HYDROGEN ION CONCENTRATIONS LIMITING AUTOMATICITY IN DIFFERENT REGIONS OF THE FROG'S HEART[1].

By DOROTHY DALE, *Fellow of Newnham College*, AND C. R. A. THACKER, *Downing College, Cambridge.*

(*From the Physiological Laboratory, Cambridge.*)

It is now generally accepted, chiefly from the work of Gaskell and Engelmann, that the different parts of the heart are of varying automaticity, and that the normal beat is due to the more rapid rhythmic contraction of the venous end of the heart, the contraction set up being propagated to the other parts in sequence. And it is known that in various circumstances the order of beat may be reversed.

The work of Herlitzka, Mines, Clark and others has shown the great importance of the *reaction* of the fluid perfusing the heart in connexion with the origination of rhythm, while Mines (1) has pointed out that the limits of hydrogen ion concentration (C_H.) within which the development of spontaneous beats is possible differ for the hearts of different kinds of animals.

In experimenting with the auricle-ventricle preparation of the frog we found that this preparation would not beat spontaneously in a slightly acid Ringer's solution in which the sinus would beat regularly, but that if a small amount of Na_2CO_3 solution was introduced into the cannula the auricle at once started a regular, spontaneous rhythm. This observation led us to make experiments to ascertain whether there is a difference in the optimal C_H. for the development of rhythm in the different chambers of the heart, and, if so, to determine the limits of C_H. between which each chamber will originate spontaneous contractions.

Methods. Medium-sized or large specimens of *Rana temp.* were used for these experiments which were carried out chiefly in the

[1] A preliminary communication of this research was published in the *Proc. Physiol. Soc.* p. i. 1913 (this *Journal*, XLVII.).

summer months. In investigating the automaticity of the sinus, the whole heart was perfused from the inferior vena cava below the liver, the perfusion pressure being kept constant by the use of a five-way cannula[1]. In some cases a fine silk thread tied to the tip of the ventricle was attached to a light lever and graphic records taken.

In making the auricle-ventricle preparation we followed the technique described by Symes (2). For the recording lever, a V-shaped piece of aluminium sheet, mounted at the end of one arm on a pivot, bore a writing point on the other arm. A thread tied to the tip of the ventricle was attached to the most dependent part. The drops of fluid escaping from the cut aortæ collected here and ran down to a vessel below. When the perfusion fluid was changed, the cannula was emptied by a side tube, and washed out with the new solution before the perfusion was continued. As the perfusion with any given solution lasted at least several minutes any temporary effects due to alterations in pressure on changing the solution were eliminated. A perfusion pressure of 4·5 to 5·0 cms. of the solution was found by experiment to be the most suitable.

In making the ventricle preparation, a Symes cannula was inserted through the left auricle into the ventricle and a ligature tied round the auriculo-ventricular junction. The perfusion fluid escaped through a small vertical cut 1 to 2 mm. long made in the ventricle wall with a very fine scalpel. An attempt was made to use a two-way cannula with inlet and outlet tubes so as to remove the necessity of damaging the ventricle wall, but it was found more satisfactory to use the simple cannula. The ventricle and auricle-ventricle preparations were set up in the same way, but rather higher pressures (6·5 to 7·0 cm.) were needed for the former than the latter.

Since equilibrium was attained only slowly, in some cases after 15′ to 30′, the experiments were necessarily of long duration, lasting usually from two to four hours. The slow attainment of equilibrium was not due to the methods of perfusion, for experiments performed in this laboratory have shown that the replacement of fluid occurs very rapidly, equilibrium with certain solutions being attained within 30″.

In dealing with the intact heart, experience showed that the mechanical records might be misleading and were of little use in giving information as to the condition of the sinus venosus. In some cases

[1] For description and method of insertion of cannula, see Mines, this *Journal*, XLVI. p. 190. 1913.

when the auricle and ventricle were beating vigorously, the most careful observation of the heart through a lens failed to show any sign of movement in the sinus. In the later experiments on the whole heart, therefore, no mechanical record was taken but the heart was observed directly throughout the experiment, a procedure which, if laborious, resulted in a much truer idea of the condition of the heart than could be gained from the mechanical record with direct observation only at intervals, for we were dealing with the rhythm, not with the force of the contractions.

The water used in making up the solutions was distilled in a tinned vessel and condensed and stored in glass. Kahlbaum's purest chemicals were used throughout. The solutions of sodium chloride, potassium chloride, sodium acetate and boric acid were prepared by weighing, the calcium chloride by titration against standard silver nitrate, and the hydrochloric acid and caustic soda by titration against standard alkali and acid respectively. All solutions which were kept for any length of time were stored in bottles of resistance glass or in Jena flasks.

