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THE ANAEROBIC ACTIVITY OF THE ISOLATED FROG'S HEART.

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A study of the literature regarding the effect of oxygen lack on the heart showed that different workers had obtained very contradictory results. Some authors stated that oxygen lack produced an immediate and progressive depression, whilst others found that isolated frog's heart could maintain fair activity for more than a day when deprived of oxygen.

Preliminary experiments showed us that the effects produced by oxygen lack on the isolated frog's heart depended on two independent variables, namely the amount of carbohydrate available for use by the heart and the reaction of the perfusion fluid. The experiments described below were designed to determine the influence of these two variables. It was found that, by adjustment of the two variable factors mentioned above, it was possible to reproduce almost all the widely varying effects of oxygen lack reported by previous workers.

We have shown [Clark, Gaddie and Stewart, 1931] that under aerobic conditions at least half the material metabolized by the isolated frog's heart is non-carbohydrate. The experiments described below indicate that under anaerobic conditions the only important source of energy available for the heart is the conversion of carbohydrate to lactic acid. We have therefore studied in detail the production of lactic acid by the isolated frog's heart.

Methods.

The method used for the estimation of lactic acid was one recommended by Dr P. Eggleton, and was founded on the usual oxidation to acetaldehyde by potassium permanganate in presence of sulphuric acid and manganous sulphate, absorption of the distilled aldehyde in bisulphate and titration of the bound bisulphite with iodine. The iodine was standardized every day by estimation of lithium lactate, and the method was

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periodically checked by estimation of lithium lactate, the iodine being then standardized by one of the usual methods. The actual recovery of lactic acid was found to be between 94 and 96 p.c. of that known to be present.

The following two methods were used for perfusion of the hearts:

Method 1. Perfusion of the whole frog's heart with cannulæ in the sinus and the aorta [cf. Clark, Gaddie and Stewart, 1931].

Method 2. Perfusion of the isolated ventricle with a cannula in the auricolo-ventricular opening, and arranged so that isochoric records could be taken when desired. (An apparatus of this type is illustrated by Clark [1927, Fig. 1].) We stirred the fluid in the cannula either with air or nitrogen, and in the latter case closed the cannula with a rubber stopper fitted with a jet for the escape of gas. The nitrogen was rendered oxygen-free by passing through a heated quartz tube containing copper filings.

The standard Ringer's fluid used had the following composition p.c.: NaCl 0·65, KCl 0·015, $CaCl_2$ 0·012, Na phosphate 0·02, *p*H 7·6. In the experiments with alkaline Ringer's fluid $NaHCO_3$ 0·05 p.c. was substituted for the phosphate, and when gas was bubbled through this the *p*H rose to about 8·5. No glucose was added except in those cases where its addition is mentioned.

(i) The lactic acid content of the fresh heart of the frog.

Table I shows figures given by various authors for the lactic acid content of the fresh hearts of various animals.

Table I. The lactic acid content of fresh hearts.

	Author		Lactic acid content p.c.
Tortoise and turtle	Redfield and Medearis [1926]	Ventricle	0·04
	Gemmell [1928]	Auricle	0·036
		Ventricle	0·026
	Boyland [1928]	Ventricle	0·22–0·31
Rabbit and cat	Schenk [1924]	Heart	0–0·07
	Katz and Long [1925]	Heart	0·028

We measured the lactic acid content of the excised frog's heart both in the fresh condition and after keeping, in order to discover how readily the lactic acid production could be induced.

Our results, which are shown in Table II, agree with those already quoted (except those of Boyland). Perfectly fresh frogs' hearts frozen

Table II. The lactic acid content of the isolated frog's heart.

