stimulus the "refractory phase" was completely abolished. Next Marey shewed that a rapid succession of minimal stimuli applied to the heart were responded to by a very much smaller number of contractions, these latter being quite discrete, but that, as the intensity of the stimuli was increased, the rapidity of succession being maintained, the number of contractions increased till finally adjacent contractions overlapped each other and fusion more or less complete obtained. The conclusion drawn was that of the close series of weak (minimal) stimuli the greater number fell during refractory periods and therefore were ineffectual, but that, as with growing strength of stimulus the refractory periods lessened, the number of ineffectual stimuli lessened pari passu, and hence in the final stages the fusion arose. Underlying fusion then we should have, according to this, "diminution" of the refractory period, and the question now to be put is, have we, underlying the potash effect, which shews us the reverse of the above, viz. the undoing of fusion, a "lengthening out" of the refractory period? In addition, the present series of experiments includes the influence of the bases sodium and ammonium; these were examined in like manner to the potassium base.

Dr Burdon-Sanderson very kindly superintended the arrangement of an apparatus whereby break shocks alone might be used to stimulate the frog's heart. The chronograph was placed in the primary circuit, whilst, by means of a metronome, the pendulum of which carried a wire which was made to dip into a pool of mercury, the primary was short circuited once for every to and fro movement of the pendulum; the moments of make and break were thus recorded by the chronograph. By means of the hammer of the induction apparatus, employed as a magnet key in the secondary circuit, the make shocks were cut off.

Hence the moment of break recorded on the cylinder alone corresponded to the moment of stimulation.

Such an apparatus then records accurately the moment of stimulation, at the same time it allows us, by means of the metronome, to regulate at will the interval between two successive stimuli.

To avoid criticism it may be here stated that the exact measurement of changes in duration was not attempted, the question which it was sought to answer was whether shortening or lengthening of certain periods did occur; this the apparatus here described enabled one definitively to determine.

Since lengthening or shortening, rather than by exactly how much the one or other obtained, was that which concerned us, it was sufficient to note the position of the indicator on the stem instead of taking the actual number of beats as marked on the metronome scale for each position of the indicator, or indeed instead of timing the rate accurately by the watch for each such position. Hence metronome 1, i.e. with the indicator at the topmost mark, indicated the slowest rate, metronome 32 the quickest rate. This range in the positions of the indicator from 1 to 32 gave a considerable range in rate, amounting approximately to a multiplication of the rate by two, or a little in excess of this.

For the rest the apparatus used consisted of a Roy's tonometer and revolving drum. The heart was fed with a mixture of dried bullock's blood dissolved in water and further diluted with two and a half times its volume of saline solution 0·75 per cent. Of this mixture 100 c.c. were used in each experiment.

The common English frog (*R. temporaria*) was throughout employed.

It was important in these experiments that the heart should not beat spontaneously or at any rate that the spontaneous beats should be infrequent, the ligature was hence tied low so as to include part of the ventricle, the upper third where possible.

The experiments were made during the months of June, August and September, 1882.

The mode of experimentation was as follows:—the metronome being set at some given rate, the cylinder was started and two successive stimuli thrown into the heart. The result might be a single contraction or two contractions; if two contractions, these might be quite separate, the diastole of the first contraction being completed before the second systole commenced, or there might be fusion more or less complete. If but one contraction resulted the metronome was set at a slower rate, if two discrete or but partially fused contractions it was set at a quicker

rate. In this way the earliest point at which a second stimulus was effective was found. As a rule such earliest stimulation gave fusion complete or all but complete. (See Figs. *A*, *B*, *C*, Trace I.)

An arbitrary but fixed position of the secondary coil was maintained during the above determination.

The length of the "period of diminished excitability"[1] was thus obtained and was seen to comprise:

1. The latent period.
2. A certain portion of the "beat" itself, i.e., of that time during which the heart is undergoing visible change of form.

The position of the second stimulus on the contraction curve enables one to determine the duration of this second element.

Note that the essential in the present mode of experimentation consists in the application of the stimuli in pairs and the ability to regulate at will the length of the interval separating the stimuli of each couple.

