LACTIC ACID METABOLISM IN THE ISOLATED HEART (HEART LUNG PREPARATION).

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It is well known that sugar can be utilised by muscle as a source of energy and the main product of its activity is carbon dioxide. Rohde(1) and Evans(2) found that the gaseous metabolism of the isolated heart is nearly the same as that of the entire animal. Locke and Rosenheim(3) showed that glucose disappears from the fluid used to perfuse an isolated heart. Patterson and Starling(4) confirmed their results and have shown that when hearts are treated with adrenalin glucose disappears much more quickly from the circulating blood than it does in the case of normal hearts without such addition. Evans(5) ascribed this disappearance of glucose from the circulating blood to its oxidation to the end product, CO_2. Evans and Ogawa(6) found that the total gaseous exchange of the isolated heart is increased by the addition of adrenalin and that the power of the tissue to utilise carbohydrate is not interfered with by its addition.

The present experiments were undertaken to determine in what conditions the lactic acid, known as the intermediate metabolite of muscle tissue, increases or diminishes in the case of the isolated heart, and its relation with the soluble nitrogenous constituents (amino-acid, urea and ammonia) contained in the circulating blood.

With regard to the origin of lactic acid formed in the tissues, various views are held. Some authors ascribe it to the disintegration of carbohydrate (glucose), while others suggest the deaminisation of amino-acid (alanine) as its source. This question remains still undecided.

However, that the formation of lactic acid in the tissue is due to the vital processes of the tissue cells is evident from the observations of Gaglio(7), Salkowski(8), Fletcher(9) and others. Of importance is the observation of Minkowski(10), who found that the lactic acid in the urine of geese increases after the extirpation of the liver in the

same proportion as the increased ammonia and the decreased uric acid. v. Frey(11) and Schöndorff(12) have shown that if muscle be kept alive by perfusion with defibrinated blood, a slightly increased amount of ammonia is formed; the former author added that there is neither increase nor decrease of urea in the blood after circulation. Asher and Jackson(13) found that the increase of lactic acid in the blood circulated through live muscle is associated with the formation of soluble nitrogenous constituents. In opposition to these results Parnas and Wagner(14) found in their experiments on muscle autolysis that the increase of lactic acid is not accompanied by any rise in the amount of nitrogenous constituents.

The most special notice must be taken of the recent works of Embden and his co-workers. Embden and Almagia(15), Norden and Embden(16), Embden and Kraus(17), carried out experiments on the artificial circulation through the liver, and came to the conclusion that lactic acid is formed in a liver free from glycogen and that the lactic acid is markedly increased by adding alanine or sugar to the circulating blood. The experiments of Embden and Kraus(18) and Embden, Kalberlah and Engel(19) on the compressed fluid of muscle, and the experiments of Fletcher on minced muscle, indicate that the lactic acid in tissue is produced neither from carbohydrate nor from alanine and might be derived from an unknown substance contained in the cell protoplasm. Tuerkel(20), who investigated the formation of lactic acid in autolysed liver, came to the same conclusion.

Methods. Dogs were used throughout the experiments. The heart-lung preparation was made by the method described by Knowlton and Starling(21). The blood employed for circulation was obtained from the carotid of one or two other dogs under chloroform-ether anæsthesia. For the estimation of lactic acid Embden's method(22) was used, Hurtley's(23) apparatus being employed for ether extraction. Ammonia was determined by Folin's method, urea by urease method, and the total amino-acid nitrogen by van Slyke's method(24). In the two former cases the protein in the blood was removed by dialysed iron and a small amount of hydrochloric acid was added to the filtrate which was then evaporated on the water-bath; in the latter case alcohol was used for the removal of protein and the filtrate was evaporated *in vacuo*, the trace of protein remaining being removed by a small amount of dialysed iron.

1. *The lactic acid formation in the normal heart.*

In the first place it was sought to determine whether, when the heart is beating and well supplied with air, there is any increase of lactic acid in the blood flowing through it.

Exp. 1. Dog. Heart 63 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·2° C. Periods of 90 minutes.

