# THE METABOLISM OF THE MAMMALIAN HEART

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Papers by Author:

1. The Influence of Adrenaline on Cardiac Glycogen.
2. Asphyxial Arrest of the Isolated Rabbit's Auricle.
3. The Metabolism of the Isolated Mammalian Heart under Partial Anoxia(in collaboration with Bogue, J.Y. and Gregory,R.A.).
4. Effect of Asphyxia on the Adenosinetriphosphate Content of the Rabbit's Heart ( in press ).
5. The Action of Iodoacetate and Fluoride upon the Isolated Rabbit's Auricle ( to be published).

# I. INTRODUCTION

During the past five years knowledge regarding the metabolism of the mammalian heart has been greatly advanced by the intensive work carried out by Evans and his associates on the dog's heart-lung preparation and isolated heart.

This work has shown that the metabolism of the heart-lung preparation is extremely complex because the lungs actively glycolyse sugar and the lactate thus formed is oxidized by the heart. Furthermore there is extensive glycolysis in the circulating blood. In consequnence of the extent of these errors, most of the older work on the heart-lung metabolism is of doubtful significance.

Workers in Edinburgh have analysed the metabolism of the cold-blooded heart and have reached certain important conclusions.

These two series of researches require to be correlated with the older work regarding the metabolism of skeletal muscle, and also with the complex theory of the carbohydrate metabolism in muscle which has been built up by Embden, Meyerhof and Parnas chiefly on the basis of the reactions which occur in muscle-brei.

The correlation between skeletal muscle metabolism and cardiac metabolism is difficult because the theories of skeletal muscle metabolism are based chiefly on the/

the metabolism of muscles completely or partially deprived of oxygen or on muscle-brei experiments. On the other hand the work on cardiac metabolism has been based on experiments in a condition of functional activity.

The author found that the isolated auricle of the rabbit offered the following advantages for the study of cardiac metabolism.

(1) The tissue maintains good activity in Locke's fluid and hence the numerous complications associated with the presence of blood are avoided.

(2) The auricle can be alternately depressed and revived in a manner that is impossible in the case of the heart in situ or even in the case of the heart-lung preparation.

(3) By means of asphyxia, iodoacetate poisoning etc., it is possible to exclude certain of the metabolic processes of the auricle and thus to simplify the system.

The author has used the rabbit's auricle chiefly as a means for analysing the apparent differences between the results obtained from studies on the frog's heart and those obtained from studies on the dog's heart or heart-lung preparation. This general purpose has made it most convenient to present the problems of cardiac metabolism in a continuous manner and to intersperse those experiments which have been made by the author to elucidate points of difficulty.

The original work done by the author has partly been published and in this case it has been incorporat-

ed in the general account. Unpublished experiments are marked by a star.

The writer gratefully acknowledges his indebtness to Professor A.J.Clark for his constant advice throughout this research. Acknowledgement is also due to Drs. P. Eggleton,J.M.Robson and C.P.Stewart for their kind help.

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## 11. CARBOHYDRATE METABOLISM OF THE MAMMALIAN HEART

(1) Normal Glycogen Content and Anaerobic Glycolysis. (2) Aerobic Glycogenolysis. (3) Glycogen Resynthesis. (4) Utilization of Lower Carbohydrate, Glucose and Lactate (5) Path of Carbohydrate Breakdown.

(1) Normal Glycogen Content and Anaerobic Glycolysis: It is well known that the contraction of skeletal muscle involves the breakdown of glycogen into lactic acid and the subsequent oxidation of part of the latter. It was formerly believed that this reaction supplied the immediate energy for the process of contraction. The more recent view is that the formation of lactic acid from glycogen does no more than supply energy for the restitution of substances which are more immediately concerned with the contraction. There is, however, a general agreement that lactic acid production is the chief ultimate source of energy for the process of contraction in the skeletal muscle.

Glycogen is undoubtedly the most important carbohydrate in the mammalian heart, but until a few years ago the estimates of the normal glycogen content varied in a most remarkable manner.

Evans (1934) showed that asphyxia very rapidly removed the glycogen from the rat's heart and that the greater portion normally present was thus removed, unless special/

special precautions were taken to avoid asphyxia when the animal was killed.

I (1937 a,b) found that this was true in rabbits as well as in rats. I also found that in addition to asphyxia adrenaline also accelerated the breakdown of glycogen. These results are summarised in Table 1.

Table 1:- Influence of Adrenaline and Asphyxia on Carbohydrate Content of Rat's Ventricle.

| Description | No. of animals | Glycogen mg./100 g. | Total Carbohydrate mg./100 g. | Lactic acid mg./100 g. |
|---|---|---|---|---|
| 1. Controls | 7 | 517 (96)* | 581 (91) | 107 (20) |
| 2. Asphyxia without adrenalin: | | | | |
| a) Asphyxia in vitro | 4 | 498 (44) | 537 (46) | 145 (33) |
| b) Asphyxia in vivo after section of aorta and vena cava | 4 | 361 (72) | 461 (69) | 254 (53) |
| c) Asphyxia after clamping of adrenal vessels | 2 | 294 | 460 | 184 |
| d) After excision of adrenal glands | 4 | 290 (80) | 406 (23) | 167 (27) |
| 3. Asphyxia plus adrenaline: | | | | |
| a) Asphyxia in vivo. | 6 | 101 (48) | 226 (62) | 182 (22) |
| ⊙b) Injection of adrenaline | 4 | 145 (74) | 237 (50) | 221 (45) |

*Figures in brackets = standard deviations of mean. Data from Chang, 1937 a.

⊙ Asphyxia conducted after excision of adrenal glands and intramuscular injection of adrenaline.

Table 1 shows that asphyxia in the absence of adrenaline causes a much slower reduction in glycogen content than does asphyxia plus adrenaline. It will be shown later that adrenaline even without asphyxia can cause a temporary decrease in the glycogen content of the mammalian heart. These facts indicate that either asphyxia or adrenaline causes a reduction in the glycogen of the mammalian heart, but that a combination of the two factors produces an extraordinarily rapid breakdown of glycogen. Since asphyxia in the normal animal is always associated with the liberation of adrenaline, it is bound to lead to extensive breakdown of cardiac glycogen, unless special measures are taken to prevent the asphyxia during the removal of the heart. When such measures were taken, I found that the glycogen content of the hearts of rabbits and rats was about 500 mg./100 g. of the wet weight (Table 11).

A selection of estimates of cardiac glycogen which are believed to be reliable are shown in Table 11.

Table 11. Glycogen Content of Mammalian Hearts

| Animal | Glycogen mg./100 g. | Author |
|---|---|---|
| Dog | 560 | Visscher and Mulder (1930) |
| ,, | 668 | Evans et al. (1935) |
| Rabbit | 483 | Macpherson, Essex and Mann (1931) |
| ,, | 542 | Chang (1937 b) |
| Rats | 570 | Lawrence and McCance (1931) |
| ,, | 488 | Evans (1934) |
| ,, | 517 | Chang (1937 a) |

The normal glycogen content of the mammal's heart appears/

appears to lie between 0.5 and 0.7 per cent or 500 and 700 mg. per 100 g. of wet weight.

Recent work is therefore in good agreement in showing that the heart has a relatively high glycogen content, but that this is rapidly removed by asphyxia.

(2) Aerobic Glycogenolysis: Asphyxia and especially asphyxia plus adrenaline produces rapid and extensive breakdown of glycogen. Hence it is somewhat difficult to determine the extent to which glycogen is broken down under aerobic conditions, because such experiments have little significance unless it is certain that the whole tissue is under full aerobic conditions. Many workers found that mammalian hearts perfused with glucose-free saline solution lost their glycogen rapidly (Camis, 1911; Loewi and Weselko, 1914; Witting, Markowitz and Mann, 1930; Macpherson, Essex and Mann, 1931; Sprague, 1933). Other workers found that dog's hearts isolated from the body as heart-lung or as isolated heart-oxygenator preparations maintained their glycogen content at a normal level for periods of several hours (Visscher and Mulder, 1930; Evans et al., 1935). As the hearts in the heart-lung preparation and in the isolated heart-oxygenator preparation are working under conditions more nearly resembling those which obtain in vivo than in the saline perfused hearts and since there is good reason to believe that the latter may not receive an adequate supply of oxygen, the reduction of gly-

cogen which has been regularly observed in saline perfused hearts may reasonably be ascribed to the asphyxia to which these hearts are probably subjected.

In the year 1935 Lohmann and Weicker reviewed the literature on the metabolism of the heart and pointed out that heart glycogen is normally kept very constant. Cruickshank (1936) in a review on the same subject also concluded that the mammalian heart tended to retain its glycogen and to part with it sparingly.

The question therefore arises whether glycogen plays any part in the aerobic activity of the mammalian heart.

In the first place the following simple experiment proves that the mammalian heart can obtain energy by exidation of lactic acid. Hence if glycogen breaks down to lactic acid it will thus provide a source of aerobic energy for the heart.

Iodoacetate is known to inhibit the breakdown of glycogen into lactic acid and it has been shown to do so in the isolated rabbit's auricle(Chang,1937 b). When an isolated rabbit's auricle is set up as described by Chang,it can usually contract forcibly for a number of hours as long as it is well supplied with oxygen. But if iodoacetate is added to the perfusion fluid to make a concentration of 1-30,000, the mechanical activity of the auricle rapidly declines in the manner shown in fig. I

fig.1. After about 30 minutes, the amplitude and fre-

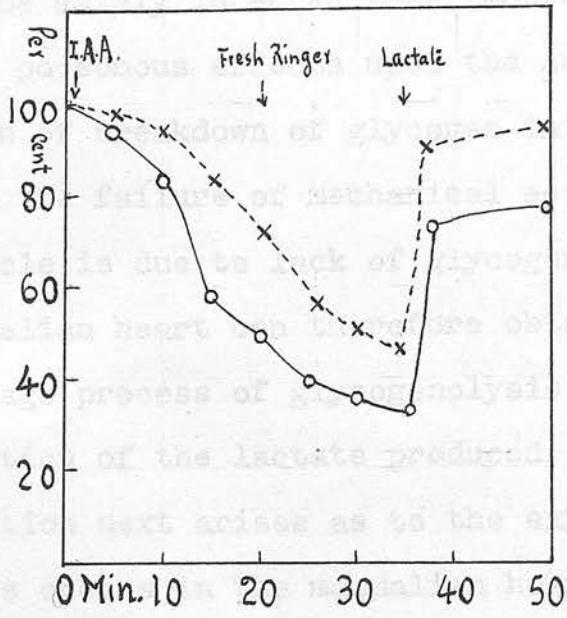



Fig.1.- Effect of I.A.A. upon aerobic activity of isolated rabbit's auricle. Abscissa: time in minutes. Ordinate: percentage of normal. °-° : amplitude. + ..+ : frequency. (Unpublished results of 6 expts)

quency of contraction falls to a level as low as 30 and 50 per cent of normal respectively. Without any other treatment, the auricle usually remains at this low activity for some 20 to 30 minutes and then goes into contracture. If lactate is added to the perfusion fluid, rapid recovery, as shown in fig.1, takes place. Within a few minutes the amplitude of contraction returns to about 80 per cent, while the frequency to almost 100 per cent of the normal. As long as lactate is present in the fluid in a sufficient concentration, the poisoned auricle can maintain an activity at a high level for a period of several hours (usually 2-4 hours) before being arrested in contracture.

Since the poisoned auricle can contract fairly

normally in the presence of lactate for a number of hours, it may be safely inferred that iodoacetate has no other serious poisonous effects upon the auricle than the inhibition of breakdown of glycogen into lactic acid and that the failure of mechanical activity of the poisoned auricle is due to lack of glycogenolysis.

The mammalian heart can therefore obtain energy by the two stage process of glycogenolysis and the subsequent oxidation of the lactate produced.

The question next arises as to the extent to which glycogenolysis occurs in the mammalian heart working under conditions approximating to normal. Experiments with the heart-lung preparation indicate that even in the case of a heart perfused with blood, the glycogen plays an important part in maintaining the activity of the heart.

Bogue, Evans and Gregory (1937) found that isolated dog's hearts could normally work for a number of hours, but that if the glycogen was exhausted by the use of adrenaline, rapid deterioration and failure of the heart ensued. These authors suggested that some glycogen was essential for the normal function of the heart.

The cause of mechanical failure in the I.A.A. poisoned auricle and in the dog's heart exhausted of glycogen is identical. In both cases no breakdown of glycogen into lactic acid can take place. These observations therefore prove that glycogen can take an important part in the aerobic activity of the mammalian heart

heart. By analogy with the skeletal muscle it may be inferred that glycogen is continuously broken down to supply energy for the contraction of the mammalian heart.

There is no question that under aerobic conditions glycogen in the mammalian heart can be broken down. I (1937 a) found that administration of adrenaline to rats caused a reduction of heart glycogen to less than half of its normal value in 25 minutes. The reduction lasted for about half an hour after which full recovery of glycogen took place. These results are shown in Table III.

Table III.-Influence of Adrenaline without asphyxia upon Carbohydrate Content of rat's ventricle.

| No. of expts. | Time after injection, minutes. | Dosage of adrenaline mg./100 g. | Glycogen mg./100 g. | Total carbohydrate mg/100 g. | Lactic acid mg/ 100 g. |
|---|---|---|---|---|---|
| 1 | 10 | 0.1 | 635 | 705 | 135 |
| 1 | 15 | 0.1 | 411 | 666 | 142 |
| 1 | 20 | 0.2 | 235 | 372 | 129 |
| 1 | 25 | 0.2 | 119 | 246 | 114 |
| 6 | 25 | 0.1 | 277 | 362 | 136 |
| 2 | 25 | 0.05 | 552 | 735 | .. |
| 1 | 40 | 0.1 | 440 | 505 | 204 |
| 1 | 45 | 0.1 | 449 | 492 | 132 |
| 1 | 60 | 0.1 | 519 | 626 | 121 |

Both earlier and recent experiments by Bogue et al. (1935,1937) show that administration of adrenaline invariably depleted the glycogen of the isolated dog's heart.

A reduction of the glycogen in the mammalian heart can occur, however, apart from the administration of adrenaline. Bogue et al.(1935) found that an increase in the amount of work performed by the heart also led to

a reduction in the glycogen of the dog's heart. Cruickshank and Startup(1933) and Cruickshank and McClure (1936) found that dog's hearts lost about one-third of their glycogen when the perfusion blood was rendered nearly free of sugar.

The results of Sprague (1933) showed that the addition of red blood cells to the perfusion fluid did not significantly prevent the glycogen loss of the isolated rabbit's heart, although such a procedure apparently improved the oxygenation of the heart.

These facts prove that glycogen in the mammalian heart can be broken down. The breakdown takes place not only with adrenaline but also without it. It occurs not only in preparations (salien perfusion)where there might be partial asphyxia of the heart but also in preparations (heart-lung and isolated heart-oxygenator preparation and saline perfusion plus red blood cells) where oxygen lack can be definitely excluded.

The fact that the glycogen of the mammalian heart can be broken down under fully aerobic conditions lends strong support to the theory that glycogen is normally broken down to supply energy for the heart's contraction.

(3) Glycogen Resynthesis:- In the mammalian heart not only is glycogen broken down but it is also readily synthesised. Reference has already been made to the recovery of glycogen after depletion by adrenaline in rats (Table III). Apparently the rat's heart can rapidly draw material from blood to built up its glycogen

store.In analogy with skeletal muscle, the obvious substances likely to be utilized for this purpose are glucose and lactic acid. Under well controlled conditions, Bogue, Evans and Gregory (1937) showed clearly that glycogen of the dog's heart was built up from glucose but not from lactic acid. In the saline perfused hearts all authors who regularly found a reduction in glycogen also found that the addition of glucose to the perfusion fluid either prevented or retarded the loss of glycogen (Camis,1911;Loewi and Weseko,1914; Macpherson et al. 1931). This fact may also be interpreted to mean that glycogen is resynthesised at the expense of glucose.

Since glycogen breakdown and resynthesis can take place in the mammalian heart, it may be inferred that under normal conditions the glycogen of the mammalian heart,like that of skeletal muscle,is constantly drawn upon but unlike that of skeletal muscle it is rapidly replenished as soon as it is withdrawn. Such a supposition would provide a sound explanation of many facts which appear somewhat paradoxical. The behaviour of the glycogen of the mammalian heart may be compared with that of the phosphorus compounds which will be seen later, also remain constant under aerobic conditions although there is evidence that they take an active part in the process of contraction.

(4) Utilization of Glucose and Lactic acid:- The lower carbohydrates appears to be next in importance to

glycogen in the metabolism of the mammalian heart. Table lV shows the amount of lower carbohydrates in the mammalian heart.

Table iV.- Lower Carbohydrates in the Mammalian Hearts.

| Animal | Mg./100 g. | Author |
|---|---|---|
| Dog | 117 | Visscher and Mulder (1930) |
| Rabbit | 46-59 | Cori, Closs and Cori (1933) |
| Rat | 64 | Chang (1937 a) |

The so called lower carbohydrates appear to include a number of substances, but for the purpose of this discussion it may be reasonably presumed that they are composed largely of glucose. I (1938) found that the normal rabbit's heart contained a considerable amount of barium soluble phosphoric esters as well as barium insoluble phosphoric esters (Table 8). If we regard the former as being composed mainly of hexose-mono-phosphate and the latter partly of hexose-di-phosphate, we may presume that part of the glucose in the mammalian heart is not free but exists in the form of phosphoric esters. Apparently the lower carbohydrates can be derived from two sources. One of these is the blood sugar. Cori, Closs and Cori (1933) showed in the rabbit's heart and Visscher and Mulder (1930) in the dog's heart that they vary with the blood sugar level. The other possible source is the cardiac glycogen. I (1937 a) found in the rat's heart that when glycogen was broken down in asphyxia, the amount of lower carbohydrates was increased, the/

the figures for lower carbohydrates in the normal and asphyxiated rat's hearts being:

Normal--------581 mg./100 g.(total carbohydrate)
517 mg./100 g.(glycogen)
64 mg./100 g.(Lower carbohydrates)

Asphyxiated --226 mg./100 g.(total carbohydrate)
101 mg./100 g.(glycogen)
125 mg./100 g.(lower carbohydrates).

Visscher and Mulder (1930) found in the dog's heart that under aerobic conditions in spite of the normal glycogen content the lower carbohydrates decreased when the blood sugar fell to a low level. This indicates that blood sugar rather than glycogen forms the predominant source of lower carbohydrates in the heart.

Since the lower carbohydrates are composed chiefly of glucose their utilization can be considered together with that of glucose.

With regard to the actual usage of glucose by the mammalian heart different authors obtained widely different values. When expressed as mg./g./hr.,the figures are as follows:(a) cat's heart: 1.9 - 3.7 (Burn and Dale, 1924); 0.8 - 3.2 (Mansfeld,1925); 7.0 (Ambrus,1927); (b) rabbit's heart: 0.87 (Hepburn and Latchford,1922); 0.54 - 1.82 (Burn and Dale,1924);(c) dog's heart: 0.8 - 3.8 (Patterson and Starling,1913);0.2 - 0.39 (Mcginty and Miller,1933);4.6 - 5.3 (Cruickshank and Startup,1933);0.7 (Evans,Grande and Hsu,1935). Apparently a number of factors can affect the usage of glucose by

the mammalian heart. Of these factors we may mention the following, which are arranged roughly in the order of their importance. (a) The concentration of sugar in the perfusion fluid: Evans,Grande and Hsu (1935) found that glucose usage by the dog's heart bears an almost linear relationship to the glucose concentration of the perfusion blood. Cruickshank and Startup (1933) also found that the glucose uptake by the dog's heart varied with the blood sugar level. (b) Lactate concentration: This is a factor which affects chiefly heart-lung and heart-oxygenator preparations,since in these cases a certain amount of lactate is always present in the perfusion blood. Evans,Grande and Hsu (1935) showed that within certain limits sugar usage varied indirectly with the lactate level. (c) Adequacy of oxygen supply: This factor affects chiefly the saline-perfused preparations. Since under aerobic conditions the mammalian heart may use other food stuffs as well as carbohydrate, but under anaerobic conditions it resorts almost completely to the carbohydrate as a source of energy,and in saline perfused preparations the oxygen supply may vary to a large extent, the amount of sugar usage under these conditions is liable to wide fluctuations. (d) Amount of work done by the heart: Bogue et al. (1935) found that sugar usage was augmented by the increase of work done by the heart.

In studying the usage of sugar by the heart one must also take into consideration many of the possible

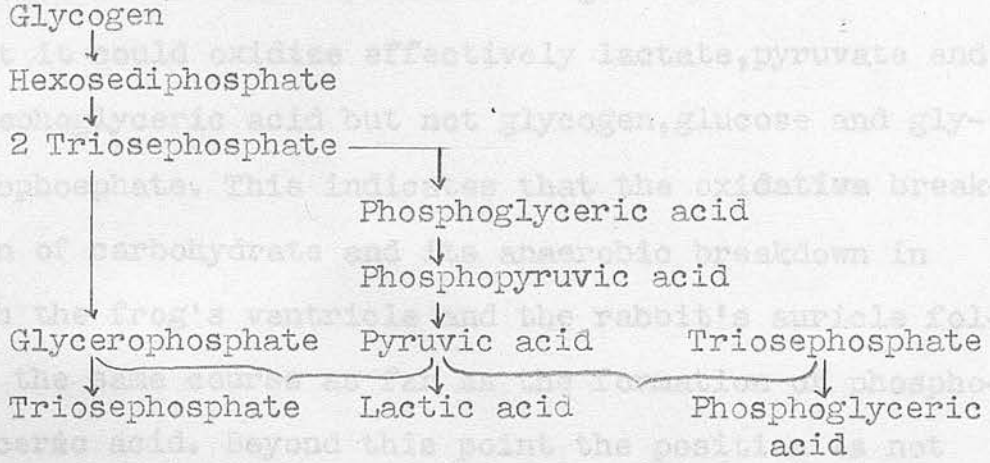
sources of error: e.g. possible losses by bacterial or blood glycolysis; possible retention or excretion of sugar by the heart and in the heart-lung preparation glycolysis of sugar by the lungs (Evans, Hsu and Kosaka, 1934). As in previous years, these factors were either neglected or not known, many studies on the sugar usage of the mammalian heart are now known to be invalid.

There is no doubt that the mammalian heart can oxidize lactic acid. It has been shown to occur in the normal dog's heart by a number of workers (Himwich, Koskoff and Nahum, 1928; Mcginty and Miller, 1933; Ruhl, 1934; Evans, Grande and Hsu, 1935). I found that rabbit's auricle poisoned and markedly depressed by I.A.A. (Fig. 1) could be revived to almost normal activity by the addition of lactic acid to the perfusion fluid. The conclusion that the I.A.A. poisoned auricle can utilise lactic acid for its contraction is supported by the work of Bogue, Chang and Gregory (1938) who showed by direct analysis that the I.A.A. poisoned dog's heart could use as much lactic acid as did the normal heart if not more. Furthermore, Bogue, Evans and Gregory (1937) have shown that lactic acid used by the dog's heart was not converted into glycogen.

(5) Path of Carbohydrate Breakdown:- Another important problem in the study of the carbohydrate metabolism of the mammalian heart is the path by which carbohydrate is oxidized.

According to the Embden-Meyerhof's scheme of

the anaerobic production of lactic acid from carbohydrate, the reaction takes place in the following steps:




It has been shown that the frog's heart poisoned with I.A.A. can oxidize lactate, pyruvate but not glycogen, glucose, hexose-diphosphate, triosephosphate and glycerophosphate (Clark et al., 1938). It has been shown by Parnas, Ostern and Mann (1934) that phosphoglyceric acid can be utilised by the I.A.A. poisoned frog's heart.

Iodoacetate prevents the formation of lactic acid by inhibiting three reactions in which mutases activity occurs, namely, (1) the conversion of triosephosphate into phosphoglyceric acid and glycerophosphate; (2)the conversion of pyruvic acid and of glycerophosphoric acid into lactic acid and triosephosphate; (3) the conversion of pyruvic acid and triosephosphate into lactic acid and phosphoglyceric acid. No substance prior to phosphoglyceric acid can be oxidized by the poisoned frog's heart; it is thus likely that the oxidative breakdown of carbohydrate and its anaerobic breakdown in the frog's heart follow the same course as far as

that point.

I found that rabbit's auricles poisoned with I.A.A. resembled the I.A.A. poisoned frog's ventricles in that it could oxidize effectively lactate,pyruvate and phosphoglyceric acid but not glycogen,glucose and glycerophosphate. This indicates that the oxidative breakdown of carbohydrate and its anaerobic breakdown in both the frog's ventricle and the rabbit's auricle follow the same course as far as the formation of phosphoglyceric acid. Beyond this point the position is not clear. Fluoride is known to inhibit the anaerobic breakdown of phosphoglyceric acid into pyruvic acid (Meyerhof and Kiessling,1933;Meyerhof and Lohmann,1934). When an isolated rabbit's auricle was poisoned with fluoride,I found that it could contract forcibly for several hours in the presence of oxygen but that it was rapidly arrested when the oxygen supply was cut off. On readmission of oxygen full recovery soon took place. Similar results were obtained on the frog's heart. Provided that oxygen was passed through the perfusion fluid,a frog's heart poisoned with fluoride could continue to contract for as long as 9 or 10 hours usually with only a small diminution in activity. Some typical results are shown in Table V.

Table V /

Table V.-Effect of fluoride Upon the Aerobic Activity of the Frog's heart.

| Concentration of NaF | Mechanical Response as Percentage of the normal after exposure to Fluoride. | | | | |
|---|---|---|---|---|---|
| | 0 hr. | I hr. | 3 hrs. | 5 hrs. | 9 hrs. |
| 1-5,000 | 100 | 100 | 86 | 76 | 86 |
| ,, | 100 | 100 | 95 | 95 | — |
| 1-3,000 | 100 | 100 | 90 | 83 | 50 |
| ,, | 100 | 100 | — | 70 | 70 |
| ,, | 100 | 77 | 41 | — | — |
| ,, | 100 | 91 | 64 | 64 | — |

It has been shown that a frog's heart perfused with large volumes of alkaline Ringer's solution can maintain a fairly normal activity for a period of one or two hours by obtaining energy from glycolysis (Clark, Gaddie and Stewart,1932). On the other hand,the results summarised in fig.2,show that in the fluoride-poisoned frog's heart oxygen lack led to rapid diminution in activity and arrest within 15 or 30 minutes in the first asphyxia and within 7 or 10 minutes in the second asphyxia.

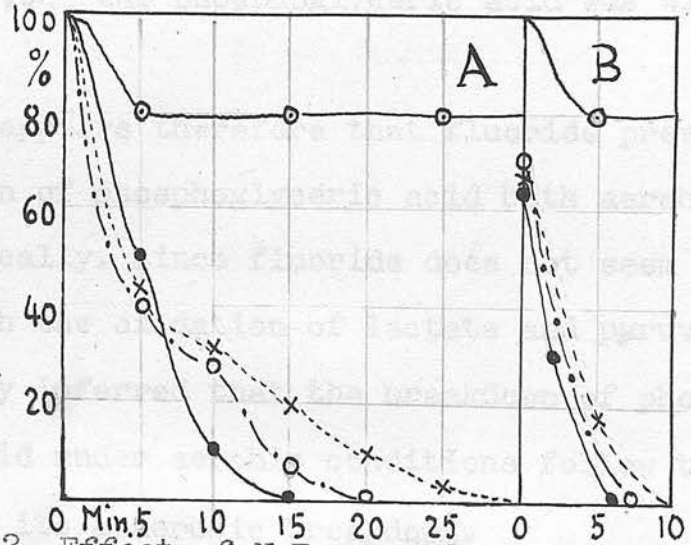



Fig.2.-Effect of NaF on anaerobic Activity of Frog's heart. Ordinate: mechanical response as per cent.normal.Abscissa:time in minutes after exposure to nitrogen. -o-: normal; --x--; NaF 1-10,000; .-o-. NaF 1-5,000; —•— NaF 1-3,000; A:first asphyxia; B:second asphyxia.

Previous experiments showed that under similar conditions as above a frog's heart exhausted of carbohydrate available for glycolysis by repeated asphyxiation was arrested within 10 or 15 minutes after the beginning of asphyxia. This indicates that most of the period elapsing between the onset of asphyxia and the arrest of the fluoride poisoned frog's heart is due to removal of oxygen from the system. It appears therefore that fluoride abolishes glycolysis almost completely and this inhibition has been shown in muscle brei experiments to be due to the prevention of the breakdown of phosphoglyceric acid into pyruvic acid.