The word excitability has in general been employed solely with reference to strength of stimulus; *diminished* and *increased* excitability implying corresponding increase and diminution in the strength of the stimulus adequate to produce a given effect. The use of the term may with advantage be extended and made to refer to the length of the "period of diminished excitability," the strength of the stimulus in this case remaining constant. In this specialized sense the statement that the excitability has been *diminished* will signify that the "period of diminished excitability" has been lengthened out, the reverse, of course, obtaining if the word *increased* be the qualifying term. The repetition of a somewhat lengthy phrase is thus avoided at the same time that there is no likelihood of confusion arising. The word excitability when made to refer to *strength* of stimulus implies a 'prepared' state of the excitable tissue, the stimuli being separated by intervals of sufficient length to permit of recovery from the effects of the one before application of the other; hence an antecedent effective stimulus has no special signification here. When however the word excitability is made to refer to the length of the "period of diminished excitability" the antecedent effective stimulus is an essential element, for the word excitability now refers to an *unprepared state* of the tissue, the result of the preceding activity, and it implies an interval for preparation. If then a longer

[1] The term "period of diminished excitability" is employed in a paper on "the time relations of the excitatory process in the ventricle of the frog's heart," by J. B. Sanderson, M.D., F.R.S., and F. J. M. Page, B.Sc., F.C.S., *Journal of Physiol.* Vol. II. Nos. 5 and 6.

interval is needed by the heart to recover its excitability, this latter is fitly described as diminished; in like manner if a shorter interval is requisite the excitability may be described as increased. Before experimenting with drugs it was necessary to determine what, if any, variation occurred normally as a result simply of the duration of the experiment and what influence preceding electric stimulation itself had on the excitability as here tested. This being determined it was possible to examine the modifying effect of drugs on the "period of diminished excitability," as a whole, and on its constituent elements corresponding to "Period of Latency" and more or less of the "Period of Active Change."

The first experiments on this point shewed that very considerable variation in the "period of diminished excitability" occurred during the experiment, even though this were not much protracted, as a rule not beyond the hour. (It must be remembered that we are throughout dealing with a heart ligatured low.) Thus, to take an example, the metronome would need to be placed say at 1, i.e., at the slowest rate, in order to give the second stimulus effective, at any quicker rate the second stimulus falling within the "refractory period" and being ineffectual; before the end of the experiment it was on several occasions possible to get the second stimulus effective with the metronome at 15. Indeed even with metronome 1 *in*effectual at the start this extent of range was obtained on more than one occasion.

As to the cause of this variation in excitability it soon appeared that whilst, as a rule, an interval of rest would shew diminution in excitability, *given such change,* stimulation would invariably increase the excitability. Thus testing the excitability, then throwing in a number of stimuli in quick succession, and again testing the excitability, this latter was found to have increased; by a repetition of this process it was possible to advance the indicator, division by division, down the scale. This result came out very definitely, clearly establishing as a fact that *stimulation increases the excitability of the ventricle,* i.e. lessens the period of diminished excitability. The same result became apparent in yet other ways, e.g., it has been stated that the ligature was placed low, a portion of the ventricle being almost invariably included within the ligature, still it was not always possible to inhibit the heart, and in some cases groups of beats and intervals of rest would alternate, i.e. phasic action occurred; in these cases the same effect as to excitability was observed to result from these spontaneous beats as occurred with artificial stimulation, viz., the heart after any such phase was found to be more excitable than before it.

Again, the heart would sometimes beat at a sufficiently slow rate to allow of successive stimulation in the intervals, it was then seen that the nearer to the last spontaneous beat one was able to place two successive stimuli, the more excitable did the heart appear, e.g., the metronome at a given figure would just fail to allow of the second stimulus being effective when no care was taken as to the position of the stimuli; when however care was taken to place these as soon as possible after a spontaneous beat, then, without altering the metronome figure, the second stimulus became effective. This result came out very constantly.

Obviously we are dealing here with one and the same phenomenon—if electric stimuli increase the excitability of the cardiac muscle, it is more than likely that natural stimuli will have the same effect, whilst the last point as to proximity to a preceding contraction normally or artificially excited is but a means of demonstrating the phenomenon in question.

Examined further, this phenomenon of lessening of the "period of diminished excitability" was found to be coincident with a lessening of both the "period of latency" and of the "whole duration of the beat" —this result came out unequivocally. The key to the lessening of the "period of diminished excitability" is at once obtained, for this period is relative to the changes latent and visible attending a contraction; if caet. par. both of these changes be shortened in duration the "period of diminished excitability" constituted from both of these must itself be shortened.