	No. of exps.	Lactic acid p.c.
Fresh hearts frozen with CO_2 snow	3	0·06
Fresh hearts dropped into acid at room temp.	5	0·09
Hearts left soaking for 60 min. in Ringer at room temp. and then frozen	3	0·09
Hearts perfused 30 min. and then frozen	1	0·085
Hearts perfused 5 hours with oxygen and then frozen	3	0·09

immediately contained 0·06 p.c. lactic acid. Under most other conditions a value of 0·09 p.c. lactic acid was obtained, but values higher than this were not found except where the conditions were definitely anaerobic.

(ii) The lactic acid maximum of the hearts.

Table III shows the figures obtained by various authors for the lactic acid maxima of the hearts of various animals.

Table III. Maximum lactic acid content of heart.

		Lactic acid (p.c.)
Turtles' and tortoises' hearts:		
Turtle ventricle strip suspended in nitrogen and stimulated to exhaustion. Redfield and Medearis [1926], Gemmell [1928]	Auricle	0·16
	Ventricle	0·137
Chloroform rigor. Arning [1927]	Ventricle	0·141
Incubation with alkaline phosphate. Boyland [1928]		0·280
		0·49–0·61
Frog's heart:		
Chloroform rigor. Arning [1927]		0·143–0·325
Rabbits' and cats' hearts:		
Stimulated to exhaustion with asphyxia. Katz and Long [1925]		0·072
Rigor mortis. Hines, Katz and Long [1925]		0·23

Boyland's figures show the effects of incubation with alkaline phosphate and are higher than the rest, but the others show that in rigor the lactic acid of the heart does not rise above 0·28 p.c., and that the cold-blooded heart is arrested when the lactic acid content reaches about 0·14 p.c. These figures are less than half the corresponding figures found with skeletal muscle.

(iii) The production of lactic acid during aerobic activity.

Nagaya [1929] found that when frogs' hearts were perfused with a Straub's cannula demonstrable quantities of lactic acid were formed. After 75 min. perfusion he found about 0·11 p.c. of lactic acid in the heart, which was equivalent to about 0·2 mg., and between 1 and 2 mg. in the perfusion fluid. The total production of lactic acid corresponded to about 4 mg. per g. per hour. When the hearts were perfused with a double cannula only a trace of lactic acid could be demonstrated in either the hearts or the perfusion fluid.

These experiments show that the method of perfusion is of importance. A heart set up with cannulæ in the sinus and in the aorta and perfused with an oxygenated fluid receives a good supply of oxygen

and no lactic acid is produced. On the other hand when the heart pumps a small volume of fluid backwards and forwards, as is the case with the Straub's cannula, it is in reality suffering from a partial oxygen lack and demonstrable quantities of lactic acid are found.

Eismayer and Quincke [1930] used the isolated ventricle, working under conditions similar to our method 2. They found an excretion of about 0·4 mg. per g. per hour in the presence of oxygen. Wertheimer [1930, Table IX] used strips of ventricle and found that after 6 hours' isolation only traces of lactic acid were present in the strips, and that the amount in the surrounding fluid corresponded to a figure between 0·06 and 0·09 mg. per g. per hour.

The most probable reason for the wide differences in lactic acid production obtained by different workers is that their methods varied as regards the efficiency of the oxygenation of the heart.

We used two methods of perfusion and found that the lactic acid production was much less when there was a circulation of fluid (method 1) than when the fluid was pumped to and fro (method 2). With method 1 we obtained the following results: nineteen hearts were perfused for 20 hours, the total weight was 3·98 g., and the total lactic acid recovered from the heart and perfusion fluid together was 2·65 mg. This corresponds to a production of 0·03 mg. lactic acid per g. per hour, a value which is of the same order but less than that obtained by Wertheimer.

Method 2 gave higher figures. Three hearts perfused for 6 hours gave a lactic acid production of 0·22 mg. per g. per hour. This is intermediate between Wertheimer's and Eismayer and Quincke's figures, but is much smaller than those given by Nagaya.

The oxygen consumption of the isolated frog's heart working under average conditions is about 1 c.c. per g. per hour [Clark and White, 1928], an amount which is sufficient to oxidize 1·3 mg. of carbohydrate. The lactic acid production, even with method 2, is therefore only a small fraction of the total metabolism.