	At beginning	At end
Lactic acid ...	·0185 ...	·0418

Exp. 2. Dog. Heart 52 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·5° C. Periods of 110 minutes.

	At beginning	At end
Lactic acid ...	·0308 ...	·0452
Nitrogenous constituents		
Amino-acid-N. ...	·00489 ...	·00524
Urea-N.	·0102 ...	·0107
Ammonia-N. ...	·00303 ...	·00513

From these two results it is seen that there is a definite rise in the amount of lactic acid in the blood circulated through the heart lung preparation under normal conditions.

That the lactic acid in the blood perfusing through the live organ (muscle, liver and lung) is always increased, was determined by many authors (Gaglio, Frey and Asher and Jackson, and others); moreover we know from many observations of previous authors that the lactic acid produced in tissues is one of the normal metabolites. We might conclude that lactic acid is produced in the normal heart lung preparation as well as CO_2. Before deciding in this sense we must be quite certain that the supply of the heart muscle with oxygen in the experiments mentioned was adequate. The production of lactic acid in the absence of oxygen is a well established fact in the case of voluntary muscle and might be expected also for heart muscle. The experiments which I shall quote later arouse the suspicion that the arterial pressure adopted in these experiments, namely 60 mm. Hg., was hardly sufficient to maintain an adequate flow of blood through the coronary vessels. It will be seen that, when the pressure was raised, so bringing about a greater flow through the coronary vessels, the lactic acid in the blood was not increased but was diminished. That the production of lactic acid can be still further increased by deficient oxygenation is shown in the next series of experiments.

With regard to the nitrogenous constituents, there is some increase of ammonia after circulation, but no great difference is seen in the amount of amino-acid or urea.

2. *The formation of lactic acid in deficient oxygenation.*

Since Araki(25) discovered that lactic acid is produced by a deficient supply of oxygen in the tissue, his results have been frequently confirmed. For this purpose the artificial respiration was stopped for six minutes in the following experiment.

Exp. 3. Dog. Heart 54 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·5° C. Artificial respiration stopped for 6 mins.

	Before	After
Lactic acid ...	·0556 ...	·0688

Although not so largely as expected, the lactic acid is apparently increased by stopping the artificial respiration. It must not be overlooked that the amount of lactic acid in the blood used for my experiments may have been much larger than that of normal blood owing to the fact that it was under chloroform narcosis. Hopkins and Fletcher(26) and others indicate that there is some final maximum in the formation of lactic acid in muscle. It is possible that when a large amount of lactic acid is contained in the blood perfused through the heart, it may more or less inhibit the production of lactic acid from the tissue.

3. *The formation of lactic acid in chloroform poisoning.*

There is definite evidence that when the tissues are poisoned by chloroform, lactic acid may be produced. The following experiment was carried out to confirm the effect of chloroform on the formation of lactic acid in the case of the heart lung preparation.

Exp. 4. Dog. Heart 80 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·4° C. Chloroform was mixed with the air of artificial respiration for 10 minutes.

	Before	After
Lactic acid ...	·0324 ...	·0440
Nitrogenous constituents		
Ammonia ...	·00616 ...	·00728

An increase of lactic acid is seen associated with a rise of ammonia.

4. *The effect of adding amino-acids to the circulating blood.*

That the formation of lactic acid in the tissues has an intimate relation with the production of soluble nitrogenous constituents is considered evident by some authors (Minkowski (liver) and Asher and Jackson (muscle)). In my experiment I attempted to determine

whether amino-acid added to the circulating blood would increase the production of lactic acid and whether the amino-acid would be consumed by the work of the heart.

(1) *Alanine.* Among the many amino-acids, alanine suggests itself for trial in view of its constitution, and there are many experiments which indicate that lactic acid may be produced by deaminisation of alanine in the tissues.

Exp. 5. Dog. Heart 62 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·5° C. The circulation was continued for 100 minutes after adding 4 gms. of L-alanine to the blood. The heart beat was markedly accelerated. Three minutes after adding alanine the blood was taken for the first estimation.

