THE ACTION OF MUSCARIN AND PILOCARPIN ON THE HEARTS OF CERTAIN VERTEBRATES, WITH OBSERVATIONS ON SEASONAL CHANGES

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Whether muscarin and pilocarpin, when applied to the heart, act directly in virtue of their depressing influence on the inherent motor activity, or indirectly as excitants of the cardio-inhibitory mechanism, is still an open question.

Schmiedeberg and Koppe¹ attributed the action of muscarin to its influence as a stimulant of inhibitory ganglia. Kobert² subsequently emphasised the same idea. Later on Schmiedeberg's general results were confirmed by Prevost and Monnier³. Since that time many investigators have published results favouring the theory of vagal stimulation, and many authorities of the present day accept the view that these drugs act by stimulating the vagus endings.

On the other side, the direct action of a comparatively weak solution of muscarin or pilocarpin as a paralysing of the cardiac muscle substance, has been emphasised by various authorities.

One of the strongest advocates of the theory of direct depression of the cardiac motor mechanism is Gaskell⁴. After the application of muscarin he found a general depression of rhythm, strength, tone and conductivity. He holds that the drugs act on the muscular tissue atonically.

1. Schmiedeberg u. Koppe, *Das Muscarin*, Leipzig, F. C. W. Vogel, 1869, S. 28; *Studien über Herzgifte*, Würzburg, 1871.

2. *Archiv. f. exper. Patbol. u. Pharmakol.*, Bd. 20, S. 92.

3. *Gaz. méd. de Paris*, 1874, p. 243.

4. *Philosophical Transactions*, 1882. *Journal of Physiology*, Vols. III, IV, and VIII. Schäfer's *Text-book of Physiology*, (1900) p. 223.

DESCRIPTION OF SAMPLES OF MUSCARIN AND PILOCARPIN
USED IN THIS INVESTIGATION

In all investigations bearing on the action of muscarin with regard to cardiac inhibition, it is obviously of primary importance that the drug should be pure. Schmiedeberg has pointed out that the samples obtained from dealers are never sufficiently purified, and are liable to contain, amongst other impurities, traces of an atropin-like base. The solution used in these experiments has been kindly supplied by Professor Schmiedeberg himself, and a similar solution has been used by Gaskell in his experiments on muscarin action. The latter observer found that the strength was such that one or two drops placed on the sinus of the frog's heart were sufficient to cause immediate standstill; placed on the sinus of the tortoise, the beat ceased immediately¹.

With this solution of muscarin the majority of my experiments were made; in suitable cases one drop or even less applied to the sinus gave the characteristic effect; if one or two drops were not sufficient, an increase in the quantity never gave an immediate effect, the result of the exhibition of a large quantity being essentially different in nature from that of a small amount.

For some experiments pilocarpin was used, and in order to insure the elimination of any possible errors arising from impurities or defects in the salts, five different specimens were used—three nitrates and two chlorides—two of these being specially certified to contain no impurities. The solutions were made up in Ringer's circulating fluid, so that 1 drop as obtained from a special pipette contained exactly 2 mgrs. of the salt; by dilution it was thus easy to apply to the heart any fraction of a mgr. of the pilocarpin. The activity and purity of the samples used, I have often demonstrated on the normal heart of the frog and eel, when a small quantity applied to the sinus produced the usual results.

1. *Journal of Physiology*, Vol. VIII, p. 407.

HEART OF FROG

On Seasonal Changes in the Frog-Heart, and their Influence on Inhibition.—When making observations on the heart of the British frog in the months of January, February and March, I was surprised to find that very considerable changes were in evidence with regard to the manner in which the heart responded to vagus stimulation. In some cases the inhibitory function of the vagus seemed to be quite inactive, while in others it was manifested only to an exceedingly slight degree. In January, certain hearts responded to faradisation applied to the 'posterior white crescent,' but in many the vagus influence seemed to be very slight indeed, the heart quickly escaping from the effects of stimulation and regaining its former rhythm; on the application of a stronger faradic stimulus there was occasionally a slight inhibitory effect, but it was always of very short duration, and soon no strength of stimulus had any effect. Stimulation of the vagus trunk or medulla did not, as a rule, alter in any way the rate of cardiac action, though in a few cases there was a rather ambiguous result produced probably dependent on the intermixture of accelerator and inhibitory fibres in the frog's vagus (*Rana temporaria*).

In February and March the same features were in evidence, only the elimination of vagus influence became gradually more pronounced, till towards the end of March it often happened that no inhibitory effect could be demonstrated. In April, also, there was little response to vagal stimulation, but in May the heart was found to be gradually assuming its normal condition, for faradisation of the posterior white crescent often resulted in a marked slowing or temporary stoppage of the heart; even in May, however, normal conditions did not by any means prevail.

In the autumn months the result of vagus stimulation on British frogs was quite different, the majority giving unmistakable evidence of an active inhibitory mechanism; some German frogs examined in October, however, gave no result, and it is interesting to note that these were distended with ova (*Rana esculenta*).

Many observers have noted certain changes in the ordinary

frog-heart during the winter and spring months, but there does not seem to be any definite account of the very marked phenomenon in regard to this diminution of the inhibitory power, and the exact cause of it is difficult to establish.