(iv) Lactic acid production under anaerobic conditions.

Nagaya [1929] measured the lactic acid production of frogs' hearts perfused with addition of KCN ($M/1000$) for periods of 45–90 min. The average of 18 experiments recorded by him is 10·3 mg. lactic acid per g. per hour. Eismayer and Quincke [1930] measured the lactic acid production of ventricles supplied with Ringer's fluid perfused with nitrogen, and their figures show a production of 4·1 mg. lactic acid per g.

per hour. Wertheimer [1930] measured the lactic acid production of ventricle strips in presence of NaCN ($M/2000$ to $M/5000$). The total of seven experiments in which the strips (total weight 0·393 g.) were stimulated showed 0·035 p.c. lactic acid in the heart strips, while from the perfusion fluid 0·85 mg. lactic acid was recovered.

This shows that most of the lactic acid formed passed out into the prefusion fluid. The lactic acid produced corresponded to about 4 mg. per g., but the duration of the experiments was not given, probably it was not much more than an hour.

We investigated the lactic acid production of ventricles perfused (method 2) with strongly alkaline Ringer's solution ($NaHCO_3$ 0·05 p.c., *p*H about 8·5) through which nitrogen was bubbled. Ten ventricles were perfused for 60 min., their total weight was 1·31 g., and the total lactic acid recovered was 0·98 mg. or 0·075 p.c.

The quantity of lactic acid found in these hearts was the same as in the control hearts, and this confirms Wertheimer's conclusion that lactic acid is rapidly excreted into the perfusion fluid. It will be shown later that this conclusion is only true when the perfusion fluid is alkaline. The quantity of lactic acid excreted into the perfusion fluid was as follows:

Duration of anaerobiosis in min.	10	20	40	60	80	120
No. of observations	6	6	4	3	5	4
Average lactic acid excretion in mg. per g. of ventricle	0·61	1·84	2·8	2·2	2·3	3·5

The lactic acid production under the above conditions was also estimated indirectly by measuring the loss of total carbohydrate in the hearts. In six experiments with ventricles rendered anaerobic by nitrogen perfusion for 120 min. the total reducing substance was found to be 0·73 p.c. The reducing substance in 20 controls measured at this period was found to be 1·23 p.c. This indicates a probable loss of 5 mg. of reducing substances per g. heart weight in 2 hours. The lactic acid estimations show an excretion of 3·5 mg. per g. in this period.

A few preliminary experiments were made with hearts subjected to a vacuum. Method 1 was used. Four hearts were put up at a time and the circulation was arranged from a common reservoir containing 200 c.c. fluid. The method was not very satisfactory because auriculo-ventricular block frequently occurred. The vacuum was maintained for 4 hours.

The following results were obtained: fifteen hearts (average weight 0·174 g.) were found to contain after exposure to the vacuum 0·77 p.c. total reducing substance. The corresponding average value in 20 control

hearts was 1·23 p.c. The calculated loss of reducing substance was 4·6 mg. per g. in 4 hours. The lactic acid recovered from the perfusion fluid was 0·95 mg. per heart = 5·45 mg. per g.

Fig. 1 shows the effect of anaerobiosis in alkaline sugar-free Ringer's fluid on the mechanical activity of the heart, and also the lactic acid production under these conditions. The lactic acid production per g. per hour is as follows: 1st hour, 3 mg.; 2nd hour, 1·3 mg.; 3rd and 4th hours, less than 0·5 mg.