In a few frog's hearts which showed considerable decrease in activity after prolonged perfusion with sodium fluoride containing fluid in the presence of air, additon of lactate and pyruvate to the perfusion fluid usually produced a slight but definite increase in its activity, whereas phosphoglyceric acid was without effect.

It appears therefore that fluoride prevents the breakdown of phosphoglyceric acid both aerobically and anaerobically. Since fluoride does not seem to interfere with the oxidation of lactate and pyruvate, it may be safely inferred that the breakdown of phosphoglyceric acid under aerobic conditions follow the same route as its anaerobic breakdown.

Since fluoride inhibits carbohydrate oxidation but does not affect the aerobic activity of cardiac tissue, it follows that both the rabbit's auricle and the frog's

heart can maintain a nearly normal activity by the oxidation of substances other than carbohydrate. Such a conclusion is in agreement with the work of Lipmann (1928) who showed in minced muscle that fluoride caused only a slight diminution in oxygen uptake although it inhibited glycolysis completely.

To sum up, the glycogen content of the mammal's heart is normally very constant, but it is readily broken down by asphyxia, especially asphyxia plus adrenaline. Under aerobic conditions, glycogen can be broken-down but it is readily built up, the theory has been advanced that glycogen of the mammal's heart is continuously broken down but is built up as soon as it is broken down. The mammal's heart normally utilizes a considerable amount of carbohydrate and the evidence at present seems to indicate that at the early stages the oxidative breakdown of carbohydrate follow the same route as its anaerobic breakdown.

## 111. NONCARBOHYDRATE METABOLISM OF THE MAMMALIAN HEART.

(1) Introduction. (2) Utilization of Fat. (3) Utilization of protein. (4) Utilization of Amino-acids.

(1) Introduction: It has been shown that isolated rabbit's auricles poisoned with I.A.A. can survive for a short time and that auricles poisoned with fluoride can contract well for a number of hours in the presence of oxygen. Since in both cases, the power to utilize glycogen or glucose is apparently abolished, it may be inferred that the rabbit's auricle can utilize other substances in addition to carbohydrate as sources of energy. It will be shown later that rabbit's auricles freed from glycogen by asphyxia in situ can be revived in vitro in glucose-free Locke's solution and that they contract fairly well in the presence of oxygen. This fact also indicates that rabbit's auricles can use substances other than carbohydrate. Further evidence pointing to the same conclusion is provided by determinations of the total oxygen usage and of the respiratory quotient of the mammalian heart. Most authors agree that carbohydrate (including lactate) usage by the mammalian heart can only account for part of the oxygen used: 25-60% (Visscher and Mulder,1930), 44 % (Mcginty and Miller, 1933),and 67-80 % (Evans, Grande and Hsu,1935), and that the R.Q.of the mammalian heart rarely attains unity: 0.85 (Starling and Evans,

1914-15),0.9 (Bayliss, Muller and Starling, 1928),c.0.7 (Mcginty and Miller, 1933), and 0.7-1.0(Crickshank and Startup, 1933;Cruickshank and McClure, 1936; Burns and Cruickshank, 1937). The mammalian heart is therefore like the frog's heart in that it normally burns a considerable amount of noncarbohydrate as a source of energy supply. In the frog's heart it has been shown that carbohydrate usage can only account for some 20 to 50 per cent of the total oxygen used (Clark et al. 1938).

The chief materials other than carbohydrates available for oxidation in the mammalian heart are fats, proteins and amino-acids.

(2) Utilization of Fat:Isolated rabbit's auricles poisoned with I.A.A. and markedly depressed in activity in the presence of oxygen provide a system on which the possibility of fat usage can be studied. Positive evidence that fatty acids can be oxidized by the frog's heart has been obtained from experiments with the isolated ventricle, poisoned with I.A.A. and stimulated to exhaustion in air (Clark, Gaddie and Stewart, 1937). Using the I.A.A. poisoned and depressed rabbit's auricle, I found that propionate produced a marked and caproate a slight restoration of the power of contraction (Fig 3). Other fatty acids, butyrate and valerate seemed to delay the arrest of the poisoned auricle but had no definite restorative effect. It may be possible/

sible that the poisoned auricle being deprived of carbohydrate as a source of energy might have already been using fat to its full extent and in this case one would not expect that the addition of an extra amount of fatty acids would produce any effect. These facts therefore strongly suggest, although they do not prove, that the rabbit's auricle can use a certain amount of fatty acids.

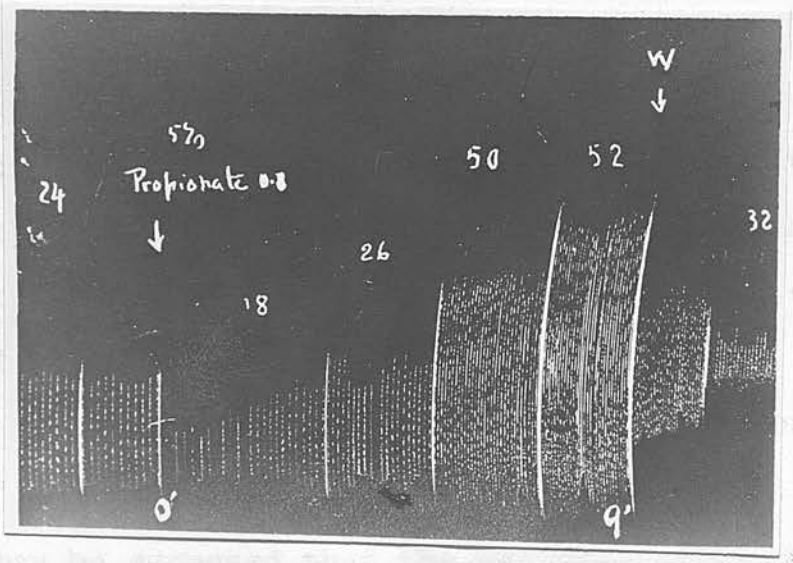



Fig.3. A. Isolated auricle poisoned and depressed by I.A.A. was partially revived by propionate but became depressed again after removal of propionate at W. Numbers above fig. = frequency of contraction per min.; numbers below fig. = min. after addition of propionate. B. Effect of caproate upon auricle poisoned and depressed by I.A.A.

Evidence obtained by other workers supports this suggestion. Cruickshank and McClure (1936) have reported that dog's heart lost a certain amount of fat when the perfusion blood was rendered free of sugar. Dittmar (1933) claimed that starvation produced steady daily losses of fat and accumulation of glycogen in the rat's heart. As the loss of fat was many times greater than the increase in glycogen, the writer concluded that the fat lost was oxidised rather than converted into glycogen.

In conclusion, one may say that fat utilisation by the mammalian heart, though it seems probable, has not been proved beyond doubt. The situation as regards fat utilisation by the frog's heart is very similar.

(3) Utilisation of Protein: Since there is no evidence that the mammalian heart can definitely utilise fat, it may be supposed that the residual oxygen not accounted for by carbohydrate is used by protein.

It has been shown that the frog's heart produces not only ammonia (Ostern, 1930; Clark, Gaddie and Stewart, 1931; Parnas and Ostern, 1931) but also urea (Clark, Gaddie and Stewart, 1931). In fact, the amount of ammonia and urea produced by the frog's heart is sufficient to account for some 30 to 60 per cent of its oxygen usage (Clark et al., 1938). With regard to the mammalian heart, Tsuji (1916) and Cruickshank and McClure (1936) found that it did not produce urea. Many work-

ers also showed that it formed no ammonia (klisiecki, 1934; Cruickshank and McClure, 1936), unless there was partial oxygen lack (Tsuji, 1916; Bornstein and Roese, 1929). The ammonia formed during oxygen lack is probably derived from adenylic acid, since I found that under the influence of asphyxia adenosinetriphosphate is readily decomposed into adenylic acid and Weicker (1934) showed that free adenylic acid in the rabbit's heart is readily deaminised and gives rise to ammonia.

(4) Utilisation of Amino-acids: Since the mammalian heart does not seem to produce urea nor ammonia, it is not likely that it can utilise amino-acids. In the I.A.A. poisoned and depressed auricle, I found that the addition of amino-acids - glycine, alanine and glutamic acid- was without effect on the contractions, although under similar conditions these same substances restored the power of contraction of the frog's heart poisoned with I.A.A. and exhausted in air. Direct estimations have shown that the mammalian heart does not use utilise amino-acids (Tsuji, 1916; Klewitz, 1923; Cruickshank and McClure, 1936). It is interesting to note that while pyruvic acid is readily utilised by the poisoned auricle, alanine which is convertible by the usual process of deamination to pyruvic acid cannot be used by the tissue. This suggests that the failure of the poisoned auricle to utilise amino-acids is perhaps due to its inability to deaminise amino-acids.

amino-acids.

The evidence at present therefore indicates that the mammalian heart differs froms the frog's heart in that it can not utilise protein or amino-acids. On the other hand the isolated rabbit's auricle resembles the isolated frog's heart and differs from skeletal muscle in that it can maintain a nearly normal activity by the oxidation of non-carbohydrate material.

## IV. ANAEROBIC CARBOHYDRATE METABOLISM OF THE MAMMALIAN HEART

(1) Anaerobic Carbohydrate Metabolism of the Frog's heart. (2) Effect of Asphyxia upon the Isolated Rabbit's Auricle: (a) Normal auricle; (b) Auricle poisoned with I.A.A.; (c) Auricle poisoned with fluoride; (3) Summary.

(1) Anaerobic Carbohydrate Metabolism of The Frog's Heart: A number of workers have shown that the anaerobic frog's heart utilizes carbohydrate and converts it into lactic acid (Nagaya,1929;Eismayer and Quincke, 1930;Wertheimer,1930). Clark,Gaddie and Stewart (1932, 1934) found that there is a close relation between the anaerobic acitivity of the frog's heart and the quantity of lactic acid which it produced. During the first hour of anaerobiosis,the lactic acid production was greatest and the activity maintained was equal to about 80 per cent of the normal aerobic activity. After one hour the lactic acid production and the mechanical response fell together. According to Clark, Gaddie and Stewart the frog's heart perfused in nitrogen with alkaline Ringer's solution produced during the first hour about 3 mg./g./hour of lactic acid when glucose was absent and about 5.7 mg./g./hour of lactic acid when glucose was present. The oxygen usage of the frog's heart in moderate activity is

according to Clark and White (1928) about 0.8 cc./g./hr. which would oxidise about 1 mg. lactic acid /g./hour. Hence these figures show a ratio between anaerobic lactic acid production and carbohydrate oxidation of about 3 to 1 or 6 to 1. In the early stages of asphyxia, this ratio may be even higher, namely, about 9 to 1 (Clark, Gaddie and Stewart, 1932; 1934). These results indicate that the formation of lactic acid is an important source of energy for the anaerobic frog's heart. Gaddie and Stewart (1934) showed that a frog's heart exhausted of all carbohydrate available for glycolysis could be revived rapidly and completely by glucose or any other substance convertible to lactic acid, but not by amino-acids and fatty acids. These facts show that lactic acid production from carbohydrate forms the sole source of energy for the anaerobic frog's heart.

Since the frog's heart deprived of oxygen depends upon glycolysis as a sole source of energy, it is natural that its activity should be limited by the supply of carbohydrate. Clark, Gaddie and Stewart (1932) showed that the activity of an anaerobic frog's heart perfused with alkaline Ringer's solution began to fall after an hour, but if sugar was added its activity could be maintained much longer.

Clark, Gaddie and Stewart (1932) have further shown that the anaerobic activity of the frog's heart is dependent also upon the amount of buffer present in the system. According to them, lactic acid formed

by the anaerobic frog's heart is excreted into the perfusion fluid and thereby lowers the pH of the latter. When this reaches the value of about 6.6, glycolysis is arrested and the heart is deprived of all available sources of energy. These authors have shown clearly that at a given pH, with small volumes of perfusion fluid, asphyxia produces a much more rapid effect than with large volumes of fluid and that with large amounts of alkaline fluid anaerobic activity can be maintained for 2 or 3 hours, whereas with equally large amounts of unbuffered fluid oxygen lack leads to a rapid diminution in activity and complete arrest within an hour.

(2) Effect of Asphyxia upon the Isolated Rabbit's Auricle: (a) Asphyxia of Normal Auricles: Isolated rabbit's auricles suspended in well oxygenated Locke's solution can normally contract for many hours. Whether the fluid was acid (pH 6.8) or alkaline (pH 8.2) did not greatly influence the survival period, although in acid media the amplitude of contraction was usually smaller. But if nitrogen was bubbled through the solution, the activity of the auricles rapidly declined in a die-away curve and became totally abolished in 10 to 30 minutes, but if oxygen was readmitted full recovery soon took place. Some of these results given in Table VI show that the auricle is arrested more rapidly at pH 6.8 than at pH 8.2 and that the addition of glucose at pH 8.2 prolongs the survival period but does not do so at pH 6.8.

Table VI:- Effect of PH of perfusion fluid and of addition of glucose upon the rates of asphyxiation (numbers indicate time in minutes from the begining of asphyxia)

| | pH 6.8 | | | | pH 8.2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Without glucose | | With glucose | | Without glucose | | With glucose | |
| | To 1/2 activity. | Until arrest. | To 1/2 activity. | Until arrest. | To 1/2 activity. | Until arrest. | To 1/2 activity. | Until arrest. |
| A. | 4.0 | 7.5 | 4.0 | 8.0 | 4.5 | 9.0 | 6.0 | 19.0 |
| B. | ... | ... | ... | ... | 3.0 | 14.0 | 3.5 | 25.0 |
| C. | 4.5 | 18.5 | ... | ... | 8.0 | 36.0 | ... | ... |

These results are in general agreement with those obtained by Gottdenker and Wachstein (I933) with a similar preparation. A comparison of the response to asphyxia of the rabbit's auricle with that of the frog's heart (Clark,Gaddie and Stewart I932) shows the following points of interest.

Both preparations are rapidly depressed by asphyxia when in contact with neutral fluid with or without glucose, but when in contact with alkaline fluid their response to asphyxia is quite different.

The frog's heart perfused with alkaline fluid without glucose can maintain an activity of about 80 per cent of the normal for an hour and when glucose is added the survival may be much longer. In this case, it has been shown that the anaerobic survival is dependent upon the power of the tissue to obtain energy by glycolysis,and that this is limited by two factors namely,the supply of carbohydrate available for glycolysis,and the amount of buffer available for neutralization of the lactic acid formed.

In the case of the isolated rabbit's auricle there is no well marked difference between the rate of asphyxial arrest at pH 6.8 and at pH 8.2 or between the rates without and with glucose.

Table Vl suggests that alkali and glucose favour the asphyxial survival of the rabbit's auricle, but this effect is not very definite.

This result is in striking contrast to the manner in which the duration of the asphyxial survival of the frog's heart can be varied as desired by modifying the carbohydrate content and the buffer power of the perfusion fluid.

A similar difference is seen as regards the effect of addition of fresh glucose or of alkali to the perfusion fluid after asphyxial depression has occurred. In the case of the frog's heart nearly complete revival of mechanical activity can be produced by such addition but they produce no effect in the case of the rabbit's auricle.

These facts indicate that neither the supply of buffer nor of carbohydrate in the form of glucose is the limiting factor of the anaerobic activity of the isolated rabbit's auricle.

The next question is whether or not cardiac glycogen is a limiting factor in the anaerobic activity of the rabbit's auricle. It has been shown that the frog's heart perfused with alkaline glucose-free Ringer's solution under anaerobic conditions glycolyses

its own carbohydrates and maintain a fairly normal activity until most of the carbohydrates available for glycolysis have been used up and that arrest occurs when practically all the available carbohydrates are exhausted.

Estimations of glycogen loss as given in Table V11 show that repeated asphyxia or asphyxia in situ

Table V11.- Effect of asphyxia upon the glycogen content of the rabbit's heart.

| Description. | No. of expts. | Glycogen. Mg./100 g. |
|---|---|---|
| Isolated auricles- | | |
| (1) Controls . . . . . | 19 | 512 |
| (2) Aerobic perfusion . . . . | 3 | 446 |
| (3) Repeated asphyxiation until arrest - | | |
| (a) 3 times . . . . . | 1 | 398 |
| (b) 6-8 times . . . . | 2 | 100 |
| (4) Asphyxiated once - | | |
| (a) To 1/10 activity . . | 3 | 380 |
| (b) Until arrest . . . . | 2 | 288 |
| (c) Until arrest with adrenaline (1 in $10^7$) . . | 2 | 219 |
| Heart in situ - | | |
| (1) Auricle - | | |
| (a) Controls . . . . . | 19 | 512 |
| (b) Asphyxia until arrest . | 2 | 133 |
| (2) Ventricle - | | |
| (a) Controls . . . . . | 10 | 542 |
| (b) Asphyxia until arrest . | 4 | 109 |

can reduce the glycogen content of the auricle from about 500 to about 100 mg./100 g., and hence at least four-fifths of the glycogen is available for glycolysis.

The results with a single asphyxiation shows, however, that the mechanical response may be reduced to 10 per cent of the normal when only about one-

fifth of the total glycogen has been removed and at the time of arrest the glycogen content is reduced to only about two-thirds normal. These results show, therefore,that asphyxial arrest is not due to a simple depletion of the glycogen available for glycolysis, since it occurs long before the glycogen depletion is completed.

The results obtained with the rabbit's auricle agree closely with those obtained with the isolated dog's heart perfused with blood (Bogue,Chang and Gregory,1938). These authors found that dog's heart under partial anoxia survived slightly longer when the blood glucose was high than when it was low. Asphyxial arrest occurred,however,at a time when glucose was still present in abundance in the circulating blood. They also showed that at the time of arrest there was only partial depletion of glycogen,although much of the remaining glycogen was available for glycolysis,and that,as a rule,the amount of lactic acid produced was too small to produce any significant change in the reaction of the blood used for perfusion.

(b) Asphyxia of Rabbit's Auricles poisoned with Iodoacetate : - Iodoacetate is known to inhibit glycolysis,hence,if glycolysis were an important source of energy for the anaerobic activity of the isolated rabbit's auricle,iodoacetate poisoning should lead to more rapid arrest in asphyxia. Iodoacetate was

added to the perfusion fluid of the isolated rabbit's auricle to make a concentration of 1-50,000 to 1-30,000 the drug was allowed to remain in contact with the tissue for 15 minutes and then removed by replacing the drug-containing solution with clean fluid. This procedure establishes fully the effect of the iodoacetate but avoids any progressive toxic effect upon the tissue. As long as oxygen was passed through the solution the rabbit's auricle so poisoned could survive and maintain a fairly normal activity for many hours if lactate were added to the perfusion fluid. But if nitrogen was bubbled through the fluid, arrest of the auricle occurred in three or four minutes. The arrest was equally rapid with or without glucose and at pH 8.2 or pH 6.8. Upon readmission of oxygen, the poisoned auricle could be readily revived.

The response of I.A.A. poisoned auricles toward asphyxia is essentially similar to that of auricles rendered practically free from glycogen. When rabbit's auricles were asphyxiated in situ until complete arrest, nearly all the glycogen available for glycolysis could be removed. If these auricles were revived in well oxygenated Locke's fluid and asphyxiated for a second time, they became arrested in three or four minutes.

A comparison of the response of normal auricles suspended in glucose-containing alkaline Locke's fluid towards asphyxia and that of the auricles poi-

soned with I.A.A. or rendered free of glycogen shows the following points. All preparations rapidly decline in activity during the first few minutes of asphyxia. But the normal auricle can maintain a low activity after the initial fall for some 20 or 30 minutes more, while tha auricles poisoned with I.A.A. or rendered free from glycogen can not do so. These facts indicate that glycolysis alone can only at its best maintain a low activity of the rabbit's auricle for a relatively short period.

(c) Asphyxia of Rabbit's Auricles Poisoned with fluoride: - Lipmann (1928) showed in muscle brei that fluoride in moderate concentrations, while it inhibited glycolysis almost completely, produced little effect upon oxygen uptake. Later, the same author (1930) showed that a frog's muscle poisoned with fluoride could contract as well as a normal one, and that it produced no lactic acid but a progressive destruction of phosphagen. The writer concluded that the action of fluoride was similar to that of iodoacetic acid. Ochoa (1937) showed that fluoride inhibited the formation of lactic acid in cardiac as well as in skeletal muscle brei.

In the isolated rabbit's auricle, I found that fluoride 1 in 3,000 produced little effect upon the mechanical response, provided oxygen was passed through the solution. Indeed, fluoride in concentrations lower than 1 in 3,000 actually improved the activity. This

proves that, in the rabbit's auricle, the mechanism destroyed by fluoride poisoning is not needed provided there is adequate oxygenation. When, however, the fluoride poisoned auricle was deprived of oxygen by the passage of a stream of nitrogen, the mechanical response, as is shown in Fig.4, fell sharply and systolic arrest occurred in three or four minutes. When oxygen was readmitted, full recovery took place in a few minutes.

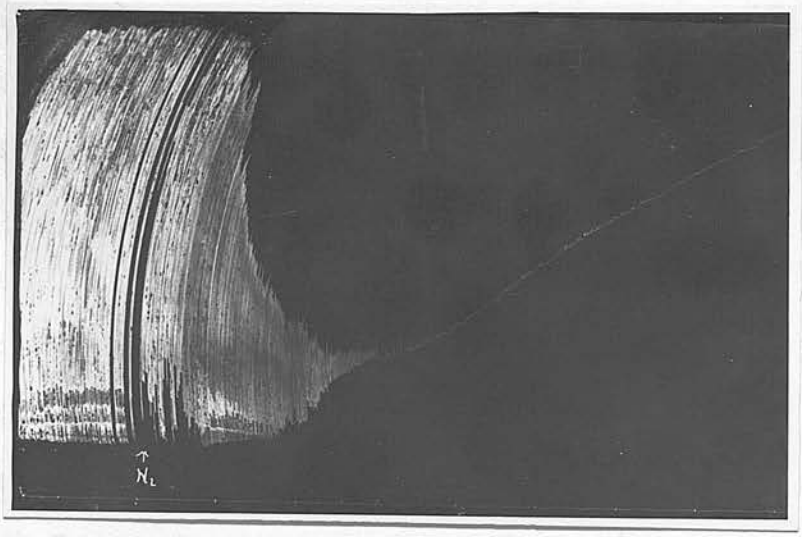



Fig. 4.- Effect of Asphyxia upon isolated rabbit's auricle poisoned with NAF. Time = 1 minute.

The response of the rabbit's auricle poisoned by fluoride towards asphyxia is therefore closely similar to that of auricles poisoned by I.A.A. or rendered free of glycogen. The obvious explanation is that fluoride inhibits glycolysis and therefore abolishes anaerobic activity.

The results obtained with the rabbit's auricle were confirmed by experiments on the frog's heart ( p.20 ); in the latter, fluoride in adequate concentrations had little effect upon aerobic activity but inhibited anaerobic contractions.

It may be pointed out that the action of fluoride is produced only when the poison remains in contact with the tissue. Furthermore, prolonged contact of fluoride with cardiac tissue does not seem to produce any progressive effect. It is also interesting to note that, while fluoride readily precipitates calcium in vitro, all hearts poisoned by fluoride showed no effects of calcium lack. It is known that fluoride has no action upon methyl-glyoxalase, yet it inhibits anaerobic activity of the heart almost completely. This indicates that the formation of lactic acid via the route of methyl glyoxal does not constitute an important source of energy for the anaerobic heart.

(d) Summary; - At first sight the effect of asphyxia upon the frog's heart and the rabbit's auricle appear to be completely different. Under favourable conditions (alkali plus glucose) the frog's heart can maintain a considerable mechanical activity for an hour or more and is not arrested until after many hours. Some authors have maintained a small mechanical activity under anaerobic conditions for 60 hours. On the other hand the frog's heart can be arrested in a few minutes by asphyxiation when in contact with

a small volume of neutral glucose-free Ringer's solution.

There is also a very striking contrast between the effect of asphyxia on the normal and on the I.A.A. poisoned frog's heart; it is very easy to arrange conditions so that the times until arrest in these cases are in a ratio of 100 to 1.

The rabbit's auricle shows no such gross difference in its response to asphyxia. At first indeed it would appear that the duration of asphyxial survival is not markedly influenced either by the presence of glucose or by the reaction of the perfusion fluid.

It can, however, be shown that asphyxial arrest does occur somewhat more rapidly in neutral than in alkaline perfusion fluid and also is somewhat delayed by the presence of glucose.

The rate of asphyxial depression is also dependent on the available glycogen content of the rabbit's auricle. Glycogen depletion by repeated asphyxiation in vitro or by asphyxiation in vivo causes the auricle to be rapidly arrested by asphyxia.

Similarly asphyxial arrest is accelerated by either I.A.A. or fluoride poisoning. In this case there is no well marked acceleration of the onset of asphyxial depression, but a marked difference is seen between normal and poisoned auricles as regards the times until asphyxia produces complete arrest.

The speed with which the fall of mechanical ac-

tivity occurs during the begining of asphyxia does not differ greatly under any of the conditions mentioned.

The different conditions show, however, obvious differences in the subsequent effects of asphyxia. When no glycolysis is possible ( I.A.A. poisoning , fluoride poisoning, glycogen depletion and neutral fluid) then the asphyxial depression rapidly passes on to asphyxial arrest. When conditions are favourable to glycolysis ( normal auricles with glycogen or in alkaline glucose-containing fluid) then the auricles maintain a small activity under asphyxial conditions for a considerable period.

The essential difference between the effects of asphyxia on the frog's heart and on the rabbit's auricle would appear to be of a quantitative rather than of a qualitative character.

In the case of the frog's heart,when conditions are favourable for glycolysis this can maintain an anaerobic activity equal to about 80 per cent of the normal activity for about an hour,whereas when glycolysis is inhibited completely arrest occurs in less than five minutes.

In the case of the rabbit's auricle even when conditions are most favourable for anaerobic glycolysis this appears to be inadequate to maintain a mechanical activity of more than 10 to 20 per cent. of the normal.

Hence under all conditions ( acid or alkaline media, glucose present or absent, normal or poisoned with I.A.A.), asphyxia causes a rapid depression of the mechanical activity to about 20 per cent of the noraml.

V. PHOSPHORUS METABOLISM OF THE MAMMALIAN HEART

(1) Phosphorus Compounds in the Normal Heart. (2) Effect of Asphyxia : (a) Asphyxia in Situ; (b) Asphyxia of Isolated Rabbit's Auricle. (3) Discussion : (a) Role of Phosphagen; (b) Role of Adenosinetriphosphate.

(1) Phosphorus Compounds in the Normal Heart : The mammalian heart contains two groups of phosphorus compounds, namely, acid-soluble and acid-insoluble. The acid-soluble phosphorus compounds which will be considered here can be divided into five fractions, namely, phosphagen, pyrophosphate (adenosinetriphosphate), orthophosphate, soluble esters and insoluble esters (Eggleton and Eggleton, 1929). The percentage distribution of these fractions and the total acid-soluble phosphorus in the rabbit's auricle and ventricle was found by the author to be as shown in Table VIII.

| Description. | Phosphagen | Pyrophosphate | Orthophosphate | Soluble esters | Insoluble esters | Total-P. Mg/100 g. |
|---|---|---|---|---|---|---|
| A. Auricle | 17.3 | 21.0 | 20.1 | 15.3 | 25.3 | c.60 |
| B. Ventricle | 16.9 | 26.6 | 20.5 | 14.0 | 22.0 | 92 |

Weicker (1934) found in the rabbit's ventricle a phosphagen content of about 4.5 per cent., while I found it to be about 17.0 . The probable reason for this difference is that Weicker killed the animal by bleeding and perfused the hearts with saline solution.

and it is probable that his heart was subjected to asphyxia. Similar discrepancies have, however, been found in the case of the dog's heart, since Pollack et al.(I934)found phosphagen values between 7.2 and 29.8 and Grande (I936) I3 per cent., while Burns and Cruickshank (I937) found a value of about 6 per cent only.

(2) Effect of Asphyxia : - (a) Asphyxia in situ : Two series of experiment were conducted. In the first series of experiments, asphyxia was produced by opening the chest and section of aorta and vena cava superior to the diaphragm. This was done in order to minimise the effect of adrenaline on the cardiac glycogen. In the second series, asphyxia was produced by clamping the trachea with the circulation intact. The results which are summarized in Table IX show that, with the aorta and vena cava sectioned (Table lX, A, (a),(1),(2) ) two minutes asphyxia produced a reduction of the phosphagen to about one quarter of its normal value but caused little change in the glycogen and adenosinetriphosphate contents. Asphyxia for five minutes caused little further change in phosphagen but the glycogen and the adenosinetriphosphate were reduced to about four-fifths of their normal values.