A striking fact is the observation that in a general way this change coincides more or less with the season of sexual activity in the frog, while such factors as temperature and the diminished activity of the animal in the cold season may also have some influence. Ringer¹ noticed that certain antagonisms were slight during the breeding season. Pandlejeff² also found that the antagonism of atropin for quinine on the frog-heart was affected by the time of year. In summer frogs, quinine arrested the heart in diastole and atropin caused the heart at once to resume its pulsations. In winter frogs, quinine arrested the heart much more slowly, while atropin, instead of obviating actually increased the arrest. Low temperature would not entirely account for the changes observed in regard to inhibition, for while the mean temperature in January was fully 6° F. lower than that in the beginning of April, yet these changes were much more pronounced in April. Sexual activity in the frog at any rate seems to be the dominant factor in the production of this change. Jordan found the action of muscarin difficult to demonstrate in winter frogs.³

The action of Muscarin and Pilocarpin on the Frog-Heart affected by Seasonal Changes.—The diminution, or in some cases, absence of any apparent inhibitory action in response to stimulation of the cardio-inhibitory apparatus in frogs affected by the seasonal changes discussed above suggested the idea that advantage might be taken of the condition in order to test the mode of action of muscarin and pilocarpin. Here was a case where in certain frogs there was a comparative elimination of the function of inhibition, while in others it was markedly less than normal, probably an expression of some profound general change.

1. *Journal of Physiology*, Vol. III, p. 115.

2. *Lancet*, July 31st, 1880, p. 176.

3. *Arch. f. exp. Patbol. u. Pharmacol.*, VIII, 1878.

If muscarin stopped or slowed the heart in these frogs, then this drug could not be acting through a medium which had been proved inactive by other means. In other words, if muscarin acted as an excitant of the cardio-inhibitory nerves, its effect on the cardiac rhythm should bear some comparatively constant ratio to the effect of electrical stimulation of the inhibitory apparatus. If, on the other hand, muscarin acted as a direct depressant of the inherent motor mechanism of the cardiac muscle, then the fact of the inhibitory apparatus being inert should not influence its action when applied to the heart substance. During a long series of experiments carried out at different seasons during the last three years, it was found invariably that when electrical stimulation of the inhibitory mechanism through the *posterior white crescent* failed to affect the rhythm of the heart, then no effect was produced by the application of weak solutions of muscarin or pilocarpin. When electrical stimulation gave a positive result (*i.e.* slowed the heart) muscarin also gave a like result in many cases; as a general rule, faradisation quickly resulted in tiring out the already attenuated inhibitory apparatus so that subsequent increased stimulation had no effect, and in such a case muscarin likewise proved ineffective; if the faradisation was immediately stopped after an effect was observed, the subsequent application of muscarin generally resulted in slowing the heart for a time; an escape from the inhibition, however, generally occurred in a comparatively short time; this setting free of the heart could be brought about by faradisation and by muscarin or pilocarpin.

Even in late summer and autumn when normal conditions apparently prevail, many variations were met with in the course of this investigation, but no instance was observed in which a heart unaffected by faradisation of the posterior white crescent gave any result in the direction of slowing or stoppage after the exhibition of muscarin.

Fig. I¹ represents a tracing from a 'normal' frog heart, showing result of a local application of a few drops 2 per cent. pilocarpin

1. All the tracings read from left to right.

solution ; heart is seen to be considerably slowed. The result of atropin is also well seen. Drum was stopped for a few seconds on each application to give drugs time to act.

FIG. I. Effect of pilocarpin on frog's heart. (Autumn).

Fig. II is from an 'abnormal' heart in which faradisation failed to cause inhibition, showing result of pilocarpin application. Here such a strong solution as 4 per cent. pilocarpin failed to influence the beat even after ten minutes.

FIG. II. Effect of pilocarpin on frog's heart. (Spring).

With large doses of muscarin or pilocarpin, direct depression of the properties of cardiac muscle became evident ; the time and amount of drug required for this varies greatly in different frogs according to the vitality of the heart and animal in general. In nearly all my experiments the depression of these special functions of cardiac muscle followed a well marked rule. The first effect of a large dose seemed to be on the muscular *tone*. Very soon after the application of a strong solution of pilocarpin the whole heart became somewhat flabby, both the auricle and ventricle exhibiting well marked distension during systole. This did not seem to influence

immediately the rate or force. Some time afterwards the rate became less, due probably to a diminution of *excitability*. A weakness of the cardiac *contractile force* was next manifested, followed often by a condition in which a considerable interval elapsed before the *conduction* of the auricular beat to the ventricle was affected. The order, therefore, in which pilocarpin seems to depress the properties of cardiac muscle is—

- Diminution in (1) Tone
(2) Rate
(3) Contraction force
(4) Conduction.

A heart slowed by direct depression by a large dose of pilocarpin acting for a comparatively long time, differs from a heart quickly arrested or slowed by a small dose in the fact that the rate of the former heart is not increased by atropin, whereas digitalin immediately caused a marked acceleration. On a heart quickly slowed by a small dose, digitalin in weak solution has little or no effect when the inhibition is strong, but atropin gives a most marked and immediate result.

Several of the above points are seen in Fig. III. In A faradisation was applied at the point marked, but without effect. B was taken half a minute after the application of a few drops 2 per cent. pilocarpin; no change is apparent. The heart was then covered with 5 per cent. pilocarpin nitrate solution and left for 25 minutes (*see* C, D, E); it was then again covered with pilocarpin solution every 5 minutes until 65 minutes had passed; gradual slowing due to direct muscular depression is seen, but this is not marked till after one hour or so. After 65 minutes, atropin was applied (E): heart still slow. Four minutes after first dose of atropin more was applied, the result being to cause more marked slowing. Digitalin in weak solution had a marked effect, the rate being instantly quickened (F).

