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METABOLIC FACTORS AND VENTRICULAR FIBRILLATION

BY

P. J. GOODFORD

From the Department of Pharmacology, University of Oxford

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Ventricular fibrillation has been studied in the isolated rabbit heart to determine the effect of factors modifying the metabolism. Sodium azide, sodium monoiodoacetate and sodium fluoride were found to cause fibrillation, the effect of sodium fluoride being neutralized by magnesium chloride. Fibrillation was also caused by lack of glucose and could be arrested by adding glucose; the effect of glucose in arresting fibrillation was facilitated by insulin. In other experiments mannose and pyruvate could arrest fibrillation due to lack of glucose, but L(–)lactate could not. The effect of temperature changes, of adrenaline and of cyanide were also studied. When all oxygen was removed from the perfusing solution fibrillation was arrested.

The belief is widely held that ventricular fibrillation occurs in circumstances in which there is interference with normal metabolic processes, and that it is in some way a consequence of a failure of the sources of energy which are ordinarily at the disposal of cardiac muscle. Investigation of this idea when fibrillation occurs in the animal is difficult because fibrillation is usually irreversible and because it is impossible to distinguish between primary and secondary factors.

A method is now available for the study of ventricular fibrillation in the isolated heart of the rabbit. The heart is perfused through the aorta by the method of Langendorff and electrical stimulation is applied to the ventricle at a high rate. Fibrillation then occurs. When the stimulation is stopped the fibrillation either reverts very soon to a normal rhythm or else it persists, according to the composition of the fluid perfusing the heart (Armitage, Burn, and Gunning, 1957).

This method has now been used to investigate the effect of metabolic inhibitors, and of other factors affecting metabolism such as the presence of adrenaline, temperature, changes in oxygen supply and absence of glucose. In the work quoted the effect of 2-4-dinitrophenol was investigated; when present in the perfusion fluid in concentrations 3 to 5×10^{-6} molar, this substance caused fibrillation for long periods.

METHODS

The normal perfusion fluid contained NaCl 7.7 g., KCl 0.42 g., $CaCl_2$ 0.24 g., $NaH_2PO_4,2H_2O$ 0.14 g., $NaHCO_3$ 2.1 g., dextrose 2 g., sucrose 4.5 g., distilled water to 1 l. The solution was saturated with 95% oxygen and 5% CO_2. The temperature was maintained within $\pm 0.5°$ using the device of Saxby (1956). The pressure head at which the hearts were perfused was 54 cm. water. The ventricles were stimulated by platinum fish-hook electrodes, one piercing the wall of the left ventricle at the apex and the other near the base midway between the coronary arteries. The stimuli were 1 mA. in strength and 0.75 msec. in duration; they were applied usually at the rate of 10/sec. but always at the same rate in any one experiment. When stimulation was not being applied the electrodes were used as leads to an electrocardiograph (Cossor model 1314) on which the rhythm was recorded.

In testing substances to see if they would cause fibrillation, use was made of the finding by Armitage *et al.* (1957) that the occurrence of fibrillation as a result of stimulation depended on the concentration of K^+ in the perfusing fluid. In some hearts persistent fibrillation (which in practice was taken to be fibrillation lasting for 15 minutes) followed electrical stimulation when the perfusion fluid contained the normal concentration of K^+, namely 5.6 mM. This fibrillation was arrested by pouring cold saline over the heart (Dirken, Gevers, Heemstra, and Huizing, 1955). If the perfusion fluid was changed to one containing a higher concentration of K^+ such as 7 mM., stimulation then usually failed to produce more than a transient fibrillation (less than 5 minutes), a normal rhythm returning spontaneously; if stimulation still caused persistent fibrillation, the K^+ concentration was increased further to 8.4 mM. Conversely if stimulation during perfusion with a solution containing K^+ in concentration 5.6 mM. failed to cause more than transient fibrillation, the heart was perfused with a lower concentration of K^+ in the expectation that stimulation would then cause persistent fibrillation.

Thus at the beginning of each experiment a concentration of K^+ was determined in which stimulation caused only a transient fibrillation and a lower con-

centration of K^+ was also determined in which stimulation caused persistent fibrillation. The substance which was to be tested was then added to the solution containing the higher concentration of K^+, and after this solution had been perfused through the heart for 20 to 30 minutes stimulation was applied to determine the effect.