Table lX /

TABLE IX

Effect of Asphyxia upon the Phosphorus Distribution and Glycogen Content of the Rabbit's Heart (Phosphorus Fractions expressed as per cent, of Total Phosphorus in this and later Tables)

| Description | No. of expt. | Phosphagen | Pyrophosphate | Orthophosphate | Soluble ester | Insoluble ester | Mg./100 g. Total P. | Mg./100 g. Glycogen |
|---|---|---|---|---|---|---|---|---|
| A. Ventricle: | | | | | | | | |
| Control | 11 | 16.9 | 26.6 | 20.5 | 14.0 | 22.0 | 92 | 542 |
| (a) Asphyxia with aorta and vena cava cut: | | | | | | | | |
| (1) 2 minutes | 3 | 4.4 | 23.5 | 35.6 | 20.2 | 16.3 | - | 522 |
| (2) 5 minutes | 3 | 3.3 | 21.2 | 36.9 | 20.8 | 17.8 | - | 411 |
| (b) Asphyxia with circulation intact: | | | | | | | | |
| (1) 2 minutes | 2 | 5.0 | 22.3 | 33.5 | 22.9 | 16.3 | 88 | 341 |
| (2) 5 minutes | 2 | 4.1 | 17.7 | 37.2 | 21.5 | 19.5 | 92 | 88 |
| (3) until arrest | 6 | 4.0 | 6.7 | 47.6 | 22.6 | 19.1 | 90 | 54 |
| (4) after arrest (30 min) | 3 | 0 | 1.2 | 56.9 | 22.9 | 19.0 | 92 | -- |
| B. Auricle: Control | 2 | 17.3 | 21.0 | 20.1 | 15.3 | 25.3 | -- | 512 |
| Asphyxia until arrest | 4 | 7.0 | 8.3 | 42.6 | 24.1 | 18.0 | -- | 133 |

The results obtained with the circulation intact ( Fig. 5; Table lX , A, (b) (1) (2) (3)) show that asphyxia for two minutes reduced the phosphagen by more than two-thirds,the adenosinetriphosphate about one-sixth and the glycogen more than one-third. Asphyxia for five minutes produced little further reduction in the phosphagen, but the adenosinetriphosphate was reduced to about two-thirds and the glycogen to about one-sixth of their normal values. Still longer asphyxia, until mechanical arrest occurred, showed little further change in the phosphagen and glycogen but the adenosinetriphosphate was reduced to only one-fourth of its normal value. The soluble esters were increased by more than one-third over the normal value,while the insoluble esters showed a small reduction.

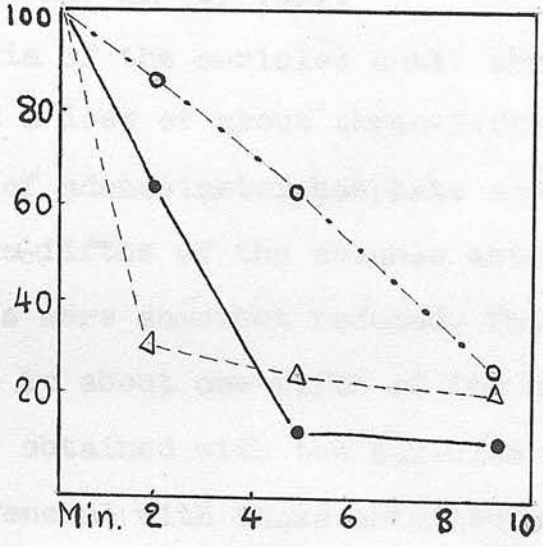



Fig. 5.- Effect of asphyxia upon the phosphagen, pyrophosphate and glycogen content of the rabbit's ventricle. Ordinate;- per cent. of normal; abscissa:- time in minutes;
— O — pyrophosphate; —●— glycogen;
--Δ-- phosphagen.

The results regarding the changes in phosphagen and adenosinetriphosphate agree in general with those recently published by Burns and Cruickshank (1937) who asphyxiated excised hearts of cats and dogs perfused with blood.

In a few experiments a piece of the ventricle was excised, after the heart had been asphyxiated until arrest, and was placed in a stoppered test tube containing a little alkaline Locke's solution, which was freed from oxygen by means of nitrogen. The tube was immersed in a water bath maintained at 36° C. and after 30 minutes, analysis of the phosphorus compounds showed that all the phosphagen had disappeared and that the adenosinetriphosphate was reduced to only a trace (Table IX. A. (b) (4)).

Asphyxia of the auricles until arrest (Table IX. B.)produced a loss of about three-fifths of the phosphagen and of adenosinetriphesphate and an increase of about two-fifths of the soluble esters. The insoluble esters were somewhat reduced. The glycogen was reduced to about one-fifth of its normal value. The results obtained with the auricles therefore agreed in general with those obtained with the ventricles.

It would appear from the above results that in the rabbit's ventricle the change of phosphagen,glycogen and adenosinetriphosphate in asphyxia follow one another in a definite order; phosphagen is first

reduced to a certain minimal value,this is then followed by the loss of glycogen and finally of adenosinetriphosphate. This sequence of events suggests that phosphagen constitutes the immediate source of energy for the contraction of the heart while the breakdown of glycogen and adenosinetriphosphate provide energy for the resynthesis of the phosphagen broken down. various authors have shown that in muscle pulp and muscle extracts phosphagen can be resynthesised by the breakdown of adenosinetriphosphate or by the conversion of glycogen into lactic acid(Meyerhof and Lohmann. I928;I932).

The intimacy of the relation between phosphagen and contraction is also shown by the following fact. With the circulation intact, the mechanical activity of the heart fell to a low level after asphyxia for two minutes, at this time the phosphagen was also reduced to a minimun. On the other hand marked reduction of adenosinetriphosphate was obvious only after gross mechanical failure had set in.

(b) Asphyxia of the isolated rabbit's auricle:

An isolated rabbit's auricle suspended in well oxygenated Locke's solution will contract at an almost steady level for many hours and its phosphagen content does not undergo any marked change, as is shown in Table X.

Table X

Effect of Isolation and Asphyxia upon the Phosphagen Content of the Rabbit's Auricle.

| Description | No. of expts. | Phosphagen index |
|---|---|---|
| (1)Controls . . . . . | 2 | 0.46 |
| (2)Aerobic perfusion . . . | 5 | 0.41 |
| (3)Anaerobic perfusion . . | | |
| (a)To 1/2 activity . . . | 1 | 0.34 |
| (b)To 1/3 activity . . . | 1 | 0.24 |
| (c)Until arrest . . . | 2 | 0.13 |
| (4)Recovery from asphyxia . | 2 | 0.50 |
| (5)I.A.A.poisoning . . . | | |
| (a)Aerobic . . . . | 2 | 0.44 |
| (b)Anaerobic arrest . . (2-3 min.) | 3 | 0.22 |

When the oxygen supply is shut off, destruction of phosphagen occurs rapidly and progressively, while the mechanical activity also declines in a die-away curve and arrest occurs in 20 or 30 minutes. As is shown in Table X and Fig. 6, the phosphagen content and the mechanical activity fall together and at the time of arrest only about one-third of the phosphagen is left. when oxygen is readmitted, the mechanical activity and the phosphagen content both recover together.

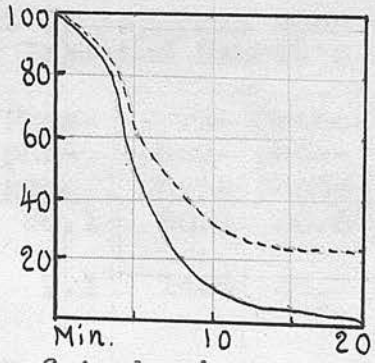



Fig.6.- Effect of Asphyxia upon mechanical activity and Phosphagen content of isolated rabbit's auricle. Ordinate: per cent. of normal; abscissa: time after exposure to nitrogen. Continuous line: mechanical response; dotted line: phosphage index expressed as per cent. of the noraml.

When an isolated rabbit's auricle was poisoned with I.A.A. so that glycolysis was inhibited, asphyxia produced arrest in a few minutes. At the time of arrest, the phosphagen index (phosphagen/phosphagen + inorganic phosphate) as shown in Table X had fallen to about one-half of its normal value. This value is apparently higher than that found at the moment of arrest in the normal auricles. As it can be seen in Table Xl the phosphate liberated from the breakdown of phosphagen in the I.A.A. poisoned auricles is partly converted into esters. Estimation of the phosphagen-P as the percentage of total acid-soluble phosphorus shows that asphyxia reduces it to a similar level in the normal and in the I.A.A. poisoned auricle, for at the time of arrest the phosphagen is 7 per cent. in the normal and 8 per cent. in the I.A.A. poisoned auricle. Hence, it appears that in both the normal and the I.A.A. poisoned rabbit's auricle, asphyxial arrest occurs when the phosphagen has fallen to a equally low value.

Table Xl.- Effect of Asphyxia upon the Phosphorus Distribution of the Isolated Rabbit's Auricle.

| Description | No. of expts. | Phospha-gen | Pyro-phos-phate | Ortho-phos-phate | Soluble esters | Insoluble esters |
|---|---|---|---|---|---|---|
| 1. Aerobic perfusion | 5 | 20.1 | 20.1 | 29.5 | 13.1 | 17.2 |
| 2. Anaerobic arrest | 2 | 7.1 | 14.6 | 45.2 | 16.1 | 17.0 |
| 3. I.A.A. poisoning: | | | | | | |
| (a) Aerobic | 4 | 14.1 | 20.4 | 20.9 | 22.0 | 22.6 |
| (b) Anaerobic arrest | 3 | 8.1 | 18.1 | 28.0 | 24.7 | 21.1 |

It will be seen in Table XI that in both the normal and the I.A.A. poisoned auricles arrest occurs at a time when there is little change in the adenosinetriphosphate.

The results obtained with the isolated auricles therefore agree closely with those obtained with asphyxia of the heart in situ and indicate that phosphagen is more closely related to mechanical activity than adenosinetriphosphate.

It may be again pointed out here that the anaerobic activity of the normal auricles and that of the I.A.A. poisoned auricles is not greatly different, except that in the latter the somewhat prolonged anaerobic activity equal to about 10 or 20 per cent of the normal is not present. Since in both the normal and the I.A.A. poisoned auricles, asphyxial arrest occurs when the phosphagen has fallen to an equally low value, it is clear that normal activity of the rabbit's auricle is more dependent upon phosphagen than glycolysis.

(c) Revival of Rabbit's Auricles Previously Arrested by Asphyxia in Situ:- Auricles arrested by asphyxia in situ when isolated in Locke's solution and supplied with oxygen could be revived either completely or partially. Such auricles provided a means for studying the relation between the recovery of mechanical activity and the recovery of phosphagen and of adenosinetriphosaphate. Table XII and Fig.7 show the results obtained.

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TABLE XII

The effect of in vitro revival of rabbit's auricles asphyxiated until arrest in situ (Phosphorus fractions expressed as per cent. of total acid-soluble phosphorus)

| Description | No. of expts. | Duration perfusion | Phosphagen | Pyrophosphate | Orthophosphate | Soluble esters | Insoluble ester |
|---|---|---|---|---|---|---|---|
| 1. Normal auricles | 5 | c.60* | 20.1 | 20.1 | 29.5 | 13.1 | 17.2 |
| 2. Auricles asphyxiated until arrest | 4 | -- | 7.0 | 8.3 | 42.6 | 24.1 | 18.0 |
| 3. Revival in fluid pH 8.2 (a) | 2 | 20 | 13.9 | 12.9 | 34.4 | 20.2 | 18.6 |
| (b) | 5 | 40 | 22.5 | 14.6 | 25.0 | 18.6 | 19.3 |
| (c) | 2 | 100 | 28.0 | 14.7 | 27.5 | 10.3 | 19.5 |
| 4. Revival in fluid pH 7.2 (a) | 1 | 60 | 20.4 | 4.7 | 28.6 | 25.0 | 21.3 |
| (b) | 2 | 90 | 20.7 | 11.6 | 27.6 | 18.3 | 21.8 |
| 5. Revival in fluid pH 8.2 plus adenylic acid (a) | 3 | 20 | 17.8 | 17.4 | 24.6 | 20.9 | 19.3 |
| (b) | 2 | 75 | 21.1 | 19.7 | 21.0 | 16.7 | 21.5 |

* minutes.

When the auricles were revived in fluids at pH 8.2(Table XII, 3). two-thirds of the phosphagen was restored in about 20 minutes; full recovery of phosphagen took place in about 40 minutes; but the adenosinetriphosphate was only restored to two-thirds of its normal value even when the revival time was prolonged to I00 minutes. In a few experiments, the auricles were revived in fluids with pH 7.2,(Table XII 4.) and in this case complete recovery of phosphagen occurred in 60 minutes, but adenosinetriphosphate resynthesis was practically absent even when the time

of revival was prolonged to 90 minutes.

Parnas and Ostern (I93I) showed in the isolated perfused frog's heart that ammonia was formed and that this ammonia was derived from the deaminization of free adenylic acid into inosinic acid(I932). The latter acid unless reaminized would not be available for the rebuilding of adenosinetriphosphate.

A few experiments were made to determine whether the imperfect resynthesis of adenosinetriphosphate was due to lack of adenylic acid. Adenylic acid (I00-200 ug./30 c.c.) was added to the Lock's fluid in which the auricle was suspended. This improved the aerobic resynthesis of adenosinetriphosphate (Table XII, 5), since the values found after 20 and 75 min. were I7.4 and I9.7 per cent. as compared with I2.9 and I4.7 per cent .after 20 and I00 min. respectively when adenylic acid was not added. The rate of recovery of the mechanical activity was not appreciably affected by the addition of adenylic acid.

Fig. 7 shows the manner in which the asphyxiated auricle recovers its activity when immersed in aerated Lockes solution. The rate of recovery varied greatly in different individuals but in most cases maximun recovery occurred in about 20 or 40 minutes. The recovery appeared to be fairly complete in some cases, but in other cases only a feeble mechanical activity was restored. There appeared to be some correlation between the extent of the recovery of the mechanical

activity and the recovery of the phosphagen content, but there was little correlation between the changes in adenosinetriphosphate content and the mechanical activity.

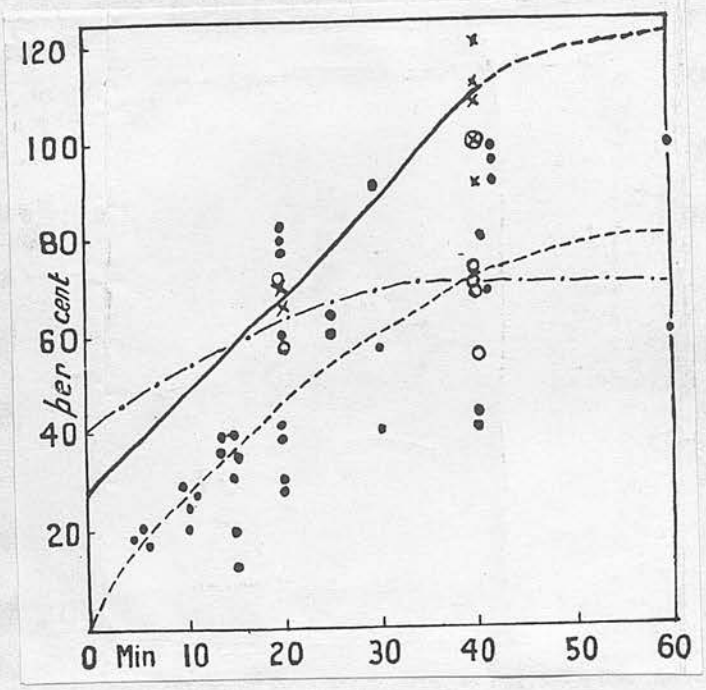


Fig. 7. - Recovery of phosphagen, adenosintriphosphate and mechanical activity of rabbit's auricles asphyxiated to arrest in situ and revived in vitro. Ordinate:- per cent. of normal; abscissa: time in minutes. x —— x phosphagen; —·-o-·— adenosintriphosphate; •---• mechanical activity.

(3) Discussion: (a) Role of phosphagen: It has been shown that when a skeletal muscle of the frog is stimulated to contract in the absence of oxygen, there occurs a progressive destruction of phosphagen. After a short tetanus or a small series of twitches, there is usually little or no lactic acid production, and the total energy produced can be nearly completely accounted for by the energy supplied by phosphagen hydrolysis alone. Toward the end of a longer tetanus

or a larger series of twitches, however, phosphagen breakdown becomes less and less while lactic acid production becomes more and more, and this latter reaction accounts for almost all the energy released by activity.(Eggleton and Eggleton I928; Nachmannsohn, I928; Lundsgaard I93I; Meyerhof and Shutz I93I).

It has been shown in the frog's heart that asphyxia produces a progressive destruction of phosphagen, until about one-quarter of the phosphagen is left. If the Ringer's solution is unbuffered, the frog's heart becomes practically arrested at this point,but if the solution is alkaline, the mechanical activity persists for an hour or two with the phosphagen practically constant at the reduced level, and lactic acid production contiues throughout the whole period and supplies the necessary energy for the activity(Clark,Eggleton and Eggleton, I932).

The effect of asphyxia upon the phosphagen content of the rabbit's heart is strikingly similar to that of the skeletal muscle and of the frog's heart. Both asphyxia of the rabbit's heart in situ and of the auricle in vitro produce progressive destruction of phosphagen and coincidently continuous fall of mechanical activity. Although it is not possible to correlate these changes in terms of energy, these results suggest strongly that phosphagen in the rabbit's heart like that in the skeletal muscle provides a source of energy for the contraction.

Since in both the skeletal muscle and the frog's heart mechanical activity can go on in the absence of oxygen with a low phosphagen content but a continuous lactic acid production, it would appear that the frog's heart and the skeletal muscle can change over from phosphagen breakdown to lactic acid production as source of energy for their anaerobic activity. A number of facts suggest, however, that phosphagen furnishes the more immediate energy for the contraction while lactic acid production serves only to resynthesize the phosphagen brokendown. In the skeletal muscle, such an anaerobic resynthsis has been shown to occur (Nachmannsohn,I928).Studies on muscle extracts have demonstrated clearly that the following reactions may serve to resynthsize phosphagen during the breakdown of glycogen into lactic acid.

I. 2 creatine + Adenosinetriphosphate = 2 phosphagen + adenylic acid.(Meyerhof and Lohmann I932)

II. 2 phosphopyruvic acid + adenylic acid = Adenosinetriphosphate +2 pyruvic acid

2 creatine + adenosinetriphosphate = 2 phosphagen + adenylic acid

---

2 creatine + phosphopyruvic acid = 2 phosphagen + 2 pyruvic acid (Parnas et al.,I934 Needham and von Heymingen I935).

III. Phosphoric acid + creatine + triosephosphate +pyruvic acid =phosphagen + phospho-

glyceric acid + lactic acid

creatine + phosphaglyceric acid = phosphagen

+ pyruvic acid

---

2 creatine + phosphoric acid + triosephosphate

= 2 phosphagen + lactic acid.

(Meyerhof et al., I937).

Since the frog's heart can maintain a fairly normal activity in the absence of oxygen for several hours by obtaining energy from glycolysis, it would appear that the latter reaction provides sufficient energy for the rebuilding of phosphagen that is broken down. On the other hand, under similar conditions, a rabbit's auricle can not maintain activity for any length of time. This suggests that lactic acid production in the rabbit's auricle is unable to sustain the phosphagen store at a level essential for normal activity. Ochoa(I937) has shown in muscle brei that under identical conditions rabbit's heart muscle forms much less lactic acid than skeletal muscle. Gottdenker and Wachstein (I933) found that asphyxiation of the isolated rabbit's auricle in alkaline-glucose containing fluid produced only about 0.4 g./I00 g./hour of lactic acid. Clark, Gaddie and Stewart (I932), however, found that the frog's heart under similar conditions produced lactic acid equal to 0.57 g./I00 g./hour. Yet there is every reason to believe that the rabbit's auricle has a higher energy requirement than the frog's heart.

Lundsgaard (I930) found that skeletal muscle of frog, when poisoned with I.A.A. and made to contract in an oxygen free medium, produced no lactic acid but that a progressive decomposition of phosphagen occurred. According to him, such muscles can contract as well as normal ones, but can only give about one-third as many contractions before being arrested. The total energy developed is proportional to the quantity of phosphagen decomposed, and arrest occurs when there is no phosphagen left. These facts have prompted the theory that phosphagen serves as the immediate energy for the process of contraction.

When a rabbit's auricle poisoned with I.A.A. is asphyxiated, arrest occurs in a few minutes and phosphagen falls to about one-third of the normal. This result is again similar to that found in the case of skeletal muscle, and suggest that phosphagen in the rabbit's auricle serves as an immediate energy for its contraction.

(b) Role of adenosinetriphosphate: Work on the muscle brei or muscle extracts has led to the theory that the breakdown of adenosinetriphosphate forms the first chemical event in the process of contraction while the breakdown of phosphagen and of glycogen into lactic acid serve only to restore the adenosinetriphosphate destroyed,(Lohmann,I934;Parnas,I937).

In the rabbit's heart in situ,after five minutes asphyxia both glycogen and phosphagen are reduced to

a minimal value, and still longer asphyxia until arrest produces little further change in either of these substances, yet the heart continues to beat, during this interval, there is a marked breakdown of adenosinetriphosphate, and this breakdown takes place at a rate much more rapid than that which occurs in a piece of quiescent heart muscle. These facts indicate that adenosinetriphosphate can serve as a source of energy for cardiac contraction. The fact that destruction of adenosinetriphosphate is not evident until most of the phosphagen and glycogen have decomposed may be interpreted to mean that, when phosphagen and glycogen are available, adenosinetriphosphate is resynthesised as soon as it is broken down. These facts are therefore not in disagreement with the theory that the destruction of adenosinetriphosphate forms the primary chemical process in muscle contraction, while the breakdown of phosphagen and glycogen serve only to restore the adenosinetriphosphate broken down.

On the other hand, asphyxia of the rabbit's heart in situ reduced the mechanical activity to a low level long before there was an appreciable reduction of adenosinetriphosphate. Asphyxia of the isolated rabbit's auricle caused arrest even at a time when no significant breakdown of adenosinetriphosphate had occurred. When an arrested auricle was revived in oxygen in many cases, an apparently normal mechanical response could be regained, even when the adenosinetriphosphate

was low. Addition of adenylic acid seemed to have accelerated the recovery of adenosinetriphosphate but had little influence upon the recovery of mechanical activity. The changes in adenosinetriphosphate content therefore appear to bear no close relation to the changes in mechanical activity.

In the frog's heart, it has been shown that asphyxia produced extensive breakdown of phosphagen but much less change in adenosinetriphosphate (Clark, Eggleton and Eggleton, 1932; Clark and Eggleton, 1937).

Weicker (1934) found that asphyxia of the isolated rabbit's ventricle produced nearly complete disappearance of both phosphagen and adenosinetriphosphate. My results show, however, that neither of these substances disappear completely. Burns and Cruickshank (1937) obtained similar results with the isolated dog's and cat's heart, On the other hand I found that when asphyxia was continued beyond the time at which it produced arrest, an almost complete disappearance of both phosphagen and adenosinetriphosphate was observed. This suggests that a small amount of phosphagen and adenosinetriphosphate are essential for the maintenance of resting metabolism.

(c) Hexosephosphate: In the above experiments, certain changes in phosphoric esters were observed. Asphyxia and aerobic perfusion decreased the insoluble esters, while I.A.A. poisoning increased them. The nature of insoluble esters is not clear, but we

may reasonablely presume that they are composed partly of hexosediphosphate. It seems likely that the changes in insoluble esters are due to hexosediphosphate, since Weicker (1934) has shown that changes in'hexosediphosphate'corresponding to those in insoluble esters takes place under the conditions mentioned above.

The soluble esters showed an increase in asphyxia and I.A.A. poisoning but tended to decrease when arrested auricles were revived in oxygenated Locke's solution. The soluble esters appear to be composed mainly of hexosemonophosphate,we may therefore regard changes in soluble esters to be due to hexosemonophosphate. These changes are interesting ,since similar changes have been observed in the skeletal muscle. Eggleton and Eggleton (1928) and Cori and Cori (1932) showed that anaerobiosis produced an accumulation of hexosemonophosphate; Cori and Cori (1936) found that I.A.A. poisoning caused an increase in hexosemonophosphate in the skeletal muscle; Cori et al. (1937) also demonstrated that hexosemonophosphate accumulated in asphyxia disappeared when the muscle was recovered in oxygen.

## VI GENERAL DISCUSSION

The primary aim of the author has been to correlate the metabolism of cold-blooded and of mammalian hearts.

The evidence available suggested that these were very different because the work of Clark,Gaddie and Stwart showed that under anaerobic condition the cold-blooded heart sustained mechanical activity by means of glycolysis until either the available carbohydrate was exhausted or until glycolysis was arrested by accumulation of acid.

Gottdenker and Wachstein(I933) showed on the other hand that lack of oxygen arrested the isolated rabbit's auricle before there had been suffient production of lactic acid to produce any measurable change in reaction. Hence the reason for the asphyxial arrest of the mammalian heart was not known.

Analysis of the changes produced by asphyxia on the phosphate fractions of the mammalian heart showed that in both the frog's heart and in the mammalian heart the primary change caused by asphyxia was a rapid disappearance of most of the phosphagen. When the phosphagen fell to a low level the mammalian heart was arrested, but the frog's heart differed in that it could maintain activity for a long time after the phosphagen content had fallen to a low level. The source of energy under these conditions was glycoly-

sis.

The peculiar aptitude for anaerobic survival in the frog's heart may reasonably be accounted for as a special character necessitated by the fact that the frog spends long periods of its life hibernating in mud under conditions which must be practically anaerobic.The remarkable rapidity with which asphyxia in situ causes disappearance of glycogen in the mammalian heart suggests that a very high rate of glycolysis is necessary to maintain an effective activity. The presence of adrenaline, the liberation of which is part of the normal response to asphyxia is essential for this sudden breakdown of glycogen.

The maintenance of activity of the mammalian heart by glycolysis may therefore be regarded as an emergency mechanism which only sustain activity for a short time.

The disappearance of phosphagen is the most obvious change produced by asphyxia in the mammalian heart,and all the evidence agrees in showing a close correlation between the phosphagen content and the mechanical activity.

The relation between mechanical activity and the adenosinetriphosphate content is much less obvious.

As far as the evidence from the mammalian heart is concerned it indicates that phosphagen is the phosphate fraction which is essential for the maintenance of mechanical activity and the same is true in the

case of the frog's heart. It may indeed be said that the studies of cardiac metabobism do not indicate that adenosinetriphosphate plays any important part in such metabolism. On the other hand no clear evidence has been found which proves that adenosinetriphosphate is not an essential link in cardiac metabolism. Since experiments with muscle-brei indicate that the energy liberated by phosphagen breakdown is utilized to resynthesise ademosinetriphosphate it is simplest to accept this hypothesis, since it is not disapproved by the studies of cardiac metabolism.

It is obvious that the hypothesis in question is extremely difficult to disprove and it can not be regarded satisfactory since all the evidence in its support comes from experiments on muscle brei and little direct evidence in its support has been obtained from studies of intact skeletal or cardiac muscle.

The process of asphyxial arrest in the mammalian heart appears therefore to be as follows:

a) In the intact animal asphyxia causes adrenaline release and adrenaline plus asphyxia cause a very rapid glycolysis of the cardiac glycogen. This release of energy can only sustain cardiac activity for a minute or two.

b) When adrenaline is excluded (heart in situ with suprarenals cut off or isolated auricle) asphyxia does not provoke such rapid glycolysis, and the energy supply from this source is inadequate to maintain car-

diac activity. Phosphagen depletion results and the mechanical activity falls at the same rate as does the phosphagen content.

In a few minutes the mechanical activity falls to about 20 per cent of the normal, and then, if condition permit glycolysis, this reduced activity is maintained for about 20 minutes; if no glycolysis is possible (glycogen exhaustion, I.A.A. or fluoride poisoning etc.) there is no stage of activity maintained at a reduced rate but the auricle rapidly passes to complete arrest.

The facts observed could all be explained on the simple assumption that phosphagen breakdown is the immediate source of energy for the contraction process. These facts can however be reconciled with the hypothesis (based on muscle brei experiments) that adenosinetriphosphate breakdown is the immediate source of energy for the contractile process and that phosphagen breakdown supplies the energy necessary for the resynthesis of adenosinetriphosphate.

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# APPENDIX

## PAPPERS BY AUTHOR

World-List Abbreviation.—Quart. J. exp. Physiol.

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## THE INFLUENCE OF ADRENALINE ON CARDIAC GLYCOGEN.