In a strong frog the heart may often be soaked with pilocarpin nitrate, from 5 to 10 per cent. solution, and yet be able to beat quite strongly after an hour or more, though in direct contact with the

FIG. III.

Direct weakening action of pilocarpin after long period, with effect of atropin and of digitalin.

drug the whole time ; in general a diminution in rate is observed in much less than an hour.

Weaker frogs vary considerably in regard to the time and amount of drug required to cause depression, but they never give an indication of depression unless the dose is much greater than is usually necessary to produce a result in a normal frog-heart—also the time necessary for the occurrence of appreciable direct depression is much greater than is generally required for the causation of slowing or stoppage in a normal heart.

The following experiments serve as an indication of the condition obtaining in certain frogs during the months when sexual activity is most manifest ; it will be noticed that in the cases quoted, inhibition was often found to be practically absent ; such cases serve best to bring out a certain phase of the parallel action of muscarin and faradic stimulation. In many experiments there was, of course, a certain amount of inhibitory power present, but as a general rule, as the result of a great number of experiments repeated during several years, it was found to be markedly diminished during the spring season. In all experiments mentioned in this paper, faradisation was applied to the 'posterior white crescent.'

EXPERIMENTS

Experiment I.—January.

Remarks on heart	Drug	Dose	Faradisation	Rate before (per minute)	Rate after (per minute)	Condition of heart
Exposed in situ ; fairly vigorous	Muscarin	—	Negative	22	—	—
		1 drop	...	...	22	Strong beat
		1 „	...	...	22	„ „
		2 drops	...	...	20	—
		4 „	...	...	21	Heart quite strong after 8 drops
						After 10 minutes rate 18 per minute and fairly strong ; gradually became weaker
	Atropin	2 drops 1 % sol.	...	...	14	Weaker still
	Digitalin	Few drops of weak solution	...	...	23	Slightly stronger beat

Experiment II.—February.

Remarks on heart	Drug	Dose	Faradisation	Rate before (per minute)	Rate after (per minute)	Condition of heart
Exposed in situ ; strong beat	Pilocarpin nitrate (3 %)	—	Stopped heart for 10 seconds ; afterwards failed to affect it	30	—	—
		1 drop	...	...	30	—
		I „	...	...	31	—
		I „	...	...	27	Quite strong
		I „	...	...	30	—
		I „	...	...	29	—
		2 drops	...	...	25	—
		3 „	...	...	26	—
		4 „	...	...	26	—
		5 „	...	...	25	Quite strong
						After $\frac{1}{2}$ hour beating strongly 18 per minute
						Digitalin increased rate to 25 per minute

Experiment III.—March.

Excised ; feeble heart	Muscarin	—	Negative	18	—	—
		$\frac{1}{2}$ drop	...	...	18	Weaker
		I „	...	...	18	Still getting weaker
		I „	...	..	16	—
		I „	...	...	14	Very weak ; soon only faint contractions noticed passing over heart ; then stopped
						Digitalin no effect

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Experiment IV.—April.

Remarks on heart	Drug	Dose	Faradisation	Rate before (per minute)	Rate after (per minute)	Condition of heart
Excised ; fairly vigorous heart	Muscarin	—	Negative	16	—	—
		$\frac{1}{5}$ drop	...	...	16	—
		$\frac{2}{5}$ "	...	...	14	—
		$\frac{3}{5}$ "	...	...	14	—
		$\frac{4}{5}$ "	...	...	16	—
		1 "	...	...	16	—
		1 "	...	...	13	—
		1 "	...	...	12	Beat rather weak
		1 "	...	...	15	—
	Atropin (1 % sol.)	3 drops	...	...	12	Beat still weaker ; heart placed in muscarin solution, continued to beat though weakly for several minutes ; washed and treat- ed with Atropin —no change Helped slightly by digitalin

Experiment V.—May.

Exposed in situ ; strongly beating	Muscarin	—	Slowed to average of 10 per minute	30	—	—
		$\frac{1}{2}$ drop	...	...	24	Strong
		1 "	...	...	13	—
		1 "	...	...	21	Strong
		1 "	...	...	25	—
		1 "	...	...	20	Slightly weaker
		2 drops	...	...	20	—
		1 drop	...	...	18	Somewhat weaker but beating after other 6 drops Atropin—no effect Digitalin—beat quicker and stronger

ON THE ANTAGONISM OF MUSCARIN AND PILOCARPIN

Many years ago Ringer¹ concluded that pilocarpin antagonizes the action of extract of muscarin on the frog-heart, a curious result in regard to two drugs exhibiting such closely allied pharmacological action as pilocarpin and muscarin. Judging from the general action of these two drugs it would be expected that a heart slowed by a certain amount of muscarin would be further slowed by an additional dose of pilocarpin. Instead of this, however, Ringer has found that in certain cases, a heart slowed by muscarin is accelerated by the application of pilocarpin, and he therefore concludes that pilocarpin acts as an antagonist to muscarin.