	At beginning	At end
Lactic acid ...	·0486 ...	·0248
Nitrogenous constituents		
Amino-acid-N. ...	·0532 ...	·0532
Urea-N. ...	·0112 ...	·0112
Ammonia-N. ...	·00560 ...	·00560

Contrary to my expectation, the lactic acid markedly diminished after adding alanine. There was no difference in the amounts of all three nitrogenous constituents.

(2) *Glycine.*

Exp. 6. Dog. Heart 68 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·4° C. Periods of 20 minutes after adding 5 gms. of glycine. The blood for the first estimation was taken three minutes after adding glycine. The effect of glycine on the heart beat was not so remarkable as that of alanine, but the apparent acceleration of the heart beat was still observed.

	At beginning	At end
Lactic acid ...	·0222 ...	·0201

Exp. 7. Dog. Heart 55 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·4° C. Periods of 15 minutes after adding 5 gms. of glycine. Otherwise the same as the previous experiment.

	At beginning	At end
Lactic acid ...	·0472 ...	·0457

Owing to insufficiency of the blood used for circulation these two experiments could not be continued so long as the previous experiments. However it is seen that the lactic acid was again found diminished by adding glycine.

(3) *Ereptone.*

Exp. 8. Dog. Heart 71 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·5° C. Periods of 30 minutes after adding 5 gms. of ereptone. The heart beat was remarkably accelerated. The blood for the first estimation was taken three minutes after adding ereptone.

	At beginning	At end
Lactic acid ...	·0571 ...	·0454
Nitrogenous constituents		
Amino-acid-N. ...	·0454 ...	·0432
Urea-N.	·0207 ...	·0211
Ammonia-N. ...	·0134 ...	·0139

As regards the three nitrogenous constituents the difference found may quite possibly be due to errors of estimation.

The question may arise, what is the cause of the diminution of lactic acid after the addition of amino-acids? Is it due to the acceleration of the heart beat or to the special action of amino-acids?

5. *The effect of adding adrenalin to the circulating blood.*

In order to ascertain whether the diminution of lactic acid was caused by the acceleration of the heart beat, adrenalin was added to the circulating blood.

Exp. 9. Dog. Heart 47 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·5° C. Periods of 50 minutes after adding 2 c.c. of adrenalin ($\frac{1}{10000}$). The effect of adrenalin on the heart beat was almost the same as that of alanine and ereptone. The blood for the first estimation was taken three minutes after adding adrenalin.

	At beginning	At end
Lactic acid ...	·0293 ...	·0253
Nitrogenous constituents		
Amino-acid-N.	·00650 ...	·00701
Ammonia-N. ...	·00735 ...	·00700

Exp. 10. Dog. Heart 62 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·4° C. Periods of 35 minutes after adding 2 c.c. of adrenalin ($\frac{1}{10000}$). The effect of adrenalin on the heart beat was the same as in the previous experiment. The blood was taken before adding adrenalin for the first estimation and 15 minutes after adding adrenalin for the second estimation.

	Before	After	At end
Lactic acid ...	·0281	·0175	·0176
Nitrogenous constituents			
Ammonia-N. ...	·00336 ...		·00336

It will be seen that a marked diminution in the amount of lactic acid occurs as a result of adding adrenalin to the circulating blood. From the results of this experiment the diminution of lactic acid in the previous experiment must be ascribed to the acceleration of the heart beat brought about by the amino-acid. The amount of ammonia still remains negligible.

Why acceleration of the heart beat should cause diminution of the lactic acid in the blood is not at first sight evident. We may think that since the total metabolism, as shown by Evans, goes up *pari passu*

with the heart rate, the supply of oxygen would be more deficient in the accelerated heart and the formation of lactic acid increased. But we know that adrenalin, in addition to its action on the rate of the heart beat, causes marked dilatation of the coronary vessels. It seems possible that some action might be produced by the amino-acids; if this were the case, an extra supply of oxygen to the heart muscle might more than counterbalance the effect of the increased rate so that the production of lactic acid would be diminished and this substance actually utilised for the work of the heart and completely oxidised to CO_2.

