THE LACTIC ACID IN THE BLOOD OF A RESTING MAN. By C. N. H. LONG[1], M.Sc.

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A study of the work done on the lactic acid content of the blood of a resting man shows that there is some difference of opinion as to whether this acid is really present under true resting conditions. A number of observers employing several different methods have investigated the question. Berlinblau(1) estimated the acid as zinc salt and says it is a constant constituent of human blood. He gives the figure 8 mgrs. per 100 c.c. as the usual amount found. Salomon(2) using the same method states that lactic acid is absent during life but rapidly appears in the blood after death. Most of his determinations were made on hospital patients. Jerusalem(3) estimated the acid by oxidation with dilute permanganate solution, after the acid had been extracted with ether; the aldehyde formed was caught in bisulphite solution and estimated. Although he used large amounts of blood he could detect no lactic acid. This is remarkable as all other observers who have used an oxidative method have obtained positive results. Fries(4) using the same method as Jerusalem but chiefly on the blood of hospital patients found values varying from 9–63 mgrs. per 100 c.c. Ryffel(5) estimated the acid colorimetrically, by oxidation with strong sulphuric acid and the use of Schiff's reagent. His values were about 15 mgrs. per 100 c.c. Barcroft(6) using Ryffel's method found values in healthy young men of 12–19 mgrs. per 100 c.c. Clausen(7) greatly improved the technique of the permanganate method and states the usual amount in man is 15–32 mgrs. per 100 c.c. Barr, Himwich and Green(8) using Clausen's method found values of 14–25 mgrs. My own values using the same method show a value ranging from 10–20 mgrs. per 100 c.c.

At this point the criticism of Clausen(7) on the estimation of lactic acid in blood by oxidative methods should be noted. He says: "It must be emphasised that biological fluids probably contain substances other than lactic acid which are extracted by ether and which yield bisulphite binding compounds on oxidation."

The bulk of the above evidence is in favour of lactic acid being a

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normal constituent of human blood, even under resting conditions, but in addition to Clausen's statements as to the possibility of other substances giving rise to aldehydes and also forming similar zinc compounds we have to consider the work of Lovatt Evans(9) on the formation of lactic acid in drawn blood by glycolysis. This glycolysis is easily prevented by the addition of small amounts of sodium fluoride, but none of the above workers except Barr, etc.(8), used this precaution and it is very likely that some at any rate of the lactic acid present may have had its origin from the blood sugar after the withdrawal of the blood sample.

Again some workers used the blood of hospital patients for their determinations. The lactic acid found under these conditions may have appeared as the result of some pathological changes in the subject and indeed the high values obtained by Fries(4) seem to suggest this.

Lastly, in all the foregoing investigations no special precautions were taken to see that the subject was in a true resting condition when the blood was withdrawn, since, in most cases, the results were comparative ones only. The movements of everyday life would probably account for the appearance of a certain amount in the blood. The possibility of this is emphasised by the work of Hartree and Hill(10) on the recovery heat production. They found that the recovery process in the isolated muscle appears to go on at a rate roughly proportional to the square of the concentration of the bodies produced during the preceding activity. Thus when the concentration of lactic acid produced by the stimulus is small the recovery process will be very slow. Now all muscular exertion liberates some lactic acid in the muscles, from which it tends to diffuse into the blood; its final or oxidative removal probably occurs mainly or entirely after its diffusion back again into the muscle. When the concentration of lactic acid in the muscles and blood is low the speed of the recovery process tending to remove it—being proportional to its square—will be extremely small, and the last phase of complete recovery will take a very long time. It is necessary therefore, in such experiments, that the subject should have been completely at rest for a long period before the sample of blood is taken.

Use of the thiophene test. Since the permanganate method estimates other substances as lactic acid besides the acid itself it was necessary to make use of a more specific test for lactic acid. The one used was that devised by Fletcher and Hopkins(11). These authors say that this test will detect 1 mgr. of lactic acid, but I have found that about $\frac{1}{2}$ mgr. of the acid can be detected in the form of its zinc or lithium salt.