Now the C_H· of a solution containing only strongly dissociated salts is changed by one passage through the frog's heart and is readily altered by the absorption of CO_2 from the air. It was therefore necessary to have "buffers" present in the solutions to stabilise their hydrogen ion concentrations. The C_H· of a Ringer's solution containing ·005 mol. boric acid and sodium acetate is not changed appreciably in one passage through the heart and the presence of these substances does not appear to be harmful (3).

Two stock solutions were prepared, and by mixing these in definite proportions a solution of approximately the desired C_H· between $C_H \cdot 10^{-2}$ and $C_H \cdot 10^{-12}$ could be obtained. In each experiment, the determinations of the C_H· of the solutions used were made by Sörensen's colorimetric method (4). The stock solutions were:

Solution I	*Solution II*
Hydrochloric acid M/10 400 c.c.	Caustic soda M/10 400 c.c.
Boric acid	M/10 100 c.c.
Sodium acetate	M/10 100 c.c.
Potassium chloride	M/10 60 c.c.
Calcium ,,	M/10 40 c.c.
Sodium ,,	M/8 to 2000 c.c.

The objection might be raised that the presence of these "buffers" might alter the C_H· limits of the heart, but such a possibility does not affect the main issue of this paper. The interest of the experiments

lies in the physiological differentiation between the chambers of the heart by solutions differing materially only in their C_H.[1]. The use of different "buffers" would in all probability change the C_H. limits for a given part of the heart. The observation that an acid solution containing glycocoll is much less toxic to Paramœcium (5) than a solution of the same C_H. containing sodium acetate as "buffer" would suggest that the heart might perhaps stand a greater C_H. if the sodium acetate in the solution were replaced by glycocoll. Experiments were carried out to test this point, and the results confirmed this suggestion (see Fig. 1). But the number of experiments was too small to be entirely convincing.

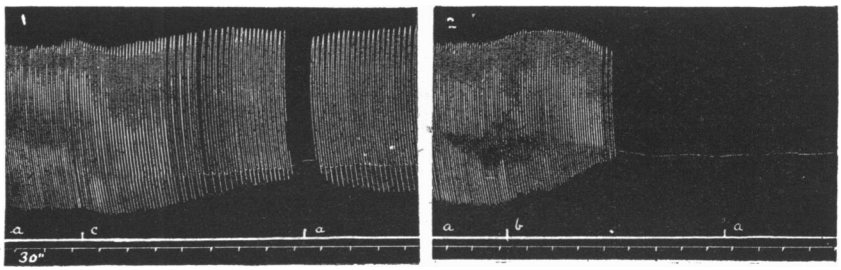

Fig. 1. Intact heart perfused inf. vena cava: (*a*) boric acetate Ringer $C_H \cdot 10^{-7.5}$; (*b*) boric acetate Ringer $C_H \cdot 10^{-3.5}$; (*c*) boric glycocoll Ringer $C_H \cdot 10^{-3.5}$.

Perfusion of the heart with acid and alkaline Ringer's fluid.

In perfusing the whole heart, a solution of $C_H \cdot 10^{-5}$ to $C_H \cdot 10^{-5.5}$ was never sufficiently acid to stop the rhythm of the sinus, although in some cases the auricle and ventricle did not respond to the sinus after prolonged perfusion (20′ to 30′) of the acid solution. In no case was rhythm maintained for any length of time in an auricle-ventricle preparation by a solution of $C_H \cdot 10^{-5}$ to $C_H \cdot 10^{-5.5}$ (see Fig. 2). It was usually found that a solution of $C_H \cdot 10^{-8}$ was needed to start contractions in an auricle-ventricle preparation, but that when once started, they could be maintained fairly well in a solution of $C_H \cdot 10^{-6.5}$, but that the preparation was stopped by $C_H \cdot 10^{-5.5}$ after 5′ to 15′ perfusion. In the same way, a more alkaline solution was needed to start a rhythm in the ventricle than was necessary to maintain that rhythm when once started. A solution of $C_H \cdot 10^{-5.5}$ stopped the ventricular rhythm in about 15″,

[1] It is theoretically impossible to have two solutions differing only in their C_H., but the proportional changes in ionic concentrations other than those of the H and OH ions are so small as to be negligible in the solutions we have used.

while a solution of $C_H \cdot 10^{-6.5}$ in almost every case brought the ventricle to rest in 5′ to 15′ (see Fig. 3).

The determination of the alkaline limits for the sinus and auricle was complicated by the great tendency of the ventricle to initiate the

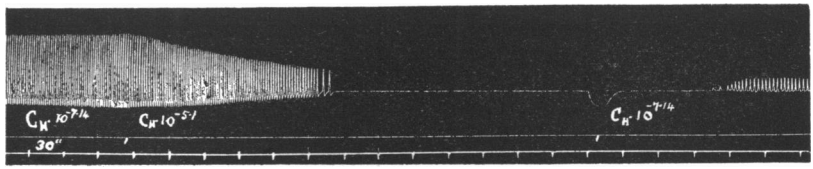

Fig. 2. Auricle-ventricle preparation, showing arrest in solution $C_H \cdot 10^{-5.1}$ (boric acetate Ringer).