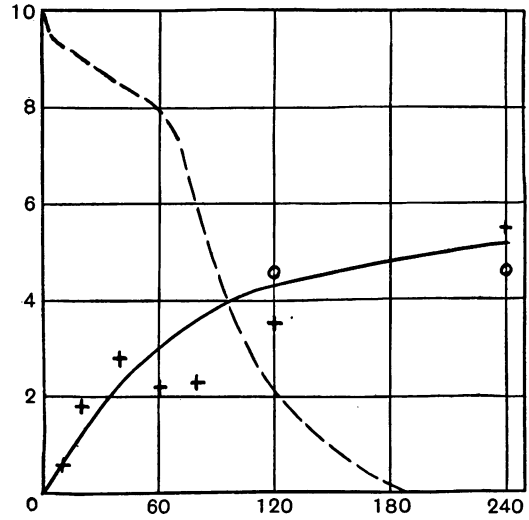

Fig. 1. The effect of anaerobiosis on the isolated frog's ventricle perfused with alkaline Ringer's fluid (*p*H 8·5). Abscissa: time in minutes. Dotted line: mechanical response (normal = 10). Continuous line: lactic acid production in mg. Crosses = direct estimations: circles = production as calculated from carbohydrate loss.

This rapid decrease in lactic acid production is accompanied by a corresponding decrease in the mechanical activity of the heart as estimated by the amplitude of the isometric response. The amplitude of the mechanical response of the heart during the first hour is, however, about 80 p.c. of the normal, and the lactic acid production that occurs during this period (3 mg.) is surprisingly low.

The data shown in Fig. 1 were for the most part obtained with the isolated ventricle, and we have no measurements of the oxygen consumption of the ventricle of *R. hung.* under these conditions; moreover our data do not show the actual work done by the ventricle. The results are therefore unsuitable for quantitative treatment in terms of energy,

but they certainly suggest that the energy liberated under anaerobic conditions is used more efficiently than that liberated under aerobic conditions.

(v) Lactic acid production in hearts perfused with neutral Ringer's fluid.

The effects produced by lack of oxygen on heart tissue are partially antagonized by the presence of alkali. This fact was noted by Martin [1905] in the case of strips of turtle's ventricle and by Drury and Andrus [1924] in the isolated dog's heart. We found that oxygen lack in hearts perfused with unbuffered Ringer's fluid produced an immediate and powerful action on the heart and caused complete arrest in less than an hour. The following analyses of the hearts and fluids showed that relatively small quantities of lactic acid were excreted into the perfusion fluid, but that it accumulated in the heart.

Time in min.	10	20	40
No. of exps.	8	4	12
Lactic acid excreted in mg. per g.	0·5	0·54	0·63

In ten hearts analysed after 40 min. anaerobiosis the lactic acid content was 1·66 mg. per g. Allowing for a control value of 0·66 mg. per g., this corresponds to an accumulation in 40 min. of 1·0 mg. per g. in the heart, and a total production of lactic acid of 1·63 mg. per g. in 40 min.

Table IV shows the outstanding effects produced by anaerobiosis in alkaline and in neutral Ringer's fluid. It would appear that in neutral Ringer's fluid the lactic acid produced cannot be excreted, and hence accumulates in the heart and produces paralysis fairly rapidly. The lactic acid content found after 40 min. perfusion is similar to the values shown in Table III for the lactic acid content of turtle's heart strips suspended in nitrogen and stimulated to exhaustion.

Table IV. Effect of anaerobiosis for 40 min.

	Alkaline Ringer's fluid (*p*H 8·5)	Unbuffered Ringer's fluid (*p*H 6·8–7·0)
Decrease in mechanical response as p.c. of normal	20	100
Lactic acid excreted into perfusion fluid mg. per g.	2·8	0·63
Lactic acid content of ventricle mg. per g. (control = 0·66)	0·75	1·66
Total lactic acid produced in mg. per g. in excess of control	2·9	1·63

In alkaline Ringer's fluid the lactic acid is excreted readily and the content inside the heart does not rise, hence the mechanical response is

but slightly impaired and the total production of lactic acid is considerably greater than it is in neutral Ringer's fluid.

