FURTHER EXPERIMENTS ON THE ACTION OF THE VAGUS ON THE ELECTROGRAM OF THE FROG'S HEART.

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In a recent paper[1] Miss Dale and I showed that the effects of stimulation of the vagus on the electrocardiogram of the frog depended in great measure on the amount of slowing produced. The direct effects of the vagus on the duration of the electric disturbance in the ventricle and on the A.-V. interval are precisely opposed to the effects produced on these time intervals by reduction in the frequency of the heart beat. In other words, the effect of the vagus on the ventricle is in a sense antagonised by the effect of the vagus on the sinus venosus in the spontaneously beating heart. We found that in cases where stimulation of the intra-cranial vagus caused great slowing of the heart, there was not infrequently shortening of the A.-V. interval and lengthening of the duration of the excited state in the ventricle. On the other hand, when the chronotropic effect of the vagus was less in evidence the effects on the electrogram were always a lengthening of the A.-V. interval and a shortening of the ventricular complex. We also suggested a simple explanation of the fact that the form of the final variation of the ventricular complex is very commonly modified as a result of vagus stimulation. In this paper I propose to describe some experiments designed to analyse further the action of the vagus on the ventricular electrogram, and to give a direct experimental proof of the explanation of the change in form of the ventricular complex which we deduced from our former experiments.

[1] Dale and Mines. This *Journal*, XLVI. p. 319. 1913.

The effect of atropinisation of the sinus venosus on the action of the vagus on the electrogram.

It might be thought that the simplest method of eliminating the chronotropic effect of the vagus in studying the effects of its stimulation of the heart, would be to keep the heart beating throughout the experiment by rhythmical artificial stimuli. The difficulty is however encountered that the application of such stimuli, whether to the sinus, auricle or ventricle often of itself causes excitation of the inhibitory or augmentor apparatus and so obscures the background on which should appear the effects of intentional stimulation of the vagus.

With a little care it is possible to treat the sinus venosus with atropine by direct local application in such a way as to eliminate the effect of the vagus on the sinus, and therefore on the frequency of the heart beat, without interfering with its direct action on the ventricle. If the effects of vagus stimulation on the electrogram before and after the application of atropine to the sinus are compared it is found that the results before described as characteristic of the direct action of the vagus on the junctional tissue between auricles and ventricle and on the ventricle itself are much more pronounced when the retardation is eliminated. Care is necessary in applying the atropine; any spread to the base of the ventricle interferes with the success of the experiment. I have performed the experiment with stimulation of the intra-cranial vagus and with stimulation of the intra-cardiac vagus, and both methods have demonstrated the essential fact. Naturally, stimulation of the intra-cranial vagus is a better experiment, since there is no danger of exciting any but inhibitory fibres under these conditions; while stimulation of the sinus venosus usually quickens the rhythm by direct action on the muscle and sometimes by stimulation of the intra-cardiac sympathetic fibres. I shall quote one experiment at length.

Exp. November. Temp. 14·8° C. *Rana temp.*, male. Central nervous system destroyed except for the spinal bulb. Stimulating electrodes on exposed medulla at origin of vagi. Heart suspended. Galv. electrodes on apex of ventr. and viscera. Deflection time = ·015". 10 mm. defl. = 20 millivolts approx.

11.22 a.m. Stimulation causes great slowing and alteration in form of ventr. complex.

11.27. Atropine sulphate (Merck) ·01 % in Ringer applied to sinus venosus on a piece of blotting paper about 3 mm. square.

11.30. Stimulation causes no slowing of auricle but produces block between auricle and ventricle.

11.35. Stimulation of vagus: *records analysed below.*
The stimulation was stopped directly block appeared.

11.40. Atropine on ventricle.

11.44. Stimulation of vagus does not alter electrogram.

Measurements of the records of a series of consecutive beats after application of atropine to the sinus venosus.