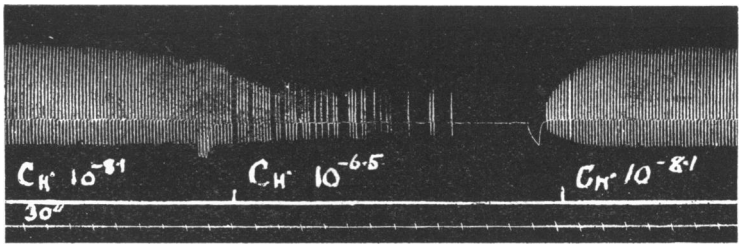

Fig. 3. Ventricle preparation, showing arrest in solution $C_H \cdot 10^{-6.5}$.

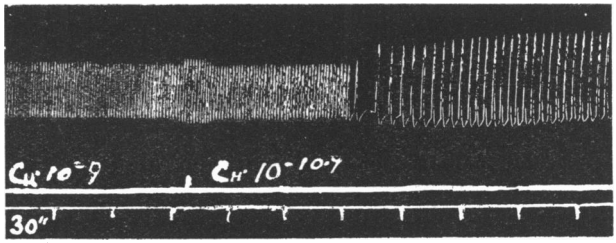

Fig. 4. Auricle-ventricle preparation, showing reversal of sequence in alkaline solution. First part of tracing, beats auricle to ventricle, latter part of tracing, slower beats ventricle to auricle.

rhythm in alkaline solutions (see Fig. 4). This fact taken by itself would certainly show that an alkaline solution is more favourable to the origination of rhythm in the ventricle than in the sinus or auricle. In a number of experiments, however, where the most careful observation failed to show any abnormal sequence, it was found that on the whole the ventricle would beat in a solution so alkaline that the auricle

was arrested. Fig. 5 shows the ventricle beating well in a solution of $C_H \cdot 10^{-11}$. Similarly, the auricle in some cases would originate a rhythm in a solution which would hold in abeyance the automaticity of the sinus. These experiments were not altogether conclusive, but taken in conjunction with others, which will be described later, seem to leave no doubt but that there is a difference in the alkaline as in the acid limits for automaticity of the different chambers of the heart.

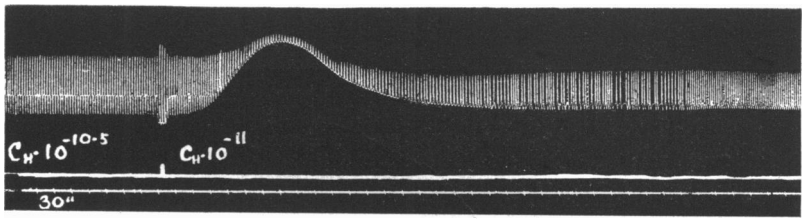

Fig. 5. Ventricle preparation beating in solution $C_H \cdot 10^{-11}$.

Table of experiments on acid limits.

	Solution		
	$C_H \cdot 10^{-5.5}$	$C_H \cdot 10^{-6.5}$	$C_H \cdot 10^{-8.1}$
Intact heart, sinus rhythm	0 expts. –		0 expts. –
	8 ,, +		8 ,, +
A.-V. preparation, auricular rhythm	15 ,, –	2 expts. –	0 ,, –
	0 ,, +	10 ,, +	17 ,, +
V. preparation, ventricular rhythm	2 ,, – (within 15")	7 ,, –	0 ,, –
	0 expts. +	2 ,, +	9 ,, +

The *minus* sign signifies that the heart was stopped in the solution in the number of experiments performed. The *plus* sign indicates that the rhythm continued after at least 15′ perfusion.

From this table we see clearly that the sinus will beat in a solution too acid for the origination of rhythm in auricular muscle, while a solution in which the auricle will beat spontaneously may be too acid for the ventricle. A similar gradation probably occurs when we consider the alkaline limits. An alkaline solution which will stop the sinus will allow the auricle to beat, and a greater alkalinity is necessary to stop the rhythm of the ventricle than that of the auricle.