Thus then we have as the effect of *stimulation*, lessening of the "period of diminished excitability" as a whole, whilst this effect analyzed into its constituent elements corresponds to a lessening of

1. The Latent Period.
2. The period of active contraction.

It may be asked, does the second element just mentioned, viz., shortening of the *whole* period of visible active change, necessarily involve shortening of the period of diminished excitability; for this *whole* period includes systole and diastole, and shortening of either of these would shorten the whole, whilst the state of diminished excitability stands in relation to the systolic element nearly exclusively. This is true, but the shortening of the duration of the contraction curve resulting from stimulation did not appear to be at the expense of one element more than the other; hence shortening of the *whole* period would involve shortening of the refractory period.

The modifying effect of drugs may now be examined. *Chloride of Potassium* will be first considered. The method of examination was as follows:—the metronome was adjusted to the quickest rate giving the second stimulus uniformly effective, this being found by trial, then an interval of three, four, or five minutes was allowed to pass and the excitability was again tested, the metronome being stationary at the above found figure. Supposing the second stimulus still to be effective this process was repeated, a second, and a third time. Then the drug was added and at the end of a fourth interval of equal duration with the others the excitability was again tested. Thence on through the experiment this method of procedure was maintained. The necessity for this mode of proceeding was that it is impossible to avoid intervals of rest coming in here and there, thus the addition of the drug takes a certain time and then it has to reach the heart, this last with a slow circulation may take from one and a half to two or two and a half minutes, intervals sufficient in some hearts to produce considerable effect, for in some cases the excitability falls quickly on ceasing to stimulate. The above method of examination, in which a definite time interval is fixed on and maintained, obviates the source of error which time introduces, for the kind of change and rate of change due to rest is thus determinable, what remains over and above must be the "Drug effect."

Examined thus it was found that Chloride of Potassium *increased* the "period of diminished excitability"; this result came out uniformly. A moderate dose, it is true, did not always increase this "period" at once, e.g., 0·2 c.c. of a 10 per cent. solution of the salt, and in one or two instances this dose did not produce the effect at all. An increased dose, e.g., 0·4 c.c. of the same solution, given at once, always brought the effect out speedily and strongly.

Examined further, it was found that the "period of Latency" was always increased as an effect of the potassium salt, and further that, as a rule, the duration of the period of visible change, i.e., the breadth of the contraction curve, was shortened.

It is obvious that these two results are antagonistic so far as the "whole period of diminished excitability" is concerned, for, whilst the increased period of latency will, caet. par., pitch the second stimulus early on the contraction curve, the diminished duration of the contraction itself will have an opposite effect. In the slighter degrees of toxic action, it may be one would need to consider both of these elements in the result obtained, but when a well-marked toxic effect has been produced

one has no need to balance the above two in order to determine whether or no the diminution in excitability is apparent or real; that it is real, and that we have to deal with a diminished excitability of the cardiac tissue which can not be explained by the position of the second stimulus on the contraction curve is certain, for we may increase the interval between the stimuli to the extent even of throwing the second stimulus after completion of diastole and yet this stimulus remains ineffective. See Figs. *A, B, C, D, E, F,* Trace II.

Potassium chloride then increases the "period of diminished excitability" as a whole, and it does so by increasing both constituents of this period, viz.,

1. The Period of Latency.
2. The Refractory period attending the contraction.

This latter element, from occupying a certain portion of the contraction curve as it does in the normal, comes to occupy more and more of the curve till finally it may stretch out beyond the beat to a greater or less extent. Together with these changes there goes as a rule diminution in the duration of the beat, though in some cases this effect is apparent rather than real and due to the apex of the beat having become more peaked, the base however remaining of the same breadth. With these changes there is of course a gradual fall in the height of the curve.

Another point which comes out constantly with potassium chloride is that, supposing one starts with a metronome rate giving complete fusion of the two beats, the first effect of the salt will be to lessen this fusion, a dip appearing between the two crests, then the valley deepens, till finally you may get two quite discrete beats; the next effect is that the second beat disappears; all the while the metronome rate has been fixed.