By I. CHANG. From the Departments of Pharmacology and Medical Chemistry, University of Edinburgh.

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# THE INFLUENCE OF ADRENALINE ON CARDIAC GLYCOGEN.

By I. Chang. From the Departments of Pharmacology and Medical Chemistry, University of Edinburgh.

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## Introduction.

In the course of some experiments on rats' hearts, it was observed that while asphyxia *in situ* caused an extensive breakdown of glycogen, asphyxia of the freshly excised heart produced no decrease. An attempt made to ascertain the cause of this discrepancy showed that the adrenal glands were largely responsible.

Opinions recorded in the literature are divided with regard to the action of adrenaline upon cardiac glycogen. In the intact animal, Wertheimer [1929] and Haendel and Munilla [1929] reported that adrenaline caused a fall of heart glycogen, whilst other observers found that the heart glycogen was unaltered [Lawrence and McCance, 1931; Evans, 1934; Bong *et al.*, 1934] or even increased [Junkersdorf and Hanisch, 1927; Geiger and Schmidt, 1929] after adrenaline. In the heart-lung preparation of the dog, adrenaline was found to deplete the heart glycogen by Patterson and Starling [1913] and by Cruickshank [1913]; this finding has since been confirmed by Bogue *et al.* [1935] on the isolated dog's heart preparation.

## Methods.

Experiments were made on albino rats of a single stock which was maintained on a diet consisting of white bread soaked in milk. Males of between 110 and 230 g. in weight fasted for twenty-four hours were used. Sodium amytal (10 mg./100 g.) was given intraperitoneally as an anæsthetic to all animals, a precaution recommended by Evans to avoid asphyxia during removal of the heart. Glycogen was determined by the method of Cori [1932] with slight modifications and total carbohydrate by the method of Ochoa [1930]. The hydrolysate obtained in both cases was analysed for sugar by Shaffer and Somogyi's modification of the Shaffer-Hartman method [1933]. Lactic acid was estimated by the method of Friedemann, Cotonio, and Shaffer [1927] after deproteinisation with 4 per cent. trichloroacetic acid and removal of sugar with copper and lime. Whenever possible,

parallel determinations of the three substances were made on a single ventricle, the auricles being removed. Asphyxia of the heart *in situ* was produced simply by the opening of the chest and was always four minutes in duration.

## Results.

### (1) *Adrenaline and Asphyxia.*

The influence of adrenaline on the asphyxial depletion of heart glycogen is shown by the results recorded in Table I. The following points are of interest with regard to these figures:—

Table I.—Influence of Adrenaline and Asphyxia on Carbohydrate Content of Ventricle.

| Description. | No. of animals. | Glycogen content. | | Total carbohydrate content. | | Lactic acid content. | |
|---|---|---|---|---|---|---|---|
| | | Glycogen, mg./100 g. | Standard deviation. | Total carbohydrate, mg./100 g. | Standard deviation. | Lactic acid, mg./100 g. | Standard deviation. |
| (i) Normal controls | 7 | 517 | ±96 | 581 | ± 91 | 107 | ±20 |
| (ii) Asphyxia *in vitro* | 4 | 498 | ±44 | 537 | ± 46 | 145 | ±33 |
| (iii) Asphyxia *in vivo* | 6 | 101 | ±48 | 226 | ± 62 | 182 | ±22 |
| (iv) Asphyxia *in vivo* after section of aorta and vena cava. | 4 | 361 | ±72 | 461 | ± 69 | 254 | ±53 |
| (v) Asphyxia after exclusion of adrenal glands— | | | | | | | |
| A. Control | 4 | 529 | ±97 | 573 | ±105 | 114 | ±39 |
| B. After clamping of vessels. | 2 | 294 | .. | 460 | .. | 184 | .. |
| C. After excision of adrenal glands. | 4 | 290 | ±80 | 406 | ± 23 | 167 | ±27 |
| (vi) Adrenaline and asphyxia. | 4 | 145 | ±74 | 237 | ± 50 | 221 | ±45 |

(i) *Controls.*—In these rats the chest was rapidly opened and the heart was rapidly excised for chemical analysis. The figures for glycogen ranged from 430 to 658, with an average of 517 mg. per 100 g. of heart muscle. These results show a fair uniformity and closely resemble those obtained by Evans [1934] and by Lawrence and McCance [1931]. The figures for total carbohydrate and lactic acid show a similar regularity.

(ii) *Asphyxia in vitro.*—In order to ascertain the extent of the breakdown of glycogen and of the formation of breakdown products, namely, lower carbohydrate and lactic acid, the heart was rapidly excised and allowed to beat outside the body at room temperature for exactly four minutes. This procedure was chosen to avoid the loss of the breakdown products into the circulation. Although Evans [1934] found that asphyxia rapidly lowered cardiac glycogen, yet the results with this method showed only a small decrease of glycogen and of total carbohydrate and a correspondingly small increase of lactic acid.

(iii) *Asphyxia in vivo.*—Asphyxia of the heart *in situ* was next tried. In contrast to the findings obtained in the above experiment, a marked dimution of glycogen and an increase of lower carbohydrate and lactic acid was found, the glycogen being reduced to one-fifth, total carbohydrate to about two-fifths of their normal value, and lactic acid raised by about four-fifths of its normal value. These results therefore confirm the finding of Evans [1934], and indicate further that glycogen is broken down both into lower carbohydrate and lactic acid. A large part of the glycogen which was lost could not, however, be accounted for; presumably some of its breakdown products passed into the circulation. The gross difference found as regards glycogen disappearance between asphyxia *in vitro* and *in vivo* suggested that some factor in the intact animal actively accelerated glycolysis.

(iv) The effect of asphyxia *in vivo* after section of the aorta and vena cava was next tried, in order to determine whether the factor or factors concerned in accelerating the breakdown of heart glycogen were chemical or nervous. The vena cava and the aorta were divided just superior to the diaphragm immediately after the chest had been opened, and then the heart was asphyxiated as described above. The results show a much smaller breakdown of the heart glycogen into lower carbohydrate and lactic acid than was found with simple asphyxia *in vivo*, since the glycogen found was more than three-fifths of the normal value. It is also interesting to note in this case, in which loss of the breakdown products into the circulation could be excluded, that the glycogen lost corresponded approximately to the lactic acid formed, the figures being 156 and 147 mg. per 100 g. respectively. The glycogen figures obtained with excised hearts are higher than those obtained in the present experiment, the respective figures being 498 and 361 mg. per 100 g. This discrepancy is outside the probable limits of experimental error, but might be due to the fact that the excised heart was exposed to a lower temperature. The results show clearly, however, that rapid arrest of the circulation by section of the aorta and vena cava slowed down the process of glycogenolysis, and this suggests that the glycogenolysis is accelerated by a chemical factor,

since in this case the heart had its nervous connection intact, but could not be affected by chemical agents carried in the circulating blood.

(v) *Asphyxia after Exclusion of the Suprarenal Glands.*—A control series of experiments was made (5 A) in which the suprarenal glands were exposed, the vessels clamped, and the glands excised. This operation did not affect the glycogen content of the heart, which remained normal.

Similar operations ((v) B, (v) C) were performed and followed by four minutes asphyxia.

The cardiac glycogen found was three-fifths of the normal, and this is a much higher figure than was obtained in Experiment (iii), where asphyxia of the heart was conducted under otherwise similar conditions, except with the adrenal glands intact; indeed, the value for cardiac glycogen is nearly as high as that obtained in Experiment (iv), where asphyxia of the heart was produced after section of the vena cava and aorta. Exclusion of the adrenal glands, therefore, definitely slowed down the glycogen breakdown when the heart was subjected to asphyxia.

(vi) *Adrenaline after Excision of the Adrenal Glands.*—Four experiments were carried out in which the adrenal glands were rapidly excised and the bleeding vessels ligated. Adrenaline (0·1 mg./100 g. body weight) was then given intramuscularly. Asphyxia of the heart was produced ten minutes after the administration of the drug. This caused a reduction of the cardiac glycogen to about one-fourth of the normal value, a fall nearly as great as that obtained by asphyxiation of the normal animal.

(vii) *Asphyxia after Ergotoxine.*—The results obtained in Experiments 5 and 6 show fairly conclusively that the presence of adrenaline greatly accelerates the reduction in cardiac glycogen caused by asphyxia. In no case, however, was a value for cardiac glycogen equal to the normal value obtained after asphyxia *in situ*, and a few experiments with ergotoxine were performed to see if this could prevent asphyxia from producing any reduction in cardiac glycogen. Large doses of ergotoxine ethane sulphonate (2–4 mg./100 g.) were given intraperitoneally, the suprarenals were then excised, and finally asphyxia was produced. The average cardiac glycogen value found in three experiments was 358 mg./100 g., which is somewhat higher than the value of 290 mg./100 g. found in the comparable experiments without ergotoxine. Since this drug produced a marked toxic effect on the rats and did not appear to arrest completely cardiac glycogenolysis the experiments were not continued.

(2) *Adrenaline without Asphyxia.*

A series of experiments were next carried out to determine the effect of adrenaline in the absence of asphyxia. Adrenaline 1 : 1000 diluted to 1 : 5000 with saline was injected intramuscularly in doses varying from 0·05 mg. to 0·2 mg. per 100 g. body weight, and the animals were sacrificed for chemical analysis at various intervals of time after the injection.

Table II. shows that doses of 0·1 or 0·2 mg. adrenaline per 100 g. after 25 minutes reduce the glycogen to less than half the normal

TABLE II.—INFLUENCE OF ADRENALINE WITHOUT ASPHYXIA ON CARBOHYDRATE CONTENT OF VENTRICLE.

| No. of experiments. | Time after injection, minutes. | Dosage of adrenaline, mg./100 g. | Mg. per 100 g. | | |
|---|---|---|---|---|---|
| | | | Glycogen. | Total carbohydrate. | Lactic acid. |
| 1 | 10 | 0·1 | 635 | 705 | 185 |
| 1 | 15 | 0·1 | 411 | 666 | 142 |
| 1 | 20 | 0·2 | 235 | 372 | 129 |
| 1 | 25 | 0·2 | 119 | 246 | 114 |
| 6 | 25 | 0·1 | 277 (±107)[1] | 362 (±64) | 136 (±16) |
| 2 | 25 | 0·05 | 552 | 735 | .. |
| 1 | 40 | 0·1 | 440 | 505 | 204 |
| 1 | 45 | 0·1 | 449 | 492 | 132 |
| 1 | 60 | 0·1 | 519 | 626 | 121 |

[1] The figures in the brackets represent standard deviations of the mean.

value, but that this effect is not produced by 0·05 mg. adrenaline per 100 g. The figures also indicate that adrenaline produces little effect on cardiac glycogen until 20 minutes after the injection, and that the lowering of the glycogen only lasts for about half an hour. The short duration of the reduction in cardiac glycogen explains why it has not been noted by other authors who have studied the action of adrenaline on the cardiac glycogen of the intact animal. The discovery that adrenaline does produce this reduction, although its action is of short duration, agrees with the observations made on the heart-lung and on the isolated heart of the dog. Furthermore, the other effects produced by adrenaline in this preparation are such as would encourage glycogen breakdown. Adrenaline specifically increases oxygen consumption [Evans and Ogawa, 1913; Gremels, 1933] and sugar and lactic acid uptake by the heart [Patterson and Starling, 1913; Bogue *et al.*, 1935].

The rapidity with which the glycogen is restored after being depleted

by adrenaline appears surprising, but Evans [1934] showed that full recovery in the rat's cardiac glycogen after asphyxial depletion occurred within an hour. In the experiments described above, the glycogen recovery would be favoured by the adrenaline hyperglycæmia, which occurs in rats as in other animals [Cori, 1931]. There is a certain amount of direct evidence that hyperglycæmia and possibly also excess of lactic acid in the blood may lead to a greater or more rapid deposition of cardiac glycogen [Cruickshank and Startup, 1933; Bogue *et al.*, 1935].

## Discussion.

My results show that in the absence of asphyxia, adrenaline in large doses causes a reduction in cardiac glycogen, but that this is followed by a rapid recovery. When asphyxia is combined with the increased adrenaline production that it provokes in the normal animal four-fifths of the cardiac glycogen are lost in four minutes, but asphyxia, when the suprarenal glands are excluded, produces a much smaller reduction.

My results in the intact animal agree with those obtained in the heart-lung preparation in that they show the dominant rôle played by adrenaline in regulating the heart glycogen content. My results also confirm the results of Evans as regards the remarkable speed with which cardiac glycogen can be removed or restored.

The estimations of total carbohydrate and glycogen show that the normal ventricle contains 50 mg./100 g. of reducing substances other than glycogen, and that this value is doubled when the glycogen is rapidly depleted either by adrenaline alone or by asphyxia and adrenaline. Apparently the whole of the glycogen broken down is not converted into lactic acid, but a certain fraction forms lower carbohydrates.

## Summary.

1. Asphyxia of rats rapidly reduces the glycogen content of the heart to one-fifth of its normal value, but only to three-fifths of its normal value when the influence of the adrenal glands is excluded.

2. Large doses of adrenaline, in the absence of asphyxia, reduce the cardiac glycogen to one-half after twenty-five minutes, but full recovery occurs in the subsequent half-hour.

3. Adrenaline therefore causes depletion of cardiac glycogen in both normal and in asphyxiated rats.

The author wishes to acknowledge his great indebtedness to Professor A. J. Clark and Dr. C. P. Stewart for their advice and criticism. The expenses of these experiments were defrayed by a grant from the Moray Fund, for which the author desires to express his thanks.

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## ASPHYXIAL ARREST OF THE ISOLATED RABBIT'S AURICLE.

By I. CHANG. From the Departments of Pharmacology and Physiology, University of Edinburgh.

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# ASPHYXIAL ARREST OF THE ISOLATED RABBIT'S AURICLE.

By I. Chang. From the Departments of Pharmacology and Physiology, University of Edinburgh.

(*Received for publication* 14*th May* 1937.)

The chief object of the experiments described below was to determine the cause of asphyxial arrest in the rabbit's heart. Clark, Gaddie, and Stewart [1932] concluded that in the normal frog's heart, asphyxial arrest occurred when a quantity of lactic acid accumulated sufficient to reduce the pH to a level at which glycolysis was inhibited. Gottdenker and Wachstein [1933] found, however, that asphyxia arrested the isolated rabbit's auricle before there was any sufficient accumulation of lactic acid, and pointed out that the conclusion of Clark, Gaddie, and Stewart could not apply to the rabbit's auricle. Weicker [1934] reported that asphyxial arrest of the isolated rabbit's ventricle caused complete loss of phosphagen. Clark and Eggleton [1936] found, however, that frog's ventricles, poisoned with iodoacetic acid, showed at the time of asphyxial arrest a loss of about half of their phosphagen, and concluded that the mechanical failure of the I.A.A. poisoned frog's ventricle could not be attributed simply to phosphagen depletion.

## Methods.

Rabbits of various weights and of both sexes were used. Since it has been shown that short periods of asphyxia causes extensive glycogenolysis [Evans, 1934; Chang, 1937] as well as breakdown of phosphagen [Clark, Eggleton, and Eggleton, 1932], all animals were anæsthetised with amytal or ether and put under artificial respiration. The chest was then opened and the heart rapidly excised either for chemical analysis or for isolation in the Locke's fluid. For the first purpose, the auricles or a portion of the ventricles were dropped directly into the various solutions as described. For the latter purpose, the auricles were suspended in a bath which contained 30 c.c. of Locke's solution through which oxygen was constantly bubbled. Preliminary experiments showed that isolated auricles maintained their activity best at a temperature of 27° C., all later experiments were therefore carried out at this temperature. Contractions of the auricles were recorded on smoked paper in the usual way. Asphyxia of the auricles

was produced by passing nitrogen through the fluid. Phosphagen and other phosphorus compounds were estimated by the method of Eggleton and Eggleton [1929] except that the colorimeter was replaced by a Pulfrich photometer, which permitted the accurate estimation of smaller amounts of phosphate. Glycogen was determined by the method described previously [Chang, 1937]. Locke's solution was made up of the following composition, NaCl 9·2 g., KCl 0·42 g., $CaCl_2$ 0·24 g. per litre of distilled water. $NaHCO_3$ was used as the buffering agent.

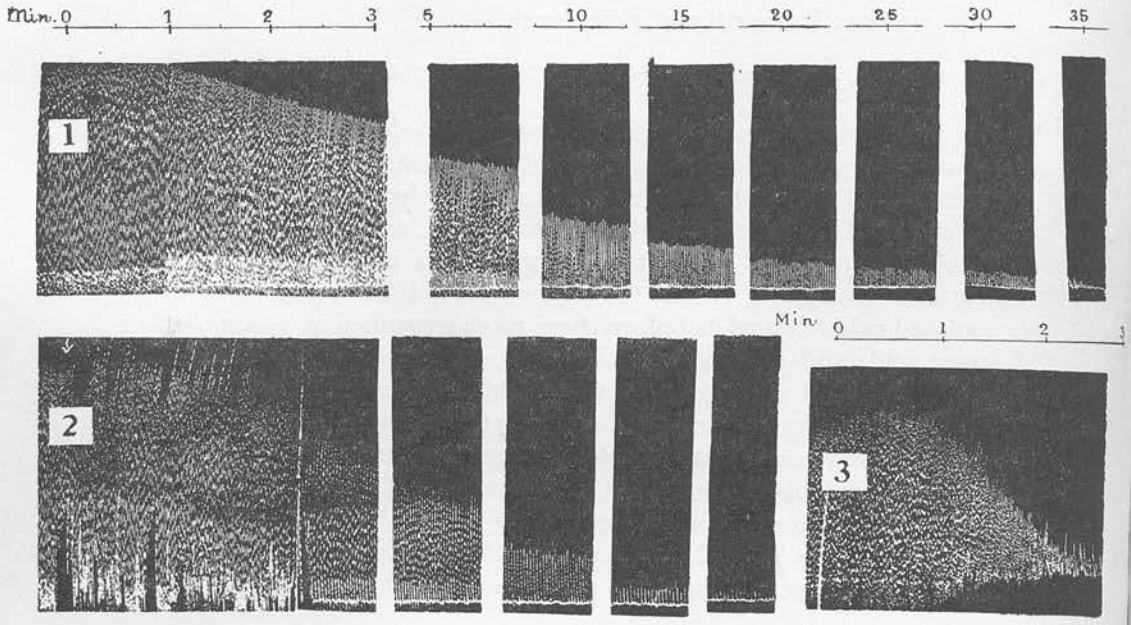


FIG. 1.—Effect of asphyxia on isolated rabbit's auricle in various Locke's solution. Nitrogen commenced at zero time. (1) Alkaline fluid (pH 8·2); (2) acid fluid (pH 6·8); (3) alkaline fluid (pH 8·2) plus 1 in 50,000 I.A.A.

## RESULTS.

(a) *The effect of pH and glucose on rate of asphyxial arrest.* The isolated rabbit's auricle when asphyxiated declines rapidly in activity and finally becomes arrested, but upon the admission of oxygen full recovery soon takes place. Although a marked individual variation exists in the time-response of isolated rabbit's auricles to asphyxia, yet the durations of a series of successive asphyxias of a single auricle are sufficiently uniform to yield comparative results. Consequently, the rates of asphyxia under different experimental conditions can be conveniently studied on one single auricle.

Using the method described, the influence of pH of the perfusion fluid and that of glucose upon the rates of asphyxia were investigated. These results are shown in fig. 1 and Table I. It will be seen that the

auricle is arrested more rapidly at pH 6·8 than at pH 8·2, and that the addition of glucose at pH 8·2 prolongs the survival period, but does not do so at pH 6·8. These results are in general agreement with those obtained by Gottdenker and Wachstein [1933] with a similar preparation. A comparison of the response to asphyxia of the rabbit's auricle with that of the frog's heart [Clark, Gaddie, and Stewart, 1934] shows the following points.

TABLE I.—EFFECTS OF pH OF PERFUSION FLUID AND OF ADDITION OF GLUCOSE UPON THE RATES OF ASPHYXIA (NUMBERS INDICATE TIME IN MINUTES FROM THE BEGINNING OF ASPHYXIA).

| Expt. No. | pH 6·8. | | | | pH 8·2. | | | |
|---|---|---|---|---|---|---|---|---|
| | Without glucose. | | With glucose. | | Without glucose. | | With glucose. | |
| | To half-activity. | Until arrest. | To half-activity. | Until arrest. | To half-activity. | Until arrest. | To half-activity. | Until arrest. |
| 21 | 4·0 | 7·5 | 4·0 | 8·0 | 4·5 | 9·0 | 6·0 | 19·0 |
| 22 | .. | .. | .. | .. | 3·0 | 14·0 | 3·5 | 25·0 |
| 23 | 4·5 | 18·5 | .. | .. | 8·0 | 36·0 | .. | .. |

Both preparations are rapidly depressed by asphyxia when in contact with neutral Ringer's or Locke's fluid with or without glucose, but when in contact with alkaline fluid their response to asphyxia is quite different. The frog's heart under such conditions can maintain an activity equal to about 80 per cent. normal without glucose for an hour and when glucose is added the survival may be much longer. The presence of alkali with or without glucose produces much less benefit on the rabbit's auricle.

In the case of the frog's heart it was shown that the anaerobic survival was dependent on the power of the tissue to obtain energy by glycolysis, and this was limited by two factors, namely, the supply of carbohydrate available, and the amount of buffer available to neutralise the lactic acid formed.

In the case of the isolated rabbit's auricles the tissue weighed about 0·2 to 0·5 g. and was suspended in 30 c.c. fluid, hence the amount of buffer present was large. It was in fact found that when asphyxial arrest occurred the fluid was still alkaline (about pH 7·6). The addition of fresh Locke's solution produced no effect upon the mechanical activity of the isolated rabbit's auricle during asphyxia, while similar treatment caused almost complete recovery of the asphyxiated frog's heart.

(b) *Asphyxial glycogen loss in normal auricles.* The above results suggest the possibility that asphyxial arrest is due to exhaustion of the cardiac glycogen.

Table II. shows analysis made to determine this point. Repeated asphyxia can reduce the glycogen content of the auricle from 500 to 100 mg./100 g., and hence at least four-fifths of the glycogen is available for glycolysis.

TABLE II.—EFFECT OF ASPHYXIA UPON THE GLYCOGEN CONTENT OF THE RABBIT'S HEART.

| Description. | No. of expts. | Average duration of perfusion in min. | | Glycogen. Mg./100 g. |
|---|---|---|---|---|
| | | Aerobic. | Anaerobic. | |
| Isolated auricles— | | | | |
| (1) Controls . . . | 19 | .. | .. | 512 |
| (2) Aerobic perfusion . . | 3 | 70 | .. | 446 |
| (3) Repeated asphyxiation until arrest— | | | | |
| (*a*) 3 times . . | 1 | *c.* 70 | *c.* 50 | 398 |
| (*b*) 6–8 times . . | 2 | *c.* 150 | *c.* 130 | 100 |
| (4) Asphyxiated once— | | | | |
| (*a*) To 1/10 activity . | 3 | 60 | 8 | 380 |
| (*b*) Until arrest . . | 2 | 40 | 20 | 288 |
| (5) Aerobic perfusion with adrenaline (1 in $10^7$) . | 2 | 70 | .. | 469 |
| (6) Anaerobic perfusion until arrest with adrenaline (1 in $10^7$) . . . | 2 | 50 | 20 | 219 |
| Heart *in situ*— | | | | |
| (1) Auricle— | | | | |
| (*a*) Controls . . | 19 | .. | .. | 512 |
| (*b*) Asphyxia until arrest | 2 | .. | .. | 133 |
| (2) Ventricle— | | | | |
| (*a*) Controls . . | 10 | .. | .. | 542 |
| (*b*) Asphyxia until arrest | 4 | .. | .. | 109 |

The results with a single asphyxiation shows, however, that the mechanical response may be reduced to 10 per cent. of the normal when only one-fifth of the total glycogen has been removed. This proves that asphyxial arrest is not due to simple depletion of the glycogen available for glycolysis.

The author has shown [1937] that asphyxia in presence of adrenaline causes a much more extensive glycolysis in the rat's heart than does asphyxia alone. The influence of adrenaline on asphyxial arrest was therefore determined. Addition of adrenaline under aerobic conditions

produced no reduction in the glycogen content (Table II., 5); moreover, asphyxia in the presence of adrenaline only caused a slight increase in the glycogen depletion (Table II., 6) as compared with the effects of similar degree of asphyxia without adrenaline (Table II., 4 (*b*)).

These results show, therefore, that asphyxial arrest occurs in auricles suspended in alkaline fluid long before complete glycogen depletion has occurred. Gottdenker and Wachstein [1933] found that under the conditions mentioned above about 240 mg./100 g. auricle of lactic acid were produced before asphyxial arrest. This result agrees with my estimations of glycogen loss.

The conclusion was confirmed by the experiments on hearts *in situ* (Table II.), which showed that the rabbit's heart resembled the rat's heart in that a few minutes asphyxia *in situ* caused the disappearance of at least four-fifths of the glycogen content.

If the rabbit's auricle were unable to excrete lactic acid, then the accumulation of this inside the auricle could be a possible cause of asphyxial arrest. Gottdenker and Wachstein [1933] showed, however, that after asphyxiation of the isolated rabbit's auricle there was little retention of lactic acid within the auricle, and most of that formed passed out into the surrounding fluid.

The results, therefore, show that neither lactic acid accumulation nor carbohydrate exhaustion can be the cause of asphyxial arrest.

(c) *Asphyxial arrest in auricles poisoned with iodo-acetic acid.* Clark, Eggleton, and Eggleton (1932) showed that the frog's heart when poisoned with I.A.A. could maintain a normal activity under aerobic conditions but was arrested in a few minutes by asphyxia.

I exposed rabbits' auricles to I.A.A. (1–50,000 and 1–30,000) for fifteen minutes, and then washed with clean Locke's fluid. This treatment produced no obvious change, except a slight depression of mechanical activity, as long as the auricle was supplied with oxygen, but asphyxia produced arrest in the poisoned auricle within 3 or 4 minutes (fig. 1). The arrest was equally rapid with or without glucose and at pH 8·2 or pH 6·8.

These results agree exactly with the results obtained with the frog's heart. I also found that asphyxia of the I.A.A. poisoned auricle first produced arrest, and this was followed by contracture. The effect was completely reversible if oxygen was introduced before the development of contracture. These effects are all similar to the effects obtained with the frog's heart.

Measurements of the glycogen content of I.A.A. poisoned auricles after asphyxia showed that this was reduced. For example, after three or four asphyxiations the glycogen content was 246 mg./100 g. heart (average of two experiments) as compared with a control value of 446 mg./100 g. The fate of the glycogen thus lost has not yet been determined, but in the case of frog's skeletal muscle it is known that

I.A.A. poisoning does not prevent the breakdown of glycogen to lower carbohydrates [Lundsgaard, 1930].

(d) *Effect of asphyxia on phosphagen content.* The total acid-soluble-P in the rabbit's auricle was found to be about 60 mg./100 g., auricle (moist weight). For estimations of phosphagen content after asphyxia it is necessary to destroy the tissue immediately on removal from the bath, hence accurate weighing is difficult, and therefore the phosphagen content has been estimated as per cent. of the total acid-soluble-P and as phosphagen index (phosphagen-P/phosphagen – P +inorganic – P). Weicker [1934] found in the rabbit's ventricle a phosphagen index of 0·16, but I found it to be 0·43. The probable reason for this difference is that Weicker perfused his hearts with saline solution, and it is improbable that the isolated heart receives an adequate oxygen supply under such conditions.

My results (Table III.) showed that isolation of the auricle in Locke's fluid under aerobic conditions caused no decrease in the phosphagen

TABLE III.—EFFECT OF ASPHYXIA ON THE PHOSPHAGEN CONTENT OF THE RABBIT'S HEART.

| Description. | No. of expts. | Phosphagen – P expressed as percentage of total acid – soluble – P. | Phosphagen index. |
|---|---|---|---|
| Auricles— | | | |
| (1) Controls . . . | 2 | 17·3 | 0·46 |
| (2) Perfusion . . . | 3 | 21·3 | 0·41 |
| (3) Anaerobic perfusion— | | | |
| (*a*) To 1/2 activity . | 1 | 17·2 | 0·34 |
| (*b*) To 1/3 activity . | 1 | 9·3 | 0·24 |
| (*c*) Until arrest . | 2 | 7·1 | 0·13 |
| (4) Recovery from asphyxia | 2 | 21·0 | 0·50 |
| (5) I.A.A. poisoning (1–30,000)— | | | |
| (*a*) Aerobic . . | 2 | 14·5 | 0·44 |
| (*b*) Anaerobic arrest (2–3 min.) . | 3 | 8·1 | 0·22 |
| Ventricles— | | | |
| (1) Controls . . . | 9 | 15·9 | 0·43 |
| (2) Asphyxia (5 min. *in situ*) . . . | 3 | 6·8 | 0·16 |

index. Asphyxiation of the ventricles *in situ* reduced the phosphagen index to 0·16 in 5 minutes, and asphyxiation until arrest of the isolated auricle produced a similar change both in the normal and in the I.A.A. poisoned tissue. When recovery was produced by oxygenation after asphyxiation the phosphagen index returned to normal. In all these respects the isolated rabbit's auricle agrees closely with the isolated heart of the frog.