The objection might be raised that the hearts of normal frogs show considerable individual variations, and that these variations might account for the differences observed. In the auricle-ventricle preparation, however, the remains of the sinus above the ligature often beat regularly when the acid solution used was holding the auricle and ventricle at

rest. In order to rule out the factor of individuality, in a number of cases the whole heart was perfused with a solution of C_H which according to the table would probably be outside the limit for the auricle-ventricle preparation. After the whole heart with rhythm originating in the sinus had beat strongly and regularly in this solution for about 30′, an auricle-ventricle preparation was made of the heart and perfusion with the same solution was continued. It was always found that the auricle-ventricle preparation would not beat spontaneously in the

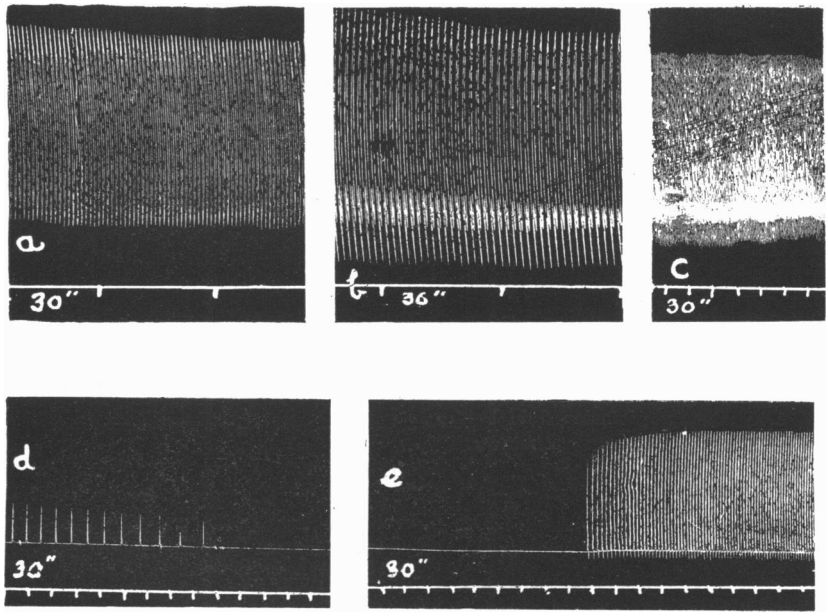

Fig. 6. (*a*), (*b*) and (*c*) heart perfused through inf. vena cava. (*d*) and (*e*) auricle-ventricle preparation. (*a*) after 5′ perfusion of solution $C_H \cdot 10^{-6.4}$; (*b*) after 4′ perfusion of solution $C_H \cdot 10^{-4.2}$; (*c*) after 6′ perfusion of solution $C_H \cdot 10^{-6.4}$ (20′ after beginning of experiment); (*d*) after 7′ perfusion of solution $C_H \cdot 10^{-6.4}$. Record (*d*) followed by perfusion of solution $C_H \cdot 10^{-8.3}$ for 24′ during which time the heart was quiescent. (*e*) record 24′ after (*d*) perfusion of solution $C_H \cdot 10^{-9.2}$ started at beginning of record (*e*).

slightly acid solution which had maintained the sinus rhythm, and that a rhythm started by a more alkaline solution was abolished on reverting to the acid solution. Similar experiments were carried out in which a heart previously perfused through the vena cava was made into a ventricle preparation, and the results here also were confirmatory (see Fig. 6).

Similar confirmatory evidence was obtained from ten experiments in which the heart perfused through the vena cava was observed the whole time. The records of two such experiments are given below in detail.

EXP. 1.

11.15	♂ frog pithed; cannula in inferior vena cava.	
11.25	Perfuse boric acetate Ringer, $C_H \cdot 10^{-7.2}$. T. = 19·5° C.	
11.43	Rate, 41 beats per minute.	
11.50	,, 39 ,, ,,	
12.1	,, 38 ,, ,,	
12.8	,, 40 ,, ,,	
12.13	,, 40 ,, ,,	
12.15	,, 40 ,, ,,	
12.16	Change perfusion fluid to $C_H \cdot 10^{-5.5}$.	
12.17	Rate, 37 beats per minute.	
12.21	,, 36 ,, ,,	
12.23	,, 33 ,, ,,	Heart very distended. Ventricular beat weak.
12.25	,, 30 ,, ,,	
12.29	,, 28 ,, ,,	
12.31	,, 28 ,, ,,	S.-A. interval = 1″, A.-V. interval = 1″.
12.40	,, 26 ,, ,,	
12.49	,, 29 ,, ,,	S.-A. interval = $1\frac{2}{5}$″, A.-V. interval = $1\frac{1}{5}$″.
12.52	,, 29 ,, ,,	Sinus beat vigorous, aur. and ventr. beats very weak.
12.58½	,, 29 ,, ,,	S.-V. interval = $2\frac{2}{5}$″.
1.0	Change perfusion fluid to $C_H \cdot 10^{-7.2}$.	
1.1	Rate, 29 beats per minute.	
1.3	,, 31 ,, ,,	Ventr. beat much improved.
1.5	,, 34 ,, ,,	S.-V. interval = $1\frac{4}{5}$″; both S.-A. and A.-V. intervals much reduced.
1.10	,, 39 ,, ,,	Strong regular beat.
1.12		S.-V. interval = $1\frac{1}{5}$″.
1.22	,, 42 ,, ,,	
1.27	,, 42 ,, ,,	S.-V. interval = $1\frac{1}{5}$″.
1.38	,, 41 ,, ,;	
1.45	,, 41 ,, ,,	
1.46	Change perfusion fluid to $C_H \cdot 10^{-10.3}$.	
1.47	Rate, 42 beats per minute.	
1.49	,, 36 ,, ,,	
1.51	,, 35 ,, ,,	S.-V. interval = $\frac{4}{5}$″, relaxation incomplete.
2.0	Sinus has stopped, conduction A. to V. very rapid.	
2.1	Rate, 24 beats per minute.	
2.11	,, 24 ,, ,,	Bulbus beating strongly and regularly. A. and V. beat about once a minute.
2.24	,, 24 ,, ,,	Do. Do.
2.40	,, 24 ,, ,,	Do. Do.
3.6	,, 24 ,, ,,	Beat of bulbus followed by ventricle and auricle in sequence.
3.12½	Change perfusion fluid to $C_H \cdot 10^{-7.2}$.	