The above effects may be readily summed up in the statement that potassium chloride lessens excitability from the very commencement. For, to enumerate: a given strength of stimulus takes longer to manifest itself, "increased period of latency"; when it does manifest itself, the manifestation is a diminished one, "lessened height and duration of the beat"; the refractory state, normally but slightly involving the period of diastole, now, under the potassium salt, comes to implicate the whole period of the beat or even to extend beyond this; all these are surely but expressions of the above general statement that Potassium lessens excitability. To these may be added the effect noted in former experiments that, as the toxic effect deepens, e.g. in the stage

after inhibition, the strength of the stimulus, to be effective, must be increased. There yet remains a somewhat remarkable phenomenon which must not be omitted, and which apparently is witnessed at all stages of the potassium effect, it is that, whether the diminution in excitability effected by the drug be small or great, the effect of stimulation appears to deepen the effect, not to antagonize it, the latent period increasing, the height and duration of the beats growing smaller, the period of diminished excitability lengthening out; thus, a complete reversal of the effect noted in the case of the undrugged heart. Have we not herein some clue to the reading of the phenomenon mentioned at the outset? It is noted, viz., that continuous faradization not only lost its power to produce fusion as the dose of potash was increased, but that in the final stages a reverse effect was observed, viz., the actual suppression of spontaneous beats during the time of faradization. Though perhaps no explanation of the latter, the above facts appear at any rate to be of the same order; both indicating that stimulation would seem to develop or intensify the potash effect, the tissue actually becoming less excitable.

The following experiments were made with a view of determining this point:—Into the secondary circuit of the arrangement which has been described as here employed the two terminals from the secondary coil of another du Bois Reymond induction apparatus, set for faradization, were brought. It was possible now to faradize the heart for a short interval between the two successive break shocks of the original apparatus. For explanation we may refer to any of the diagrams; the process consisted simply in faradizing the heart for a shorter or longer period between the stimuli a_1, a_2 of each couple. It was not possible to begin the faradization directly after a_1 or to continue it directly up to a_2 for fear of touching these boundary limits and so short circuiting the heart, so that the middle period between a_1 and a_2 was the period of faradization. Having then found an effective couple by the ordinary method a series of such couples was taken, but for every alternate couple the heart was faradized between the stimuli. These faradic shocks of course fell during the refractory period and the question to be determined was whether the already existing state of lessened excitability would be intensified by such faradization or uninfluenced; if the former then each faradized couple should shew either a diminution in the fusion of the two beats or a suppression of the second beat. The results obtained were negative, the faradization appeared to be without effect.

This point was however still further tested—the same apparatus was

kept—the drug was added and pushed till a marked effect had been produced, till in fact powerful faradic stimulation continuously applied gave but a single initial contraction instead of a rapid succession of beats blending by their proximity such as obtains for the undrugged heart. On this stage of *lowered* excitability it was possible to advance yet a step further, for by repeatedly faradizing, allowing only very short intervals for rest, it was possible even to abolish this initial beat, i.e. the rapid succession of shocks were without effect; when this stage had been reached and immediately after such an ineffectual series, a single break shock was thrown into the ventricle and this invariably gave a contraction. As to the relative strength of the shocks, two separate induction apparatus being employed, the coils were tested for us for given positions, and the secondary coil used for obtaining break shocks only was then moved so as to give decidedly weaker shocks than the coil of the apparatus used for faradizing (we refer here only to the opening shocks of the faradic series). The results then are these—a series of shocks give no effect, a single weaker shock gives an effect; it must then be that shocks falling during a certain part or during all of the period of latency tend to inhibit or interfere with the initial shock of the series. This of course applies only to the frog's ventricle under the influence of a potassium salt.

We may now pass on to consider the other bases.

Ammonium chloride similarly tested gave very different results; here it was necessary to distinguish two stages, a primary and a secondary. The primary stage occurred early in the action of the drug, indeed it set in almost as soon as the drug had reached the heart, its duration however was but short.

During this primary and passing stage the effect on the period of diminished excitability was a variable one; in some cases it was lessened, in others slightly increased. On the period of latency the effect was probably always one of diminution; in some of the cases in which before the addition of the drug, the period of latency was but short, no noticeable diminution could be made out, certainly there was no lengthening. In others in which a very well-marked latent period existed, slight but undoubted shortening obtained; it is more than probable that more delicate means would have detected the same in the previous cases just mentioned. In addition this primary stage was marked by increase in the height of the contraction and increased duration of the beat with broadening of the apex of the beat; the effect on duration was the one chiefly marked. See Figs. *A'*, *B'*, Trace III.

This primary stage, then, with its diminished period of latency and its increased height and breadth of trace, contrasts very markedly with the potassium effect, which on all these points shews the exact reverse.