When the composition of the perfusing solution was modified by the inclusion of a substance, such as mannose, in sufficient amount to alter the tonicity of the solution, the sucrose concentration was adjusted accordingly.

RESULTS

Sodium Azide.—Results with sodium azide are set out in Table I, which shows the duration of fibrillation after stimulation in 6 experiments. In expt. 1, stimulation during perfusion without sodium azide caused fibrillation for 1.5 min., when a normal rhythm returned. Sodium azide in a concentration 3×10^{-3} M was then added to the same perfusion fluid, and, after the perfusion had continued for 25 min., stimulation was applied. Fibrillation occurred and continued for 19 min. after stimulation before reverting to a normal rhythm. The perfusion was then continued for 20 min. without sodium azide, and when stimulation was applied there was no period of fibrillation afterwards.

TABLE I

EFFECT OF SODIUM AZIDE (3×10^{-3}M) IN CAUSING FIBRILLATION

An asterisk indicates that fibrillation was arrested by cooling.

Expt. No.	Duration of Fibrillation after Stimulation (min.)		
	Before Azide	In Presence of Azide	After Removing Azide
1	1·5	19	0
2	0	54	7
3	0	27	0
4	9	22*	0
5	1	29*	0
6	0	6	0

The results made it clear that fibrillation occurred more readily and for a longer time when the metabolism was depressed by the presence of sodium azide, the change being fully reversible when the sodium azide was removed.

Sodium Monoiodoacetate. — The effect of sodium monoiodoacetate on fibrillation was more difficult to determine than that of sodium azide, since it had no action until nearly toxic concentrations were used, when its action was not readily reversible. The concentrations used were 2.1 or 3.2×10^{-5} M. The results in Table II show that the presence of sodium monoiodoacetate caused fibrillation to follow stimulation in 7 out of 12 hearts. In the remaining 5 hearts, there was no evidence that monoiodoacetate interfered with metabolism sufficiently to cause fibrillation.

TABLE II

EFFECT OF SODIUM MONOIODOACETATE (IAA)

An asterisk indicates that fibrillation was arrested by cooling.

Expt. No.	Duration of Fibrillation after Stimulation (min.)		Molar Concentration of IAA
	Before IAA	In Presence of IAA	
7	0	13*	$3·2 \times 10^{-5}$
8	0	5*	$3·2 \times 10^{-5}$
9	0	6*	$3·2 \times 10^{-5}$
10	0	6*	$3·2 \times 10^{-5}$
11	0	4·5*	$3·2 \times 10^{-5}$
12	0	0	$3·2 \times 10^{-5}$
13	0	0	$3·2 \times 10^{-5}$
14	0	31	$2·1 \times 10^{-5}$
15	0	0	$2·1 \times 10^{-5}$
16	0	18	$2·1 \times 10^{-5}$
17	0	0	$3·2 \times 10^{-5}$
18	3	0	$3·2 \times 10^{-5}$

Sodium Fluoride.—The action of sodium fluoride was much clearer than that of sodium monoiodoacetate, for it increased the duration of fibrillation in all trials, as shown in Table III.

TABLE III

EFFECT OF FLUORIDE ($2·5 \times 10^{-4}$M) ON FIBRILLATION

An asterisk indicates that fibrillation was arrested by cooling.

Expt. No.	Duration of Fibrillation after Stimulation		Time Required for $MgCl_2$ to Arrest Fibrillation (min.)	Molar Concentration Mg++
	Before Fluoride (min.)	In Presence of Fluoride (min.)		
19	0	63	Not arrested	2×10^{-4}
20	0	16*	—	—
	0	10*	—	—
21	0	9*	—	—
	7	15	0·5	2×10^{-4}
	—	11	0·5	2×10^{-4}
22	—	13	1·0	10^{-5}
23	0	6*	—	—
	—	43	0·5	10^{-5}
	—	30	Not arrested	10^{-6}

The effect of fluoride was reversible, and moreover the fibrillation induced in its presence was promptly arrested by magnesium chloride. Thus in expt. 21 there was no fibrillation after stimulation in the absence of fluoride, but in its presence there was fibrillation for 9 min.; this was arrested by cooling the heart. The heart was again perfused without fluoride, and on stimulation there was fibrillation for 7 min. which reverted to normal rhythm spontaneously. The heart was then perfused with fluoride and after stimulation fibrillated for 15 min. The perfusion fluid was changed to one containing $MgCl_2$ as well as fluoride, and fibrillation was arrested in 0.5 min. The heart was again perfused with fluoride without $MgCl_2$, and after stimulation it fibrillated for

11 min. On changing to perfusion with fluoride and $MgCl_2$, the fibrillation was again arrested in 0.5 min.