Examination of the unbuffered perfusion fluid after arrest of the heart had occurred showed that its reaction was only very slightly acid (about *p*H 6·9). The frog's heart when supplied with oxygen can maintain a fair activity for long periods when perfused with a fluid with this reaction. The arrest of the heart that occurs in anaerobiosis with unbuffered Ringer's fluid must therefore be due to the accumulation of lactic acid in the heart itself rather than to any change in the reaction of the perfusion fluid caused by the acid that is excreted.

(vi) The effect of glucose on the asphyxiated heart.

Freund and König [1927] found that hearts perfused with oxygen-free alkaline Ringer's fluid were arrested in 2 to 3 hours, but that when glucose was present the hearts maintained good contractions for at least 6 hours. They also showed that hearts arrested with oxygen lack could be revived by addition of glucose. Backmann [1927] found that hearts perfused with a solution containing sugar could survive anaerobically for as long as 61 hours. We confirmed Freund and König's conclusions, for we found that the addition of glucose enabled the heart to maintain a good activity for 6 hours, and that a heart arrested by lack of oxygen could be revived by glucose. The addition of as small a quantity as 2 mg. to 10 c.c. perfusion fluid produced an immediate stimulant effect on the exhausted anaerobic heart. The tracings published by Freund and König show that the stimulant effect produced by addition of sugar to the frog's heart exhausted by lack of oxygen is very dramatic as regards the rapidity and completeness of the recovery. Our results showed effects of similar intensity. This effect was only produced in alkaline Ringer's fluid, and the addition of glucose to a heart arrested by oxygen lack in neutral Ringer's solution produced no benefit. The figures given in Table V show that the anaerobic heart converts considerable quantities of glucose to lactic acid.

Table V. Effects of perfusion with oxygen lack for 6 hours with 0·1 p.c. glucose in the Ringer's solution.

No. of exps.	Average heart weight in g.	Average total carbohydrate p.c.	Lactic acid recovered from Ringer's fluid in mg. per g. per hour	Nitrogen excreted in mg. per g. per hour
6	0·076	1·59	5·7	0·02

The total carbohydrate content of the hearts after anaerobic perfusion with glucose for 6 hours, namely 1·59 p.c., was actually higher than that of the controls taken at the same time (average of 10 controls = 1·55 p.c. total carbohydrate). Therefore when the heart is perfused with an alkaline fluid containing glucose under anaerobic conditions it does not exhaust its own carbohydrate but obtains energy by converting the glucose present in the perfusion fluid to lactic acid. The nitrogen excretion is much lower in anaerobic than in aerobic conditions. Under aerobic conditions a heart perfused for 6 hours excreted 0·52 mg. nitrogen per g. [Clark, Gaddie and Stewart, 1931, Table III], which is about 25 times the value we obtained under anaerobic conditions. The trace of nitrogen obtained in the latter case cannot all be derived from the adenylic acid of the heart, if it be assumed that the pyrophosphate found by Clark, Eggleton and Eggleton [1931] represents the whole of the adenylic acid. It is noteworthy, however, that almost the whole of the nitrogen excreted by the anaerobic hearts was in the form of ammonia, whereas under aerobic conditions two-thirds of the nitrogen recovered was in the form of urea. It is of course possible that other substances than adenylic acid may undergo deamination without oxidation, especially under the abnormal condition of anaerobiosis.

(vii) Discussion.

Our results show that under anaerobic conditions the only important source of energy available for the isolated frog's heart is the conversion of carbohydrate to lactic acid. The heart, however, differs very widely from the skeletal muscle as regards the effect produced upon it by accumulation of lactic acid. The skeletal muscle can neutralize considerable amounts of lactic acid, whereas accumulation of lactic acid rapidly impairs the mechanical response of the heart and arrest occurs when the lactic acid content rises to 0·15 p.c. Consequently the heart is unable to function anaerobically, or to incur oxygen debt unless it is able to excrete rapidly the lactic acid it produces. The excretion of lactic acid depends on the reaction of the perfusion fluid. Excretion occurs freely at *p*H 8·5 and very slowly at *p*H 7·0. We have not yet investigated the intermediate range of reaction.