The effect on the period of diminished excitability has been stated to have been inconstant, the variation either way was however in no case considerable; and since the shortening of the period of latency would have a tendency the opposite of that due to the increased duration of the beat, it seemed not improbable that the predominance of the one effect over the other might account for the variation observed.

So far the primary stage has alone been considered, this however was transitory, gradually the secondary stage developed which, shortly described, presented exactly the potassium effect, the period of diminished excitability as a whole being greatly lengthened out, the latent period being also much increased and the duration of the beat diminished. Here too, as for potassium, the phase of diminished excitability from occupying but a limited portion of the contraction curve, as in the normal, came finally to involve the whole of the curve, both systole and diastole. See Figs. *C, D, E, F,* Trace III.

Sodium Chloride. The dosage with this salt had to be increased according to the approximate toxic ratio already determined in previous experiments, the doses employed were, viz., ten times as large as for the two preceding salts. As to the effects they are not quite so unequivocal as for the potassium and ammonium salts.

The difficulties with respect to the period of diminished excitability were these:—if, subsequently to the addition of the drug, one threw in a series of double stimuli, such as before the dosage had given both stimuli effective, the trace obtained might appear as if the result of a single contraction only; if however an occasional single stimulus were thrown in, comparison of the resulting contraction with that obtained by double stimulation would shew the latter to be both higher and of longer duration. Had one here to do with a case of blending or not? If of blending the fusion was complete, there being not the slightest break in the curve to indicate its composite nature, see Fig. *B,* Trace IV. However that this apparently simple trace is really compound is more than probable, for we get transitional forms from such to a trace which is quite obviously double, see Fig. *B'* and Fig. *C.*

The question of fusion or not is at best of no great importance for it does not touch the point at issue, viz., the effectiveness of the second stimulus, for whether this second stimulus shew itself by causing an obvious second contraction or simply cause the line of systole to rise

higher and prolong the period of active contraction, in either case the second beat *is* effective. Moreover it is effective after a shorter interval of time as Fig. *C*, Trace IV. shews.

The latent period is distinctly increased by the sodium salt, making all allowance for the more gradual rise in the line of systole which renders it somewhat difficult to determine exactly when the systole does commence (see Trace IV.).

The effect on the duration of the beat was not very decided; as a rule there was shortening till in the later stages, when the beats became lengthened out, sometimes considerably.

We have seen in the case of the potassium salt and also in the second stage with the ammonium salt that the refractory state gradually encroaches on the line of diastole till finally the whole curve may be involved; if now we compare Fig. *A* with Fig. *C*, Trace IV., we shall note in this latter how early the second stimulus falls as compared with the former, in fact the reverse condition obtains here; under the influence of the sodium salt the refractory state has come to occupy less and less of the period of active contraction. Yet another point of contrast may be instanced; under the potassium salt we have seen that repeated stimulation, instead of increasing the excitability as it does for the undrugged heart, always lowers it; under the sodium salt the influence of stimulation is to increase the excitability. Thus, after a large dose of the sodium salt the first contraction may be very small and the latent period very long; as stimulation is repeated the beats grow in height and the latent period shortens.

The results obtained may be briefly summed up as follows:

In the case of the undrugged ventricle, stimulation shortens the period of diminished excitability; analyzed, this effect shews shortening of the period of latency and likewise of the duration of the period of active contraction.

Passing to the drugged heart we find, that *Potassium chloride* increases markedly the period of diminished excitability; that the period of latency is also considerably increased; that the duration of the beat is as a rule diminished; but that in spite of this the refractory state encroaches more and more on the period of diastole till finally the whole of the contraction curve may be involved and indeed beyond this to greater or less extent; that stimulation appears to intensify the potassium effect, diminishing the excitability still further, the reverse effect to that obtaining with the undrugged heart.

That *Ammonium chloride* shews two stages, a primary stage corre-

sponding to increased height and breadth of beat, here the period of latency is *diminished*, but the period of diminished excitability variously affected, in some cases slightly lessened, in some slightly increased; a secondary stage in which effects similar to those of potassium obtain, viz. increase of the period of latency and of the period of diminished excitability with diminished duration of the beat.

That *Sodium chloride*, when the drug effect is pronounced, shews marked diminution of the period of diminished excitability, and this in spite of prolongation of the latent period; that the duration of the beat is variously affected, except in the final stages when it is lengthened out; that the refractory state hence comes to occupy less of the period of active contraction; finally that repeated stimulation increases the excitability as it does for the undrugged heart.