The effect of asphyxia in presence of alkali (Table III., 3) is of particular interest, since it will be seen that the phosphagen index and the mechanical response fall together. This result is different from the effect of asphyxia on the frog's heart in presence of alkali, for in the

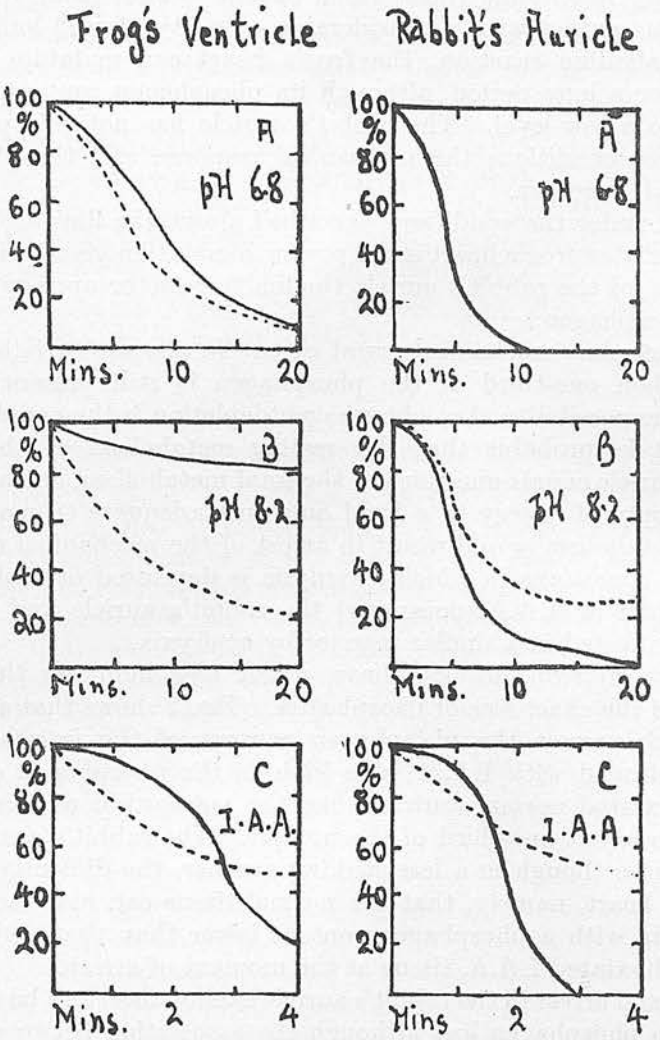


FIG. 2.—Ordinate: percentage of normal; abscissa: time after exposure to nitrogen. Continuous line, mechanical response; dotted line, phosphagen index expressed as percentage of the normal value. (A) Results with acid fluid; (B) results with alkaline fluid; (C) results with alkaline fluid plus I.A.A.

latter case asphyxia causes a rapid fall of phosphagen index but a much slower decrease in the mechanical response.

The changes in the mechanical activity and the phosphagen content of the frog's and of the rabbit's heart produced by asphyxia are depicted in fig. 2.

## Discussion.

Fig. 2 shows one outstanding difference between the effect of asphyxia on the frog's heart and the rabbit's auricle. Under the conditions most nearly resembling those which obtain *in situ*, namely, when the tissue is in contact with a considerable quantity of well-buffered fluid with an alkaline reaction, the frog's heart can maintain anaerobic activity for a long period, although its phosphagen content is rapidly reduced to a low level. The rabbit's auricle has not this power, and under these conditions the mechanical response and the phosphagen content fall together.

Hence under the conditions described above the limiting factor for the asphyxiated frog's heart is the power to maintain glycolysis, whereas in the case of the rabbit's auricle the limiting factor appears to be the loss of phosphagen.

The fact that mechanical arrest occurs in the asphyxiated rabbit's auricle when one-third of the phosphagen is still present does not exclude the possibility that phosphagen depletion is the cause of arrest, because it is probable that the resting metabolism of the isolated rabbit's auricle equals one-third of the total metabolism, and a reduction of the supply of energy to a level only just adequate to maintain the resting metabolism would result in arrest of the mechanical response.

Under conditions in which glycolysis is depressed or inhibited (*i.e.* acid medium or I.A.A. poisoning) the rabbit's auricle and the frog's heart are affected in a similar manner by asphyxia.

My experiments do not throw much new light on the difficult problem of the exact rôle of phosphagen. Fig. 2 shows that at the time of asphyxial arrest the phosphagen content of the isolated rabbit's auricle poisoned with I.A.A. is as high as the phosphagen content of the asphyxiated normal auricle when the mechanical response is only reduced to about one-third of the normal. The rabbit's auricle therefore presents, though in a less striking manner, the difficulty shown in the frog's heart, namely, that the normal tissue can maintain activity in asphyxia with a phosphagen content lower than that which occurs in the asphyxiated I.A.A. tissue at the moment of arrest.

Asphyxial arrest in the rabbit's auricle cannot therefore be attributed directly to phosphagen loss although the association between decrease in phosphagen content and in mechanical activity is closer in the rabbit's auricle than in the frog's heart.

## Summary.

1. Under asphyxial conditions, normal mechanical activity of the rabbit's auricle is not maintained for longer than a few minutes even when the conditions are most favourable for glycolysis, the mechanical activity and the phosphagen index seem to fall together, it is therefore

suggested that phosphagen breakdown rather than glycolysis energises the contraction of the rabbit's auricle.

2. At asphyxial arrest the glycogen content of rabbit's auricle is reduced to two-thirds and the phosphagen content to one-third of their normal value.

3. Poisoning with I.A.A. produces changes in the response of the rabbit's auricle to asphyxia essentially similar to those observed with the frog's heart.

The writer gratefully acknowledges his indebtedness to Professor A. J. Clark for his suggestions and advice throughout the experiments. Acknowledgment is also due to Dr. P. Eggleton and Dr. A. Deutsch for their kind help in connection with the chemical techniques employed.

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## THE METABOLISM OF THE ISOLATED MAMMALIAN HEART UNDER PARTIAL ANOXIA.

By J. YULE BOGUE, I. CHANG, and R. A. GREGORY. From the Department of Physiology, Pharmacology, and Biochemistry, University College, London.

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# THE METABOLISM OF THE ISOLATED MAMMALIAN HEART UNDER PARTIAL ANOXIA.

By J. Yule Bogue, I. Chang, and R. A. Gregory.[1] From the Department of Physiology, Pharmacology, and Biochemistry, University College, London.

(*Received for publication 5th November* 1937.)

Previous work from this laboratory has dealt with the aerobic metabolism of the isolated dog's heart, and certain quantitative and perhaps qualitative differences from skeletal muscle have been pointed out [Evans *et al.*, 1933, 1934, 1935; Bogue *et al.*, 1935]. In the main the results are in agreement with those of Clark and collaborators who obtained similar evidence concerning the carbohydrate mechanisms in the frog's heart, although measurements of the actual usages of glucose or lactate were not feasible with such a frog-heart preparation.

The study of anaerobic conditions with and without iodoacetate has proved so valuable in the investigation of the metabolism of striated muscle, and of the frog's heart by Clark *et al.* and Gaddie and Stewart, that their extension to include the mammalian heart should prove of interest. For brevity, iodoacetate will be referred to as I.A.A.

## Methods.

These experiments were made with the heart oxygenator preparation as described by Evans, Grande, and Hsu [1934], using the same apparatus and procedure as described in earlier papers from this laboratory. In each experiment, frequent observations were made of the right and left ventricular outputs and coronary flow, the temperature and heart rate. The aortic, pulmonary, and both auricular pressures were recorded continuously. Samples of reservoir blood were taken at intervals for the estimation of glucose and lactate; calculation of the hearts' usage being made from these figures after making allowance for blood glycolysis in the same manner as described by Evans, Grande, and Hsu [1935]. Blood glycolysis was estimated by analysis of part of the reservoir sample, which was placed in a rotating saturator immersed in a bath at 38° C. until the next sample was taken. Where addition of lactate, glucose, or iodoacetate was made to the blood,

[1] Sharpey Scholar.

an interval of not less than ten minutes was allowed for distribution throughout the system to occur before withdrawing a portion of this blood for control. Such controls were kept in equilibrium with the same gas mixture as was being passed through the oxygenator during the period they controlled. The gas mixtures were either 5 per cent. carbon dioxide in oxygen or 5 per cent. carbon dioxide in nitrogen.

The chemical methods were similar to those previously used. Blood sugar was estimated by means of the Shaffer-Hartmann reagent after removal of the protein with copper sulphate/tungstate reagent. Blood lactate was estimated by a modification of the Friedmann-Cotonio-Shaffer method. Glycogen determinations were made on the whole ventricular portion of the heart as described by Evans *et al.* [1935]. Quadruplicate estimations upon the original extract were made. Blood oxygen determinations were made using the manometric van Slyke apparatus.

*State of the Heart.*—During the course of a four- to six-hour experiment with the heart-oxygenator preparation, the condition of the heart slowly declines. A reliable indication of the extent of this decline is given by the height of the venous pressure at any time. During the first hour or so after completion of the preparation, the venous pressure remains sensibly constant and near to zero. This initial level is arbitrarily called +1 cm. of water. After an hour or so it usually rises slowly; the arterial pressure, being dependent on an unchanging resistance, shows very little change throughout the experiment, until signs of impending failure occur, with a gradual diminution of cardiac output, dilatation of the heart, and a sudden mounting of the venous pressure to a very high level. The ultimately inevitable occurrence and the magnitude of such changes in the condition of the heart in this preparation must be borne in mind when considering the effects upon the heart of any experimental variables. Graphs are shown of the course of the venous pressure in typical experiments from each series (figs. 1 and 4).

During all the experiments, the venous reservoir level, throttling of the inlet, and other mechanical conditions which could alter the rate of inflow of blood into the heart were unchanged, and the arterial resistance and temperature kept constant. Any subsequent alteration in inflow or output of the heart or in arterial pressure would therefore be an expression of alteration in the condition of the muscle, due, for example, to the deterioration discussed above, and which seems to result chiefly from a reduction in the power of systole, to a lesser extent from a reduction of aortic output by increased short-circuiting through the coronary circulation (which can always be checked by measuring the latter), or, in addition to these, to the effect of oxygen lack and/or I.A.A. on the heart. Such changes were always reflected in the venous pressure recorded in the inferior vena cava; thus reduced output of

the ventricles was always accompanied by a rise of venous pressure, and sometimes, though by a much less evident, fall of arterial pressure.

Although the usages of glucose and lactate have been shown to have a prime importance in relation to the condition of the heart and to the work it performs, yet certain factors unfortunately complicate the assessment of the effects produced upon their usage by such conditions as I.A.A. or anoxæmia. One of these is that, other conditions being the same, the usage of lactate or of glucose varies with its concentration in the blood, as has been shown by Evans, Grande, and Hsu [1935]. Further, these authors have shown that increase in the concentration of blood lactate, while increasing the usage of lactate, decreases that of glucose, although increase in the level of glucose in the blood while increasing the glucose usage, had but little effect upon the lactate consumption. Again, although the usage of lactate and of glucose may be expected to vary with the work performed by the heart, the heart may not be solely dependent upon the blood lactate or glucose for its sources of energy if, as perhaps often happens, it is able to draw upon its glycogen reserves and perhaps also upon non-carbohydrate substances of an unspecified nature [Evans, Grande, and Hsu, 1935]. As will be seen in our experiments, the usages of fuel by the heart depend closely upon other conditions than that of the rate of work; for example, the usage/unit work ratio rises markedly after addition of I.A.A., with anoxia, but not during anoxia alone. In arriving at our conclusions, allowances have had to be made as far as possible for such factors as these on the basis of previous and current work in this laboratory.

## Experiments.

These fall into two main groups:

(*a*) Anoxæmia with $N_2/CO_2$ mixture. Such anoxæmia is necessarily gradual in character, its rate of onset depending upon such factors as the speed with which the oxygen in the saturator ("oxygenator") is replaced by $N_2/CO_2$ mixture, the rate at which the heart is taking oxygen from the blood, the total blood volume, and the area of the blood exposed to the nitrogen mixture. The output of the right ventricle was between 300 and 500 c.c./min., and the surface area of the oxygenator 5000 sq. cm. Anoxæmia was produced in the following manner. Fifty or sixty litres of the gas mixture (5 per cent. $CO_2$ in $N_2$) were washed into the oxygenator (capacity 34 litres) in two minutes, and the rate of flow of gas then adjusted to about 1 litre per minute. Analysis of the gas mixture showed that this procedure was adequate to replace the oxygen.

In four of the experiments asphyxia was produced simply by short-circuiting the oxygenator; in these, a surface of 350 sq. cm. of

blood remained exposed to the air, so that the deoxygenation produced in this manner was much slower than when using the nitrogen mixture in the oxygenator as outlined above.

(*b*) I.A.A. added to the blood followed by anoxia with $N_2/CO_2$ mixture, the pre-anoxial period with I.A.A. alone varying between 43 and 126 minutes.

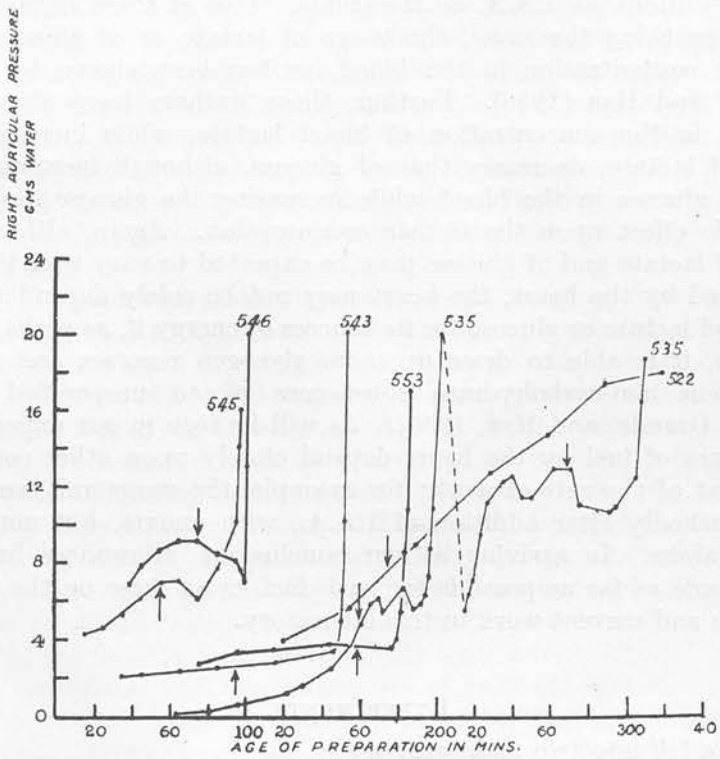


FIG. 1.—Venous pressure curves plotted from continuous records taken during plain anoxial experiments. Arrows indicate the beginning of anoxial period with $N_2/CO_2$ mixtures, with the exception of expts. 522, 546, and 553, in which the oxygenator was short-circuited.

In both types of experiment one or more normal or control periods of at least 30 minutes each preceded the anoxic or I.A.A. periods.

The resistance to anoxic conditions was deduced from the length of survival observed.

In the anoxæmic periods, the heart survived from 32 to 106 minutes when $N_2/CO_2$ alone was used; from 29 to 212 minutes when the oxygenator was short-circuited, and from 14 to 58 minutes when I.A.A. and $N_2/CO_2$ were used together. Frequent extra systoles were often seen during the latter half of the anoxic period. In either series, the rate of rise of the venous pressure was, until shortly before failure, not much greater than that during normal experiments (figs. 1 and 4).

The onset of failure was usually indicated by a sudden rise of venous pressure ultimately accompanied by a sharp decline in the arterial pressure.

When such failure had occurred from oxygen lack without I.A.A. and the heart had been arrested, it could still be recovered by prompt and abundant reventilation of the oxygenator with the $O_2/CO_2$ mixture and careful hand massage. This was done in one or two cases; a second anaerobic period was obtained in expt. 535 after recovery by this means from the first period of anoxia (fig. 1, Tables I. and II.).

Reference to fig. 1 shows that in spite of the above differences in the rates of deoxygenation of the blood in the two types of oxygen deprivation, the rise of venous pressure is just as sudden in either case; in fact, the short-circuited oxygenator experiments usually show a venous pressure which remains at a low level until a few minutes before failure (2 min. in expt. 553), whereas the $N_2/CO_2$ experiments commonly show a moderate rise some time before the end. In all the experiments with I.A.A., anoxia was produced by the use of $N_2/CO_2$ mixture; the venous pressure curve did not differ significantly from those experiments without I.A.A. (fig. 4).

## Results.

In imposing upon the heart such a condition as gradually increasing oxygen deficiency, with or without previous addition of I.A.A., an observation of primary importance is that of the *time of survival* of the heart. This may be expected to vary as the resultant of a number of factors, prominent among which would be the reserves available for survival, and the rate of usage of these reserves. Several of these factors can be assessed or controlled, as for instance:

(1) The sources of energy for the work. Glucose and lactate levels have been determined, and the amount of heart glycogen at failure estimated. Non-carbohydrate sources cannot be assessed at present and remain an unknown and perhaps considerable quantity. It is always possible that the reserves may be different in oxygen lack and still again in I.A.A. poisoning.

(2) The rate of disappearance of oxygen. This was gradually diminishing during the anoxæmic period. The presence of oxygen must as a first approximation be accounted a factor of reserve. Besides affecting the heart's immediate sources of energy, oxygen lack might also produce damage of an irreversible nature to the cells, so that the entry of some toxic factor was facilitated.

(3) The presence of a toxic substance. For example, a drug essentially toxic in its action may be debarred from exerting its effects under certain conditions among which, for all we know, the presence of oxygen may be one. Or again, toxic intermediaries might arise

TABLE I.—EFFECT OF GRADUALLY INCREASING ANOXIA ON THE CARBOHYDRATE METABOLISM AND CONDITION OF THE HEART.

| Expt. | Time, mins. | Concentration in blood, mg./100 c.c. start/finish. | | Usage, mg./100 g. heart/hr. | | Sum usage, lactate and glucose, mg./100 g./hr. | Kg.-m. work per hr./100 g. heart. | Usage, mg./kg.-m./hr./100 g. heart. | | Sum usage, lactate and glucose, mg./kg.-m./hr./100 g. | Survival time from start of anoxial period, min. | Outputs, c.c./min. | | | Aortic pressure, mm. Hg. | Total blood volume, c.c. | Heart muscle glycogen, per cent. | Conditions. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lactic acid. | Glucose. | Lactic acid. | Glucose. | | | Lactic acid. | Glucose. | | | L.V. excluding coronary. | R.V. | Coronary. | | | | |
| 522 | 0–45 | 75/64 | 100/90 | 92 | 138 | 230 | 20·9 | 4·40 | 6·60 | 11·00 | | 198 | 332 | 143 | 87 | 1125 | | Normal. |
| | 45–90 | 64/52 | 90/86 | 200 | 0 | 200 | 17·0 | 11·80 | .. | 11·80 | | 158 | 300 | 110 | 87 | 1080 | | Oxygenator short-circuited. |
| | 90–135 | 52/44 | 86/73 | 236 | 32 | 268 | 14·0 | 16·90 | 2·30 | 19·20 | | 67 | 268 | 137 | 70 | 1045 | | " " " |
| | 135–165 | 44/44 | 73/60 | 93 | 124 | 217 | 10·8 | 8·60 | 11·60 | 20·20 | | 50 | 230 | 117 | 64 | 1010 | | " " " |
| | 165–175 | 44/38 | 60/58 | 120 | 0 | 120 | 8·1 | 14·80 | .. | 14·80 | 145 | 59 | 172 | 65 | 75 | 975 | .. | " " " |
| 526 | 0–43 | 21/12 | 140/128 | 103 | 154 | 257 | 18·0 | 5·70 | 8·60 | 14·30 | | 211 | 273 | 195 | 64 | 920 | | Normal. |
| | 70–105 | 20/14 | 128/118 | 112 | 75 | 197 | 16·0 | 7·00 | 4·70 | 11·70 | | 176 | 349 | 218 | 60 | 820 | | Normal. 0·25 g. of glucose and lactate added. |
| | 105–145 | 14/17 | 118/92 | 21 | 170 | 191 | 13·1 | 1·60 | 13·00 | 14·60 | | 154 | 306 | 203 | 57 | 780 | | $N_2/CO_2$ mixture on at 115 min. |
| | 145–170 | 17/17 | 92/70 | .. | 69 | .. | .. | .. | .. | .. | 55 | 109 | 273 | 195 | 53 | 690 | 0·43 | $N_2/CO_2$ failed. |
| 534 | 0–41 | 89/68 | 106/97 | 327 | ? | 327 | 13·8 | 23·70 | .. | .. | | 107 | 396 | 250 | .. | 900 | | Normal. |
| | 41–71 | 68/56 | 97/82 | 141 | 164 | 305 | 11·9 | 11·90 | 13·80 | 25·70 | | 117 | .. | 246 | 60 | 850 | | " |
| | 71–99 | 56/46 | 82/66 | 138 | 207 | 345 | 8·7 | 15·90 | 23·90 | 39·80 | | 75 | .. | 200 | 60 | 780 | | $N_2/CO_2$ on at 76 min. |
| | 99–116 | 46/53 | 66/71 | −35* | −69* | −104* | 6·5 | .. | .. | .. | 40 | 60 | .. | 162 | 45 | 710 | 0·33 | $N_2/CO_2$ failed. |
| 535 | 0–52 | 93/72 | 151/139 | 192 | 73 | 265 | 21·4 | 9·00 | 3·40 | 12·40 | | 340 | 555 | 258 | 56 | 1154 | | Normal. |
| | 52–111 | 72/49 | 139/124 | 170 | 68 | 238 | 19·3 | 8·80 | 3·50 | 12·30 | | 260 | .. | 326 | 58 | 1105 | | " |
| | 111–136 | 49/34 | 124/112 | 214 | 92 | 306 | 16·9 | 12·70 | 5·50 | 18·20 | | 204 | .. | 319 | 58 | 1055 | | $N_2/CO_2$ on at 121 min. |
| | 136–161 | 34/34 | 112/104 | 113 | 113 | 226 | .. | .. | .. | .. | 41 | 172 | .. | 273 | 58 | 975 | | $N_2/CO_2$ failed. |
| | 161–206 | 34/37 | 104/79 | 15 | 89 | 104 | 11·9 | .. | .. | .. | | .. | .. | .. | 54 | 920 | | Recovery. $O_2/CO_2$. |
| | 206–229 | 37/31 | 79/72 | 109 | 82 | 191 | 11·9 | 9·20 | 6·90 | 16·10 | | 166 | .. | 182 | 58 | 863 | | " " |
| | 229–257 | 31/25 | 72/61 | 153 | 55 | 208 | 12·3 | 12·30 | 4·40 | 16·70 | | 158 | .. | 182 | 60 | 843 | | $N_2/CO_2$ mixture at 230 min. |
| | 257–276 | 25/25 | 61/49 | 0 | 137 | 137 | .. | .. | .. | .. | 51 | 200 | .. | 187 | 58 | 793 | 0·35 | $N_2/CO_2$ mixture failed at 281 min. |
| 542 | 0–63 | 212/167 | 185/157 | 396 | 127 | 524 | 26·0 | 10·30 | 4·90 | 15·20 | | 230 | 334 | 250 | 68 | 1000 | | Normal. |
| | 63–111 | 167/143 | 157/137 | 400 | 86 | 486 | 21·9 | 18·30 | 3·90 | 22·20 | | 176 | .. | 273 | 66 | 970 | | " |
| | 111–145 | 143/125 | 137/123 | 390 | 104 | 494 | 19·6 | 19·90 | 5·30 | 25·20 | | 154 | .. | 273 | 61 | 932 | | $N_2/CO_2$ mixture on at 113 min. |
| | 145–186 | 125/117 | 123/101 | 236 | 113 | 349 | 16·1 | 14·70 | 7·00 | 21·70 | | 150 | .. | 240 | 58 | 892 | | " " " |
| | 186–219 | 117/125 | 101/88 | 0 | 71 | 71 | 7·6 | 0 | 9·40 | 9·40 | 106 | 125 | .. | 172 | 53 | 831 | 0·49 | $N_2/CO_2$ mixture failed. " |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 543 | 0–60 | 138/99 | 166/151 | 397 | 28 | 425 | 14·8 | 26·80 | 1·90 | 28·70 | | 294 | 268 | 112 | 63 | 940 | | Normal. |
| | 60–100 | 99/74 | 151/128 | 326 | 20 | 346 | 13·4 | 24·30 | 1·50 | 25·80 | | 204 | .. | 164 | 54 | 885 | | $N_2/CO_2$ mixture on at 62 min. |
| | 100–123 | 74/71 | 128/117 | 147 | 49 | 196 | .. | .. | .. | .. | 66 | 120 | .. | 224 | 46 | 845 | 0·85 | $N_2/CO_2$ failed at 128 min. |
| 544 | 0–64 | 160/126 | 111/95 | 369 | 51 | 420 | 16·8 | 21·90 | 3·40 | 25·30 | | 226 | 300 | 129 | 54 | 1060 | | Normal. |
| | 64–97 | 126/112 | 95/85 | 314 | 55 | 369 | 15·4 | 15·40 | 2·60 | 18·00 | | 146 | 334 | 176 | 45 | 970 | | $N_2/CO_2$ mixture on at 67 min. |
| | 97–112 | 112/101 | 85/77 | 427 | 232 | 659 | 13·5 | 31·80 | 17·30 | 49·10 | | 131 | 326 | 167 | 46 | 940 | | |
| | 112–141 | 101/95 | 77/66 | 337 | 194 | 531 | 8·2 | 40·80 | 11·40 | 52·20 | 74 | 125 | 250 | 120 | 44 | 880 | 0·69 | $N_2/CO_2$ mixture about to fail. |
| 545 | 0–46 | 244/215 | 119/100 | 282 | 87 | 369 | 19·1 | 14·80 | 4·60 | 19·40 | | 300 | 340 | 140 | 68 | 815 | | Normal. |
| | 46–77 | 215/202 | 100/85 | 231 | 70 | 301 | 17·5 | 13·20 | 4·00 | 17·20 | | 137 | 417 | 230 | 60 | 765 | | $N_2/CO_2$ mixture on at 47 min. |
| | 77–111 | 202/186 | 85/64 | 282 | 124 | 406 | 15·4 | 18·30 | 8·10 | 26·40 | 68 | 84 | 500 | 272 | 56 | 735 | 0·56 | $N_2/CO_2$ mixture about to fail at 115 min. |
| 546 | 0–45 | 296/264 | 59/45 | 260 | 58 | 318 | 33·8 | 7·70 | 1·70 | 9·40 | | 357 | .. | 218 | 76 | 560 | | Normal. |
| | 45–75 | 264/272 | 45/32 | 254 | 46 | 300 | 30·0 | 8·50 | 1·90 | 10·40 | 29 | 268 | .. | 250 | 74 | 525 | 0·45 | Oxygenator short-circuited, failed suddenly. |
| 547 | 0–46 | 308/236 | 0/0 | 504 | 0 | 504 | 24·2 | 20·80 | 0 | 20·80 | | 227 | 428 | 200 | 74 | 930 | | Normal. Blood obtained aseptically day before, kept overnight to remove sugar. |
| | 46–81 | 236/213 | 0/0 | 354 | 0 | 354 | 23·0 | 15·40 | 0 | 15·40 | | 192 | 385 | 231 | 70 | 900 | | Normal. |
| | 100–126 | 260/225 | 0/0 | 515 | 0 | 515 | 21·0 | 24·40 | 0 | 24·40 | | 150 | 428 | 263 | 70 | 860 | | $N_2/CO_2$ mixture on at 101 min. |
| | 126–133 | 225/219 | 0/0 | 320 | 0 | 320 | .. | .. | .. | .. | 32 | 148 | 385 | 246 | 68 | 835 | 0·57 | $N_2/CO_2$ mixture. Sudden failure. |
| 553 | 0–46 | 240/228 | 62/40 | 216 | 67 | 283 | 22·0 | 9·83 | 3·04 | 12·87 | | 88 | 417 | 356 | 74 | 990 | | Normal. Aseptic bleeder as in Expt. 547. |
| | 46–79 | 228/187 | 40/28 | 423 | 22 | 445 | 20·9 | 20·20 | 1·05 | 21·25 | | 106 | 406 | 320 | 74 | 950 | | Normal. |
| | 79–101 | 187/171 | 28/24 | 243 | 0 | 243 | 20·7 | 11·70 | 0 | 11·70 | | 63 | 400 | 366 | 72 | 920 | | Oxygenator short-circuited. |
| | 101–113 | 171/167 | 24/24 | 116 | 0 | 116 | .. | .. | .. | .. | 33 | .. | .. | .. | .. | 900 | 0·55 | Failure. |
| 554 | 0–30 | 80/32 | 0/0 | 424 | 0 | 424 | 23·2 | 18·30 | 0 | 18·30 | | 157 | 750 | 600 | 82 | 1080 | | Normal. Aseptic bleeder as in Expt. 547. |
| | 30–50 | 32/14 | 0/0 | .. | .. | .. | 20·1 | .. | .. | .. | | 131 | 880 | 680 | 76 | 1062 | | Normal. |
| | 50–78 | 14/15 | 0/0 | .. | .. | .. | 16·3 | .. | | .. | | 131 | 750 | 625 | 70 | 1042 | | $N_2/CO_2$ mixture on at 52 min. |
| | 78–87 | 15/15 | 0/0 | .. | .. | .. | .. | .. | .. | .. | 35 | 100 | 600 | 485 | 62 | 1020 | 0·10 | $N_2/CO_2$ mixture failed. |
| | | | | | | | | | .. | | | | | | | | | |

[a] Expt. 534. Production of lactic acid and reducing substance.

or become potent under such conditions, and such an occurrence has in fact been mooted by Gaddie and Stewart (1934).