Lack of Glucose.—Since it is known that glycolysis is inhibited by fluoride and that this inhibition is neutralized by magnesium, the foregoing results led to the investigation of the effect of a lack of glucose. In 4 out of 5 hearts it was found that perfusion with a glucose-free solution for 30 min. made no difference to the effect of stimulation, but in one heart persistent fibrillation was observed. Experiments were therefore carried out in which the perfusion fluid was without glucose for a longer time, and in these it was found that the lack of glucose always caused fibrillation after stimulation, and that fibrillation due to the absence of glucose was usually arrested by adding glucose. The following experiment provides an example. A heart perfused with glucose-free solution was stimulated for 2 min. and fibrillation was produced. After 5 min. this fibrillation was arrested by cooling the ventricles. The K^+ concentration was then raised from 5.6 to 7.0 mM., and 12 min. later stimulation was repeated. Fibrillation did not occur. Nor did it occur when stimulation was repeated after a further 15 min. However, when the heart was stimulated 20 min. later still, by which time it had been perfused with a glucose-free solution for 70 min., fibrillation began and continued for 39 min. At this point perfusion was continued with a fluid containing 11 mM./l. glucose, and after 4 min. fibrillation stopped and a normal beat was recorded on the drum and on the electrocardiograph.

The results of this series of experiments are given in Table IV. In expts. 24 (see Fig. 1), 31 and 34 fibrillation occurred spontaneously although the K^+ concentrations were 8.4, 7.7, and 7.7 mM.

TABLE IV

EFFECT OF GLUCOSE-FREE SOLUTION IN CAUSING FIBRILLATION

An asterisk indicates that fibrillation occurred spontaneously.

Expt. No.	Time of Perfusion with Glucose-free Solution before Stimulation (min.)	Duration of Fibrillation after Stimulation (min.)	Time Taken for Arrest of Fibrillation by Glucose (min.)
24	99*	12	2
25	104	16	9
26	145	18	8
27	85	15	4
28	77	17	4
29	105	38	0·5
30	70	39	4
31	48*	34	(No arrest)
32	55	35	4
33	160	39	1
34	43*	37	(No arrest)

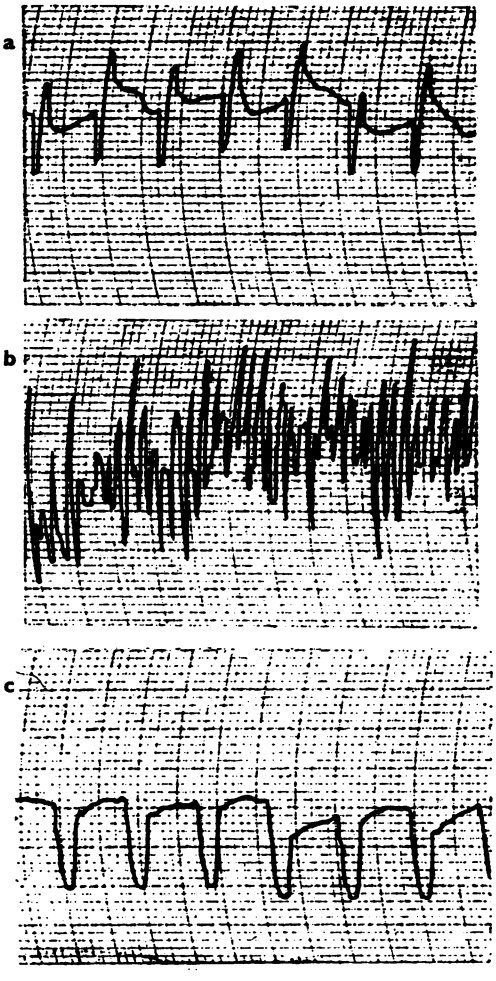

FIG. 1.—Electrocardiograms recorded from the isolated rabbit heart. (a) Shows the regular rhythm when the heart was first perfused with glucose-free solution. (b) Shows spontaneous fibrillation. (c) Shows the restoration of regular rhythm on changing to a solution containing glucose.

respectively. Spontaneous fibrillation had otherwise only been observed when the K^+ concentration was as low as 1.4 mM. In expts. 31 and 34 readmission of glucose did not arrest the fibrillation.