These results have been given at length, not because they represent the individual effects of the three drugs, potassium, sodium and ammonium chloride, but because they represent probably the effect of potassium, sodium and ammonium salts generally. (For the reasons for this statement see the results of previous experiments recorded in the *Medico-Chir. Trans.* loc. cit. and in the *Practitioner*, Aug. 1882.) As class effects they would naturally possess the greater value. We know from the teachings of physiology that during the excitatory process which results from stimulation of a contractile tissue, this tissue for the greater part of the duration of this process is relatively insusceptible of responding to further stimulation[1]. The interest of the present paper centres around the modification of this refractory state which certain drugs are capable of effecting, which modification is both marked and definite for the particular drug taken. The striking contrast which obtains between the potassium and the sodium salt with respect to this modification and indeed with respect to nearly every point mentioned in relation to this subject, is of the greatest interest, not only because, from a chemical point of view, such contrast would be quite unlooked for in two elements apparently so nearly akin, but also because we are here dealing with fundamental properties of tissues and also because both these elements are important constituents of the animal organism. It can scarcely be doubted but that such marked differences as here appear between the sodium and the potassium salts must indicate their subservience in the organism of functions proportionally distinct in their

[1] With reference specially to the Frog's heart consult the previously mentioned paper by Dr Sanderson and Mr Page, *Journ. of Phys.* Vol. II. Nos. 5 and 6.

nature. The strength of this conclusion is scarcely weakened by the fact that we have throughout been dealing with toxic doses.

In conclusion it may be stated that the results obtained with the potassium salt are compatible with Marey's explanation of the mode of action of a succession of stimuli in producing continuous spasm of the ventricle.

EXPLANATION OF PLATE XXI.

TRACE I. Figs. *A*, *B*, *C* illustrate simply the effect of double stimulation. In *A*, the two stimuli, thrown in at the points a_1 a_2, cause but a single contraction—the second stimulus a_2 falls therefore too early, i.e., during the refractory period.

In *B*, the two stimuli have each had effect, the two beats are however quite discrete.

In *C*, two tracings are given, II. shews the effect of double stimulation with both stimuli effective; fusion is here all but complete. I. is the result of a single stimulus, it is for the sake of comparison.

TRACE II. shews the modifying effect of the potassium salt.

In *A* both stimuli are seen to be effective; note the period of latency and the position of the second stimulus, a_2, on the contraction curve.

In *B* the like is seen; this trace was taken after the heart had been allowed a rest of five minutes.

During the five minutes rest following trace *B*, the potassium salt was added, 0·4 c.c. of 10 per cent. potassium chloride solution. *C*, *D*, *E*, *F* shew the tracings now taken.

Therefore *A* and *B* are before the addition of potassium salt.

C, *D*, and *E* after ,, ,,

The metronome stood at 22 in *A*, *B*, and *C*, in *D* it was pushed up to 5, in *E* to 1. Note the enormous lengthening out of the latent period; the great variation in the refractory period, from metronome 22 to 1, the position of the second stimulus in *E*, where alone it becomes effective; note also that the second stimulus when it does become effective does not yield fusion.

TRACE III. shews the ammonium effect in its early stage.

A is before the addition of the drug.

B subsequent to the addition of 0·3 c.c. of a 10 per cent. solution of ammonium chloride.

A single spontaneous beat is in each case given for comparison with the effects of double stimulation.

Note in *B* the greatly increased duration of the beat, and the greater completeness of the fusion; the effect on the latent period is not marked, but if in any direction it is in that of diminution.

Note that the increased duration of the beat, resulting from the ammonium chloride, tends to pitch the second stimulus, a_2, earlier on the contraction curve.

The later stage of ammonium chloride action is not shewn, it is similar to that of the potassium salt but less pronounced.

In both *A* and *B* the metronome stood at the same figure, viz., 20.

Figs. *A'*, *B'* illustrate again the primary stage of ammonium effect (metronome at 25 in either case), *B'* being subsequent to the addition of the drug 0·3 c.c. of NH_4Cl 10 per cent., the shortening of the period of latency is here well marked.

Figs. *C*, *D*, *E*, *F* shew both stages; *D* shews the primary stage after 0·3 c.c. of NH_4Cl 10 per cent.; *E* and *F* the secondary stage, compare potassium; the metronome in *C* and *D* stood at 15, in *E* it stood at 10, in *F* at 5. The secondary stage will be seen to be exactly like that for potassium chloride.