Duration of cycle, secs.	A.-V. interval, secs.	Duration of ventr. complex, secs.	Amplitude of mechan. ventr. record, mm.	Height of ventricular "spike," mm.
2·03	0·34	1·02	25	22
2·04	0·35	1·02	25	22
2·03	0·35	1·02	25	22
2·03	0·36	1·02	25	22
(Stimulation of intra-cranial vagus begins.)				
2·03	0·35	0·96	24	22
2·04	0·38	0·82	16	22
2·04	0·78	0·66	7	21
2·04	∝	none	none	none
(Stimulation of vagus ends.)				
2·04	0·50	0·56	2	20
2·03	0·37	0·58	2·5	20
2·03	0·35	0·60	4	20
2·03	0·35	0·64	6	21
2·03	0·35	0·70	8·5	21
2·03	0·35	0·76	12	21
2·03	0·36	0·84	15	21
2·03	0·36	0·90	18	21·5
2·03	0·36	0·94	20	22
2·03	0·36	0·98	21·5	22
2·04	0·36	1·00	22	22
	0·36	1·00	22·5	22
(Interval of 10 seconds in record.)				
2·04	0·36	1·00	23	22
(Interval of 30 seconds in record.)				
2·04	0·36	1·02	24	22

The measurements of the A.-V. intervals, the duration of the ventricular complex and the amplitude of the mechanical record are plotted in Fig. 1. The curves are very similar to those in Figs. 2 and 3 of the paper[1] by Miss Dale and myself. The elimination of change in frequency of sinus and auricles allows us to see in this case what are the direct effects of the vagus on the A.-V. interval and the ventricular complex when the dynamic equilibrium of the heart is undisturbed by other influences. The shortening of the duration of the excited state in the ventricle and the lengthening of the A.-V. interval, leading in this case to actual block, are both very strongly marked. The difference in rate of recovery of these processes is striking. The A.-V. interval was more than doubled before block was produced, yet within 5″ of the cessation of stimulation the A.-V. interval

[1] *Loc. cit.*

had recovered its normal length. The duration of the ventricular complex had not quite returned to its original value even after 20″. The relation between the duration of the excited state in the ventricle and the amplitude of the mechanical response appears again most clearly in this experiment. The last column of figures in the table shows that the potential difference set up between the apex and base of the ventricle was affected only to a slight extent during this great change in the mechanical response.

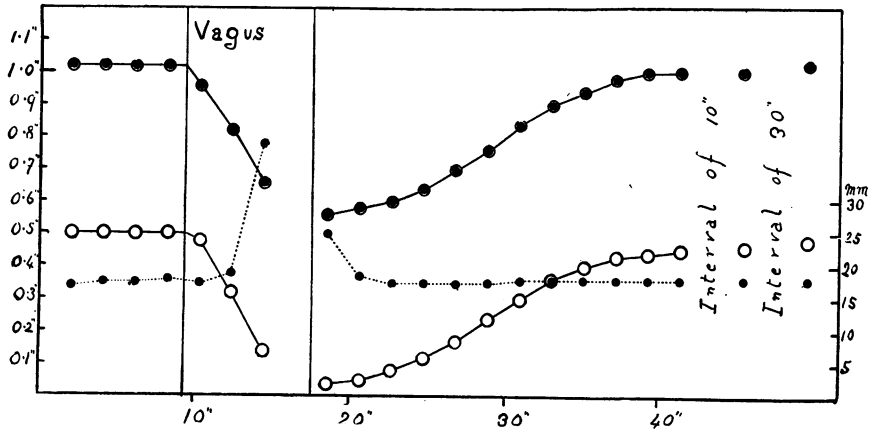

Fig. 1. ◉ = duration of ventricular complex (scale on left).
• = auriculo-ventricular interval ,, ,,
○ = amplitude of mechanical record of ventricle (scale on right).
The points representing these characteristics of each beat are placed in the same vertical line.

If the time of ascent of the "spike" may be taken as evidence of the rate of transmission of the excited state in the ventricle, this rate was not changed appreciably. Throughout the record the time occupied by the ascent of the "spike" (*R*-wave) was 0·08″.

An experiment made in the same way but with the region immediately under the apical electrode crushed so as to give a monophasic electrogram, showed closely similar results.

Stimulation of the vagus increased the A.-V. interval from 0·52″ to 0·78″ (in this experiment A.-V. block was not produced). The duration of the ventricular electrogram was reduced from 0·84″ to 0·44″. Complete recovery of the normal value of the A.-V. interval occurred within 4 beats (8″) after cessation of vagus stimulation. Recovery of the normal duration of the ventricular electrogram took more than 18 beats (36″).