3.17 Rate, 38 beats per minute. Beats start in sinus; auricle and ventricle beat at half the rate (half-block at S.-A. junction).
3.20 ,, 43 ,, ,, Whole heart beating regularly.
3.30 ,, 42 ,, ,,
3.47 ,, 40 ,, ,,
3.50 ,, 40 ,, ,,
3.54 Perfusion discontinued; Symes cannula tied in sino-auricular junction and perfusion continued with fluid $C_H \cdot 10^{-7.2}$. Perf. pressure = 45 mm.
4.2 Heart has not begun beating; change perfusion fluid to $C_H \cdot 10^{-10.3}$. Slow rhythm starts at once. Beat is V. to A. Very rapid transmission.
4.20 Bulbus beating 19 per minute. Vent. and aur. responding to 1 in 4 bulbus beats.

Exp. 2.

1·7 ♂ frog pithed; cannula in inferior vena cava.
1.15 Perfuse boric acetate Ringer, $C_H \cdot 10^{-7.17}$. T. = 19° C.
1.22 Rate, 39 beats per minute.
1.34 ,, 38 ,, ,,
1.42 ,, 35 ,, ,,
1.48 ,, 35 ,, ,,
1.52 ,, 35 ,, ,,
1.53 Change perfusion fluid to $C_H \cdot 10^{-5.14}$.
1.54 Rate, 32 beats per minute.
2.1 ,, 28 ,, ,, Ventricle has stopped.
2.8 ,, 31 ,, ,, Sinus beating regularly, auricle occasionally follows.
2.14 ,, 30 ,, ,, Auricle has stopped.
2.17 ,, 30 ,, ,,
2.19 Change perfusion fluid to $C_H \cdot 10^{-7.17}$.
2.23 Rate, 24 beats per minute. Aur. following many sinus beats. Slow transmission.
2.28 ,, 28 ,, ,,
2.33 ,, 35 ,, ,, A. following sinus regularly. V. beating occasionally.
2.39 ,, 44 ,, ,,
2.44 ,, 44 ,, ,, Regular, strong beat. Conduction still slower than normal.
2.48 ,, 44 ,, ,,
2.49 Change perfusion fluid to $C_H \cdot 10^{-10.6}$.
2.50 Rate, 49 beats per minute.
2.52 ,, 46 ,, ,,
2.53 ,, 37 ,, ,, Sinus has stopped beating.
2.56 ,, 28 ,, ,, Heart does not relax completely.
3.0 ,, 26 ,, ,,
3.10 Sinus is beating again feebly, 32 beats per min.
3.13 Sinus has stopped again.
3.15 Rate, 25 beats per minute.
3.18½ ,, 20 ,, ,,
3.23 ,, 17 ,, ,,
3.26 ,, 16 ,, ,, T. = 19° C.

3.28 Rate, 14 beats per minute.
3.31 ,, 14 ,, ,, Reversed beat, V. to A.
3.34 ,, 15 ,, ,,
3.37 ,, 14 ,, ,,
3.49 ,, 11 ,, ,,
3.51 Change perfusion fluid to $C_{H\cdot}\ 10^{-7.17}$.
3.52 Rate, 10 beats per minute. Reversed beat still continues.
3.54 ,, 15 ,, ,, Normal sequence A. to V.
3.56 ,, 25 ,, ,, Sinus and ventr. have been slowly relaxing since change in perfusion fluid to $C_{H\cdot}\ 10^{-7.17}$.
3.58 ,, 28 ,, ,,
4.1 ,, 33 ,, ,, Sinus is beating feebly and rest of heart follows sinus beats.
4.13 ,, 34 ,, ,,
4.17 ,, 33 ,, ,,
4.20 Change perfusion fluid to $C_{H\cdot}\ 10^{-10.6}$.
4.27 Rate, 43 beats per minute.
4.31 ,, 35 ,, ,,
4.40 Sinus has stopped.
4.41 Rate, 16 beats per minute.
4.44 ,, 11 ,, ,, Reversed sequence. V. to A. Very rapid transmission.
4.48 ,, 33 ,, ,, Sinus beating again.
5.0 Whole heart has stopped. Ventricle much more excitable to prick than auricle, and auricle more than sinus.
5.5 Ventricle beating again followed by auricle.
5.7 Rate, 11 beats per minute.