The effect of lack of oxygen on the frog's heart therefore depends primarily on the reaction of the perfusion fluid, for unless this is alkaline the heart is fairly rapidly arrested by lactic acid accumulating in its cells. If the perfusion fluid is alkaline the duration of activity depends on the amount of carbohydrate available. When the heart is perfused

with glucose-free alkaline Ringer's fluid its available carbohydrate begins to fail after about 2 hours, but if glucose is added the heart can maintain a good activity for many hours. Table VI shows the manner in which

TABLE VI. The duration of survival of the anaerobic frog's heart.

	Arrest from lack of oxygen min.	Mg. per g. lactic acid formed at time of arrest
Hearts poisoned with iodoacetic acid	<10	0
Hearts perfused, unbuffered Ringer's fluid	40	1·63
Hearts perfused, alkaline Ringer's fluid	120–180	5
Hearts perfused, alkaline Ringer's fluid + glucose	>400	>25

various modifications of the conditions of perfusion affect the duration of the survival of the anaerobic heart. It is interesting to note that a heart exhausted by perfusion for 3 hours with alkaline glucose-free Ringer's fluid still contains 0·77 p.c. total carbohydrate. This figure is considerably higher than that found in hearts after prolonged perfusion under aerobic conditions. For example, the authors in a previous paper [1931] recorded the following figures.

Method of perfusion	Total carbohydrate p.c.
Serum + Ringer's fluid 24 hours	0·480
Serum + glucose 24 hours	0·403
Serum + glucose + insulin 24 hours	0·280

The "availability" of the reducing substance in the frog's heart appears therefore to vary. About 0·3 or 0·4 p.c. cannot be utilized at all, whilst about 0·8 p.c. cannot be used under anaerobic conditions. About 0·15 p.c. reducing substance is non-fermentable [Clark, Gaddie and Stewart, 1932].

Under aerobic conditions the heart metabolizes both proteins and carbohydrates, whereas under anaerobic conditions it metabolizes carbohydrates alone. Our figures suggest that the protein metabolism in some manner makes available for metabolism a portion of the total carbohydrates.

In our previous work [Clark *et al.* 1931] we were puzzled by the fact that the isolated frog's heart, well supplied with oxygen, did not utilize glucose added to the perfusion fluid, whereas there was evidence that the isolated mammal's heart perfused with Ringer's fluid used considerable quantities of glucose, and other workers had reported glucose usage and stimulation by addition of glucose in the isolated frog's heart. These divergent results we believe to be due to variations in efficiency of oxygen supply, since any shortage of oxygen appears to stimulate the heart to break down carbohydrate.

The behaviour of the heart in regard to glucose in aerobic and anaerobic conditions forms a remarkable contrast. Under aerobic conditions the heart does not consume glucose added to the perfusion fluid and uses its own carbohydrate very slowly. Moreover, the addition of mono-iodo-acetic acid in concentrations sufficient to arrest all breakdown of carbohydrate to lactic acid does not produce any demonstrable effect on the activity of the heart as long as this is supplied with oxygen. Hence there is no direct evidence that the rapid breakdown of carbohydrate to lactic acid that occurs in anaerobiosis necessarily forms any part of the carbohydrate metabolism of the aerobic heart.

CONCLUSIONS.

1. The effect of oxygen lack on the heart depends on two variables, namely, alkalinity and sugar supply. The heart is rapidly arrested unless the perfusion fluid is alkaline. If the *p*H is 8·0 or over, the heart deprived of oxygen converts both its own carbohydrate or glucose added to the perfusion fluid into lactic acid, and thereby can maintain a good mechanical activity for a period of many hours.

2. In the presence of oxygen the heart uses much less carbohydrate, but even a slight oxygen deficiency can cause the production of some lactic acid.

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