The time of ascent of the monophasic ventricular curve (0·08″) was unaffected by the vagus stimulation, while the maximal potential difference developed was reduced but slightly, from 55 to 50 millivolts. The mechanical record of the ventricle was cut down from 10 mm. to 0·5 mm. The progress of its fall and recovery followed the same course as that of the duration of the ventricular electrogram.

From the data given it is evident that the contour of the curve expressing the monophasic variation is greatly altered by vagus stimulation. I propose to discuss this change in form in a subsequent paper dealing generally with the interpretation of the monophasic response.

Explanation of the influence of vagal stimulation on the form of the ventricular complex.

Samojloff[1] in 1910 described a change in the form of the ventricular complex resulting from stimulation of the vagus: a change in form which he has since insisted on as being absolutely characteristic of the action of the vagus on the frog's ventricle[2]. The final variation of the ventricular complex (the "*T*-wave") may be described as "positive" when it is in the same direction as the initial deflection of the ventricular complex (*R*-wave), negative when it is in the opposite sense. Samojloff's effect is this:—when the vagus is stimulated the final variation of the ventricular complex (*T*-wave) becomes altered in the sense of negativity. If, as is most common, the wave is positive at the start, it becomes less positive and may become negative on stimulation of the vagus. If negative at the start it becomes more strongly negative. Samojloff's experiments were made on *Rana esculenta*. As I have already stated elsewhere[3], I have had opportunities of confirming the frequent occurrence of Samojloff's effect in *R. temporaria*. If there is any change in form of the final variation on stimulation of the vagus, the change described by Samojloff is the most usual. But in at least six cases out of 20 or 30 experiments, stimulation of the vagus has produced marked increased positivity of the final variation. In other instances the wave has been unaltered in spite of well marked inhibition, and, as Miss Dale and I pointed out[4], we had two cases in which the first record of vagus stimulation showed an effect on the final variation opposite in sign to that produced by a subsequent stimulation. We suggested that the effect of the vagus on the form of

[1] Samojloff. *Pflüger's Arch.* CXXXV. p. 417. 1910.
[2] Samojloff. *Zntrlb. f. Physiol.* XXVII. p. 7. 1913.
[3] Mines. *Proc. Camb. Philosoph. Soc.* XVI. p. 615. 1912.
[4] *Loc. cit.*

the electrogram depended on its affecting different parts of the ventricular muscle to different extents in different cases; always shortening the duration of the excited state, but sometimes shortening it more in one region than in another and so leading to predominance of basal or apical regions towards the end of the ventricular complex. The most usual effect (Samojloff's) would represent the case where the basal region of the ventricle is more affected by the vagus than is the apical region.

An extension of the method described in the first section of this paper offers a simple method of testing our hypothesis. If we can prevent the action of the vagus in one part of the ventricle without upsetting it in another region, it will be possible, if our hypothesis is correct, to determine the direction of change in the final variation which shall be set up by vagal stimulation. The localised application of atropine enables this experiment to be carried out. A 0·1 % solution of atropine sulphate in Ringer's solution may be applied to the surface of the recently exposed ventricle without as a rule altering the form of the electrogram (unless the ventricle is already under vagal influence or under the influence of some drug antagonised by atropine). Now if atropine is applied to the base of the ventricle alone and allowed a few minutes to soak in, stimulation of the vagus always causes increased positivity of the final variation if it produces any effect at all. Naturally the experiment is most striking when the change produced by vagal stimulation before application of the atropine was diminished positivity (Samojloff's effect). The chronotropic effect of the vagus may be eliminated by applying a little dilute atropine to the sinus without disturbing Samojloff's effect. But when atropine is applied to the base of the ventricle, avoiding spread of the solution to the apex, within a few minutes stimulation of the vagus causes increased positivity of the final variation. The slight lag in the onset of the effect of application of the atropine solution is a control indicating that the result is in no way due to accidental change in local temperature or electrical conductivity of the preparation. Once developed the effect (that is to say, increased positivity of final variation on vagal stimulation) can be repeated a number of times and only disappears when owing to spread of atropine to the apical regions or through fatigue of the vagus, stimulation of the nerve fails to produce any effect whatsoever on the electrogram. Fig. 2 illustrates the above statements.