(4) The rate of consumption of the reserves, *i.e.* the cardiac metabolism as conditioned by oxygen. This would be expected to depend on the rate of beat (which varied but little), temperature (constant) and on work done. The maximal limit of work which the heart may perform is indicated by the mechanical conditions of inflow and of arterial resistance. These were not altered during the course of an experiment (though they were not the same for each experiment), and it follows from this that variations in the work actually performed during an experiment will depend upon:

(5) The condition of the muscle, *i.e.* the efficiency with which it performs its work. Some indication of this efficiency is given by the venous (auricular) pressure. Another indication is given by the usage/work ratio. An efficient ventricle will work at a smaller diastolic volume, and lower filling pressure and use relatively less of its reserves, including oxygen, than will a ventricle of lower efficiency doing work at the same rate.

### *Anoxia without I.A.A.*

*Survival Time.*—In all of these experiments, the time of survival of the heart was found to be proportional to the level of the blood sugar

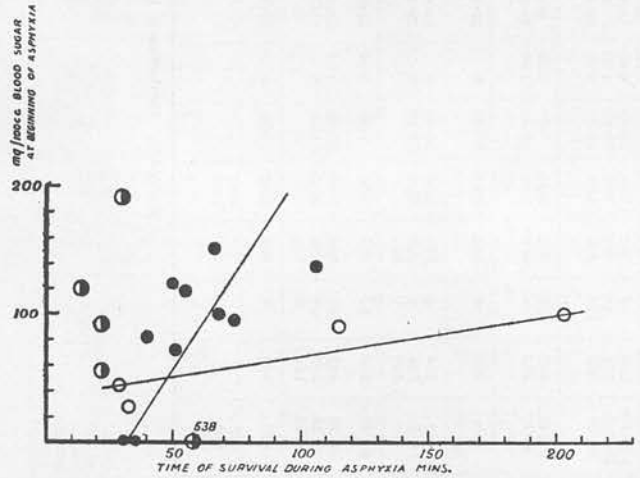


FIG. 2.—The effect of blood-sugar concentration at the beginning of the anoxial period on the survival time of the heart. Filled-in circles—$N_2/CO_2$ anoxia. Open circles—oxygenator short-circuited. Half-filled circles—I.A.A. and $N_2/CO_2$ anoxia. Expt. 538, no sugar in blood.

at the beginning of anoxia, higher initial sugar concentrations tending to prolong the survival time (fig. 2). It should be noted also that there is no direct relation between the age of the preparation and the

resistance to anoxæmia (Table II.). Attention is drawn to this because of the tendency, when glucose is not added, for the blood of older preparations to contain less glucose than that of fresh preparations, and for that reason to resist anoxæmia less well.

TABLE II.—ANOXIA EXPERIMENTS: RELATION BETWEEN BLOOD SUGAR AND LACTATE LEVELS TO TIME OF SURVIVAL.

| Exp. | Concentrations in blood at start of asphyxia, mg./100 c.c. | | Age of preparation at which oxygen lack began, in min. | Time of survival, in min. | Heart glycogen, per cent. |
|---|---|---|---|---|---|
| | Glucose. | Lactate. | | | |
| 547 | 0 | 260 | 100 | 32 | 0·57 |
| 554 | 0 | 14 | 50 | 35 | 0·10 |
| 534 | 82 | 56 | 71 | 40 | 0·33 |
| 535 *a* | 124 | 49 | 111 | 41 | .. |
| 535 *b* | 72 | 31 | 229 | 51 | 0·35* |
| 526 | 118 | 14 | 105 | 55 | 0·43 |
| 543 | 151 | 99 | 60 | 66 | 0·85 |
| 545 | 100 | 215 | 46 | 68 | 0·56 |
| 544 | 95 | 126 | 64 | 74 | 0·69 |
| 542 | 137 | 143 | 111 | 106 | 0·49 |

* Glycogen value taken after second anoxial period, heart revived after first period of anoxia.

No direct relationship between the blood lactate level and the survival time can be found, nor does high lactate appear to inhibit the effect of sugar in delaying failure, as might have been possible from the evidence concerning the inhibiting effect on sugar usage of increased lactate [Evans, Grande, and Hsu, 1935].

For example, in expts. 544 and 545 the survival times are similar, and the sugar levels are almost the same, but the lactate levels were 126 and 215 mg./100 c.c. respectively (Table II.). In the four experiments in which the oxygenator was short-circuited (see fig. 2, not shown in Table II.), a similar relation of the blood-sugar level to the time of survival is apparent, but since the rate of anoxia is more gradual the times are all much longer in proportion to the blood-sugar levels as compared with the $N_2/CO_2$ experiments. There is insufficient data to discuss the lactate relations in these experiments.

*Heart Glycogen.*—The glycogen of the heart is potentially a source of energy, particularly in the anoxial period, and by analogy, with skeletal muscle it might be expected to be readily depleted in such conditions.

It was found, however, that anoxial arrest frequently occurred when

the glycogen content of the heart was still high. When both sugar and lactate were low glycogen depletion occurred under anoxia (*e.g.* expt. 554), but the presence of either lactate or glucose tended to maintain the glycogen content of the heart.

This fact proves that the cardiac glycogen is not so easily accessible a source of energy under anaerobic conditions as is the glycogen of skeletal muscle. This is possibly due to the difference in rate of enzyme breakdown of glycogen in the two tissues.

The factors determining anaerobic glycogen usage were found to be complex. When both the sugar and the lactate were low, considerable glycogen loss occurred before anaerobic arrest (*e.g.* expt. 554, cardiac glycogen 0·1 g./100 g.). Less depletion occurred when either sugar or lactate was present, but, curiously enough, lactate apparently had a greater glycogen sparing action than sugar. For instance, in expt. 526 the sugar was normal and the lactate low and the cardiac glycogen was 0·43 g./100 g., whereas in expt. 547 sugar was absent, but the lactate high and the cardiac glycogen was nearly normal 0·57 g./100 g.

It has already been shown that the presence of sugar prolongs the period of anoxial activity before arrest occurs.

It would seem, nevertheless, that during this more prolonged period of survival glycogen is being used, since there is partial exhaustion of it at the finish, *cf.* experiments in Table I.

Lactate does not prolong anoxial survival, and presumably its glycogen sparing action depends upon its providing a source of energy during the period when the oxygen tension is falling but is still at a level at which some lactate utilisation can occur.

Apart from this experiment, the lowest glycogen of the series was 0·33 g./100 g. where both the sugar and the lactate were only present in moderate concentration. The highest glycogen was 0·86 g./100 g. in expt. 543, in which high sugar and fairly high lactate were present. Thus it appears that the survival time tends to be prolonged in proportion to the concentration of glucose present at the beginning of the anoxial period, and that this effect is not inhibited by the presence of lactate. This latter, however, determines in part the rapidity and ultimate degree of glycogen depletion. With sugar at normal levels, but very low lactate (expt. 526), a moderate depletion of glycogen will occur. Fig. 3 shows the sparing effect of the glucose concentration in the blood on the heart glycogen at failure.

It is not possible with the evidence at our disposal to dissociate the glycogen sparing effects of glucose and of lactate as completely as we could wish. But other things being equal it would be expected that the longer a preparation survived under conditions of progressive anoxæmia while using glycogen in the process, the more its glycogen would be drawn upon. The fact that the glycogen contents of the

hearts were still high after prolonged oxygen lack, provided that glucose was present in high concentration at the beginning of anoxia, but might be low after even short periods of anoxia when the lactate was low, does seem to indicate that lactate as well as glucose may play a part, but not necessarily the same part, in sparing glycogen.

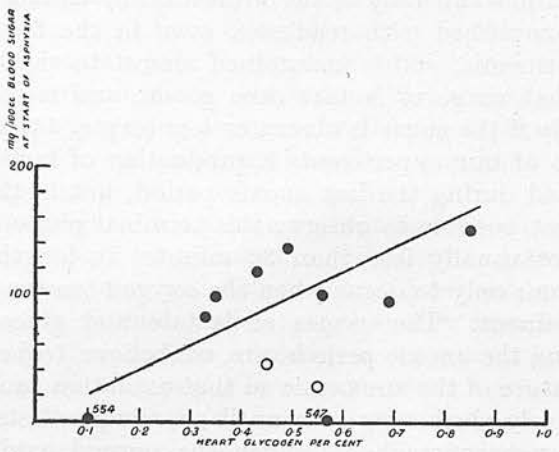


FIG. 3.—The effect of blood-sugar concentration at the beginning of anoxia on the glycogen content of the heart at failure. Filled circles—$N_2/CO_2$ experiments. Open circles—oxygenator short-circuited. High blood lactate 260 mg./100 c.c. blood in expt. 547 and low blood lactate 14 mg./100 c.c. blood in expt. 554 at beginning of anoxial period.

*Sugar and Lactate Usages.*—Previously unpublished observations by Mulder, Lee, Grande, and Hsu upon the effect of partial oxygen lack produced by ventilation with $N_2/CO_2$, and also of cyanide anoxia, indicated that in the former condition the usage of sugar was increased and that of lactate diminished. (This, however, was not invariably the case, sometimes the effect of partial oxygen lack was to increase the usage of both lactate and sugar or, more rarely, to decrease the usage of both) [Evans in *Recent Advances in Physiology*, 1936, p. 76]. In their experiments with cyanide, the usage of lactate was abolished altogether, and a large production of lactate occurred instead (240 mg./hr. in one case), Evans *et al.* [1933]. The similarity of these results, using cyanide to those obtained in the frog's heart by oxygen deprivation, is probably because only by the use of such agents as cyanide is it possible in the blood-fed heart-oxygenator preparation to obtain as rapid and complete a state of anoxia as can be obtained in the frog's heart simply by the removal of the oxygen supply.

The sensitivity of the frog's heart to acidity which Clark and co-workers [1934] found is not seen in our experiments with the blood-fed heart, since the blood is so well buffered, and also because any natural or added lactic acid is present in the blood as the sodium salt, and after usage of the lactic acid by the heart, there is a moderate alkalosis

providing excess of base available for combination with any lactic acid which may be produced in the terminal stages of anoxæmia.

Our results with regard to the usages are in agreement with the above. In the majority of the anoxic periods, the usage of lactate has been found ultimately to decrease, while that of sugar increases. However, so rapid and easy is the utilisation of lactate by the heart that it is accomplished with readiness, even in the face of a rapidly developing anoxæmia, and is maintained almost to the last. We find accordingly that usage of lactate does go on, and is sometimes very large, especially if the sugar is absent or low (expts. 544, 547, Table I.). In one or two of our experiments a production of lactic acid by the heart was noted during the last anoxic period, but in the majority of cases it was not possible to observe this terminal phase, since the last period was not usually less than 30 minutes in length, and lactate production seems only to occur when the oxygen tension is so low that failure is imminent. The usages of lactate and glucose, which we observed during the anoxic periods, are, we believe, to be explained by the gradual nature of the anoxæmia so that oxidation can go on though at a progressively slackening rate until an advanced stage of oxygen deprivation has been reached. When the normal oxidations are no longer possible, the heart may rely upon the breakdown of glucose or glycogen to lactic acid which escapes into the blood. Direct analysis of coronary venous blood taken at the stage of acute dilatation and failure does in fact invariably show an accumulation of lactate in the blood together with an almost complete removal of oxygen.

Although under aerobic conditions addition of lactate to the blood results in a reduced sugar utilisation, yet, as pointed out above, a high lactate level under anoxic conditions, while apparently checking glycogen breakdown, neither lowers the sugar usage nor shortens the survival period of the heart. It may be, however, that the reduction of sugar utilisation is dependent upon lactate being oxidised in its stead, so that as lactate oxidation becomes more difficult with advancing anoxæmia more glucose is used. In the final stages, glycogen may be broken down, but the time available is short and the amount is probably not significant.

The increased usages of lactate often noted in the earlier periods of anoxia may to some extent be explained by the findings of Rühl [1934], who suggested that the rate of lactate uptake by the heart is primarily a function of the coronary flow and so of the state of dilatation of the coronary arteries, an increase in the effective surface increasing the absorption of lactate into the cells. Further, the heart, like skeletal muscle, is no doubt more permeable to lactate than to glucose, which would partially account for the readier usage of lactate by the heart. We may perhaps imagine that when the oxygen tension falls below some critical value, oxidation of lactic acid whether from blood

lactate or from sugar ceases, and sugar-lactate breakdown replaces it. Calculations based on the oxygen saturation of the blood show that in most cases failure occurred when the oxygen tension in the blood was between 15–30 mm. Hg. pressure. There is no evidence of which we are aware, as to whether this glycolysis can serve as a source of energy to the mammalian heart, but if it can it is inadequate to maintain cardiac activity for more than a few minutes. In the frog's heart such glucose-lactate breakdown as is suggested above, with diffusion of the acid into the perfusion fluid where it is neutralised, is apparently adequate for the energy requirements of the ventricle, since anaerobic contraction at a slow rate can continue for many hours in an alkaline fluid containing sugar.

*Work of the Heart.*—This shows a progressive decline as anoxæmia proceeds, and the sum of the usages of lactate and glucose fall comparably. The usage/work ratios do not show great changes as compared with the preceding normal periods; that is, the economy with which the heart employs these sources of energy is not greatly altered, though, arguing teleologically, we might have expected a decrease in the ratio (Table I.). In our experiments the mechanical response and condition of the heart as measured by the outputs and venous pressure is a sensitive indication of oxygen lack, as Clark and co-workers also found in the frog's heart. This is rather against the existence of any important or readily available anaerobic sources of energy other than those we already know, namely, sugar and glycogen. If there was any readily available anaerobic source of energy, then the mechanical condition of the heart should not be so sensitive to oxygen lack.

The changes we have found in the heart during asphyxial conditions without I.A.A. all appear to be a function of the oxygen pressure, that is, of the period during which anoxæmia has progressed, and to be reversible in character, so that even when the heart has been arrested by oxygen lack it can be completely restored again merely by raising the oxygen pressure. The same cannot be said of asphyxia with I.A.A., for we have never succeeded by similar means in restarting a heart poisoned with I.A.A. and then asphyxiated.

*I.A.A. Experiments.*—Gaddie and Stewart [1934], working on the frog's heart, found that in concentrations of 1 : 20,000 or less I.A.A. in the presence of oxygen had no effect on the mechanical response of the ventricle, although greater concentrations exerted a toxic effect, even in the presence of oxygen. The withdrawal of oxygen from the ventricle previously treated with I.A.A. resulted in a rapid loss of activity. There are various possible ways of explaining this effect. Thus, it may be that when oxygen is withdrawn the I.A.A., even when present only in concentrations of 1 : 20,000, can get into the cells and poison them, just as it can do if the concentration is high even in the presence of oxygen. Or, again, there might be a toxic intermediary,

normally removed by oxygen, which accumulates when the oxygen is withheld.

In our experiments the concentrations varied between 1 : 40,000 and 1 : 7800. In hearts so poisoned the usages of lactate and glucose were usually of the same order as those in the control period immediately preceding the first I.A.A. period. The figures (Table III.) show that moderate variations in usages occur, and that the sugar usage is by no means completely inhibited. Further glycolysis in the blood continues and is discussed later.

In these I.A.A. periods there is little or no falling-off in the mechanical

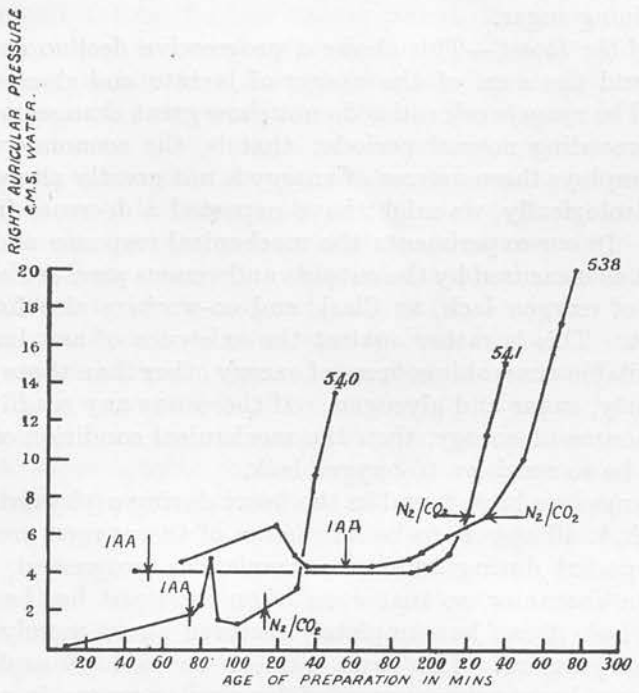


FIG. 4.—Venous pressure curves plotted from continuous records taken during I.A.A. and $N_2/CO_2$ anoxia.

response of the heart, and the usage/unit work ratios do not show significant changes. This fact is clearly shown in expt. 539 (Table III.), where the concentration of I.A.A. was gradually increased over a period of three hours until it reached 1 : 7800. The work of the heart only fell off 25 per cent. of that of the last normal period until the last 30 minutes of I.A.A., when the decrement was 50 per cent.

*I.A.A. and Anoxia.*—After one or two periods of I.A.A. alone the $N_2/CO_2$ mixture was turned on, and the usual observations continued for one or two periods to failure. As in plain anoxia, the usage of lactate continued almost until the end, the mechanism probably being

TABLE III.—THE EFFECT OF GRADUALLY INCREASING ANOXIA ON THE CARBOHYDRATE METABOLISM AND CONDITION OF THE I.A.A. POISONED HEART.

| Expt. | Time, min. | Concentration in blood, mg./100 c.c. start/finish. | | Usage, mg./100 g. heart/hr. | | Sum usage, lactate and glucose, mg./100 g./hr. | Kg.-m. work per hr./100 g. heart. | Usage, mg./kg.-m./hr./100 g. heart. | | Sum usage, lactate and glucose, mg./kg.-m./hr./100 g. | Survival time from start of anoxial period. min. | Outputs, c.c./min. | | | Aortic pressure, mm. Hg. | Total blood volume, c.c. | Heart muscle glycogen, per cent. | Conditions. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lactic acid. | Glucose. | Lactic acid. | Glucose. | | | Lactic acid. | Glucose. | | | L.V. excluding coronary. | R.V. | Coronary. | | | | |
| 528 | 32–61 | 73/58 | 94/74 | 187 | 167 | 354 | 25·0 | 7·48 | 6·68 | 14·16 | | 167 | 428 | 300 | 72 | 757 | | Normal. |
| | 61–91 | 58/37 | 74/68 | 182 | 55 | 237 | 20·8 | 8·75 | 2·64 | 11·39 | | 122 | 370 | 268 | 69 | 680 | | I.A.A. 15 mg. |
| | 91–111 | 37/22 | 68/56 | 208 | 104 | 312 | 16·3 | 12·80 | 6·4 | 19·20 | | 110 | 300 | 220 | 67 | 630 | | I.A.A. |
| | 111–137 | 22/14 | 56/51 | 97 | 48 | 145 | 9·1 | 10·65 | 5·27 | 15·92 | 22 | 33 | 174 | 89 | 47 | 610 | 0·62 | I.A.A. and $N_2/CO_2$ } $N_2/CO_2$ on at 155 min. Heart failed suddenly. |
| 529 | 0–37 | 59/41 | 185/174 | 224 | 53 | 227 | 16·1 | 13·90 | 3·29 | 17·19 | | 212 | 222 | 139 | 69 | 960 | | Normal. |
| | 37–69 | 41/33 | 174/161 | 133 | 48 | 181 | 14·4 | 9·24 | 3·33 | 12·57 | | 174 | 190 | 116 | 66 | 940 | | " |
| | 69–112 | 33/24 | 161/135 | 105 | 97 | 202 | 5·2 | 20·40 | 18·80 | 39·20 | (49)[2] | 50 | 73 | .. | 50 | 920 | 0·55 | I.A.A. 22 mg. Heart failed before asphyxial period. |
| 531 | 0–37 | 67/45 | 181/159 | 219 | 122 | 341 | 21·0 | 10·42 | 5·81 | 16·23 | | 197 | .. | 250 | 87 | 770 | | Normal. |
| | 37–77 | 45/26 | 159/136 | 159 | 122 | 281 | 17·1 | 9·45 | 7·15 | 16·60 | | 147 | .. | 214 | 84 | 740 | | " |
| | 77–115 | 26/8 | 136/120 | 140 | 74 | 214 | 18·0 | 7·80 | 4·12 | 11·92 | | 93 | .. | 228 | 78 | 720 | | I.A.A. 20 mg. |
| | 115–134 | 8/12 | 120/82 | −14 [1] | 275 | .. | 10·1 | .. | 22·74 | .. | 14 | 21 | .. | 245 | 48 | 670 | 0·59 | I.A.A. and $N_2/CO_2$. Failure $N_2/CO_2$ on at 120 min. |
| 538 | 0–67 | 91/42 | 62/36 | 285 | 0 | 285 | 13·7 | 20·80 | 0 | 20·80 | | 230 | .. | 350 | 65 | 1200 | | Normal. 1·5 g. lactic acid added. |
| | 128–163 | 117/86 | 20/8 | 309 | 25 | 334 | 11·0 | 28·10 | 2·27 | 30·37 | | 53 | .. | 147 | 60 | 1080 | | I.A.A. 1·5 g. lactic acid added and 30 mg. I.A.A. |
| | 163–193 | 86/61 | 8/0 | 298 | 75 | 373 | 12·6 | 23·60 | 5·95 | 29·55 | | 97 | .. | 400 | 58 | 1030 | | I.A.A. |
| | 193–227 | 61/26 | 0/0 | 303 | 0 | 303 | 14·0 | 21·70 | 0 | 21·70 | | 92 | .. | 435 | 62 | 970 | | I.A.A. and $N_2/CO_2$. $N_2/CO_2$ on at 195 min. |
| | 227–253 | 26/5 | 0/0 | 204 | 0 | 204 | .. | .. | .. | .. | 58 | 65 | .. | 375 | 62 | 890 | 0·19 | I.A.A. and $N_2/CO_2$. |
| 539 | 0–59 | 133/103 | 163/156 | 181 | 0 | 181 | 18·6 | 9·73 | 0 | 9·73 | | 285 | 460 | 250 | 65 | 1100 | | High lactate and sugar. 1·5 g. lactic acid, 1·0 g. glucose added. |
| | 59–80 | 103/87 | 156/148 | 204 | 10 | 214 | 16·0 | 12·80 | 0·63 | 13·43 | | 250 | 400 | 225 | 64 | 1070 | | I.A.A. 30 mg. 1·5 g. lactic acid, 1·0 g. glucose added. |
| | 122–149 | 191/153 | 210/195 | 442 | 57 | 499 | 17·9 | 24·80 | 3·19 | 27·99 | | 270 | 430 | 250 | 65 | 1020 | | I.A.A. 15 mg. |
| | 149–184 | 153/139 | 195/186 | 393 | 51 | 444 | 15·6 | 25·20 | 3·27 | 28·47 | | 210 | 380 | 240 | 67 | 990 | | I.A.A. 15 mg. 1·5 g. lactic acid, 1·0 g. glucose added. |
| | 202–229 | 224/209 | 244/246 | 230 | 21 | 251 | 16·8 | 13·70 | 1·25 | 14·95 | | 220 | 455 | 250 | 69 | 940 | | I.A.A. 26 mg. |
| | 229–267 | 209/186 | 246/236 | 190 | 126 | 316 | 12·6 | 15·07 | 10·00 | 25·07 | | 155 | 325 | 210 | 68 | 890 | | I.A.A. 35 mg. 1·5 g. lactic acid, 1·0 g. glucose added. |
| | 292–307 | 271/242 | 315/324 | 378 | −100* | .. | 9·2 | 41·10 | .. | .. | (193)[2] | 120 | 150 | 150 | 70 | 830 | 0·47 | |
| 540 | 0–53 | 111/88 | 184/170 | 278 | 62 | 340 | 29·2 | 9·50 | 2·13 | 11·63 | | 150 | 350 | 235 | 82 | 1000 | | Normal. 1·0 g. lactic acid., 1·0 g. glucose added. |
| | 53–100 | 88/70 | 170/150 | 416 | 124 | 540 | 27·4 | 14·95 | 4·46 | 19·41 | | 65 | 400 | 300 | 78 | 970 | | I.A.A. 50 mg. |
| | 126–158 | 128/116 | 191/187 | 208 | −60* | .. | 25·3 | 8·20 | .. | .. | 30 | 75 | 366 | 268 | 74 | 875 | 0·56 | I.A.A. and $N_2/CO_2$. $N_2/CO_2$ on at 132 min. Failed at 162 min. 0·5 g. lactic acid., 0·5 g. glucose added. |
| 541 | 0–60 | 190/172 | 128/112 | 292 | 34 | 326 | 20·9 | 14·02 | 1·63 | 15·65 | | 260 | 260 | 112 | 75 | 1070 | | Normal. 2·0 g. lactic acid, 1·0 g. glucose added. |
| | 60–115 | 172/145 | 112/98 | 304 | 0 | 304 | 19·7 | 15·40 | 0 | 15·40 | | 135 | 350 | 230 | 65 | 1022 | | I.A.A. 50 mg. |
| | 115–175 | 145/120 | 98/92 | 254 | 32 | 286 | 18·2 | 13·95 | 1·75 | 15·70 | | 157 | 313 | 215 | 63 | 992 | | I.A.A. |
| | 175–195 | 120/105 | 92/89 | 208 | 69 | 277 | 12·5 | 16·60 | 5·50 | 22·10 | | 120 | 242 | 145 | 58 | 962 | | I.A.A. and $N_2/CO_2$. $N_2/CO_2$ on at 175 min. |
| | 195–210 | 105/109 | 89/88 | 119 | 29 | 148 | .. | .. | .. | .. | 22 | 94 | .. | 130 | 50 | 932 | 0·60 | Attempted recovery with $O_2/CO_2$ failed. $O_2$ saturation 78 per cent. |

[1] Expt. 531. Possible production of lactic acid. Difference small.

[2] Expts. 529 and 539. Duration of I.A.A. poisoning only.

* Expts. 539 and 540. Production of reducing substance.

the same and dependent upon the oxygen pressure. The sugar usage was variable, tending on the whole to diminish. It is interesting to note that in expt. 539 (Table III.), when the I.A.A. concentration reached 1 : 7800 (without anoxia), the usage of lactate suddenly increased, an apparent production of sugar or some reducing substance occurred, and the heart then failed. An apparent sugar production under I.A.A. (and adrenaline) was indicated by Evans *et al.* [1933]. If there is an increase of reducing substance, it is by no means certain that the substance is glucose, it might be some intermediary formed from glycogen.