This curious phenomenon I have observed on several occasions, and the idea suggested itself that if muscarin acts on the cardio-inhibitory nerves, and not on the muscle directly, an explanation of this action might be afforded by the fact that the primary dose of muscarin resulted in a stimulation of the nervous mechanism, and thus brought about a slowing of the heart, whilst the subsequent acceleration of cardiac action by pilocarpin might be due to the increased dose paralysing the inhibitory mechanism to a greater or less extent and thus increasing the rhythm. In a heart where the inhibitory function was but little marked, this would probably happen. If this was the case the same result should be brought about by the application of more muscarin after the preliminary slowing by that drug. In the course of my experiments it was very obvious at certain seasons that a heart slowed by muscarin in small dose was, on the application of a little more of the drug with a view to increasing its inhibitory action, accelerated instead of slowed, and the same result was even more frequently obtained with pilocarpin. This was not so much in evidence with muscarin, at any rate in the normal frog-heart, and here it would seem that a dose of the drug sufficient to paralyse the inhibitory apparatus is so great as to directly depress the muscle. In this case no result could be expected. Again, at the season of increased sexual activity, when

1. *Journal of Physiology*, Vol. II, p. 135.

the inhibitory mechanism was practically functionless, this effect was not obtained ; any depression of rhythm in this case, however, must have been due to the effect of a large dose in depressing the cardiac muscle, and so no subsequent acceleration could be expected ; the phenomenon was best seen in a heart where inhibition was feeble but still active. The above observations do not account for many of the results obtained by Ringer, and are not inconsistent with the idea of the existence of a real antagonism between the two drugs ; on the other hand, it is obvious that the phenomenon described above would probably be accepted as a true antagonism, whereas it really is but an apparent one.

Here the escape of the heart from the effects of muscarin while the drug is still in contact with the cardiac tissue admits of practically the same explanation, and the fact that within certain limits this escape can often be brought about by the application of an increased amount of the original drug, seems to be inconsistent with the idea of direct muscular depression ; if, however, too much muscarin be added, depression of the muscle may of course be evident, and so prevent increase in the rhythm, while if the increased dose is too small to paralyse the mechanism, the inhibitory effect is more marked, as generally occurs in the normal frog-heart.

In view of these observations, it would seem that in certain hearts at any rate the apparent antagonism of muscarin and pilocarpin is due to the condition of the cardio-inhibitory apparatus.

Again, if muscarin produces its effects by its direct action on the cardiac muscle, it is difficult to understand how it should sometimes have its apparent effects considerably lessened by an additional dose.

ON A TENDENCY FOR THE ESTABLISHMENT OF IMMUNISATION TOWARDS MUSCARIN AND PILOCARPIN IN THE FROG-HEART

A very important factor exhibited in the frog-heart is a tendency for the production of a certain amount of immunisation to muscarin or pilocarpin after the exhibition of these drugs. When the heart recovers from the effect of a small dose of muscarin, as often happens

in certain hearts, it was found that a very much greater dose of the drug was required to produce slowing or stoppage a second time ; in many cases, as previously stated, it was not possible to produce a second effect, but in a fairly normal heart this could sometimes be done. I have never seen an instance of any heart escaping from the effect of a second dose being slowed by the application of more of the drug, except in cases where, after a comparatively long time, direct muscle weakening set in. It is interesting to note that many of the above results agree with observations made by Marshall on the mammalian heart¹. In view of what we know of the behaviour of muscle towards depressant drugs, such immunisation would be difficult to explain if muscarin (or pilocarpin) is in small doses a direct muscle depressant, but is perfectly intelligible if the drug is a stimulant to nerve endings²—repeated doses leading to exhaustion of their function. That the action of pilocarpin is on nerve-endings, however, has been comparatively recently disputed by Matthews³, who still advocates direct action on the animal cell, at least in the case of gland-cells.

HEART OF EEL

The heart of the eel, as shown by MacWilliam⁴ many years ago, possesses a very marked susceptibility to vagus inhibition, and differs from the frog-heart both as regards the state of the cardiac tissue while under the influence of vagal stimulation and in the peculiar manner in which spontaneous cardiac rhythm becomes re-established.

On Seasonal Variations in the Eel-Heart.—Here, as in the heart of the frog, great variations in susceptibility are met with at different seasons. In some experiments done on eels

1. *Journal of Physiology*, Vol. XXXI, p. 127. The above observations were presented as part of a thesis to the Senatus of the University of Aberdeen before I knew of Marshall's paper.

2. In this paper the term 'nerve endings' is used to indicate the peripheral terminations of the cardiac inhibitory apparatus. The present investigation shows that the drugs mentioned act indirectly by stimulation of a peripheral inhibitory mechanism and not directly by simple depression of the contractile mechanism ; whether the exact portion of this inhibitory apparatus is of nervous or muscular origin is not within the scope of the investigation. It is very possibly intra-cellular.

3. *American Journ. of Physiol.*, Vol. VI, p. 207, 1901.

4. *Journal of Physiology*, Vol. VI, p. 218.

obtained about the beginning of March, it was found that in every case faradisation of the sinus or vagus nerve had little or no effect on the cardiac rhythm or contractile force; in twelve eels experimented upon not one was definitely influenced by faradisation; here again muscarin and pilocarpin proved equally ineffective, and comparatively large doses not only failed to influence the rhythm, but a heart covered with such strong solutions as 4 per cent. pilocarpin nitrate or chloride for several minutes, showed no appreciable diminution in the force of the cardiac contraction.