Conversely, the application of atropine to the apex of the ventricle ensures the appearance of Samojloff's effect when the vagus is

stimulated if it has any action on the ventricle at all: even when the untreated heart gives a change in the direction of increased positivity of the final variation on stimulation of the vagus, after application of atropine to the apex, stimulation produces diminished positivity of the final variation.

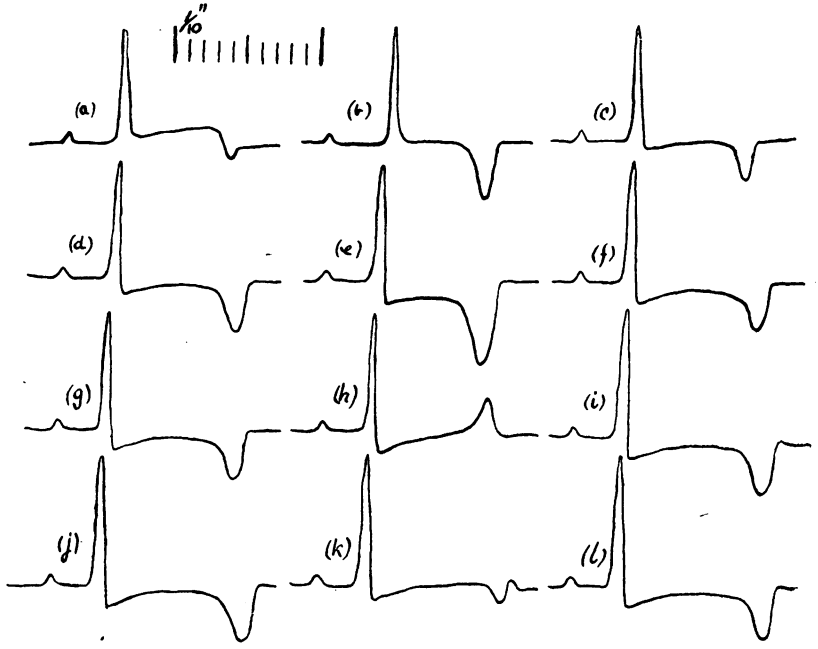

Fig. 2. Traced from the photographic records. (*a*) before, (*b*) vagus stimulation, (*c*) two minutes later, (*d*) one minute after application of 0·1 % atropine to base of ventricle, (*e*) stimulation of vagus, (*f*) three minutes later, (*g*) immediately after (*f*), (*h*) stimulation of vagus (four minutes after application of atropine), (*i*) one minute later, (*j*) two minutes later, (*k*) vagus stim., (*l*) one minute later.

Discussion of results.

The various effects of vagal stimulation on the characteristics of the co-ordinated heart beat have been recognised, since the work of Gaskell and of Engelmann, as due in large measure to the fact that the vagus terminations lie in several parts of the heart, alteration of the functions of which parts produce different effects. Thus slowing of the sinus venosus of necessity produces slowing of the beat of the whole heart, since the rhythm originates in the sinus. Diminution of the rate of conduction in the junctional tissues causes an

increase in the interval between the beginning of contraction in one chamber and the next: reduction in force of contractions in auricle or in ventricle depends on a local effect of the vagus on these chambers[1]. Zwaardemaker[2] has pointed out that experiments made by Wolterson show that the threshold values for the various effects of the vagus differ and that the maxima of the various effects observable in the mechanical response differ in their time of arrival. The experiments of Muskens[3] showed that in the frog's heart not only was the frequency of the sinus altered by vagus stimulation, but also the force of the contractions of sinus was reduced. The study of the electrogram of the beating heart confirms the observation made by Engelmann and by Muskens that the rate of conduction of the excited state from auricle to ventricle is diminished by vagal action: it facilitates the quantitative investigation of the time relations of this change. But the most important information to be gleaned from the electrogram of the frog's heart is in connection with the changes which go on in the ventricle.

Samojloff[4] has recently shown that the alteration in the strength of the demarcation current of the injured frog's ventricle is changed by stimulation of the vagus in just the same fashion as Gaskell[5] showed was the case in the quiescent auricle of the tortoise. Samojloff[6] also recorded the fact that on stimulation of the vagus the duration of the electric response of each excitation of the frog's ventricle was diminished. Miss Dale and I[7] confirmed this observation and showed that the same holds also for the tortoise auricle.