The influence of $C_{H\cdot}$ *on the heart beat.*

(*a*) *The origination of rhythm.* The experiments made comparing the action of alkaline solutions on the intact heart, the auricle-ventricle preparation and the ventricle preparation made it probable that there is a difference in the liminal values of $C_{H\cdot}$ for the origination of rhythm between the different regions of the heart. This suggestion is amply confirmed by the experiments just described. These experiments show clearly that the power of originating a rhythm in alkaline solution is present in the highest degree in the bulbus, and in the least in the sinus. As we pass from the venous to the arterial side of the heart, we find a gradual increase in the liminal $C_{OH'}$ for the origination of rhythm.

As has already been stated, on the acid side the limiting values of $C_{H\cdot}$ which will maintain an automatic rhythm differ for the different regions of the heart. The range of $C_{H\cdot}$ therefore within which automatic activity is possible is not of very different extent for the different chambers of the heart, but is shifted more and more towards the alkaline side as we pass from the venous to the arterial end of the heart.

This is indicated in Fig. 7 which shows diagrammatically the limits for automaticity of the different chambers and the effects of alterations in C_H on the durations of the sinus-auricle and auricle-ventricle intervals and of the whole heart cycle.

The differences between the different regions of the heart are similar to those existing between the hearts of different species of animals. Mines (1) has shown that there is a difference in the optimal C_H for the hearts of the elasmobranch fish and Pecten, and quite recently Clark (6) has shown that the heart of the snake is less sensitive to increase both of C_H and of $C_{OH'}$ than the heart of the frog. Mines ascribes the difference in the optimal C_H to a difference in the iso-electric points of the tissues concerned. The present experiments may therefore indicate a difference in the iso-electric points of the tissues forming the various chambers of the heart, such as might depend, for instance, on slight differences in the constitution of certain of their proteins.

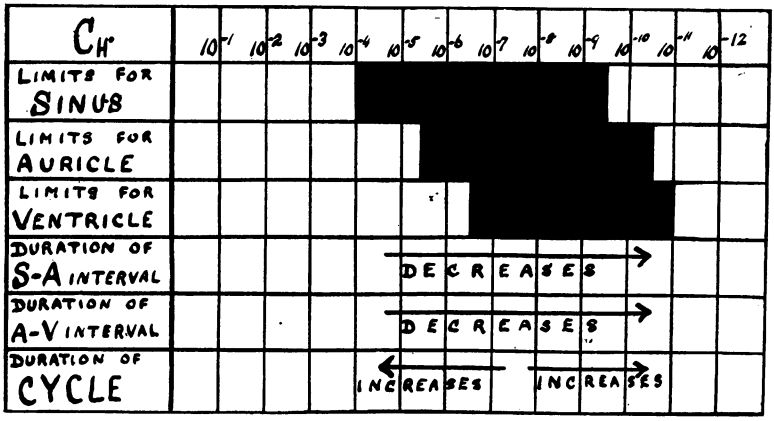

Fig. 7. Diagram showing C_H limits for the origination of rhythm in different regions of the heart, and the influence of changes in C_H on the durations of the S.-A. and A.-V. intervals and of the heart cycle.

(*b*) *The frequency of rhythm.* In perfusion of the whole heart there were well-marked changes in the frequency of the sinus with changes in the C_H of the fluid. In both acid and alkaline solutions the rate of the heart-beat was greatly diminished (see Figs. 8 and 9). In alkaline solution the slowing was in most cases preceded by a small temporary rise in frequency. The attainment of equilibrium was slow, and the solution had to be perfused for 15′ to 30′ before the rate had become steady in the new solution. As we have already shown, this

slow attainment of equilibrium was not due to the method of perfusion being inadequate. The lag in response to small changes in C_H appears to be very characteristic. Gaskell(7) insisted on the fact that after perfusion with an acid solution, recovery did not occur in a neutral

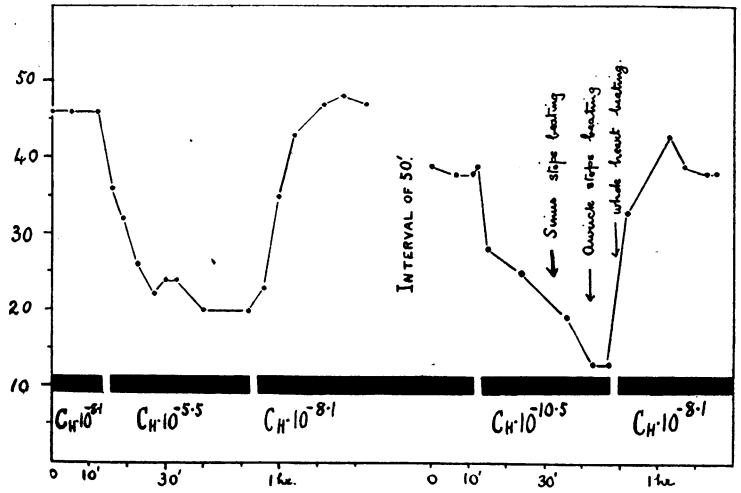

Fig. 8.