In eels obtained early in April the same phenomenon was in evidence in some of them; in a certain number of these, however, fairly definite results could be obtained by faradisation as well as by the application of muscarin and pilocarpin, but here the heart could not be always brought to a standstill by faradisation, though a considerable slowing was often observed; if the faradisation was applied for even a comparatively short time, the heart generally succeeded in escaping from the inhibitory influence and regained its normal rate; the application of a stronger stimulus sometimes gave a result, but it was always less than that obtained from the primary stimulation, and soon the heart escaped and could not be again influenced by vagal excitation. After this faradisation the heart was quite uninfluenced by muscarin or pilocarpin even in large amounts, and the fact that in these hearts the usual effects of the drugs can be prevented by previous faradisation of the vagus seems to prove that muscarin and pilocarpin are directly dependent on a functionally intact inhibitory mechanism for their action in slowing or stopping the heart.

Again, the effect of muscarin and pilocarpin always ran parallel with that of faradisation; in several cases a heart which had escaped from fairly weak faradisation was slowed by pilocarpin, but the effect very soon passed over, the heart regaining its former rhythm; when this happened, pilocarpin, even in large doses, was quite ineffective.

Fig. IV shows a tracing from such a heart. In A is seen a stoppage from weak faradisation, which was applied at the point marked on the left. After a short initial stoppage, beat escaped though current still applied. B is a continuation of the same tracing showing

result of application of pilocarpin. (The difference in level is due to a reflex movement of the eel).

In C the pilocarpin is almost recovered from; atropin has been applied at part marked.

FIG. IV. Effect of faradisation and pilocarpin on a heart in which inhibitory action was present to a slight degree.

In these hearts it was obvious that vagal inhibitory power was present only to a slight extent, and this inhibition seemed to be still less in evidence if the heart was injured in the process of exposure; an excised heart at this season seldom gave any definite indication of inhibition either as the result of faradisation or drugs, and it would seem that even in hearts in which inhibition is very pronounced, mechanical injury tends to diminish the phenomenon.

More eels were obtained at different seasons later on, and it was found that normal conditions became gradually established. In May, however, many hearts were obtained that seemed to possess no very marked inhibitory mechanism; here the same relationship as above stated obtained as regards faradisation and the application of muscarin and pilocarpin. In July, some eels were examined and found to be almost normal, though inhibition was not so marked in all as it appeared to be later.

In September and October, inhibition was found to be much more active, and both faradisation and muscarin or pilocarpin instantly stopped the heart for a long period; the application of atropin immediately restored cardiac action.

Fig. V shows a tracing taken from a normal eel-heart showing effect of pilocarpin: drug was applied at part marked on left and drum

stopped for a few seconds ; single ventricular beat in middle shows result of mechanical stimulation. On the right is seen the effect of atropin ; here drum was also stopped for a few seconds.

FIG. V. Effect of pilocarpin on normal heart.

At all seasons of the year a heart may occasionally be found in which faradisation of the vagus gives no result, and in such a case muscarin and pilocarpin are likewise incapable of slowing or arresting the rhythm. This close relationship between the effects of faradisation and the application of muscarin or pilocarpin can only be rationally explained by assuming that these drugs act by stimulation of the cardiac inhibitory nerves.

As in the case of the frog-heart this difference in regard to inhibition at different seasons is difficult to account for, but it is likely that the same general causes may be present as factors in both cases.

On the Condition of the Eel-Heart when brought to a standstill by Faradisation of the Vagus and by Muscarin or Pilocarpin.—In the normal eel-heart arrested by faradisation of the vagus, the ventricle generally remains quite responsive to stimulation, while the auricle remains inexcitable: the same thing commonly obtains in the eel-heart arrested by moderate doses of muscarin or pilocarpin; the ventricle responds readily to stimulation while the auricle remains quiescent. In many other points also, the eel-heart behaves in a peculiar manner while under the influence of vagus inhibition, and the same conditions seem to obtain under the

influence of muscarin and pilocarpin; these points are at present being investigated.

The following are notes of a few of the experiments done at different seasons of the year:—

EEL I.—March—

- 12.0 Heart exposed in situ : rate 28 per minute.
- 12.5 Faradisation applied to sinus : no result even when strong.
- 12.8 1 drop muscarin applied to sinus : no result ; 21 per minute.
- 12.12 Another drop applied : rate 20 per minute.
- 12.20 4 drops applied : heart beating fairly strong ; 18 per minute.
- 12.25 4 drops applied : slightly weaker ; 19 per minute.
- 12.30 Atropin applied, but no change in rate or force ; 19 per minute.
- 4.0 Heart still beating : very weak ; 17 per minute.

EEL II.—March—

- 11.10 Heart exposed in situ : rate 25 per minute.
- 11.15 Faradisation slowed heart slightly, but only for about 1 minute.
- 11.20 Pilocarpin nitrate (20 %)—1 drop applied : no result ; 25 per minute.
- 11.24 " " 2 drops applied : no result ; 23 per minute.
- 11.33 " " several drops applied : no result ; heart quite strong.
- 11.37 *Heart excised.*
- 11.40 Pilocarp. applied, many drops : no result ; 25 per minute.
- 11.45 Heart much weaker : 22 per minute.
- 11.55 Heart getting weaker : 19 per minute ; after little time 16 per minute.
- 11.55 Heart getting weaker : 19 per minute ; after little time 16 per minute.
- 12.5 Atropin applied : no result ; 18 per minute and weak.
- 12.8 Digitalin solution applied : beat stronger ; 16 per minute.
- 12.30 Heart beating, but very feeble : 13 per minute.