6. *The effect of increasing the work of the heart.*

This experiment was carried out to determine whether the lactic acid contained in the circulating blood might be diminished by increasing the work of the heart by means of alterations in the blood-pressure.

Exp. 11. Dog. Heart 55 gms. B.P. 100 mm. V.P. 70 mm. Temp. 36·5° C. Periods of 15 minutes.

	At beginning	At end
Lactic acid ...	·0380 ...	·0362

Owing to the entrance of air into the circulation, this experiment could not be continued. It will be seen however that there is a slight diminution in the amount of lactic acid. In this experiment again, the result seems at first sight the reverse of what might be expected. The heart, doing more work, requires a greater supply of oxygen, but the effect of the increased arterial pressure on the coronary circulation is sufficient to more than counterbalance the increased oxygen needs of the heart, so that the condition of the organ is actually improved by the rise of arterial pressure.

7. *The effect of stimulating the vagus.*

Since adrenalin, in accelerating the heart beat, caused a diminution in the lactic acid, it seemed of interest to determine whether, when the heart beat is inhibited by stimulating the vagus, there is an increase in the lactic acid.

Exp. 12. Dog. Heart 82 gms. B.P. 60 mm. V.P. 70 mm. Temp. 36·6° C. The vagus was stimulated by Faradisation for six minutes and then the blood-pressure was altered from 60 mm. to 150 mm. and the circulation continued for 30 minutes.

	Before	After	At end
Lactic acid ...	·0527	·0644	·0360
Nitrogenous constituents			
Ammonia-N. ...	·00896	·01064	

As was expected, the lactic acid is apparently increased by stimulating the vagus and again diminished by increasing the work of the heart. As is seen from the table, with the increase in lactic acid there is some rise in the amount of ammonia.

We can hardly interpret these results as showing any qualitative effect of the vagus on the metabolism of the heart. The results are comparable with those I have already recorded on the effect of changes in the arterial pressure on the production or utilisation of lactic acid by the heart muscle. I am inclined to ascribe the whole effect obtained here on stimulation of the vagus to the diminution of pressure thereby induced and the consequent lessened flow through the coronary vessels.

Discussion.

It has been argued by several observers that there is a formation of lactic acid in mammalian muscles kept alive by artificial circulation, and that when the same muscles are excised the production of lactic acid may be increased. In my experiments also this formation of lactic acid occurs in the normal heart lung preparation. It is however of interest that on increasing the work of the heart or on accelerating its beat, not only is there no further production of lactic acid, but that which has already been formed disappears from the circulating blood. Hopkins and Fletcher have shown that if a fatigued muscle be exposed to pure oxygen, the lactic acid may disappear within two hours, that is, the muscle possesses in itself a chemical mechanism for the removal of lactic acid. Evans and Ogawa have shown that when the heart beat is accelerated by adding adrenalin to the circulating blood, there is a definite rise in the respiratory quotient accompanied by an increasing consumption of sugar. The disappearance of lactic acid from the circulating blood on increasing the work of the heart must be ascribed to the fact that the lactic acid may be consumed by the heart muscle; that is to say, when the heart muscle is exercised in a sufficient supply of oxygen, the foodstuff (carbohydrate) is completely oxidised to the end product of metabolism (CO_2) as the source of energy, instead of leading to the formation of lactic acid. On the other hand, any diminution in the blood flow through the heart muscle, even such a diminution as may accompany a lowering of the blood-pressure almost within physiological limits, may lead to a marked formation of lactic acid. Under normal circumstances, however, it would seem that the metabolism leads to the production of CO_2 and

that the production of lactic acid with its accumulation in the blood must be regarded as a morbid condition.