During the anoxial periods with I.A.A. the work of the heart does not show a diminution which is any greater than that shown in plain anoxia, but the usage/unit work ratios for lactate are higher than their counterparts in plain oxygen lack, though the ratios for sugar are about the same. This suggests that in the early stages of I.A.A. anoxia the heart comes to rely to an increasing extent upon lactate, perhaps because the usage of other sources of energy has been restricted. It must be borne in mind that these ratios depend partly upon the level of the glucose or lactate in the blood which affects the usage/unit work ratio.

*Time of Survival.*—The same general considerations hold as for the plain anoxial experiments, so that comparison between the two groups may be expected to show the effects of I.A.A. over and above those due to anoxia. The time of survival of the heart is much shorter in these experiments than in those with plain anoxial conditions (Table IV. and fig. 2), though the rate of work in the two groups was about the same. This apparently arises either from the additional restriction of the heart's sources of energy or its additional handicap owing to the presence of a toxic substance, *e.g.* I.A.A. or an intermediary over and above that seen in plain anoxia due to the reduction in oxygen pressure. As in plain anoxia, the glycogen is not greatly affected unless both the sugar and lactate in the blood are low. The average glycogen contents of the hearts in both groups of experiments are very similar, but in each group there is one experiment where both the blood glucose and lactate were low and in which the glycogen was also very low (0·1 per cent.). It appears, therefore, that, despite the presence of I.A.A., glycogen can break down in anoxial experiments just as readily as in plain anoxial conditions when the blood glucose and lactate are low.

Table IV. shows the relationship between the blood sugar and lactate levels at the beginning of the asphyxial period and the time of survival when poisoned with I.A.A. Expts. 531, 528, and 538 suggest that the time of survival is proportional to the blood-lactate level and inversely proportional to the blood-sugar level. A comparison of expt. 538 with expt. 540 shows the effect of sugar, for though the

level of lactate in the former was only about half that in the latter there was no sugar present, and the survival time in this case was the longest of all. It appears that glycogen depletion is prevented by the presence of sugar; the only case in which such depletion occurred was where the sugar was zero in expt. 538. Such an effect of lactate upon the survival time is in accordance with the view previously expressed, that the heart relies more upon lactate than on sugar. But

TABLE IV.—I.A.A./ANOXIA EXPERIMENTS: RELATION BETWEEN BLOOD SUGAR AND LACTATE LEVELS TO TIME OF SURVIVAL.

| Expt. | Concentrations in blood at start of asphyxia, mg./100 c.c. | | Time of survival, in min. | Heart glycogen, per cent. |
|---|---|---|---|---|
| | Glucose. | Lactic acid. | | |
| 531 | 120 | 8 | 14 | 0·59 |
| 528 | 56 | 22 | 22 | 0·62 |
| 538 | 0 | 61 | 58 | 0·19 |
| 541 | 92 | 120 | 22 | 0·60 |
| 540 | 191 | 128 | 30 | 0·56 |

the inhibiting effect of sugar on the survival time and the depletion of glycogen is not so easily explained. It is contrary to the findings in the plain anoxial experiments, where it was the glucose which tended to prolong the survival time, the lactate having no such effect, although it prevented the depletion of glycogen. However, the observations of Gaddie and Stewart [1934] perhaps afford some explanation. They found that after the frog's heart had been asphyxiated in the presence of I.A.A. it could be partially recovered by the addition of reduced glutathione to the perfusion fluid. This partial recovery was at once abolished by further addition of glucose. They suggested that since the I.A.A. reacts with glutathione, the latter being necessary for the breakdown of glucose *via* methyl glyoxal (one of the paths suggested from this and other work), the addition of glucose resulted in accumulation of a toxic intermediary, the removal of which was prevented by the I.A.A. Some such mechanism may be concerned in our experiments. The fact that sugar prolongs the anoxic survival of the normal dog's heart but shortens the anoxic survival of the I.A.A. poisoned heart, is in accordance with the hypothesis that some toxic intermediary product of carbohydrate metabolism is produced in the latter case.

*Glycolysis in the Presence of I.A.A.*—We have also obtained further evidence of the combination of I.A.A. with some substance in the heart. We find that glycolysis does continue in blood samples taken

from the apparatus during the course of an experiment, and which should therefore have contained I.A.A. in a concentration of 1 : 20,000. If, however, the same concentration of I.A.A. is added to blood which has not passed through the heart but is equilibrated with the gas mixture (5 per cent. $CO_2$ in $O_2$ or $N_2$) in a rotating saturator, then glycolysis is inhibited; from this we must conclude that the heart in some way removes or inactivates I.A.A.

## Discussion.

Bogue, Evans, Grande, and Hsu [1935] suggested that lactic acid was burned by the heart to yield energy, and that sugar, though also burned, was mainly used to replace any glycogen used. This has since been confirmed by Bogue, Evans, and Gregory [1937], who also showed that the heart is unable to build up glycogen from blood lactic acid, whatever the conditions. The heart can, however, oxidise glucose or glycogen as well as lactate.

The preferential usage of lactate is now shown to continue even in partially anaerobic conditions. The experimental method used did not permit of the rapid alternation of full aeration with complete deprivation of oxygen, hence the hearts were exposed to a steadily decreasing oxygen tension which finally produced cardiac failure; this occurred when the oxygen tension had fallen to between 15 and 30 mm. Hg. In these conditions it is found that the usage of lactate continues only so long as the oxygen tension is sufficient; but when this is too low the heart changes over to glucose usage with the production of lactic acid, which cannot be oxidised or converted into glycogen. This is in agreement with the findings of Evans *et al.* [1933]. Assuming that the energy set free by glycolysis of blood glucose is available for energy purposes in the heart, the onset of asphyxia will thus cause a gradual shift over from oxidative to glycolytic energy production, which process must therefore be the major source of energy for contraction in the absence of oxygen, so that the survival time may be expected to depend, at least in part, upon the concentration of glucose in the blood.

We would not expect high lactate to prolong anoxic survival, since it is only used oxidatively. If, however, the initial lactate concentration is so low that it is all exhausted before the oxygen pressure becomes too low to permit of oxidation, then it may be supposed that glucose will be used oxidatively instead. That the breakdown of glycogen does not occur rapidly when there are adequate amounts of glucose and lactate present in the blood is apparently due to the greater facility with which the heart is able to use these two substances. A high lactate content has, however, a definite sparing action on the cardiac glycogen. This effect is unexpected, and suggests that the

oxidation of lactate can proceed at a lower oxygen tension than the oxidation of sugar, and hence that the presence of abundant lactate postpones glycolysis to a later stage in the process of gradual asphyxiation.

That lactate alone is able to conserve the glycogen is in agreement with the work of Bogue, Evans, Grande, and Hsu [1935], who showed that the keeping up of either glucose or lactate alone was able to prevent the loss of glycogen under prolonged high work. The sparing of glycogen in the latter stages of asphyxia may be due to the presence of glucose or to the accumulation of lactic acid in the cells.

It seems that extensive glycogen depletion will only occur (*a*) if adrenaline be present, in which case the mobilisation of glycogen is facilitated, and its depletion then occurs in spite of high concentrations of lactate and glucose [Bogue, Evans, and Gregory, 1937]; or (*b*) when the glucose and lactate are low. If both lactate and glucose are low during anoxia, then the glycogen reserve will be used and the glycogen content of the heart at failure will be low, as we have shown. A comparison of expts. 547 and 554 (Tables I. and II.) shows this very clearly. In expt. 547 the lactate was high, glucose absent, glycogen average, and the survival time short (32 min.). Here the heart apparently failed when the oxidative energy from lactate oxidation failed. This seems to indicate that the glycolysis of glycogen provides energy at a rate inadequate for further activity. In expt. 554 the lactate was low, glucose absent, and glycogen low, but the survival time (35 min.) was about the same as in expt. 547. Here we may suppose that the lactate was used up before the oxygen pressure became too low for oxidations to function so that glycogen was used oxidatively. Low glycogen values in anoxia experiments both with and without I.A.A. (expts. 538 and 547) suggest that glycogen is used oxidatively until in the final stages lactic acid or intermediaries accumulate.

Our anoxial experiments are not comparable with those in which cyanide was used [Evans *et al.*, 1933] since in the latter oxidative processes are abolished at once, and consequently no lactate is used, glycolytic processes are called upon, and there is a production of lactic acid.

These results provide certain important negative evidence regarding the cause of cardiac failure; this occurred in many cases when glucose was present in the blood and the cardiac glycogen was nearly normal, and therefore failure was not simply due to depletion of substances for glycolysis.

The buffer power of the blood is high in comparison with the amount of lactic acid produced, and hence inhibition of glycolysis by acidity cannot have occurred. The evidence also shows that lack of oxygen may cause increased breakdown of both blood sugar and glycogen. Chang [1937], working with the isolated rabbit's auricle, found that

this has only a feeble power of maintaining anaerobic activity, even when immersed in alkaline glucose—containing Locke's fluid. He also found that asphyxial arrest occurred when only a small glycogen loss had been sustained although most of the auricular glycogen was glycolysable, since it could be removed by repeated asphyxiation. He suggested that mechanical arrest might be due to phosphagen loss. The authors' results with the dog's heart perfused with blood are in general accordance with the results obtained by Chang with the rabbit's auricle immersed in Locke's fluid, and it is possible that the cause of asphyxial arrest is similar in both cases.

Our results accord equally well with the hypothesis that the cause of cardiac arrest is phosphagen depletion [Chang] or that the glycolytic powers of the heart cannot provide energy adequate for the maintenance of activity under anoxic conditions. Ochoa [1937] has shown that the glycolytic powers of the heart are not so great as those of skeletal muscle. The fact that anoxic survival is prolonged by glucose is opposed to the hypothesis that arrest is due to the production of some unknown toxic metabolite, but accords with the hypothesis that arrest is due to a deficient supply of energy. The presence of glucose presumably spares glycogen to some extent, but since it also prolongs the period before arrest it is not surprising that the cardiac glycogen content at the moment of arrest is similar when the blood glucose is high and when it is low.

Our experiments suggest that falling oxygen tension produces effects in the following order:—

(*a*) A transient increase in the usage of lactate and glucose probably due to increased coronary flow.

(*b*) Reduction of oxidation of glycogen and sugar.

(*c*) Reduction of oxidation of lactates.

(*d*) Increased glycolysis.

In the anoxic I.A.A. poisoned heart failure would be expected to occur as soon as the oxygen pressure is too low to allow of oxidative changes of lactate or of such glucose intermediaries as may be formed. It was, in fact, found that the time of survival in such hearts was comparable with that in anoxia without I.A.A. but with low glucose. In either case glucose is not available for energy production. The effect of high glucose concentration in anoxia in the presence of I.A.A. in still further decreasing the survival time may be explained by the more rapid accumulation of some toxic intermediary or the depletion of some necessary co-enzyme. I.A.A. prevents the formation of lactic acid, but it does not prevent the breakdown of glycogen to intermediate stages; the same probably applies to glucose.

If it is supposed that glycogen produces lactic acid, either (*a*) *via* methyl glyoxal, where I.A.A. combines with glutathione preventing further change to lactic acid, either the accumulation of methyl

glyoxal or the depletion of glutathione (both taking place rapidly if high concentrations of glucose are present) may cause failure—Gaddie and Stewart suggest that glutathione, necessary for the breakdown of glucose *via* methyl gloxal, is inactivated by the I.A.A. as in skeletal muscle, but Ochoa [1937] is of the opinion that, as in skeletal muscle, the methyl-glyoxal pathway is of little physiological importance; or (*b*) *via* phosphorylated intermediaries, when according to Meyerhof and Kiessling's [1935] scheme the presence of I.A.A. would cause an accumulation of dihydroxyacetone phosphate and prevent the formation of adenosine triphosphate from adenylic acid. Either of these, both accumulating faster with high concentrations of glucose, might be toxic.

The finding that I.A.A. alone has no apparent effect on the glucose or lactic acid usage of the heart in conditions as nearly anaerobic as is compatible with continued functioning is in agreement with the work of Clark, Eggleton, and Eggleton [1932] and Gaddie and Stewart [1934]. Clark, Gaddie, and Stewart [1934] have in fact shown that addition of lactate caused revival of the frog's ventricle poisoned with I.A.A. and exhausted by stimulation in air.

The fact that the presence of I.A.A. does not greatly shorten the anoxic survival of the dog's heart indicates that the last stage is a relatively short one in the normal heart, and the simplest explanation for this is that the glycolytic processes do not provide an adequate quantity of energy. Because of the inadequate glycolytic processes the true anoxic period is relatively short, therefore whether the glycolytic processes are curtailed or not by I.A.A. the total time of survival will not be greatly affected.

*Glycolysis in the Blood in the Presence of I.A.A.*—It must be supposed that I.A.A. combines with something in the heart, possibly glutathione or tissue sulphydryl, and is removed from the circulation, so that when samples of blood are afterwards withdrawn for control purposes the blood enzyme systems are still free to cause glycolysis in the blood. Later, when the oxygen pressure becomes too low for the heart to obtain energy by oxidative means it must turn to glycolytic processes, its own enzyme systems become inactivated in turn by the admission to them of I.A.A., so that this source of energy is excluded and rapid failure results. In anoxia with I.A.A. it will be seen that the usage/work ratios are higher for lactate (see Tables I. and III.). This may be because here only oxidative breakdown of lactate is of importance as a source of energy, whereas in asphyxia without I.A.A. oxidative and glycolytic changes go on simultaneously, the latter beginning whilst some lactate is still being oxidised, the change over from the one to the other occurring gradually as anoxia proceeds.

The production of a reducing substance, not necessarily glucose, by the heart in asphyxia presumably from glycogen, since it usually

coincides with lower glycogen values, has been noticed in this (*e.g.* expt. 539) and other work, and is of interest. Possibly during the abnormal conditions in the heart just prior to failure an enzyme is freed which can act upon the heart glycogen with the production of this substance, which under such conditions diffuses out of the muscle into the surrounding blood.


## Summary.

1. In gradually increasing anaerobiosis the heart continues to use glucose and lactate. Failure occurs when the oxygen tension has fallen to between 15 and 30 mm. Hg. The onset of failure is sudden.

2. There is evidence of a production of lactic acid in the terminal stages of asphyxia.

3. The survival time of the heart is prolonged by increased glucose concentration in the blood. The presence of lactate does not appear materially to affect the survival time.

4. Glycogen depletion is prevented by moderate concentrations of glucose and lactate. High lactate alone prevents the depletion of glycogen. But with low lactate and normal glucose some glycogen breakdown can occur.

5. The heart is able to immobilise I.A.A., since the addition of I.A.A. to the circulating blood in the heart-oxygenator preparation does not inhibit glycolysis.

6. Glycolysis is inhibited by the addition of I.A.A. to blood which is not afterwards circulated through the heart.

7. Concentrations of I.A.A. up to 1 : 10,000 have no apparent effect on the usage of glucose and lactate by the heart in aerobic conditions.

8. The time of survival in anaerobiosis is shortened by the presence of I.A.A. The presence of lactate appears to prolong the survival time, while the presence of glucose tends to shorten this time. But glucose prevents the depletion of glycogen under these conditions.

9. The heart, when poisoned with I.A.A. and asphyxiated, continues to utilise glucose and lactic acid, and glycogen if the blood glucose and lactate is low.



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# EFFECT OF ASPHYXIA UPON THE ADENOSINETRIPHOSPHATE CONTENT OF THE RABBIT'S HEART

## EFFECT OF ASPHYXIA ON THE ADENOSINETRIPHOSPHATE CONTENT OF THE RABBIT'S HEART

The writer showed in a previous paper (Chang,1937) that asphyxia in nitrogen of isolated rabbit's auricles suspended in alkaline Locke's fluid caused at the moment of arrest a loss of about one-third of their glycogen and a loss of about two-thirds of their phosphagen content. The results suggested that both glycogen and phosphagen constituted sources of energy for the anaerobic auricles. The writer also noted that under asphyxial conditions, normal mechanical activity of the rabbit's auricles could only be maintained for a few minutes even when the conditions were most favourable for glycolysis, e.g. the supply of alkaline fluid and the presence of glucose, The mechanical activity and the phosphagen fell together and this suggested that phosphagen breakdown rather than glycolysis energized the contraction of the rabbit's auricles.

Work on muscle brei or extract has directed attention to the importance of adenosinetriphosphate in muscle contraction.Lohmann (1934) suggested that in the contraction of skeletal muscle adenosinetriphosphate was first broken down, and this was followed by the breakdown of phosphagen,which provided energy and phosphate for the resynthesis of the adenosinetriphosphate. On the other hand Parnas (1935) believed that phosphagen breakdown constituted the primary process which energized the contraction, while the decomposi-

tion of adenosinetriphosphate only served to restore the phosphagen broken down.

METHODS.

The experiments described below were a continuation of some previous work and were planned to determine the probable role of adenosinetriphosphate in the contraction of the heart muscle. Experimental and chemical methods described previously (Chang,1937) were employed. Glycogen and total acid-soluble-phosphorus values are expressed as mg/100 gm. of heart muscle (wet weight).

The phosphorus compounds were estimated in five fractions: phosphagen, adenosinetriphosphate (pyrophosphate), orthophosphate, soluble and insoluble esters and are expressed as percentages of the total. The nature of the insoluble esters is not clear, the group appears to contain hexosedi- and hexosemonophosphate besides other phosphorus compounds, the soluble esters consist in the main of hexosemonophosphate.

Rabbits were anesthetized with ether and asphyxia was produced by clamping the trachea. In order to avoid any inhibitory influences of the vagi upon the heart that might be provoked by asphyxia, both vagi were cut prior to asphyxiation. The condition of the heart was observed by palpation through the chest wall at the beginning and then by opening the chest cavity at the later stages of asphyxia. The heart usually contracted forcibly for about two minutes after the onset of asphyxia, thereafter, it gadually lost its strength.

After about five minutes, the heart usually became widely dilated, and the contractions were very feeble. Very often heart block and sometimes ventricular fibrillation were observed. After about nine minutes all contractions disappeared in the ventricles and after about fourteen minutes in the auricles. After a certain period of asphyxia, the heart was rapidly cut out and the whole auricle or a portion of the left ventricle was analysed for glycogen and phosphorus compounds.

## RESULTS

Effects of asphyxiation. The results, which are summarised in Table 1 and in Fig. 1, show that two minutes asphyxia caused a reduction of more than two-thirds of the phosphagen, of about one-sixth of the adenosinetriphosphate (pyrophosphate) and of about one-third of the glycogen content. Asphyxia for five minutes produced little further reduction in the phosphagen, but the adenosinetriphosphate was reduced to about two-thirds and the glycogen to about one-sixth of their normal values. Still longer asphyxia until arrest showed little further change in phosphagen and glycogen but the adenosinetriphosphate was reduced to only one-fourth of its normal value. The soluble esters were increased by more than one-third over the normal value, while the insoluble esters showed a small reduction. The results

TABLE I.

Effect of Asphyxia upon the Phosphorus Distribution and Glycogen Content of the Rabbit's Heart ( Phosphorus Fractions expressed as per cent. of Total Phosphorus).

| Description | No. of expt | Phosphagen | Pyrophosphate | Orthophosphate | Soluble ester | Insoluble ester | Mg./100 g. Total P. | Glycogen |
|---|---|---|---|---|---|---|---|---|
| A. Ventricle: | | | | | | | | |
| 1. Control | 11 | 16.9 | 26.6 | 20.5 | 14.0 | 22.0 | 92 | 542 |
| 2. Asphyxia, 2 min. | 2 | 5.0 | 22.3 | 33.5 | 22.9 | 16.3 | 88 | 341 |
| 3. Asphyxia, 5 min. | 2 | 4.1 | 17.7 | 37.2 | 21.5 | 19.5 | 92 | 88 |
| 4. Asphyxia, until arrest | 6 | 4.0 | 6.7 | 47.6 | 22.6 | 19.1 | 90 | 54 |
| 5. Asphyxia after arrest, 30 min. | 3 | 0 | 1.2 | 56.9 | 22.9 | 19.0 | 92 | -- |
| B. Auricles: | | | | | | | | |
| 1. Control | 2 | 17.3 | 21.0 | 20.1 | 15.3 | 25.3 | -- | 512 |
| 2. Asphyxia until arrest | 4 | 7.0 | 8.3 | 42.6 | 24.1 | 18.0 | -- | 133 |

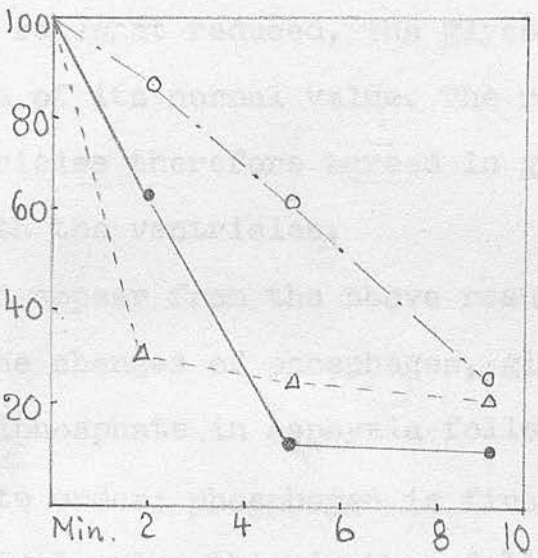


Fig.1.- Effect of asphyxia on phosphagen, pyrophosphate and glycogen content of the rabbit's ventricle. Ordinate: per cent. of normal; abscissa: time in minutes; -o- pyrophosphate; -●- glycogen; -△- phosphagen.

regarding the changes in phosphagen and adenosinetriphosphate agree in general with those just published by Burns and Cruickshank (1937) who asphyxiated excised hearts of cats and dogs perfused with blood. In a few experiments, one piece of the ventricle was excised after the heart was asphyxiated to arrest, and placed in a stoppered test tube containing a little alkaline Locke's solution and freed from oxygen by means of nitrogen. The tube was immersed in a water bath maintained at 36° C. After thirty minutes, analysis of the phosphorus compounds showed that all the phosphagen disappeared and adenosinetriphosphate was reduced to only a trace. Asphyxia of the auricles until arrest (Table 1.B,2.)produced a loss of about three-fifths of the phosphagen and of adenosinetriphosphate and an increase of about two-fifths of the soluble esters. The insoluble esters were somewhat reduced. The glycogen was reduced to one-fifth of its normal value. The results obtained with the auricles therefore agreed in general with those obtained with the ventricles.

It would appear from the above results that in the ventricle the changes of phosphagen, glycogen and adenosinetriphosphate in asphyxia follow one another in a definite order; phosphagen is first reduced to a certain minimal value this is then followed by the loss

of glycogen and finally of adenosinetriphosphate. This sequence of events suggests that phosphagen constitutes the immediate source of energy for the contraction of the heart while the breakdown of glycogen and adenosinetriphosphate provide energy for the resynthesis of the phosphagen broken down. Various authors have shown that in muscle pulp and muscle extracts phosphagen can be resynthesised by the breakdown of adenosinetriphosphate or by the conversion of glycogen to lactic acid (Meyerhof and Lohmann, 1928; 1932). The chemical changes produced by asphyxia of the heart show the following facts of interest.

After five minutes asphyxia, both glycogen and phosphagen are reduced to a minimal value, and still longer asphyxia until arrest produces little further change in either of these substances, yet the heart continues to beat, during this interval, although the adenosinetriphosphate is reduced to about one-fourth of its normal value, while when a piece of quiescent heart muscle is asphyxiated, the breakdown of adenosinetriphosphate proceeds at much slower rate than in the beating heart. These facts indicate that adenosinetriphosphate can serve as a source of energy for the anaerobically beating heart, when other forms of energy are not available. Table 1 (series A and 4) shows however that at the moment of asphyxial arrest the ventricle contains about one-fourth of its normal content of adenosinetriphosphate and since most of this quantity can be removed by

further asphyxia there is no simple relation between asphyxial arrest and depletion of adenosinetriphosphate. On the other hand, both phosphagen and mechanical activity are reduced to a low level after two minutes asphyxia and this coincidence of events suggests that mechanical failure may be due to depletion of phosphagen.

The increase of 'hexosemonophosphate' is interesting because the fact that anaerobiosis leads to an accumulation of hexosephosphate has been repeatedly observed in the skeletal muscle (Eggleton and Eggleton, 1929; Cori,1932). Cori and Cori (1936) believed that hexosemonophosphate was formed from inorganic phosphate, and hexosediphosphate from phosphagen breakdown. The results on the ventricles do not permit one to ascertain how the heoxsemonophosphate was formed in the heart muscle, because asphyxia produced changes simutaneously in all the five fractions of the phosphorus compounds. In the skeletal muscle it is known that iodoacetic acid poisoning produces an accumulation of hexosediphosphate and also of hexosemonophosphate (Lundsgaard,1930; Cori and Cori,1936). The author found that when isolated auricles, suspended in Locke's solution and supplied with oxygen were treated with I.A.A., a reduction of both phosphagen and inorganic phosphate and an increase of both groups of esters occurred (Table XI)*. The reduction of inorganic phosphate corresponds approximately to the increase in soluble esters, while the amount of

*See Table XI, p. 50.

phosphagen broken-down is about equal to the increase of insoluble esters. These results suggest that hexose-monophosphate in the heart muscle, like that in the skeletal muscle, is derived from inorganic phosphate. Since I.A.A. poisoning produces an accumulation of hexosediphosphate in the skeletal muscle, and hexosediphosphate would be included in the fraction of insoluble esters, it appears reasonable to suppose that the increase in insoluble esters in the I.A.A. poisoned auricle is due to hexosediphosphate formed. Weicker (1934) showed that in the isolated rabbit's ventricle I.A.A. poisoning led to an increase in the phosphorus fraction hydrolysable after 180 minutes, a result which agrees with the above supposition. It has been shown that I.A.A. poisoning of the rabbit's auricles abolishes anaerobic activity. The fact that I.A.A. leads to an accumulation of hexosephosphate leads one to conclude that the mode of action of I.A.A. upon the carbohydrate metabolism of the heart and of the skeletal muscle is essentially the same.

Experiments with isolated auricles: Asphyxia in vivo is a very effective method for depleting the heart of glycogen and for reducing the labile phosphorus fraction to a low level. It was found that auricles arrested by asphyxia in situ when placed in a bath of Locke's solution and supplied with oxygen could be revived either completely or partially. Such auricles provided a means for studying the relation between the recovery

of mechanical activity and the recovery of phosphagen and of adenosinetriphosphate. Table III* and Fig. 2* show the results obtained.

When the auricles were revived in fluids at pH 8.2 two-thirds of the phosphagen was restored in about 20 minutes; full recovery of phosphagen took place in about 40 minutes; but the adenosinetriphosphate was only restored to two-thirds of its normal value even when revival time was prolonged to 100 minutes. In a few experiments, the auricles were revived in fluids with pH 7.2, and in this case complete recovery of phosphagen occurred in 60 minutes. But adenosinetriphosphate resynthesis was practically absent even when the time of revival was prolonged to 90 minutes. These results show that alkaline fluid (pH 8.2) is a more favorable medium for the resynthesis of adenosinetriphosphate than nearly neutral fluid (pH 7.2). Parnas and Ostern (1931) showed in the isolated perfused frog's heart that ammonia was formed and that this ammonia was derived from the deaminization of free adenylic acid into inosinic acid (1932). The later acid unless reaminized would not be available for the rebuilding of adenosinetriphosphate.

A few experiments were made to determine whether the imperfect resynthesis of adenosinetriphosphate was due to lack of adenylic acid. Adenylic acid (100-200 ug./30 c.c.) was added to the Lodke's fluid in which the auricle was suspended. This improved the aerobic resynthesis of adenosinetriphosphate since the values

*See Table XII, p. 52, Fig. 7, p. 54.

found after 20 and 75 min were 17.4 and 19.7 per cent. as compared with 12.9 and 14.7 per cent. after 20 and 100 min. respectively when adenylic acid was not added. The rate of recovery of the mechanical activity was not appreciably affected by the addition of adenylic acid.

Fig. 2* shows the manner in which the asphyxiated auricle recovers its activity when immersed in aerated Locke's solution at pH 8.2. The rate of recovery varied greatly in different individuals but in most cases maximum recovery occurred in about 20 or 40 min. The recovery appeared to be fairly complete in some cases, but in other cases only a feeble mechanical activity was restored. There appeared to be some correlation between the extent of the recovery of the mechanical activity and the recovery of the phosphagen content. The correlation was not exact and in many cases the restoration of the mechanical activity was poor althogh the phosphagen was completely restored.

The results show that there is no close correlation between the changes in adenosinetriphosphate content and the mechanical activity. During asphyxia the former fell more slowly than the latter (fig. 1), and similarly during recovery the adenosinetriphosphate

* See Fig.7, p. 54, thesis.

content increased more slowly than did the mechanical response (fig. 2).