EEL I.—April—

- 10.30 Heart exposed : 19 per minute.
- 10.33 Faradisation effective ; only tried for $\frac{1}{4}$ minute.
- 10.38 Pilocarpin applied : beat slowed ; rate 14 per minute.
- 10.41 strong beat ; rate 10 per minute.
- 10.46 More pilocarpin applied : strong beat ; rate 12 per minute.
- 10.55 strong beat ; rate 16 per minute.
- 11.0 beat rather weaker ; rate 16 per minute.
- 11.3 Atropin applied : beat 22 per minute.

Heart continued to beat for over 6 hours.

EEL I.—July—

- 11.15 Heart exposed : reflex inhibition from gill easily obtained ; heart stopped for 65 seconds. Another arrest for 65 seconds occurred when gill again pressed. Faradisation of sinus : ready arrest of whole heart for 1 minute.
- 11.27 *Heart excised.*
- 11.29 Faradisation : arrest of whole heart for 2 minutes—10 seconds ; then one beat occurred followed after an interval of 40 seconds by another.
- 11.37 Faradisation : inhibition for over 3 minutes.
- 12.5 Faradisation of auricle : arrest of whole heart for 3 minutes (not tried long). Went on all right when current stopped. Heart dull : Ringer's fluid applied.
- 12.30 Beating very slowly ; $4\frac{1}{2}$ per minute.
Ringer's fluid removed to try faradisation : heart did not beat.
,, ,, re-applied : heart again gave auricular beat. Ventricle not beating.
- 12.35 Faradisation of auricle stopped it for 1 minute (not tried longer) : sinus went on beating.
- 12.42 Faradisation of sinus readily arrested sinus and auricle.
- 12.46 Pilocarpin readily arrested and atropin restored the beats.

EEL II.—July—

- 10.16 Heart exposed.
- 10.19 Reflex inhibition from pressure on gill (1 minute) easily got.
- 10.25 Faradisation of sinus : arrest of whole heart (for 1 minute).
- 10.29 Piece of auricle ligatured off with some blood on it.
- 10.30 *Heart excised.*
- 10.31 Inhibited from faradisation of sinus.
- 10.35 Separated piece of auricle began to beat some little time ago—now 6 per second.
- 10.40 Inhibitory effect of faradisation of excised heart is absent even with fairly strong current.
- 10.41 Faradisation of separated (autom. beating) auricle does not inhibit. Some exciting effect at beginning.
- 10.43 Pilocarpine chlor. (1 %) applied to (a) excised heart and (b) isolated piece of auricle : many drops poured on.
- 10.47 Pilocarpine has not arrested or apparently depressed (a) or (b).
- 10.50 Pilocarpine still no effect.
- 11.0 Heart still beating at good rate though weaker ; separated piece of auricle still beating.
- 11.5 Fresh blood from another eel (very large) has been applied to separated piece of auricle ; now beating rapidly 23 per minute and vigorously : pilocarpine again applied—no arrest.
- 11.6 Atropin applied to excised heart : no improvement.

- 11.20 (a) and (b) still beating; (a) weaker and ventricle responds only to every second auricle beat.
- 12.45 (a) and (b) still beating though weakly, Pilocarpine no effect.

EEL I.—October—

- 11.30 Heart exposed: rate 26 per minute.
- 11.33 Faradisation gave marked result.
- 11.35 Pilocarpin applied: heart stopped after $\frac{1}{2}$ minute.
- 11.38 Heart beating slowly: 5 per minute; strong.
- 11.39 More pilocarpin applied: 3 per minute; strong.
- 11.42 Atropin applied: 23 per minute (after $\frac{1}{2}$ minute).
- 11.48 Faradisation negative after atropin.
- 11.55 Heart at 26 per minute.

After 20 hours—

Heart examined. Sinus 16 per minute (weak); ventricle 6 per minute (also weak). Pilocarp. nitrate applied (3 drops 2 % sol.). Heart: 3 per minute (ventricle); 9 per minute (sinus).
Atropin sulphate sol. applied: 3 per minute; sinus 9 per minute. Heart very much weaker, and only shows feeble wave of contraction.

EEL II.—October.

- 11.15 Heart exposed: 28 per minute.
- 11.16 Faradisation gave marked result—(tried for 1 minute only).
- 11.20 Applied 1 drop (2 % sol.) pilocarp. nitrate: 12 per minute: strong beat: 4 per minute.
- 11.23 Applied another drop pilocarp. sol.: 4 per minute.
strong beat: 3 per minute.
fairly strong beat: 3 per minute.
- 11.41 Applied 2 drops pilocarpin. sol.: 3 per minute.
- 11.46 Applied 3 drops „ 3 per minute.
- 11.48 Heart covered up with blotting paper saturated with pilocarp.
- After 2 hours. Solution (2 %). Also many drops poured on heart.
- 1.48 Beat not very strong: rate 26 per minute.