The blood samples were taken from healthy young men who had had no strenuous exertion for some hours previously and who had lain down at least ½ hour before the blood was withdrawn. The blood (venous) was fluorided and oxalated as soon as drawn. 10 c.c. of the sample were taken and proteins removed by the method of Folin and Wu(12). Glucose was then removed from the filtrate by the method of Van Slyke(13). The solutions were then slowly evaporated to dryness on the water bath and the residue extracted with hot absolute alcohol. The excess of alcohol was removed and the test performed on the residue.

Blood, however, contains substances other than lactic acid which are extractable by alcohol and which might yield a positive thiophene test. In order to see if this was occurring the test was performed on the following substances which are usually present in normal blood, viz.: urea, uric acid, cholestrol, amino-acids, glucose, creatine aceto-acetic acid, and acetone, etc. None of these substances either in the solid state or in the alcohol gave a positive reaction. It appears then that in the case of blood, at least, a positive thiophene test points to the presence of lactic acid itself.

Experiments have been carried out in this manner on four different subjects and lactic acid appears to be a constant constituent of the blood even under resting conditions. In several of the experiments an attempt was made roughly to estimate the amount of lactic acid indicated by the thiophene test and to compare the value obtained with that given by Clausen's method. To do this the thiophene test was performed on known amounts of lactic acid (as zinc lactate) at the same time as it was carried out on the blood filtrate. The colour obtained in the latter was then quickly matched against the standards and a rough estimate of the amount of lactic acid present was made.

The figures found seem to indicate that one-half to three quarters of the values given by Clausen's method are due to the lactic acid itself, the remainder being due presumably to other hydroxy acids.

TABLE I.

Subject	Lactic acid content of blood. Mgrs. per 100 c.c. (Clausen)	Thiophene test (Qualitative)	(Quantitative) Mgrs. per 100 c.c.
H. L.	17·5	Positive	12–15
A. S. P.	35·6	Positive	—
S. S.	21·4	Positive	10–15
C. N. H. L.	17·5	Positive	10–15
C. N. H. L.	27·5	Positive	6–8

Effect of oxygen and carbon dioxide on the resting lactic acid. If lactic acid be indeed a normal constituent of resting blood, as these thiophene

determinations suggest, it is desirable to ascertain whether any agency is capable of reducing or altering this lactic acid concentration.

The first method attempted was that of breathing pure oxygen which might be expected to accelerate the oxidation and removal of lactic acid. This appears to be completely unsuccessful as the sequel shows. The next method tested was that of breathing a fairly high percentage (8–11) of carbon dioxide in pure oxygen. This mixture was chosen for two reasons, firstly Anrep and Cannan(14) have shown that in the heart-lung preparation the lactic acid content of the circulating blood may be greatly reduced by increasing the hydrogen ion concentration, the best way to do this being to add CO_2 to the respired air; secondly by breathing the CO_2 in pure oxygen it is easier to continue for much longer than would be the case if the gas was breathed in air. Even in oxygen half an hour's breathing of 8 p.c. CO_2 produces severe headache lasting several hours, in addition to nausea and giddiness while the CO_2 is being inhaled. The alterations produced in the blood by breathing such a mixture are shown in the experiment quoted below. I have to thank Mrs R. Conway-Verney for the determinations of the CO_2 content and the *p*H. The *p*H was determined by Dale and Evans' dialysis method(15).

Subject C. N. H. L. Mixture breathed 8 p.c. CO_2, 92 p.c. O_2 for 30 mins.

Blood sample taken	Thiophene test	CO_2 content of venous blood c.c. p.c.	*p*H
After breathing air ½ hour	Positive	46·2	7·50
After breathing mixture ½ hour	Negative	54·5	7·40
After breathing air for a further ½ hour	Positive (faint)	42·8	7·40

This result shows that the *p*H of the blood can be changed by CO_2 breathing and also that the thiophene test which is positive in air becomes negative on breathing the mixture for 30 minutes. It should be noted that in a blood sample taken 30 minutes after the mixture had been breathed the thiophene test had become faintly positive again.