Trace IV. shews the sodium effect.

In *A*, I. and II., we have the effect before addition of the drug; between I. and II. the heart had an interval of three minutes rest.

B, *B'*, and *C* are subsequent to the addition of chloride of sodium; between *A*, II., and *B*, in the interval of three minutes allowed, 3 c.c. of a 20 per cent. solution of sodium chloride were added. Note the effect on the period of latency. In *B* two of the curves are the result of double stimulation, two of single stimulation, but for this comparison, the double stimulation here would appear to yield but a single contraction, whilst in all probability there is fusion here, i.e., both stimuli are effective (compare *C*).

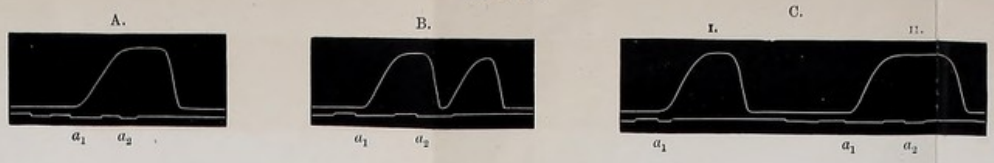
In *A*, *B*, and *B'* the metronome stood at the same figure, viz., 15. *B'* was taken after 2 c.c. more had been added, in all 5 c.c. of the 20 per cent. solution, the trace from double stimulation here shewn is distinctly compound.

In *C* we have the effect some 15—20 minutes later, in all 5 c.c. of the 20 per cent. NaCl solution had been added. The metronome stood at 27.

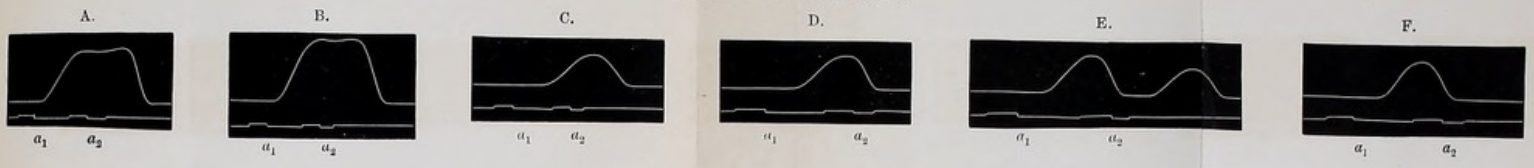
Note therefore that the refractory period has grown less, for the curve from double stimulation is obviously double. Double and single stimuli alternate here for comparison.

Note how early the second stimulus a_2 falls on the curve and compare with the potassium trace, in which the second stimulus falls low down on the diastole curve before it is effective.

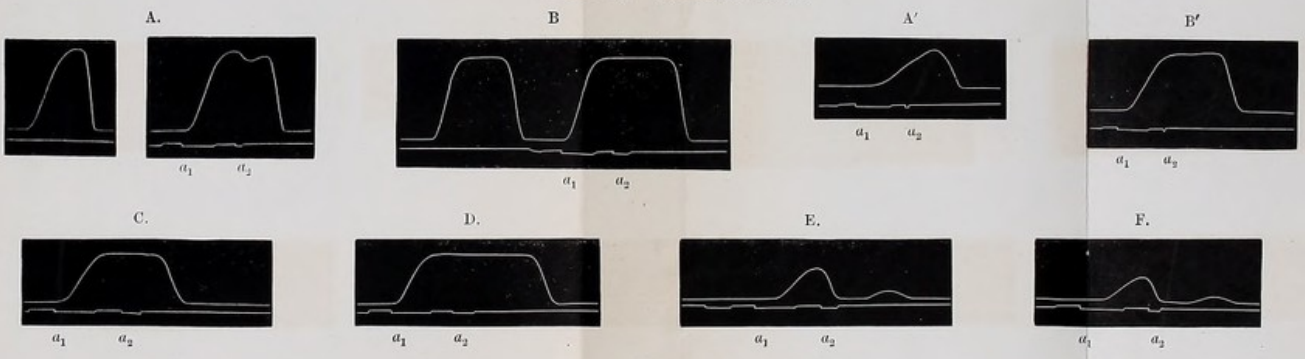
TRACE I.



TRACE II. Potassium effect.



TRACE III. Ammonium effect.



TRACE IV. Sodium effect.