ON THE DEPRESSANT ACTION OF MUSCARIN AND PILOCARPIN ON THE EEL-HEART

Muscarin.—The sample of muscarin obtained from Professor Schmiedeberg was used, and it was found that the direct depressant effect of the drug on the cardiac muscle, though much greater than pilocarpin, was not very marked, at least for some considerable time,

unless a comparatively large amount had been applied. A normal heart could be arrested by the application of one drop to the sinus region, but hearts in which inhibition was absent were often not affected by many drops, either as regards rhythm or contractile force—in fact it generally required a considerable time (from five to ten minutes or more) for any appreciable diminution to become apparent in the cardiac rate or force. The contrast between the comparatively sudden stoppage or slowing of the normal heart caused by say one drop of muscarin applied to the sinus region, and the very gradual diminution of force and rate noticed only after a considerable time in a heart in which the inhibitory apparatus is functionless, is very marked and suggestive; this gradual weakening is not helped by atropin, but the application of digitalin in weak solution increases the force. On the other hand, digitalin applied to a heart stopped by a drop or two of muscarin in the ordinary way has little effect. These points indicate that the drug must necessarily possess two different modes of action. As the supply of muscarin was limited the majority of eel-heart experiments were performed with pilocarpin.

Pilocarpin.—As the result of very many experiments with pilocarpin, it was found that this drug in doses sufficient to produce profound cardiac inhibition, proved to have only very feeble muscle depressant power; different hearts require different strengths within certain limits, but generally a few drops of a 1 per cent. or 2 per cent. solution of the salt was quite sufficient to produce a marked result in the normal heart. In a heart possessed of but feeble inhibitory power the initial slowing consequent on the application of a few drops of pilocarpin solution was quickly recovered from, and in these cases the application of the drug in quantities sufficient to cover up the heart so that it was beating in a solution of pilocarpin, did not seem to depress it, at least for a very considerable time: the same held true when as much as 30 minims was injected into the circulation. In some hearts covered with 2 per cent. pilocarpin nitrate, the heart was wrapped up with blotting paper saturated with the same solution and left over night; next day it was sometimes found to be beating fairly strongly, though naturally very much weaker than at first;

at other times there was no spontaneous contraction, but there was quite a ready response to mechanical stimulation of the ventricle. In this case, as with muscarin, atropin had no effect, but digitalin in Ringer's solution often revived it. In short, pilocarpin, though undoubtedly possessing muscle depressant action in large doses, does not seem by any means to be very active in this respect, especially when used in moderate amount. Were the primary stoppage the result of direct cardiac muscle depression, it is impossible to conceive how the muscle could so often overcome the result of this depression while still in actual contact with a strong solution of the drug.

ON GASKELL'S ELECTRICAL EXPERIMENT ON THE TORTOISE HEART

Gaskell showed that in the heart of the tortoise, when the auricle was cut away from the sinus and the coronary nerve left intact, the isolated auricle and ventricle preparation would, after a time, beat with its own rhythm, and then stimulation of the right vagus nerve in the neck would diminish the size of the beats of the auricle¹, this showed that the vagus must be active during the period of quiescence, the non-manifestation of that activity being merely due to the want of suitable means of making it visible. Later on, he describes a method by means of which he ascertained that stimulation of the vagus in the neck caused some alteration in the non-beating muscle of the auricle, which was manifested by an electrical change of an opposite sign to that which accompanied contraction of the muscle², this change in the quiescent auricle he did not get on stopping the sinus by muscarin directly applied, and from this it is argued that muscarin does not act through the inhibitory nerves.

Under normal conditions the sinus acts as the leader of the heart, the auricular and ventricular beat following in direct sequence to the lead of the sinus. When the heart is inhibited by the application of muscarin to the sinus, standstill is observed on account of the fact

1. *Journal of Physiology*, Vol. IV, p. 85.

2. *Journal of Physiology*, Vol. VIII, p. 406-407.

that the leader is stopped and no active inhibitory action is set up in the auricular or ventricular tissue as long as the drug does not come directly into contact with these parts. If muscarin acts on the nerve endings, then in Gaskell's experiment the sinus would of course stop as the result of local stimulation of the nerve endings in the sinus, but there is no reason why its influence should be apparent by the production of an electrical change in the separated auricle with which it did not come into contact; it merely acted locally at the seat of application, and no change, electrical or otherwise, could be expected in any other part of the heart.

On the other hand, this experiment affords evidence that muscarin does not indirectly influence the auricle by acting on the sinus ganglia or on the vagus fibres passing through the sinus (pre-ganglionic or post-ganglionic). The experiment, however, is quite in accord with the view that muscarin acts on nerve endings.

HEART OF NEWT

Newts were examined in April and June. In both cases ova were in evidence, and more particularly in the June newts. In April, several hearts were found to respond to faradisation and to pilocarpin, but as a rule it was not possible to prolong inhibition for any considerable period; in general the heart gradually escaped from the effects of stimulation and seemed unaffected by a stronger application of the faradic current or pilocarpin. Some hearts gave no response when acted on by an interrupted current, and in these no pilocarpin effect was obtained. In the June newts it was practically impossible to elicit any trace of inhibition.

Under normal conditions, as proved by the researches of Professor MacWilliam, the heart of the newt is most susceptible to vagus stimulation, very weak excitation causing arrest of whole or part of the heart according to the nerve acted upon; local inhibition is also easily procured as the result of direct local application of the faradic current, the part arrested being inexcitable to direct stimulation. Several features of vagal stimulation peculiar to the newt's heart seem to be reproduced when the heart is arrested by pilocarpin.