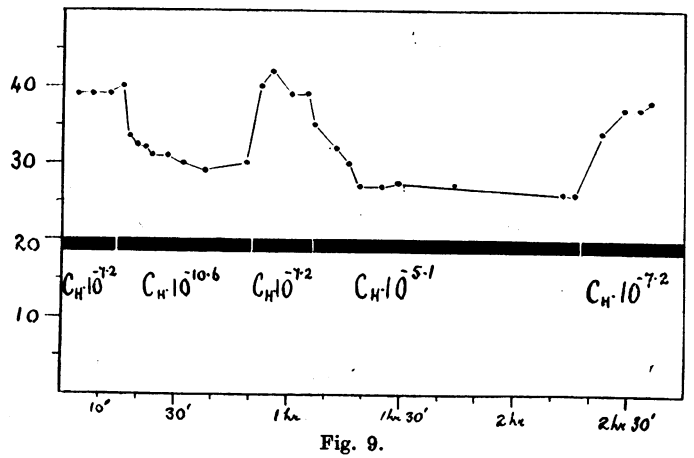

Fig. 9.

Figs. 8 and 9. Curves showing relation of C_H and frequency of the rhythm of the sinus. Ordinates = time in minutes. Abscissæ = frequency in beats per minute.

solution for a very long time. Mines(3) also calls attention to the slow attainment of equilibrium with changes in the C_H of the perfusion fluid, and his figures (Figs. 22–25) show clearly the gradual changes in the behaviour of the heart. A closely similar lag appears in the behaviour

of an artificial gelatine membrane (8),(9). The change in its electric charge produced by acid is only very slowly removed by washing in a neutral solution free from polyvalent ions.

The experiments seemed to show that the optimal C_H. for the frequency of the sinus rhythm was slightly on the alkaline side of the neutral point.

The effects of changes in C_H. on the frequency of rhythms originating in the auricle and ventricle varied in different experiments.

The change from a solution of $C_H . 10^{-8}$ to a more acid solution was always accompanied by a slowing of the rhythm. In going to a more alkaline solution, *e.g.* $C_H . 10^{-10.5}$, sometimes quickening, sometimes slowing was observed. This is a point which requires further experiment before any definite statement can be made, but a small majority of experiments showed optimal values of C_H. for the origination of rhythm in auricle and in ventricle.

(*c*) *Conduction in junctional tissue.* All the experiments showed clearly the typical diastolic conditions of the heart in acid solution. A very marked feature of the beat in acid solution was the slow conduction of the excited state from one chamber of the heart to another. This was such that in changing from a solution of $C_H . 10^{-7}$ to one of $C_H . 10^{-5.5}$ the duration of the interval between the beginnings of the sinus and ventricular contractions was approximately doubled. Both the sinus-auricle and auricle-ventricle intervals were lengthened. In some cases a solution of $C_H . 10^{-5.5}$ served to stop the contractions of auricles and ventricle after 10′ to 20′ perfusion; in others, however, the whole heart continued to beat fairly vigorously in the more acid solution but with a change in the character of the beat (which became diastolic) and with very slow transmission. In some cases a C_H. of about 10^{-4} was necessary to stop the auricles and ventricle after short perfusion, and such a solution was also inadequate to keep up the automaticity of the sinus for any length of time.

The general result of the experiments seems to show that the sinus will beat in a solution in which the auricles and ventricle are at rest. This standstill might be due either to a decreased excitability of the auricles and ventricle, so that they do not respond to the stimuli reaching them from the sinus, or to a block at the sino-auricular junction preventing the transmission of the excited state from the sinus to the auricles. No determinations were made of the excitability of auricular and ventricular muscle in acid solutions, but there appeared to be an increased difficulty in eliciting a response to a prick when the

ventricle was at rest in acid solution. On the other hand, the increased length of the sinus-auricle and auricle-ventricle intervals is considerable evidence in favour of the blocking of the impulse at the sino-auricular junction. Probably decrease both in conductivity and in excitability plays parts in the diastolic arrest of auricles and ventricle in acid solution.