Addition of adenylic acid accelerated the recovery of adenosinetriphosphate but did not accelerate the recovery of mechanical activity. On the other hand, in one particular preparation (Table III*, 4 (a) ), in which the adenosinetriphosphate was particularly low, the auricle failed to contract; while in another one with complete adenosinetriphosphate resynthesis the mechanical activity was normal.

Table III* shows that the fraction of soluble esters, composed largely of hexosemonophosphate, varies greatly in different experiments, but the amount tends to decrease with the time of perfusion. The fate of the hexosemonophosphate thus disappearing has not yet been determined, but in the skeletal muscle it has been shown that the hexose is reconverted to glycogen (Cori et al, 1937).

The reponse of revived auricles to asphyxia was studied in a few experiments and the results are shown in Table IV. Auricles when asphyxiated a second time were arrested in a few minutes, either in acid or alkaline fluid, and the addition of glucose to the fluid in the bath, did not prolong the period of survival. In one experiment, the revived auricle was asphyxiated until arrest several times. During the first asphyxia a slight'contracture' was noticed at the moment of arrest but on readmission of oxygen the'contracture' was abolished.

*See Table XII, p. 52, main thesis.

Table IV.- Periods of Anaerobic Survival of Rabbit's Auricles, previously asphyxiated to arrest in situ, but revived in oxygen in vitro.

| | Without Glucose | With Glucose |
|---|---|---|
| pH 6.8 | 3.5 min. | 3.5 min. |
| pH 8.2 | 4.0 min. | 4.5 min. |

At the third asphyxia the 'contracture' was very marked The responses of these auricles are therefore essentially similar to those shown after I.A.A. poisoning. The inability to sustain anaerobic activity in both cases could be attributed to a common cause, namely, absence of lactic acid formation, which is essential for anaerobic activity. The development of 'contracture' in both conditions, is of interest, and in view of the close resemblance in anaerobic metabolism between the revived and I.A.A. poisoned auricles, it is tempting to ascribe the 'contracture' to a common cause. In both cases, the only readily available source of energy in asphyxia is phosphagen, which is rapidly depleted. Although in the case of I.A.A. poisoning the production of abnormal metabolites is possible, in the case of those auricles arrested in situ and revived in vitro, no glycogen was present and any lower carbohydrate would have been removed by repeated asphyxia, there is

therefore no possibility of any abnormal carbohydrate metabolite being produced during asphyxia. It was found moreover that very little 'contracture' occurred in a subsequent asphyxia after addition of glucose, These facts are clearly opposed to the theory that 'contracture' is due to accumulation of abnormal metabolites (Ledebur, 1934). On the other hand, the results suggest that the 'contracture' is due to lack of available energy and is in agreement with the theory of Ritchie (1933). that relaxation rather than contraction of the muscle requires energization.

## DISCUSSION.

The results obtained with asphyxia of the rabbit's heart in situ are in general agreement with those obtained by Weicker(1934) on the isolated rabbit's heart perfused with saline solution and by Burns and Cruickshank (1937) on the isolated hearts of cats and dogs. At the moment of asphyxial arrest, phosphagen, glycogen and adenosinetriphosphate were all found to be reduced to a low level. This result suggested that asphyxial arrest of the rabbit's heart in situ was probably due to depletion of available energy material to a level insufficient to support anaerobic activity. When the asphyxia was continued beyond the time at which it produced arrest, an almost complete disappearance of both phosphagen and adenosinetriphosphate was observed, apparently some phosphagen and adenosinetriphosphate was essential for the maintenance of resting metabolism.

Asphyxia in situ produced mechanical failure in about two minutes, during this interval phosphagen was also reduced to a low level. This coincidence of events suggests that phosphagen loss is responsible for the mechanical failure. The previous finding (Chang,1937) that asphyxia of the isolated rabbit's auricles produced apparently parallel loss of phosphagen and mechanical activity is in agreement with this supposition. The fact that in auricles asphyxiated to arrest in situ and recovered in vitro, the restoration of phosphagen generally coincided with the restoration of mechanical activity also lends support to such a view. On the other hand, the changes in the adenosinetriphosphate content appear to bear no close relation to the changes in the mechanical activity. Asphyxia of the heart produced mechanical failure long before there was an appreciable reduction of adenosinetriphosphate. When an arrested auricle was revived in oxygen in many cases a normal mechanical response could be regained, even when the adenosinetriphosphate was low. On the other hand, addition of adenylic acid accelerated the recovery of adenosinetriphosphate but did not accelerate the recovery of mechanical activity.

There is therefore a definite correlation between the changes in phosphagen content and mechanical activity both during asphyxia and during recovery from asphyxia, but there is less correlation between changes in adenosinetriphosphate content and mechanical ac-

tivity in these conditions. This fact suggests that the mechanical activity is more closely dependent on phosphagen than on adenosinetriphosphate. In contrast to the rabbit's heart in which asphyxia until arrest showed a loss of three-quarters of its adenosinetriphosphate, asphyxia of iodoacetic acid poisoned frog's heart in nitrogen only produced a reduction of about one-fourth of its adenosinetriphosphate(Clark and Eggleton, 1937). This indicates that adenosinetriphosphate of the rabbit's heart is in some way more labile than that of the frog's heart. A greater lability of the adenosinetriphosphate in the mammalian skeletal muscle over the frog's muscle has also been observed (Cori and Cori, 1934).

## SUMMARY

1. Asphyxia of the rabbit's heart in situ produced at the time of arrest a loss of three-quarters of its phosphagen, nine-tenths of its glycogen and three-quarters of its adenosinetriphosphate content. The reduction of the three substances to their minimal value occurred in the following order: phosphagen, glycogen and adenosinetriphosphate. Asphyxial arrest of rabbit's heart in situ can therefore be ascribed to depletion of available sources of energy.

2. When auricles asphyxiated to arrest in situ were revived in vitro, phosphagen was rapidly restored to normal but the restoration of adenosinetriphosphate was much slower and less complete.

3. As regards both asphyxial [asphyxial] changes and changes during recovery after asphyxia the mechanical activity and the phosphagen content varied in a similar manner. The changes in the adenosinetriphosphate content were slower and less extensive and no close relation could be demonstrated between these latter changes and the changes in the mechanical response.

4. 'Hexosemonophosphate' accumulated both during anaerobiosis in the normal heart and in the I.A.A. poisoned auricles under both aerobic and anaerobic conditions.

5. Auricles asphyxiated to arrest in situ, revived in oxygen in vitro and again asphyxiated, responded to asphyxia like auricles poisoned with I.A.A. since they were arrested in two or three minutes and a 'contracture' followed.

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# THE ACTION OF IODOACETATE AND FLUORIDE ON THE ISOLATED RABBIT'S AURICLE.

THE ACTION OF IODOACETATE AND FLUORIDE ON THE ISOLATED RABBIT'S AURICLE. By I. Chang,

Iodoacetate and fluoride act as specific poisons which inhibit certain enzymes essential for the conversion of glycogen to lactic acid. This process is now regarded as being very complex; most of the evidence for the elaborate schemes set forward are derived from experiments with muscle brei or muscle extracts. Such experiments provide only indirect evidence regarding the events which actually occur in an intact muscle, but chemical evidence is so difficult to obtain from the latter that it is necessary to formulate schemes from muscle extract experiments and then to determine whether these account for the behaviour of the intact muscle.

Meyerhof and Kiessling (1935) using muscle extracts showed that, in the presence of glycogen, phosphopyruvic acid and a little adenylic acid, esterification of free phosphate occurred which was inhibited by iodoacetate but not by fluoride.

Cori and Cori (1936) found with intact muscle that iodoacetate caused the accumulation of hexosediphosphate as the result of the anaerobic breakdown of phosphagen. Iodoacetate is believed to inhibit the activity of the mutases which convert triosephosphate into glycerophosphate and phosphoglyceric acid and also to inhibit the reduction of pyruvic acid to lactic acid with the simultaneous oxidation of glycerophosphate to triose-

phosphate or of triosephosphate into phosphoglyceric acid (Green et al, 1937).

Hence iodoacetate causes accumulation of hexose-and triose-phosphates.

Fluoride does not inhibit the formation of phosphoglycerate but prevents its conversion to phosphopyruvic acid (Lohmann and Meyerhof, 1934).

The complex energy exchanges of the combinations of phosphates with intermediate products of carbohydrate metabolism have been summarised by Dorothy Needham (1937).

Little heat is given out as a result of the sum of the glycogenolytic reactions as far as the formation of phosphopyruvic acid; the hydrolysis of phosphopyruvic acid and the formation of lactic acid from pyruvic acid each provides about 8,000 cals per mol lactic acid, and the total of 16,000 cals is equal to the heat liberated when 1 mol of lactic acid is formed from glycogen.

Iodoacetate and fluoride therefore arrest glycogenolysis at different stages but as regards energy release there is no difference between the two stages.

The object of the experiments described in this paper was to compare the effect of iodoacetate and of fluoride on the isolated frog's heart and on the rabbit's isolated auricle under aerobic and anaerobic conditions.

The effect of iodoacetate on the frog's heart is summarised by Clark et al (1938). Iodoacetate causes

irreversible and progressive poisoning which can be arrested by washing out the drug after 20 or 30 min. In this condition the heart can maintain a nearly normal mechanical activity for some hours provided full aerobic conditions are maintained but is arrested in a few minutes by anaerobiosis.

Chang (I937) showed that iodoacetate abolished glycolysis in the isolated rabbit's auricle and caused rapid anaerobic arrest, [but that aerobic survival lasted for less than an hour].

Although numerous experiments on the action of fluoride upon metabolism have been made with the skeletal muscle, particularly the muscle brei, similar studies on the cardiac tissue are few. On the basis of muscle brei experiments, Ochoa(1937) concluded that fluoride inhibited glycolysis in cardiac muscle as well as skeletal muscle and that in both cases the mechanism was the same.

## METHODS

Rabbits of about I kg. in body weight and of either sex were used. Isolated auricles were set up as described previously (Chang, 1937). Locke's solution buffered with $NaHCO_3$ with pH 8.2 was employed. sodium iodoacetate, I:30,000, was added to the perfusion fluid and allowed to remain in contact with the tissue for 20 -30 min. The poisoned fluid was then changed and replaced by clean solution. This procedure establishes fully the effect of the iodoacetate but avoids any progressive

toxic effect upon the tissue. Sodium fluoride was used in a I in 3,000 concentration; unlike I.A.A. the action of fluoride is not progressive but is freely reversible by washing out. Hence when the action of fluoride was studied a constant concentration was maintained throughout the experiment.

In the experiments on the frog's heart, isolated frog's ventricles were set up according to the method described by Gaddie and Stewart (1934) and stimulated electrically at 10-12 per min. Five c.c. of Ringer's fluid buffered with $NaHCO_3$ with pH 8.2 were used for the perfusion. Isochoric records were taken intermittently.

## RESULTS

I. Effect of Iodoacetate upon Aerobic Activity of Isolated Rabbit's Auricle : - Fig. I is a record of the action Of I.A.A. upon the rabbit's auricle in the presence of oxygen. About 10 minutes after the addition of I.A.A., the amplitude and frequency of contraction both decline steadily; 60 minutes later, all contractions disappear. The rate of this depression, however, varies with the individuals; whilst some auricles resemble that shown in Fig. I which can maintain an activity for about an hour, many others cease to contract in 30 or 40 minutes. The rapidity with which iodoacetate inhibits the activity of the isolated auricle is striking; since the chief action of iodoacetate is the inhibition of the breakdown of glycogen into lactic acid, these results immediately suggest that the breakdown of gly-

cogen into lactic acid forms an important source of aerobic energy for the rabbit's auricle. Evidence obtained by other workers supports this suggestion. witting, Markowitz and Mann (1930), Macpherson,Essex and Mann (1931) and Sprague (1933) found that rabbit's heart perfused with saline solution lost their glycogen rapidly. Bogue, et al. (1935) found in isolated heart-oxygenator preparation that dog's hearts showed considerable reduction in glycogen when the blood sugar and lactate were low. Cruickshank and Startup (1933) and Cruickshank and McClure (1936) reported that dog's heart isolated as heart-lung preparation lost about one-third of its glycogen when the blood was rendered nearly free of sugar. While the loss of glycogen in the saline-perfused heart may be partly due to asphyxia, experements with the heart-oxygenator and heart-lung preparation indicate that glycogenolysis takes place even when conditions are fully aerobic.

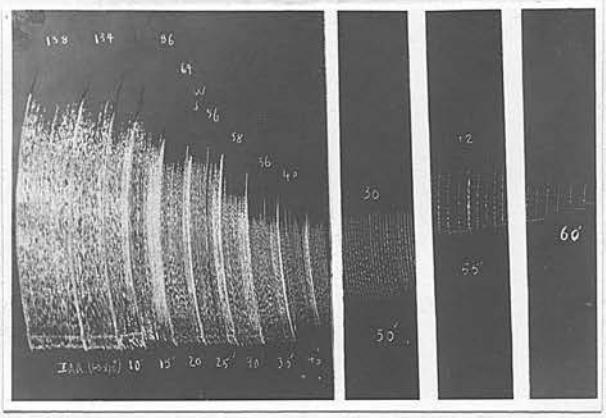


Fig. 1.- Effect of I.A.A. (1-30,000) upon the aerobic activity of the isolated rabbit's auricle. Numbers above fig. = frequency per minute; numbers below fig. = minutes after addition of I.A.A.

2. Effect of carbohydrate and its breakdown products upon the iodoacetate poisoned auricle.

Since the inhibition of aerobic activity of the rabbit's auricle by iodoacetate appears to be due to the inhibition of glycogenolysis, the heart muscle like the skeletal muscle may be expected to obtain energy by the oxidation of lactic acid formed from the breakdown of glycogen. Fig 2 shows the effect of lactic acid upon a rabbit's auricle poisoned and nearly arrested by I.A.A. Lactic acid not only restored the contraction of the auricle but also maintained it at a steady level for as long as 100 minutes.

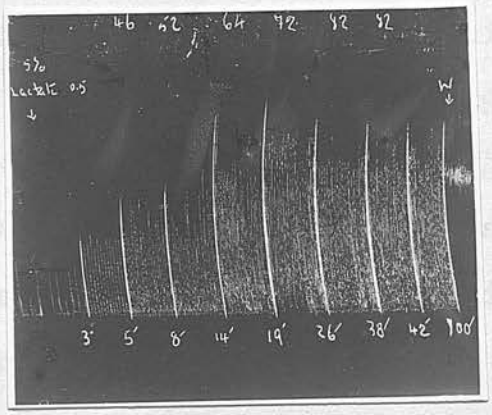


Fig. 2.- Isolated rabbit's auricle poisoned and depressed by I.A.A. in oxygen, revived by lactate but again depressed after removal of lactate at W.; numbers above fig. = frequency of contraction per minute; numbers below fig. = minutes after addition of lactate.

By changing the fluid, so that lactate was removed from the system. the auricle became once more depressed and arrested, while further addition of lactic acid (not shown in the fig.) again revived the auricle to activity. Indeed such a process of alternate arrest and revival could be repeated a number of times on the same auricle. As long as lactate was present in the perfusion fluid, a poisoned auricle could be maintained in fair activity for a period of two to four hours before being arrested finally in irreversible contracture.

Since Bogue, Chang and Gregory (1938) have shown by means of direct analysis that dog's heart poisoned with I.A.A. can use as much lactic acid as does the normal heart and Bogue, Evans and Gregory (1938) have found that lactic acid used by the dog's heart is not converted into glycogen, the above results can only be interpreted to mean that lactic acid is directly utilized by the poisoned auricle as a source of energy for its contraction. The rabbit's auricle can therefore obtain energy by the two stage process of glycogenolysis and the subsequent oxidation of the lactic acid formed, and iodoacetate inhibits the former but not the latter process.

In addition to lactate, fig. 3 shows that pyruvate is also capable of reviving the auricle poisoned and depressed by I.A.A. The effect of pyruvate upon the poisoned auricle closely resembles that of lactate.

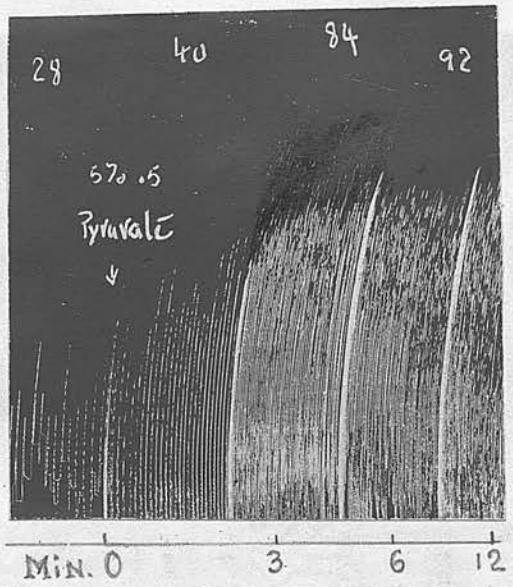


Fig. 3.- Revival effect of pyruvate upon I.A.A. poisoned and depressed rabbit's auricle; numbers above fig. = frequency of contraction per minute.

In both cases, the effect sets in rapidly and restores the power of contraction to about 70 or 80 per cent. of the normal in a few minutes, and maintains the contraction at a fairly steady level for an hour or two. These facts indicate that pyruvate and lactate can be used by the poisoned auricle with equal efficiency.

Of other intermediates in the formation of lactic acid from carbohydrates, phosphoglyceric acid seems also to have a revival effect (Fig. 4), (The sample of phosphoglyceric acid was kindly supplied by Dr.A. Deutsch). Glycerophosphate and glucose were tested but had no effect.

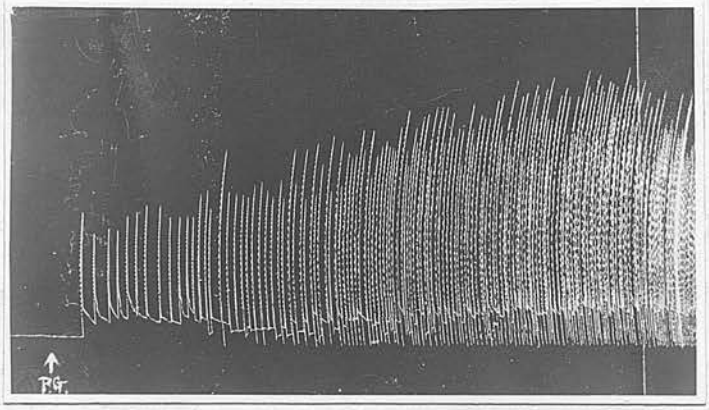

Fig. 4.- Effect of phosphoglyceric acid upon the isolated rabbit's auricle poisoned and arrested by I.A.A. in the presence of oxygen.

A number of substances, namely, succinic acid, fumaric acid and malic acid, which have been suggested as possible intermediates in the formation of lactic acid from glycogen were examined but they gave uniformly negative results.

Clark et al (1938) have shown that the frog's heart poisoned by I.A.A. can oxidise lactate, pyruvate and phosphoglyceric acid but not glycogen, glucose, hexosediphosphate, triosephosphate and glycerophosphate, and have suggested that the aerobic breakdown of carbohydrate at its early stages follows the same route as the anaerobic breakdown. The metabolic activity of the rabbit's auricle poisoned by I.A.A. as far as the oxidation of the intermediates in the breakdown of carbohydrate into lactic acid is concerned, is strikingly similar to that of the frog's heart poisoned by I.A.A.; the above suggestion is therefore also applicable to

to the rabbit's auricle.

3. Effect of fatty acids upon the iodoacetate poisoned auricle.

Several fatty acids were tested, but only propionic acid had a definite revival effect upon the auricle poisoned and depressed by I.A.A. (Fig. 5)*. Other fatty acids, such as caproate, butyrate and valerate, seemed to delay the arrest of the poisoned auricle but had no revival effect. These results suggest that the poisoned auricle can only oxidise a few fatty acids.

4. Effect of amino acids upon the iodoacetate poisoned auricle.

Glycine, alanine and glutamic acid had no effect upon the auricle; these results are in sharp contrast to those obtained with the frog's heart poisoned and exhausted by I.A.A., since the frog's heart under these conditions can oxidise effectively a number of amino-acids, including alanine and glutamic acid which proved to be ineffective in reviving the rabbit's auricle poisoned by I.A.A. Experiments with the dog's heart-lung preparation have proved, by quantitative estimations, that the mammalian heart unlike the frog's heart does not utilize amino-acids (Tsuji, 1916; Cruickshank and McClure; 1936). In this connection, it is interesting to note that while pyruvic acid is readily utilised by the poisoned auricle, alanine which is convertible by the usual process of deaminization to

* See Fig. 3, p. 25, thesis.

pyruvic acid cannot be used by this tissue . This suggests that the failure to utilise amino-acids is perhaps due to the inability to deaminise amino-acids.

5. Effect of Fluoride Upon the Frog's heart and Rabbit's auricle: - Sodium fluoride, I-3,000 was added to the perfusion fluid bathing the rabbit's auricle, this produced little effect upon the mechanical activity which remained practically steady for two to three hours, provided oxygen was passed through the solution. This result is in agreement with that obtained by Ferrannini (1936) who found that sodium fluoride in concentrations of 1 in 10,000 and 1 in 3,000 actually improved the activity of the isolated rabbit's auricle and never observed arrest within a period of one hour. This proves that the mechanism destroyed by fluoride is not essential for the activity of the auricle provided there is adequate oxygenation. When the fluoride poisoned auricle was deprived of oxygen by the passage of a stream of nitrogen into the solution, the mechanical response, as is shown in Fig. 6*, fell sharply and systolic arrest occurred in three or four minutes.

Similar results were obtained with the frog's heart. Provided oxygen was passed through the solution, a frog's heart poisoned with fluoride, 1-3,000, could continue to contract for a period as long as 9 to 10 hours usually with only a small diminution in activity. Some typical results are shown in Table 1. These results are, however, not in agreement with those obtained by

* See Fig.4, p.38, thesis.

Table I. Effect of Fluoride Upon the Aerobic Activity of the Frog's heart.

| Concentration of NaF. | Mechanical Response as percentage of the normal after addition of fluoride | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 3 | 5 | 7 | 9-10 hrs. |
| 1-5,000 | 100 | 100 | 86 | 76 | 76 | 86 |
| ,, | 100 | 100 | 95 | 95 | - | - |
| 1-3,000 | 100 | 100 | 90 | 83 | 80 | 50 |
| ,, | 100 | 100 | - | 70 | 70 | 70 |

Gottdenker and Rothberger (1935) who found that fluoride in concentrations as low as 1 in 100,000 or 1 in 50,000 produced irregular effects upon the frog's heart and that these effects included marked diminution of mechanical activity and arrest. On the other hand, the same authors found that fluoride at a higher concentration (1-8,000) improved the activity of the isolated rabbit's auricle. Since Gottdenker and Rothberger perfused their hearts beating at their own rhythm with Straub cannula containing only 1 c.c. of fluid, it is probable that their hearts were subjected to asphyxia. The author found that asphyxia of frog's heart poisoned by fluoride invariably led to rapid diminution in activity and arrest. These results are summarised in Fig.7*. The rate of asphyxial depression of the poisoned frog's heart varied with the concentration of fluoride employed, but in all cases arrest occurred in 15 to 30 minutes. On readmission of oxygen to the perfusion fluid, about half of the arrested hearts could be revived, and the activity regained was about 70 per cent. of the normal. When these hearts were asphyxiated for

* See Fig.2, p.20, thesis.

a second time, arrest took place in 7 to 10 minutes, and the rate of arrest was again proportional to the concentration of fluoride used. In all cases, the addition of glucose had no revival effect. Previous experiments indicated that under similar conditions a frog's heart exhausted of carbohydrate available for glycolysis by repeated asphyxiation was arrested within 10 or 15 minutes after the begining of asphyxia. This shows that most of the time elapsing between the onset of asphyxia and the arrest of the fluoride poisoned frog's heart is due to removal of oxygen from the system.

The response of the hearts poisoned with fluoride towards asphyxia is in sharp contrast with that of the normal hearts. Under similar conditions the normal rabbit's auricle can continue to maintain a feeble activity for 20 or 30 minutes, while the frog's heart, in alkaline glucose-Ringer's fluid, can beat with a mechanical activity equal to about 80 per cent. of the normal for an hour or even longer. Since it has been shown that under anaerobic conditions hearts obtain their energy from glycolysis and glycolysis in the cardiac tissue is inhibited by fluoride (Clark, Gaddie and Stewart; 1932,1934; Ochoa, 1937), the most obvious explanation for the effect of fluoride upon anaerobic activity of the rabbit's auricle and frog's heart is that fluoride inhibits glycolysis and therefore abolishes anaerobic activity.

## Discussion.

Table 2 summarises the effects observed with cardiac tissue of the frog and the rabbit. The normal frog's heart can, under favourable conditions, maintain anaerobic activity for a long time but the rabbit's auricle does not possess this power. Anaerobic glycogenolysis appears to be a relatively more effective process in the frog's heart than in the rabbit's auricle, but this difference is not of importance for the purposes of the present discussion.

Fluoride poisoning produces similar effects in the two hearts, they can maintain aerobic activity but are rapidly arrested by anaerobic conditions. Iodoacetate produces the same effects as fluoride in the frog's heart but in the rabbit's auricle there is a striking difference in that this is fairly rapidly arrested under aerobic conditions.

The most important fact shown by these results is that under aerobic conditions the frog's heart survives longer with fluoride than iodoacetate and this difference is still more clearly shown with the rabbit's auricle. If the theories based on muscle extract experiments are accepted and if it be assumed that aerobic and anaerobic degradation of carbohydrates follow the same initial stages this difference implies that cardiac tissue can oxidise phosphoglyceric acid.

Table 2. Approximate survival periods of isolated frog's ventricle and rabbit's auricle.

| | Frog's Ventricle | | Rabbit's Auricle | |
|---|---|---|---|---|
| | Aerobic | Anaerobic | Aerobic | Anaerobic |
| Normal with glucose | days | hours | >10 hours | <60 min. |
| Fluoride Poisoned | 10 hours | 10 min. | some hours | 10 min. |
| Iodoacetate | 4-5 hours | 10 min. | 30 min. | 10 min. |

On the other hand it appears that the iodoacetate poisoned mammalian heart cannot oxidise the triosephosphates although it can oxidise phosphoglyceric acid (fig. 4). It would appear probable that the frog's heart can oxidise triosephosphates to a certain extent since the iodoacetate poisoned frog's heart survives aerobically so much longer than the iodoacetate poisoned mammal's heart.

It must be noted however that the action of iodoacetate appears to be less specific than that of fluoride. Experiments on muscle brei have shown that fluoride in moderate concentration inhibits glycolysis almost completely but has little effect on the total oxygen uptake whilst iodoacetate in concentrations sufficient to arrest glycolysis also produces a progressive inhibition of respiration (Lipmann, 1928; Meyerhof and Boyland, 1931). Therefore iodoacetate

appears to interfere with non-carbohydrate metabolism in addition to abolishing glycolysis.

As regards the action of fluoride, the concentration used 1 in 3,000 should suffice to abolish glycolysis. It is true that the Ringer's fluid contains calcium and that the solubility of calcium fluoride is only 1 in 20,000, but Gottdenker and Rothberger (1935) have shown that calcium fluoride produces similar effects to sodium fluoride on the isolated frog's heart. The rapidity of the anaerobic arrest induced by fluoride indicates that its effectiveness in abolishing glycolysis is equal to that of iodoacetate.

It has been shown that fluoride does not inhibit the activity of methyl glyoxylase (Lohmann,1933) and since it abolishes the anaerobic activity of both the frog's and the rabbit's heart, therefore it appears that the formation of lactic acid via methyl glyoxal is not an important source of energy for the anaerobic heart.

In general the results described can be accounted for on the supposition that the aerobic and anaerobic degradation of carbohydrate follow the same initial stages but that phosphoglyceric acid can be oxidised directly by the heart without being first converted to lactic acid.

## SUMMARY

1. Iodoacetate inhibits aerobic as well as anaerobic activity of the isolated rabbit's auricle. Under aerobic conditions, a rabbit's auricle inhibited by I.A.A. can be revived by lactate, pyruvate, phosphoglycerate and propionate. Glucose, glycerophosphate, caproate, butyrate, valerate, malate, succinate, fumarate, and amino-acids, including glycine, alanine and glutamic acid have no revival effect.

2. Fluoride has no action upon aerobic activity of the frog's heart and the isolated rabbit's auricle but inhibits their anaerobic activity. It is suggested that fluoride, while it inhibits glycolysis, has no action upon the oxidation of non-carbohydrate material.

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