As regards the extractive nitrogenous constituents there is a slight rise in the amount of ammonia associated with the increase in lactic acid. As the formation of ammonia has no relation to the work of the heart, we may suppose that the muscular activity does not consume the protein. Moreover, the fact that no change was found in the amount of amino-acid nitrogen contained in the blood or added to the blood as the result of the work of the heart, may indicate the same conclusion. The question may arise, whether the ammonia formation is a primary product of the disintegration of cell protein together with the formation of lactic acid, or whether it is a secondary result required naturally for the protection of the organism against acid poisoning. Although my experiments are not sufficient to decide this question, it seems quite probable that the formation of ammonia is secondary in character since it appears that muscular activity does not at all increase protein metabolism, the lactic acid, known as an intermediate metabolite, being produced from other foodstuffs, mainly from carbohydrate.

Conclusions.

1. Lactic acid is produced in the circulating blood of a heart lung preparation under conditions approaching normal.
2. These results may indicate that lactic acid is one of the normal metabolites of muscular activity.
3. The formation of lactic acid is increased in poisoning by chloroform and in the presence of a deficient supply of oxygen in a heart lung preparation.
4. When the heart beat is accelerated by adding adrenalin or amino-acid (alanine, glycine or ereptone) to the circulating blood, or the heart work is increased by alterations in the blood-pressure, lactic acid is not only not produced, but the lactic acid previously contained in the circulating blood disappears. On the other hand lactic acid is produced when the heart beat is inhibited by means of stimulating the vagus.
5. These effects may probably all be explained as due to changes in the coronary circulation and in the oxygen supply to the heart.
6. There is a slight rise in the amount of ammonia associated with the increase of lactic acid.

7. The amino-acids contained in the circulating blood or added thereto cannot be utilised by the heart.

8. There is no evidence of the formation of urea in the heart.

In conclusion I desire to express my thanks to Professor Starling for suggesting this work and to Professor Bayliss and Dr Plimmer for their kindness in supplying the amino-acids used in this investigation.

REFERENCES.

(1) Rohde. Ztsch. f. physiol. Chem. LXVIII. S. 181. 1910.
(2) Evans. This Journal, XLV. p. 213. 1912.
(3) Locke and Rosenheim. Proc. Physiol. Soc. p. xiv. 1904. This Journal, XXXI.
(4) Patterson and Starling. This Journal, XLVII. p. 137. 1913.
(5) Evans. Ibid. XLVII. p. 406. 1913.
(6) Evans and Ogawa. Ibid. XLVII. p. 446. 1913.
(7) Gaglio. Du Bois Reymond's Archiv, S. 400. 1886.
(8) Salkowski. Ztsch. f. klin. Med. XVII. S. 77. 1890.
(9) Fletcher. This Journal, XLIII. p. 286. 1911.
(10) Minkowski. Arch. f. exp. Path. u. Pharm. XXI. S. 41. 1886.
(11) v. Frey. Du Bois Reymond's Archiv, S. 533. 1885.
(12) Schöndorff. Pflüger's Archiv, LIV. S. 420. 1893.
(13) Asher and Jackson. Ztsch. f. Biol. XLI. S. 393. 1901.
(14) Parnas und Wagner. Biochem. Ztsch. LXI. S. 381. 1914.
(15) Embden und Almagia. Zntrlb. f. Physiol. XVIII. S. 832. 1905.
(16) Norden und Embden. Zntrlb. f. Stoffw. N.F. 1. S. 1. 1906.
(17) Embden und Kraus. Biochem. Ztsch. XLV. S. 1. 1912.
(18) Embden und Kraus. Kongr. f. inn. Med. 1909.
(19) Embden, Kalberlah und Engel. Biochem. Ztsch. XLV. S. 58. 1912.
(20) Tuerkel. Ibid. XX. S. 431. 1909.
(21) Knowlton and Starling. This Journal, XLV. p. 146. 1912.
(22) Embden. Embden's Hdb. d. biochem. Arbeitsmeth. v. 2. S. 1254. 1911.
(23) Hurtley. Plimmer's practical Organic and Bio-chemistry, p. 598. 1915.
(24) Ibid. pp. 136, 146, 548, 550, 558, 575, 578. 1915.
(25) Araki. Ztsch. f. physiol. Chem. XV. S. 335 u. 546. 1891.
(26) Hopkins and Fletcher. This Journal, XXXV. p. 298. 1906–7.