Insulin and Glucose.—Since insulin facilitates the entry of glucose into the cells (see Stadie, 1954), observations were made with glucose and insulin. In 3 out of 4 experiments evidence was obtained that the addition of insulin arrested fibrillation when the heart had been perfused with a low-glucose solution for some time. In one experiment the heart was perfused with $\frac{1}{8}$ normal

glucose, that is with 1.4 mM. for 175 min., and stimulation then produced fibrillation which was allowed to continue for 34 min. The perfusate was changed to one also containing insulin in a concentration of 2 units/l. The normal rhythm returned in 2 min. The results in a second experiment were similar, the time of perfusion before stimulation being 145 min., the time of fibrillation after stimulation being 31 min. and the normal rhythm returning on addition of insulin after 10 min. In a third experiment, however, the addition of insulin failed to stop the fibrillation. In a fourth experiment fibrillation was produced in a glucose-free medium and observed for 38 min. The addition of glucose (11 mM.) failed to stop this fibrillation during 11 min., but when 1 unit of insulin was injected the fibrillation stopped in 1 min.

Substitutes for Glucose.—Observations were next made to see whether the fibrillation produced by stimulation in a glucose-free medium could be arrested by substances other than glucose. The first to be tried was mannose. When added to the normal fluid perfusing a heart, mannose in 11 mM. concentration caused a progressive diminution of the beat; this diminution was still more rapid after removing the glucose. The amplitude was not restored by readmitting glucose as always happened in the absence of mannose. In spite of this effect on the heart, mannose arrested fibrillation in a glucose-free medium in 3 out of 6 hearts. Of the 3 hearts in which mannose failed to arrest fibrillation the addition of glucose was effective in one.

Observations were also made with pyruvate. When fibrillation was produced by stimulation in a glucose-free medium, the addition of sodium pyruvate to the perfusion fluid arrested fibrillation in one heart and converted fibrillation to a tachycardia in a second; in 4 hearts it had no effect. However, fibrillation which was produced as a result of stimulation in a glucose-free medium, could not be produced in the presence of pyruvate in 3 hearts. The evidence indicated that pyruvate had some effect, but was less effective than glucose.

Observations were also made with lactate. I am grateful to Professor H. A. Krebs for supplying me with a sample of pure L(+)lactic acid free from the D-isomer, from which sodium L(−) lactate was prepared. This was tested in 5 hearts; each heart was fibrillating in glucose-free perfusate, and the addition of sodium L(−)lactate to the perfusate so that the concentration was 22 mM. did not arrest the fibrillation. In 2 of these hearts the fibrillation was arrested later by the addition of glucose and in another by the addition of glucose and insulin. Thus no evidence was obtained that L(−)lactate was effective in arresting the fibrillation.

The Effect of Temperature.—While the foregoing results were consistent with the view that fibrillation occurs in circumstances in which metabolism is disturbed, some observations are now described which at first sight appear to conflict with this view. That they may not do so will be considered in the discussion.

Observations on the effect of temperature were made in 10 hearts and the results are illustrated in Table V. The procedure in expt. 35 was to stimulate the heart electrically for 1 min. at 37° when the perfusion fluid contained 5.6 mM. K^+. After the stimulation stopped the ventricles did not fibrillate, and the beat was regular. The K^+ concentration in the perfusion fluid was then reduced to 4.2 mM., and after 20 min. stimulation was repeated. When the stimulation was stopped, the ventricles fibrillated for 6 min. and at this point the temperature of the perfusion fluid was lowered to 32°. After 5 min. more the fibrillation ceased, having continued for 11 min. When stimulation was applied for as long as 10 min. at this lower temperature fibrillation did not occur. The temperature was then raised once more to 37°, and stimulation for 1 min. caused fibrillation which was observed for 36 min. Similar results were obtained in the other experiments shown in Table V, and in 6 experiments not shown. Thus under conditions in which fibrillation occurred at 37° it did not occur at 32°.