Owing to the general unsatisfactory condition of the newt-hearts examined it was difficult to arrive at definite conclusions about some of the points examined, but there was a certain amount of evidence showing the possibility of obtaining local inhibition by careful circumscribed application of the drug ; again, during strong pilocarpin inhibition, there was a distinct diminution in the tendency of the heart to respond to direct stimuli, a response being readily elicited only as the drug effects began to wear off. It is hoped that a fuller investigation of these points may be made later, when the newt-heart becomes more normal with regard to inhibition.

HEART OF SALAMANDER

A few experiments performed on the salamander-heart in June elicited the fact that inhibition was but little in evidence at this season. In this heart there was practically a reproduction of what was noted as occurring in the frog-heart during the spring and summer months. In several hearts stimulation of the vagus nerve seemed to cause no change, or if any change was in evidence it generally consisted of slight acceleration of the rhythm. In one heart electrical stimulation had a very slight effect, and this heart was brought to a standstill for about thirty seconds by one drop of a 2 per cent. pilocarpin solution : after this time it began to beat and very quickly regained its former rhythm. Subsequent soaking with 2 per cent. pilocarpin failed to affect it for a considerable time. After fifteen minutes it gradually became weaker and slower, but was beating weakly after one hour : the application of atropin did not help to restore the beat. In another heart faradisation of the vagus trunk gave no result, but direct stimulation of the ventricle caused a temporary stoppage ; this, however, soon passed over, and though stimulation of increased strength was now applied, no change took place in the rate or strength. In this heart pilocarpin was without effect.

A heart in which temporary slowing occurred as the result of direct stimulation to the sinus region, gave no result on the exhibition of pilocarpin. It is interesting to note that all the specimens utilised were distended with ova and obviously approaching the spawning

season. Some similar experiments were done in September and October. In the September experiments the amount of inhibition present was not great, but in general much more distinct than was found in the above hearts in June. In the October experiments inhibition was more in evidence. In the salamander it is likely that inhibition becomes still more marked towards the winter and early spring months.

As in other hearts, it was very apparent that injury to the cardiac walls or cutting of the bigger vessels tended to destroy any little inhibition that might be present, for in no case was inhibition obtained where any serious injury occurred in exposing the heart.

CONCLUSIONS

1. In the early months of the year, and especially in the season of increased sexual activity, there is a general diminution of inhibitory power in the frog-heart ; when faradisation of the inhibitory apparatus has little effect, muscarin and pilocarpin also produce but very slight effects ; when vagus stimulation slows the heart, muscarin or pilocarpin will do so also : in short, the effect produced on the heart by small doses of muscarin and pilocarpin is in direct proportion to the influence exercised on the heart by the vagus nerve.

2. Faradisation applied for some time to a heart not possessed of much inhibitory power will tire out the mechanism and the heart will escape ; muscarin or pilocarpin subsequently applied has, in this case, no effect ; consequently, after a heart escapes from the action of pilocarpin, it cannot be arrested by vagal stimulation.

3. Mechanical injury resulting in loss of blood tends to diminish the inhibitory power in even a normal heart, and often destroys it in a case where it was not originally present to any marked extent.

4. Muscarin and pilocarpin in small doses, when dropped on the heart, have no appreciable immediate depressant effect on the cardiac muscle. In very weak hearts, a comparatively small dose may cause weakening, but this takes place gradually and differs from true inhibition in not being helped by atropin ; digitalin tends to increase the force. Many hearts will beat in a strong solution

(*e.g.* 2 per cent to 5 per cent.) of pilocarpin for a long period without much apparent lessening of strength or rate.

5. In the eel-heart there were found great differences in the inhibitory power of the vagus, these differences being most marked in the spring months, when, in some cases, faradisation of the vagus or sinus gives no result. As noted for the frog-heart, in cases where vagus faradisation is ineffective, no result is obtained by the application of muscarin or pilocarpin; the converse of this holds true.

6. Contractile force is similarly affected by pilocarpin and muscarin and by vagus stimulation—diminished or annulled in the auricle while not directly influenced in the ventricle: the mode of recommencement after standstill also corresponds in the two cases—the tendency of the ‘interjugular’ part of the sinus to initiate the renewal of rhythm being evident in the eel-heart.

7. A condition corresponding somewhat to the antagonism of muscarin and pilocarpin described by Ringer, is sometimes probably the result of a primary excitation of inhibitory nerves by muscarin followed by paralysis of these same nerves on the addition of a pretty large dose of pilocarpin; the same effect in certain hearts can be obtained by using muscarin or pilocarpin alone (frog).

8. Gaskell’s muscarin experiment on the tortoise-heart does not exclude the possibility of the drug acting through the nerve endings.

9. In the heart of the newt and salamander, inhibition is absent or markedly diminished at certain periods corresponding roughly to the seasons of sexual activity. Here the same general conditions obtain as were noticed in the frog and eel heart.

10. All the available evidence shows that in different types of the vertebrate heart the local application of muscarin and of pilocarpin reproduces the special results obtained by vagal stimulation in each heart, widely different as they are in different types as regards the parts of the heart acted on, the characters of the action, etc.

11. The above observations strongly support the view that muscarin and pilocarpin in small doses cause arrest of cardiac action

indirectly through excitation of the inhibitory nerve mechanism ('nerve-endings'), and not directly in virtue of their depressant action on the contractile mechanism.

In conclusion, it affords me much pleasure to express my thanks to Professor MacWilliam for much valuable advice and assistance during the course of this investigation.