Combining the observations of Gaskell, Samojloff and Dale and myself the following statement is reached:—In the auricle of the tortoise and in the ventricle of the frog the following changes in electrical condition accompany the inhibition produced by the vagus: (*a*) the difference of potential between the inhibited muscle and an injured region is exaggerated; (*b*) if a wave of excitation is started in

[1] While this paper is in the press a paper has been published by Cohn and Lewis (*Journ. Exp. Med.* Dec. 1913) showing that in the dog's heart the left vagus has a predominant effect on conduction from auricle to ventricle and referring the differences in the effects of right and left vagi to the anatomical distribution of their endings in the heart. To demonstrate the effect on conduction, the heart was kept beating by rhythmic stimulation of an auricle.

[2] Zwaardemaker. *K. Akad. Wetensch.* Amsterdam, Dec. 29, 1906.

[3] Muskens. *Amer. Journ. Physiol.* I. p. 486. 1898.

[4] Samojloff. *Zntrlb. f. Physiol.* XXVII. p. 575. 1913.

[5] Gaskell. This *Journal*, VIII. p. 404. 1887.

[6] Samojloff. *Pflüger's Arch.* CXXXV. p. 460. 1910.

[7] *Loc. cit.*

such inhibited muscle the duration of the electrical disturbance indicating activity is abnormally short.

The experiments described in the present paper enable us to state further that the action of the vagus described under (*b*) holds good for both basal and apical regions of the frog's ventricle. This is proved by the effects of local application of atropine which abolishes the power of the vagus in one region or the other without preventing all action on the ventricular complex. It might indeed be inferred from the very fact that the total duration of the electric response of the ventricle is shortened. For so long as any considerable portion of the ventricle remains in the excited state, unless such portion is orientated with absolute symmetry in respect to the leading-off electrodes, the ventricular complex will not end. This fact is exemplified also by the atropine experiments. For after the application of atropine to one or the other region, although stimulation of the vagus alters the form of the electric response, it has little or no effect on the total duration of the ventricular complex.

The interpretation of the action of the vagus on the form of the electrogram now offers no particular difficulty. Any agency which, acting on the ventricle generally, causes a reduction in the duration of its electrical disturbance, if allowed to act on one region and not on another or one region less powerfully than on another, will tend to make the disturbance at the region less powerfully acted on outlast the disturbance in the other region. The form of the final variation of the ventricular electrogram, with the mode of derivation employed by Samojloff and by me, depends on the relative duration of the disturbance at the apical and basal regions[1]. If base outlasts apex the wave is positive and *vice versâ*. In the frog's ventricle the vagus generally acts rather more strongly on the basal region than the apical. From Samojloff's experiments it would seem that this is always the case in *R. esc.* In *R. temp.* it is most common, but sometimes the apical region is more affected than the basal region and occasionally both regions are affected to the same extent. The two instances which Miss Dale and I found in which in the same preparation stimulation of the vagus at one time gave a change in one direction and later in the experiment (without the application of any substance to the muscle) gave a change opposite in sign to the first, must probably be ascribed either to a difference in the rate of onset of fatigue in the myo-neural

[1] For literature and discussion of the final variation or "*T* wave" see Mines, This *Journal*, XLVI. p. 197. 1913.

junctions in different regions or to different fibres of the vagi being reached by the weak stimuli used in the two tests, perhaps through a shifting of the electrodes.

Finally, our experiments on the electrogram enable us to view in a new light one plausible and ingenious hypothesis advanced by Muskens to explain the reduction in strength of the contractions in the various chambers of the heart on vagal stimulation. Starting from the observation that stimulation of the vagus can cause dissociation of the contractions of various portions of the sinus venosus, Muskens[1] suggested that the vagus may regulate the force of contraction of cardiac muscle simply by changing its conducting power. The diminishing force of the contraction under vagus inhibition may mean that fewer and fewer fibres take part in the contraction because the decreasing power of conduction prevents more and more fibres from being reached by the excitation wave. Muskens found support for this view in the "similarity in the action of the vagus on the force of the contraction and on the length of the contraction interval" (from the context one understands S.-A. interval, A.-V. interval etc.)—"in both the effect rises quickly to a maximum and then slowly disappears."