TABLE V

EFFECT OF TEMPERATURE ON FIBRILLATION

An asterisk indicates that fibrillation was arrested by cooling.

Expt. No.	K^+ Concentration (mM.)	Temp.	Duration of Fibrillation after Stimulation (min.)
35	5·6	37	0
	4·2	37	11 (see text)
	4·2	32	0
	4·2	37	36*
36	5·6	37	0
	4·2	37	29*
		32	0
		37	11*
		32	2
		37	20*
37	4·9	37	13*
		32	0
		37	13*
38	5·6	37	38*
		27	0
		32	0
		37	42*
		27	0·25
		37	30*

The Effect of Adrenaline.—Since the explanation of observations with adrenaline may be related to that of the foregoing observations on temperature they are described at this point. Each heart was initially perfused with a fluid containing normal K^+ (5.6 mM.) and was stimulated electrically. The course of one experiment is shown in Table VI in which this stimulation caused the

TABLE VI

EFFECT OF ADRENALINE ON FIBRILLATION

An asterisk indicates that fibrillation was arrested by cooling.

Expt. No.	K^+ Concentration (mM.)	Adrenaline Concentration (g./ml.)	Duration of Fibrillation after Stimulation (min.)
39	5·6	—	9*
	7·0	—	1
	7·7	—	0
	7·7	4×10^{-7}	9*
	7·7	—	0
	7·7	4×10^{-7}	4*
	7·7	—	0
	7·7	4×10^{-7}	9*
	7·7	—	0

ventricles to fibrillate for 9 min.; at this time a regular beat was restored by pouring cold saline over the heart. The K^+ concentration was then raised first to 7.0 and then to 7.7 mM., at which concentration the heart did not fibrillate on stimulation. The fluid was next changed to one containing adrenaline (4×10^{-7} g./ml.), and with this perfusate stimulation caused fibrillation. Five further trials were made in which adrenaline was alternately absent and present, and, as the results in Table VI show, fibrillation did not occur when it was absent, but always when it was present. Similar results were obtained in 7 out of 8 hearts.

The Effect of Oxygen Lack.—Since lack of glucose was found to lead to fibrillation, the effect of lack of oxygen was also studied. Experiments were therefore carried out in which the perfusion fluids were prepared from well-boiled water and were aerated with nitrogen and 5% CO_2. When hearts were perfused with this solution stimulation did not cause fibrillation, but on the contrary fibrillation was arrested.

The results of a series of experiments are given in Table VII in which fibrillation was produced during aeration with oxygen. This was allowed to continue for varying lengths of time as shown in the second column. The perfusion fluid was then changed to one aerated with nitrogen or to one aerated with 90% N_2 + 10% O_2 as shown in the third column. In each of the eight experiments in which the perfusion fluid was changed to one which was O_2-free a normal rhythm was restored, though a period as long as 16 min. was required

TABLE VII

EFFECT OF OXYGEN-FREE SOLUTION IN ARRESTING FIBRILLATION

Expt. No.	Period of Fibrillation in Presence of Oxygen (min.)	Gas Mixture Used to Replace Oxygen	Time before Regular Rhythm was Restored (min.)	Size of Beat after Fibrillation was Stopped (mm.)
40	17	N_2	2	0
41	16	N_2	10	3
42	19	N_2	11	—
43	16	N_2	13	—
44	23	90% N_2 + 10% O_2	(Not restored in 20)	
	(23 + 20)	N_2	8	0
45	15	N_2	0·5	40
	17	90% N_2 + 10% O_2	(Not restored in 10)	—
46	24	90% N_2 + 10% O_2	(Not restored in 20)	—
	(24 + 20)	N_2	16	0
47	22	90% N_2 + 10% O_2	(Not restored in 16)	—
	(22 + 16)	N_2	10	0
48	20	90% N_2 + 10% O_2	3	0
49	24	90% N_2 + 10% O_2	0	0

in expt. 46. When, however, as little as 10% oxygen was present, fibrillation was arrested in only 2 out of 6 hearts. Thus the effect of oxygen lack in arresting fibrillation was seen only when the lack was complete. For example, in carrying out expt. 44 the ventricles were caused to fibrillate when the fluid was aerated with oxygen and continued to do so for 23 min. The aerating gas was then changed to one containing 10% O_2; this had no effect, the fibrillation continuing for the next 20 min. Then an O_2-free solution was perfused, and after 8 min. the fibrillation was arrested. Again in expt. 45, after fibrillation in O_2 for 15 min., the fluid was changed to one which was O_2-free. The fibrillation stopped immediately. Fibrillation was again induced in O_2 and continued for 17 min.; this time the gas mixture was changed to 10% O_2, but the fibrillation was unaffected during the next 10 min.