In changing from a neutral to an alkaline solution of $C_H \cdot 10^{-10.5}$ the earliest and most marked change was the great increase in the rate of transmission. This was so great that in many cases it required the most careful inspection to determine the sequence when all chambers of the heart were beating. In alkaline solution, the sinus was the first to come to rest, next the auricle stopped and in some cases the ventricle

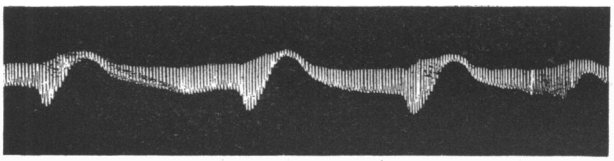

Fig. 10.

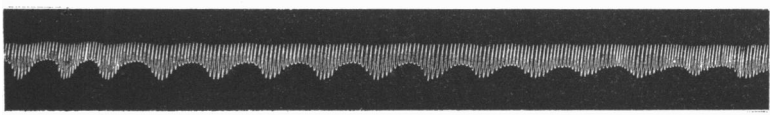

Fig. 11.

Figs. 10 and 11. Ventricle preparation. Rhythmic variations in tone in solution $C_H \cdot 10^{-10.7}$.

too, so that the bulbus aortæ alone was beating. On reverting to the neutral solution, the beat reappeared in the chambers in the reverse order to its disappearance: first the ventricle contracted, responding to the bulbus contractions, then the auricle would follow in sequence to the ventricle, so that the heart was beating strongly with reversed rhythm. Later the sinus would begin beating, but independent of the rest of the heart. Suddenly a slight pause would occur, the auricular beat would follow that of the sinus, and the whole heart resume its normal sequence. In many experiments on the auricle-ventricle preparation while perfusing a distinctly alkaline solution ($C_H \cdot 10^{-10.5}$) it was found that a reversed rhythm was very liable to occur. Both in the whole heart and in the auricle-ventricle preparation in alkaline

solution it was often a matter of uncertainty whether the ventricle was following the auricle or *vice versa*, but the change was so marked when a reversed rhythm gave place to a normal one that all doubt was then removed.

Several of the records show well-marked rhythmical variations in the tone of the ventricular muscle in alkaline solution (see Figs. 10 and 11). This effect is not due to the rhythmical dropping of the solution as it leaves the heart. The following suggests itself as an explanation: when the perfusion fluid is passing through the heart at its normal rapid rate, the acid produced by the heart muscle is insufficient to alter appreciably the $C_{H'}$ of the fluid containing "buffers." The alkalinity of the fluid causes an increase in tone of the heart muscle. While the tone is high, the amount of fluid passing through the heart is very much diminished, and it is possible that the CO_2 produced by the heart muscle is sufficient to decrease the $C_{OH'}$ of the solution, now almost stagnant in the heart cavities, in spite of the presence of "buffers." This decrease in $C_{OH'}$ of the solution in the heart leads to decrease in tone of the muscle and consequently to increase in the flow through the heart of fresh alkaline fluid, thus bringing us to the starting point of the cycle. Unfortunately no tests were made as to the $C_{H'}$ of the solutions leaving the heart at various stages of the cycle[1].

SUMMARY.

The limits of $C_{H'}$ for the origination of rhythm in different parts of the frog's heart are different. The range of $C_{H'}$ within which automatic rhythm is developed is of not very different extent for sinus, auricle and ventricle, but as we pass from the venous to the arterial end of the heart, this range is shifted more and more towards the alkaline side. The sinus-auricle and auricle-ventricle intervals are both lengthened with increasing $C_{H'}$, while there is an optimum $C_{H'}$ for the frequency of the rhythm of the sinus.

In conclusion, we wish to express our thanks to Mr G. R. Mines for the help and advice he has given throughout this research.

[1] Since going to press, our attention has been called to a paper by Clark (*Journ. Pharm. and Exp. Ther.* v. p. 215. 1914). Clark shows that if when a systolic condition has been brought about by perfusion of fluid $C_{H'}\ 10^{-9.5}$, circulation of 1 c.c. of fluid be established, the heart gradually relaxes, and the beat improves. The $C_{H'}$ of the circulating fluid is then found to be 10^{-7}. By alternate perfusion and circulation alternate waves of contraction and relaxation are produced.

REFERENCES.

(1) Mines. This Journal, XLIII. p. 467. 1912.
(2) Symes. Proc. Physiol. Soc. p. XXV. This Journal, XLIII. 1911.
(3) Mines. This Journal, XLVI. p. 188. 1913.
(4) Sörensen. C. R. Lab. Carlsberg, VIII. p. 1. 1908.
(5) Dale. This Journal, XLVI. p. 129. 1913.
(6) Clark. Journ. Pharm. and Exp. Ther. IV. p. 425. 1913.
(7) Gaskell. This Journal, IV. p. 43. 1883.
(8) Mines. Ibid. XL. p. 327. 1910.
(9) Chanoz. C. R. de l'Acad. Sci. CXLI. p. 316. 1909.

CAMBRIDGE: PRINTED BY JOHN CLAY, M.A. AT THE UNIVERSITY PRESS