Reference to the figures in the paper by Miss Dale and myself and to Fig. 1 of this paper shows very plainly that the recovery of conduction in the A.-V. bundle and the recovery of height of contraction in the ventricle follow strikingly different curves: one can base nothing on their "similarity." But the question remains, and is of extreme interest: does the weakened contraction of the ventricle under vagus inhibition mean the contraction of fewer fibres through failure of the excited state to reach the rest or does it mean the weakened contraction of all or most of the fibres? The following arguments seem to me to point to the latter alternative.

(1) Diminished power of conduction (block) is, so far as we know, always heralded by diminished rate of conduction. This is best exemplified by Gaskell's experiment on the slit auricle, where delayed conduction regularly precedes block: it is also seen in many experiments on the A.-V. junction. An agency which causes block (*e.g.* increased acidity, increased frequency of excitation, vagus excitation) always slows the rate of propagation in the earlier states of its action. Now the ventricular electrogram, as we have seen, gives evidence that the rate of propagation of the excited state in the ventricle is not slowed even during very pronounced vagal inhibition.

[1] *Loc. cit.*

(2) If the power of conduction of the ventricular muscle is diminished so as to cause certain fibres in the ventricle to escape the excitation wave, it is to be expected that those fibres will be most likely to escape excitation which are furthest removed from the region where excitation reaches the ventricle, since in order to reach them the wave would have to traverse a greater amount of poorly conducting tissue. Fibres in the apical region should therefore be cut out sooner than those in the basal (at any rate the left basal[1]) region.

If vagal stimulation caused failure at the apex rather than at the base of the ventricle, it should always make the final variation of the ventricular complex more positive. As we have seen, it usually makes the final variation more negative (Samojloff's effect).

(3) If the reduction in force of the ventricular contractions during vagus inhibition means that portions of the muscle fail to enter into the excited state, these portions must form a large proportion of the whole ventricular muscle when the reduction of the force of contractions is great. Now if a large part of the ventricular muscle fails to enter the excited state, such part will set up no electrical disturbance, but will provide paths for short-circuiting the currents set up by the still active parts. This must inevitably cause a great reduction in the strength of current derived from the muscle by the galvanometer leads. In most of our experiments, Miss Dale and I found that there was no regular relation between the height of the contraction and the strengths of the currents recorded by the galvanometer. In the table on p. 421 of the present paper it is seen that the reduction in amplitude of the ventricular contractions is accompanied by a reduction in the extent of the initial electric variation of the ventricle. But while the contractions are reduced by 92 % of their original value, the initial electric variation is reduced less than 10 %, the time of ascent of the spike being unchanged.

It would appear then that the excited state is propagated at a normal rate throughout the inhibited ventricular muscle. The difference is that in the inhibited ventricular muscle the excited state lasts a shorter time.

As I have shown before[2], other factors being equal, a diminution in the duration of the excited state commonly involves a reduction in the force of contraction.

[1] For experimental discussion of Gotch's hypothesis see Mines, This *Journal*, XLVI. p. 201. 1913. It is there shown that the looped paths of conduction assumed by Gotch cannot involve any large part of the musculature. [2] This *Journal*, XLVI. p. 375. 1913.

Conclusions.

By the local application of atropine to the sinus venosus of the frog's heart the effect of stimulation of the intra-cranial vagus on the frequency of the beat can be eliminated while the effect on the A.-V. junction and on the ventricle remains.

Changes in frequency of beat being eliminated, vagus stimulation causes reduction in the rate of transmission of excitation from auricle to ventricle and diminution in the duration of the excited state in the ventricle. The recovery curves from these effects have quite different time relations.

The effects of vagus stimulation on the form of the ventricular electrogram depend on differences in the extent to which different regions of the ventricle are influenced by the vagus. All parts appear to be influenced to some extent. By the local application of atropine to base or apex the effect of the vagus in one or the other region may be cut out and thus the effect of the vagus on the form of the electrogram modified at will.

The weakened mechanical beat of the ventricle under vagus inhibition is not generally due to failure of some muscle fibres to become excited, but to a weakening of the contraction in all the parts. This weakening is closely associated with a diminution in the duration of the excited state in the muscle.