One comment on these results should be made. When fibrillation was arrested by other means, the change in the electrocardiogram was accompanied by the restoration of mechanical beats. The arrest of fibrillation by an O_2-free perfusate was not accompanied by a return of the contractions in 6 out of the 8 experiments.

The Effect of Sodium Cyanide.—The description of observations on sodium cyanide now follows because their complexity may appear less difficult to understand in the light of the observations on lack of oxygen. More experiments were made with sodium cyanide than with any other metabolic inhibitor because it was difficult to be certain what the effect was. There were experi-

TABLE VIII
EFFECT OF CYANIDE IN CAUSING FIBRILLATION

Expt. No.	Duration of Fibrillation after Stimulation (min.)		
	Before Cyanide	In Presence of Cyanide	Molar Concentration of Cyanide
50	0	30	10^{-4}
51	0	19	8×10^{-4}
52	0	18	3×10^{-4}
53	0	16	3×10^{-4}
	4	18	3×10^{-4}
54	0	20	3×10^{-4}
55	0	16	3×10^{-4}

ments in which it seemed clear that sodium cyanide behaved like sodium azide and sodium fluoride and led to the occurrence of prolonged fibrillation. Such results are set out in Table VIII. They were obtained with concentrations of cyanide from 10^{-4} M to 8×10^{-4} M. Expt. 53 gave a very clear picture, for the effect of stimulation was twice determined in the presence of cyanide and twice in its absence, whilst in expts. 52 and 54 the irregularity stopped as soon as the cyanide was removed.

There were also other experiments in which the presence of sodium cyanide arrested fibrillation as did perfusion with an O_2-free solution. These results are set out in Table IX in which are given

TABLE IX
EFFECT OF CYANIDE IN ARRESTING FIBRILLATION
An asterisk indicates that fibrillation was arrested by cooling.

Expt. No.	Duration of Fibrillation before Addition of Cyanide (min.)	Cyanide (Molar Concentration)	Time to Arrest Fibrillation (min.)	Amplitude of Restored Beat (mm.)
56	31	6×10^{-4}	13	0
57	18	3×10^{-4}	1	10
58	17	3×10^{-4}	5	10
	17	3×10^{-4}	17*	—
59	19	3×10^{-4}	1·5	50
	18	3×10^{-4}	6	1
60	17	3×10^{-4}	17*	—

the results of 7 trials on 5 hearts. In 5 of the 7 trials the addition of cyanide to the perfusion fluid arrested fibrillation which had persisted for periods varying from 17 to 31 min. The concentrations of cyanide lay in the same range as those in Table VIII. When the electrocardiograph registered the return of a normal rhythm, the amplitude of the beat was large in the first trial of expt. 59 and appreciable in expts. 57 and 58. When the perfusion fluid was changed to one which was cyanide-free, the amplitude in expt. 58 rose from 10 to 60 mm. In the second trial of expt. 58, and in expt. 60, cyanide did not arrest fibrillation.

In addition to the results given in Tables VIII and IX there are 10 other results in which the effect of cyanide could not be stated. Since cyanide may have two opposite effects it is possible that in these experiments the two effects in some way neutralized one another.

DISCUSSION

The observations that azide, fluoride and, perhaps less conclusively, monoiodoacetate increase the occurrence and duration of ventricular fibrillation are not surprising in view of the general belief that, when there is interference with metabolic processes, fibrillation is more likely to occur. Dinitrophenol has already been shown to have a similar effect (Armitage *et al.*, 1957), and this is possibly due to an uncoupling of the linkage between oxidation and phosphorylation, leading to diminished formation of adenosine triphosphate. To what are the actions of azide and fluoride due? Since it seemed possible that in their presence less energy would be available from glycolysis, the effect of lack of glucose was investigated and it was found to exert a striking fibrillatory action. If a heart was perfused with a glucose-free solution sufficiently long, sometimes it fibrillated spontaneously, and it always fibrillated after electrical stimulation. This fibrillation was arrested by adding glucose, and the restorative power of glucose was assisted by insulin, which facilitates the entry of glucose into cells. In some experiments mannose, and in others pyruvate, had the restorative power of glucose. L(−)lactate however, had not.