This change in the thiophene test from positive to negative after $CO_2 + O_2$ breathing has been repeated three times on one subject (C. N. H. L.). Of two experiments on another subject (A. S. P.) one gave no change in the thiophene test, but the other gave a very obvious change from positive to negative, when breathing a rather higher percentage of CO_2 in O_2. The blood samples were drawn in all cases (i) after breathing air at rest for 30 minutes, (ii) after breathing the mixture for ½–¾ hour. They were treated exactly as described above although as

a rule no quantitative measurement of the lactic acid content was made.

As stated above, the breathing of a high concentration of oxygen has no effect on the lactic acid in the blood. The thiophene test remains positive and the amount determined by the Clausen technique is unchanged. This is shown in the following table. The results of the CO_2 breathing experiments are also included.

TABLE II.

(*a*) Breathing oxygen mixtures.

Subject	% O_2 breathed and time breathed	Thiophene test: before (air)	Thiophene test: after (gas mixture)	Lactic acid mgrs./100 c.c. blood (Clausen): before (air)	Lactic acid mgrs./100 c.c. blood (Clausen): after (gas mixture)
H. L.	50 % for 30 mins.	Positive	Positive	—	26·8
H. L.	Pure O_2 for ¾ hour	—	—	17·6	17·5
C. N. H. L.	Pure O_2 for ¾ hour	—	—	9·6	11·3
C. N. H. L.	Pure O_2 for 1 hour	Positive	Positive	—	—
S. S.	55 % for ½ hour	Positive	Positive	—	—

(*b*) Breathing $CO_2 + O_2$ mixtures.

C. N. H. L.	11 % CO_2, 89 % O_2 for 50 mins.	Positive	Negative	27·5	22·8
C. N. H. L.	8 % CO_2, 92 % O_2 for 25 mins.	Positive	Negative	—	—
C. N. H. L.	8 % CO_2, 92 % O_2 for 30 mins.	Positive	Negative	—	—
A. S. P.	7·5 % CO_2, 92·5 % O_2 for 30 mins.	Positive	Positive	35·6	30·2
A. S. P.	8·1 % CO_2, 91·9 % O_2 for 30 mins.	Positive	Negative	—	—

It would seem probable that the presence of lactic acid in the blood of a resting man is due to the fact, already discussed above, that the recovery process is relatively much slower after very mild exercise than after rather more severe. Corresponding to any steady state of exercise there is a certain concentration of lactic acid in the active muscles, determining the speed of the recovery process required to balance the breakdown and to maintain the steady state. Twice as much continued exertion should require twice the speed of the recovery process and therefore about $\sqrt{2}$ times the concentration of lactic acid in the active muscles. Conversely, reducing the muscular exertion from a degree requiring 1000 c.c. of oxygen per min. to one requiring 250 c.c. of oxygen should only diminish the steady state concentration of lactic acid to about $1/\sqrt{4}$, *i.e.*, to a half. Now even at "rest" there is always a certain amount of muscular activity and the steady state of lactic acid concentration corresponding to that activity will not fall as fast as the degree of activity itself so that even at rest a small but finite

concentration of lactic acid in the muscles might be expected to occur. This steady concentration of lactic acid in the muscles would be accompanied by a corresponding lactic acid concentration in the blood, owing to the diffusion equilibrium existing between them. It would seem probable therefore that the "resting" lactic acid concentration found in the blood really is a genuine physiological effect, corresponding to that in the muscle, and determining the speed of the recovery processes of which the measure is the oxygen consumption.

Summary.

1. The thiophene test of Fletcher and Hopkins has been used to show the presence of lactic acid as a constant constituent of the blood of healthy young men at rest.

2. Attempts have been made to estimate roughly this amount by means of this test, and results show that $\frac{1}{2}$ to $\frac{3}{4}$ of the resting "lactic acid" estimated by Clausen's method is lactic acid itself, the rest being other substances which yield bisulphite binding compounds on oxidation.

3. The breathing of pure oxygen for an hour does not remove this resting lactic acid from the blood.

4. If 8–11 p.c. of carbon dioxide in pure oxygen is breathed for $\frac{1}{2}$ hour the blood no longer gives a positive thiophene test. The disappearance of the lactic acid is associated with a changed hydrogen ion concentration of the blood. This is in agreement with the results of Anrep and Cannan on the heart-lung preparation.

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