Up to this point the findings seemed acceptable. An unexpected observation was then made on the effect of temperature. When hearts were caused to fibrillate at 37°, they ceased to do so at 32°. On raising the temperature to 37°, they fibrillated again. Beaulnes and Day (1957) had made a similar observation in isolated atria. It may, however, be that the effect of temperature in the isolated heart perfused with a modified Ringer solution is related to the supply of oxygen. This is the amount carried in solution, and is much smaller than is available when blood flows through the coronary vessels. It is not unlikely that the oxygen in Ringer solution is sufficient to maintain the metabolism at 32°, but not the increased metabolism at 37°. Thus stimulation at 32° is not followed by fibrillation because the oxygen supply is adequate; when, however, the temperature is raised to 37° stimulation is followed by fibrillation because the oxygen supply is no longer adequate.

The fibrillatory action of adrenaline may have a similar basis. When adrenaline is perfused through the heart it greatly augments the rate and force of the beat; this must lead to a greater demand for oxygen, which perhaps cannot be met by the amount dissolved in the perfusion fluid. As a result there is an oxygen deficiency and a greater tendency to fibrillation. It is possible that the occurrence of ventricular fibrillation in the body as a result of the injection of adrenaline during chloroform anaesthesia has a similar explanation, if chloroform diminishes the availability of the energy supplied by oxidative processes.

The observations, however, do not give direct support to this view of the importance of the oxygen supply. Yet they may do so indirectly because of some of the results obtained with cyanide. In some experiments the presence of cyanide had a similar effect to the presence of azide or of fluoride. It increased fibrillation. This may indicate the effect of a partial reduction in oxidative processes.

Direct observations on a reduction of oxygen supply were, however, confined to a total exclusion of oxygen and to a reduction of oxygen to 10% in the aerating mixture. With a total exclusion of oxygen came the unexpected result that fibrillation was arrested. In a proportion of experiments with cyanide a similar result was obtained. Thus it appeared that when the cytochrome system was completely out of action fibrillation could not continue.

It is perhaps interesting to note that there are two other circumstances in which fibrillation cannot occur in the isolated heart; the one is in the absence of calcium and the other is in the presence of twice the normal concentration of potassium in the perfusion fluid.

Is any difference known between the isolated heart when fibrillating and when beating normally? Holland (1957) first demonstrated that, in the isolated atria, fibrillation as a result of a high rate of stimulation began when the net loss of K^+/unit of time exceeded a certain figure. Later Armitage *et al.* (1957) showed that when the ventricles of the isolated heart were fibrillating there was an increased loss of K^+ as compared with the periods of normal beating preceding and following. This observation may be related to the fact that fibrillation can be arrested by increasing the K^+ concentration in the perfusing fluid.

The results in the present work may possibly indicate that to avoid fibrillation it is necessary to facilitate the active transport of K^+ into the cell. The high net loss of K^+ in fibrillation may be due to insufficient active transport, and the defibrillatory effect of an increased external concentration of K^+ may be due to an improved active transport. This conception sheds light on the fibrillatory effect of a glucose-free medium, which may be due to lack of the substance whose breakdown within the cell normally provides the energy for the active transport of K^+. The fibrillatory effect of the metabolic inhibitors which have been studied may likewise be due to their inhibition of processes which release the energy required for active transport of K^+.

It is clear that the results obtained raise many queries. The removal of glucose by an isolated heart from the fluid perfusing it can be studied when the perfusion fluid is recirculated in a closed system (Burn and Dale, 1924) and it is desirable to know whether this removal is less in the presence of metabolic inhibitors and particularly of fluoride. It would then be possible to say whether the fibrillatory action of fluoride was due to inhibition of glycolysis or whether to some other action such as the binding of intracellular, but not enzymatic, magnesium. Much might also be learnt from observations of oxygen consumption. Perhaps the chief value of the results is that they point to the ways in which a study of the biochemical aspects of fibrillation can be pursued.

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